ONLINE ISSN 2651-3579

Journal of the Entomological Research Society

Volume: 25 Part: 3 2023

JOURNAL OF THE ENTOMOLOGICAL RESEARCH SOCIETY

Published by the Gazi Entomological Research Society

Subscription information

Published by GERS in single volumes three times (March, July, November) per year. The Journal is distributed to members only. Non-members are able to obtain the journal upon giving a donation to GERS.

Papers in *J. Entomol. Res. Soc.* are indexed and abstracted in Science Citation Index Expanded (SCIE), Zoological Record, Biological Abstracts, BIOSIS Previews, Scopus, Essential Science Indicators, ProQuest, Scimago Journal & Country Rank (SJR), IPIndexing Portal, Resurchify, ORES, Academic Accelerator, CiteFactor, scinapse, Acarindex, Publons, TR Dizin.

Publication date: November 17, 2023

J. Entomol. Res. Soc., 25(3): 455-465, 2023
Doi: 10.51963/jers.v25i3.1982

Research Article
Online ISSN:2651-3579

Mapping of Spatial Behavior of *Scirtothrips perseae* (Insecta: Thysanoptera) in Avocado Orchards Using Geostatistical Techniques

Agustín David ACOSTA GUADARRAMA[1] José Francisco RAMIREZ DAVILA[2]*

Atenas TAPIA RODRIGUEZ[3] Dulce Karen FIGUEROA FIGUEROA[4]

Rodolfo SERRATO CUEVAS[5]

[1,4,5]Faculty of Agricultural Sciences, Autonomous University of the State of Mexico, Campus el Cerrillo, Piedras Blancas, Toluca State of Mexico, MEXICO

[2,3]Laboratory of Entomology Faculty of Agricultural Sciences, Autonomous University of the State of Mexico, campus el Cerrillo, Piedras Blancas, Toluca State of Mexico, MEXICO

e-mails: 1davinn@hotmail.com; [2]jframirezd@uaemex.mx; [3]atapiar938@alumno.uaemex.mx; [4]dk_figueroa@hotmail.com; [5]rserratoc@uaemex.mx

ORCID IDs: [1]0000-0001-5301-077X; [2]0000-0002-8625-4655; [3]0000-0003-4739-8469; [4]0000-0002-1482-5295; [5]0000-0002-5639-4542
*Corresponding Author

ABSTRACT

Currently, the implementation of efficient programs of integrated pest management are of vital importance, especially due to the fact that avocados produced in the study area are sold abroad. This favors the adoption of new technologies to reduce the application of agrochemicals and perform integrated pest management more effectively. The objective of the research was to determine the spatial distribution of thrips in commercial avocado orchards. To conduct the mapping of the spatial distribution, geostatistics was used, since it allows to know the location and mobility patterns of the pest, the present study was carried out in the municipality of Donato Guerra in 2019, Donato Guerra is located in the central zone of Mexico, and it is among the main avocado producers of the state. The distribution of the pest insect was incorporated for all the samples, these samples were taken every fifteen days for a year, obtaining 24 samplings in total. A semivariogram was determined for each sampling date, these semivariograms were adjusted to spherical models in most of the cases, the level of spatial dependence was determined to be high in all dates. The infested area was calculated for all the samplings and maps of pest were made through the kriging technique.

Keywords: Kriging, semivariograms, avocado, spatial distribution.

Acosta Guadarrama, A.D., Ramirez Davila, J. F., Tapia Rodriguez, A., Figueroa Figueroa, D. K., Serrato Cuevas, R. (2023). Mapping of spatial behavior of *Scirtothrips perseae* (Insecta: Thysanoptera) in avocado orchards using geostatistical techniques. *Journal of the Entomological Research Society*, 25(3), 455-465.

Received: November 23 2021 *Accepted: July 09, 2023*

INTRODUCTION

Avocado (*Persea americana* Mill.) ranks first in capital inflow from exports of agricultural products of Mexico (SE, 2019); the south-central zone of the country-particularly states like Mexico, Puebla and Morelos- have acquired in the last few years a great importance in production and commercialization of this crop, ranking second place in production at national level, (SIAP, 2019). By increasing the area under cultivation, phytosanitary problems increased as well, pest like thrips, whitefly, borers, scale insects, among others damaged not only the orchards but the commercial quality of the fruit. The insects commonly called thrips are found in the crop feeding on the small fruits (Salgado, 1993), their damage can be seen through the ridges and bumps protruding in the pericarp (González et al, 2000); these deformations become more visible as the fruit reaches maturity (Fisher & Davenport, 1989; González et al, 2000). Species like; larvae and adults of *Scirtothrips perseae*, cause damage on young foliage, creating distortion and dark scars on the underside of the leaf, along the leaf veins; something similar happens to the young fruit, which can become completely covered by a dark scar. Although, frequently more small scars can be observed, and occasionally elongated scars are observed as well, (Ascensión et al, 1999; Johansen, Mujica, & Ascensión, 1999; Hoddle, 2002), this symptom associated to thrips causes major damage, because it represents huge economical losses for avocado growers, due to the rejection of the product as exportation quality fruits.

A tool being used to monitor pests like thrips in avocado is geostatistics, because it provides a more accurate measure of behavior and spatial distribution in avocado orchards; these tools consider the bidimensional nature of the distribution of organisms through their exact spatial location, besides, this distribution may be represented through maps that are very useful in decision making (Isaaks & Srivastava, 1988; Rossi, Mulla, Journel, & Franz, 1992; Sciarretta, Trematerra, & Baumgartner, 2001; Blom & Fleischer, 2001; Ramírez, González, Ocete, & López, 2002). The objective of this research was to characterize the spatial distribution of *Scirtothrips perseae* in commercial avocado orchards that allow to know graphically the spatial distribution of the pest insect and its location in time and space.

MATERIALS AND METHODS

The study was carried out in 2019 from January to December, in Donato Guerra, State of Mexico; This site has appropriate characteristics for avocado production, such as an average altitude of 2,200 masl, an annual average temperature of 19.2 degrees Celsius, and an average rainfall of 1,000 mm. For this study, four plots were selected, one in each cardinal point of the municipality, each one with an area of four hectares, at the same time, weed control was carried out conventionally in each plot (agricultural machinery was used). Also, 200 trees were randomly selected, all of them without insecticides treatment, each tree was georeferenced using DGPS (Trimble Brand, PRO-XR model) to establish its geographic coordinates, the sampling was carried out twice a month, to obtain a total of 24 samplings throughout the year.

To count thrips, number eight yellow plastic cups (Plastifestival brand) covered with a commercial adhesive (spider-plus) were used as recommended by (Hernández, Ramos, De la Paz, & González, 1999). Sixteen glasses distributed in each tree were placed, 3,200 glasses in total for the entire experimental unit, four glasses were placed in each cardinal point of the tree, the count was done visually and manually, to later place the glass in the same place, where it was first placed. Later they were identified in the Entomology Laboratory of the Faculty of Agricultural Sciences of the Autonomous University of the State of Mexico. A statistical analysis was performed on the original data of the *Scirtothrips perseae* populations for each sampling in order to evaluate normality; for this, the asymmetry coefficient and the kurtosis test were performed. All the data was found to have a normal distribution.

The geostatistical analysis was carried out with the estimation of the semivariogram, which was done based on the acquired data from the samplings of thrips populations (Journel & Huijbregts, 1978; Isaaks & Srivastava, 1989).

The experimental semivariogram for each sampling in the experimental plots was performed using the program Variowin 2.2 (Software for Spatial Data Analysis in 2D. Spring Verlag, New York; USA).

Once the experimental semivariograms corresponding to each sampling date of the different stages were estimated, they were adjusted to a theoretical semivariogram. The level of spatial dependence was calculated in order to determine how strong the relationship between the obtained data and the theoretical model was. This value is obtained dividing the nugget effect by the sill, expressed in percentage: less than 25% is high; between 26 and 75% is moderate and over 76% is low (Cambardella et al*,* 1994; López et al, 2002). The corroboration of the adjusted models to the experimental semivariograms was carried out with the cross validation procedure (Isaaks and Srivastava, 1989), which eliminates a sample value. The geostatistical interpolation method called kriging, as well as, the model of the semivariogram being validated were used to estimate the value of the variable of interest at any given sampling point. The parameters of the model being validated are the nugget effect, the sill and the range, they are modified in a trial and error procedure until the cross validation statistics are obtained.

The statistics are the following: mean of the estimation errors (MEE), mean square error (ECM), dimensionless mean square error (ECMA); an additional statistic to validate the aptitude of the model consists in the variance of errors being less than the variance of the sample.

Once the semivariograms were obtained, mapping of density of thrips was carried out using the ordinary kriging technique (this method was used since the sample mean and sample variance had been determined). Kriging technique is an accurate estimate and its equations do not depend on the values measured, but on their positions and the semivariogram they do. This technique also allows the possibility of obtaining maps of the spatial distribution of the studied organisms, which, in turn, have wide usefulness (Samper & Carrera, 1996). Kriging was performed with the program Surfer

9.0, the estimations are represented through maps for each adult sampling date. The infested area was calculated with the obtained maps (Sánchez, Ramírez, González, & De León, 2011; Ramírez, Solares, Figueroa, & Sánchez, 2013).

RESULTS AND DISCUSSION

The presence of *Scirtothrips perseae* was registered in all 24 samplings carried out in the municipality of Donato Guerra; results show that for the first sampling in March the highest population density was found with 46.7 organisms per tree, on the other hand, November presented the lowest population density with only 2.11 organisms per tree. It is important to mention that the highest population densities were found when the phenological stage corresponded to flowering, as mentioned by Quiñones et al (2015) who argue that the highest population densities of thrips are due to the presence of flowers in the avocado tree, therefore, the damage reflected on the fruit is explained by the presence of the organisms when there were still in the flowering stage.

The semivariograms were adjusted mostly to spherical models, except the second sampling in July, which was adjusted to a Gaussian model (Table 1). In reference to the geostatistical analysis, the spatial dependence was high in all sampling dates, which is attributed to the existence of a high spatial ratio in each sampling point. Regarding the parameter nugget effect, its value was zero in all sampling dates, indicating a minimal sampling error, so the sampling scales were appropriate for this study. The simple ranges fluctuated between 25.19 in July and 58.9 in September (Table 1). The range values found, express that the validity of the adjusted models extents to reasonable distances to explain the phenomena aggregation of the insect population.

Table 1. Parameters (Pepita Effect, Plateau and Range) of the models fitted to the semivariograms of *Scirtothrips perseae* in Donato Guerra, State of Mexico.

Month	Model	Sample mean	Range	Sill	Nugget	Spatial dependence level
January 1	Spherical	4,57	45,60	17,06	0	High
January 2	Spherical	6,26	43,10	25,70	0	High
February 1	Spherical	15,00	44,39	139,50	0	High
February 2	Spherical	34,28	39,86	649,20	0	High
March 1	Spherical	46,70	38,62	728,87	0	High
March 2	Spherical	27,94	43,22	311,57	0	High
April 1	Spherical	5,44	45,32	17,40	0	High
April 2	Spherical	6,41	45,62	18,67	0	High
May1	Spherical	4,89	41,80	10,75	0	High
May2	Spherical	4,16	49,40	10,60	0	High
June 1	Spherical	3,49	44,12	9,41	0	High
June 2	Spherical	2,15	57,03	4,16	0	High
July 1	Spherical	6,39	53,47	27,77	0	High
July 2	Gaussian	10,15	25,19	114,05	0	High
August 1	Spherical	14,33	54,00	108,75	0	High
August 2	Spherical	11,18	52,20	85,44	0	High
September 1	Spherical	5,72	55,80	19,48	0	High
September 2	Spherical	4,71	58,90	11,92	0	High
October 1	Spherical	3,83	47,50	8,16	0	High

Table continued

Month	Model	Sample mean	Range	Sill	Nugget	Spatial dependence level
October 2	Spherical	4,54	55,10	9,31	0	High
November 1	Spherical	4,83	57,00	10,52	0	High
November 2	Spherical	2,11	41,80	3,18	0	High
December 1	Spherical	5,19	54,40	11,39	0	High
December 2	Spherical	7,76	45,00	11,58	0	High

Table 2. Values of the cross validation statistics of the semivariograms obtained in the samplings in the municipality of Donato Guerra: mean of the estimation errors (MEE), mean square error (ECM) and dimensionless mean square error (ECMA).

Month	Sampling	Sample mean	Sample variance	MEE	Error variance	ECM	ECMA
January	1	4,57	18,28	0,14 ns	11,65	0,13	1,11
January	2	6,26	28,91	0,11ns	19,03	0,10	1,10
February	1	15,00	145,62	0,10ns	101,29	0,11	1,13
February	2	34,28	668,43	0,09ns	388,07	0.08	1,09
March	1	46,70	749,54	0,13ns	451,04	0,11	1,06
March	2	27,94	410,85	0,10ns	214,98	0,13	1,12
April	1	5,44	19,98	0,12ns	10,52	0,14	1,10
April	2	6,41	21,90	0,07ns	16,77	0,07	1,08
May	1	4,89	24,78	0,11ns	20,53	0,09	1,11
May	2	4,16	10,99	0,08ns	8,13	0,06	1,13
June	1	3,49	9,69	0,10ns	7,48	0,11	1,11
June	2	2,15	4,48	0,12ns	2,71	0,12	1,09
July	1	6,39	23,47	0,09ns	18,51	0,10	1,06
July	2	10,15	278,51	0,14ns	176,31	0,13	1,08
August	1	14,33	130,90	0,10ns	111,90	0,08	1,14
August	2	11,18	109,14	0,13ns	96,42	0,14	1,10
September	1	5,72	22,76	0,08ns	15,06	0,11	1,12
September	2	4,71	12,31	0,07ns	9,59	0,12	1,06
October	1	3,83	8,55	0,11ns	6,22	0,08	1,33
October	2	4,54	10,26	0,14ns	7,05	0,13	1,11
November	1	4,83	11,61	0,12ns	8,59	0,07	1,14
November	2	2,11	3,72	0,09ns	1,96	0,11	1,10
December	1	5,19	11.92	0,10ns	8,37	0,06	1,08
December	2	7,76	2,32	0,08ns	1,50	0,13	1,32

To calculate the infested area, the density maps prepared by the kriging method were used. The infested area was calculated for each sampling date (Table 3), this studied area surpassed 50% in every sampling date, even in four sampling dates, it reached 99%; the lowest value found was 56%. The affected area is not directly linked to the population fluctuation, this means there can be high rates of infested area, but low population fluctuation. This calculation is used to know how the pest behaves in regards to its adaptation within the crop.

Table 3. Infested and non-infested area (%) obtained in the sample of *Scirtothrips persea* in the central zone of Mexico.

Month	Sample	% infested	% non-infested
January	1	84	16
January	2	99	1
February	1	81	19
February	2	82	18
March	1	93	7
March	2	91	9
April	1	86	16
April	2	64	36
May	1	94	6
May	2	86	14
June	1	80	20
June	2	71	29
July	1	56	44
July	2	62	38
August	1	77	23
August	2	70	30
September	1	85	15
September	2	67	33
October	1	65	35
October	2	68	32
November	1	97	3
November	2	99	1
December	1	99	1
December	2	99	1

Another key tool in the spatial analysis of pests is density mapping (Fig. 1), as mentioned before, in the present study, 24 samplings were taken, the infestation maps graphically show the pest behavior in the study zone, aggregation centers of the pest were observed, this confirms the models of the semivariograms that each sampling date was adjusted to.

Papers like the one of Maldonado et al (2016) show the spatial distribution of insects, in this case the semivariograms of the spatial distribution of thrips were adjusted to a spherical model which in biological terms implies that the insects were present in some points more than in others. This means that there were aggregation centers of the pest which was also found in the works of Jiménez et al (2013), who studied the spatial distribution of thrips in husk tomato crops. The Gaussian model found in July indicates that the pest has a continuous distribution within the plot which implies an expansion within it, (Alves et al, 2006).

The elaboration of density maps used in pest behavior studies as reported by Quiñones et al (2015) the spatial distribution and infestation maps of thrips in gladiolus crops, and Jiménez et al (2013) who did the same in husk tomato crops, found out that the insects were found in aggregation centers. Density maps may be very useful in an integrated management of thrips, since it makes possible the direct control measures towards the specific infestation points which can be located in the map, as reported by García (2004).

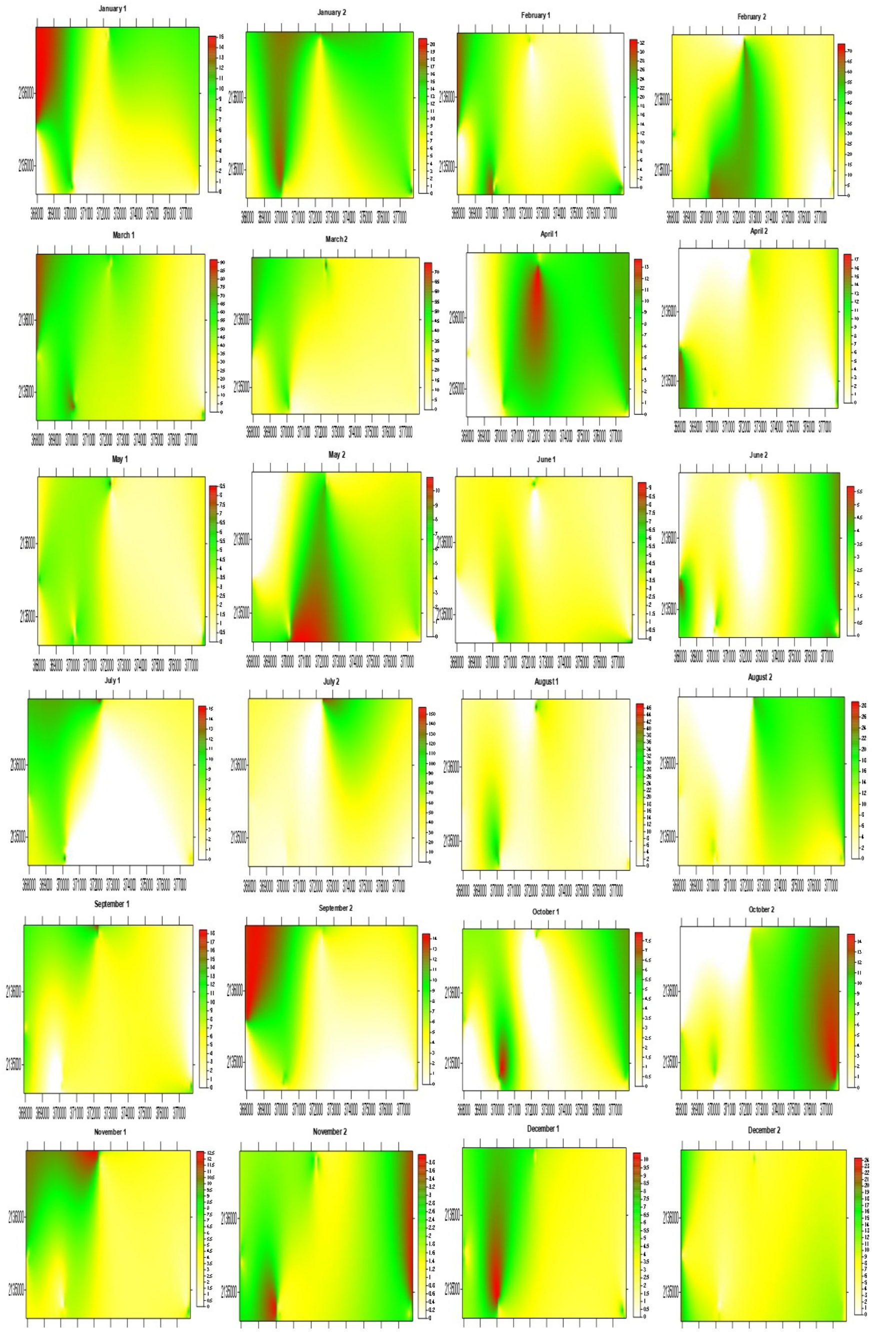

Figure 1. Density maps of *Scirtothrips perseae* populations obtained in the samplings.

The correct way to interpret the maps of each sampling is using the scale found next to the map, the color scale corresponding to a certain density within the crop can be found there. Even though, the maps seem to present the same color, it is necessary to visualize the scale because months like February and March presented the highest densities of *Scirtothrips perseae.* (Fig. 1), whereas, November and December presented the lowest densities but coloring looks the same than the rest of the months. Knowing how to interpret these maps aid within decision making that directly affect the control of this pest that causes considerable losses in the crop.

Fortnightly sampling provides an overview of the pest behavior throughout the year, so decision making can diminish the damage thrips cause to the avocado crop, which would lead to higher productions and therefore higher profits for avocado growers.

A difference was observed in the aggregation center maps obtained, compared with the ones obtained by Maldonado et al (2016) that showed a spatial behavior a little different from the thrips in general. In the second, the aggregation center was more delimited and the ones obtained in this study were more gradual and at certain points they resembled lines throughout the plot, biologically this difference can be due to the fact that in this study only *Scirtothrips perseae* was sampled and in the study mentioned above it included thrips in general.

The usage of geostadistics when studying pests behavior allows to know the fluctuation and position of the agents within the plot, which is a great aid since targeted control methods can be used; these methods can result in significant savings in the application of chemical products or alternative methods, as the control is directed to aggregation zones, which also translates in a lower environmental impact (Schotzko & O'keeffe, 1988; Bautista, Cordona, & Soto, 2013). One of the most common thrips control methods is an insecticide spinosad commercially known as Spintor, this product acts very effectively in the control of thrips. Knowing how the pest behaves spatially, targeted applications can be made on population niches, therefore, economical savings can be obtained. Another alternative is the usage of predators as reported by Ramírez et al (2018) who used *Orius insidiousus* obtaining a decrease in thrips populations, as well as, Acosta et al (2017) who used *Amblyseius swirskii* in the control of this plague.

For a better spatial analysis of pests and diseases in agricultural crops, it is important to determine the level of infestation in the area, this knowledge will allow us to determine the actions to control and prevent pests. In the present study, the infested area was determined for the 24 samplings (Table 3), infestation higher than 80% was found in most of the sampling carried out. Papers like Esquivel and Jasso (2015) also showed percentages of infestation in the studied plots; there is no relationship between the percentage of infestation and the density of infestation, levels of infestation found were high but the density was low which do not exceed the economic thresholds established for avocado. The months with the highest infestation were January in the second sampling, November in the second sampling as well as both of December samplings with a 99 percentage of infestation, in contrast the sampling in July reported the lowest infestation level with 56 of percentage. This data is in agreement with the highest and lowest densities of the pest population fluctuation within the area of study. It should be noted that the rate of infestation is not directly related with the population density of the pest, since we can have very high levels of infestation, but low densities that do not exceed the economic threshold of the pest.

The avocado growers in the study zone do not know the spatial behavior of *Scirtothrips perseae,* so the present work will allow growers and competent authorities to develop integrated management programs for this phytosanitary problem that are more efficient, timely and relevant, it will also make possible to establish more efficient strategies knowing the location and preferences of *Scirtothrips perseae.* The importance of studies like the current one lies in the fact that they determine ecological niches occupied and played by this kind of insect pest, which allows a better understanding of their behavior and in this manner, establish strategies that give better results.

More research on the spatial behavior of pest insects is required to conclude that the spatial distribution is the same, year after year, the spatial distribution of pest insects is limited or potentiated by; climate conditions, and biotic factors in orchards such as; weeds, and other crops. Thrips population densities are not related to aggregation centers since aggregation centers are conserved at different densities. The maps presented show when a greater population fluctuation of thrips was obtained. There are limitations to apply this type of research in Mexico since most farmers do not have the appropriate technologies. This research meets the objective of bringing these technologies closer to farmers so that they can determine the most appropriate management for their orchards. The flowering times of the avocado crop (February, March, April, August, September) showed the highest densities, it would be the right time to carry out some control over the pest insect.

CONCLUSIONS

In this study, *Scirtothrips perseae* presented an aggregated spatial behavior within the avocado production orchards in the State of Mexico. The application of geostatistical techniques allowed to know such behavior through population density maps.

Therefore, targeted control measures can be carried out in the aggregation centers of the pest, and thereby reduce economic and environmental costs.

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J. Entomol. Res. Soc., 25(3): 467-480, 2023 *Research Article*
Doi: 10.51963/jers.v25i3.2337 *Online ISSN:2651-3579*

Plant Extracts for the Control of *Sitophilus zeamais* (Motschulsky, 1895) (Coleoptera: Curculionidae)

Juliana NAZARÉ ALVES SOUZA[1,2,a] Marcos ROBERTO POTENZA[2,b] (in memoriam)

Márcio MARTINS DE ARAUJO[1,c*] Jamile ICASSATTI SAUD ROMANO[1,d]

Valter ARTHUR[3,1,e]

[1]Institute of Energy and Nuclear Research (IPEN), Department of Radiation Technology Center, University of São Paulo, Av. Professor Lineu Prestes, 2242, 05508-000, São Paulo, SP, BRAZIL
[2]Biological Institute of São Paulo, Center of Research and Development of Plant Health, Av. Conselheiro Rodrigues Alves,1252, 04014-002, São Paulo, SP, BRAZIL
[3]Center for Nuclear Energy in Agriculture (CENA/USP), Department of Radiobiology and Environment, University of São Paulo, Av. Centenário, 303, 13416-000, Piracicaba, SP. BRAZIL
e-mails: [a]julianaalvesbiologia@gmail.com, [c*]marcio_araujo @usp.br, [d]jisaud@usp.br, [e]arthur@cena.usp.br
ORCID IDs: [a]0000-0002-9265-1083,[b]0000-0002-5836-3774, [c]0000-0002-6367-6126, [d]0000-0001-8075-2023, [e]0000-0003-3521-9136
*Corresponding Author

ABSTRACT

Brazil is one of the largest grain producers in the world, but its productive potential is threatened by the presence of stored product pests that compromise quality and quantity. The use of synthetic insecticides and selective pressure make many of these pests increasingly resistant to control. Concern for health and the environment requires the development of less toxic and persistent products for the management of these undesirable organisms. The insecticide activity of aqueous extracts of 15 vegetal species was evaluated to control grain beetles, *Sitophilus zeamais* Motschulsky in maize stored. The adult insects came from the laboratory at Biological Institute of São Paulo. They were fed with sterilized grains of maize and kept in a room at 25±2°C and 70±% of relative humidity. The plants for the aqueous extracts were crushed in distilled water and the vegetal mass was filtered next. The liquid resulting from the filtering process was stored in plastic containers and frozen for later use. Two tests were conducted: impregnation of filter paper and treatment of grains. Ten insects were evaluated in each batch. Only the aqueous extract of *Dahlia pinnata* Cavanilles was satisfactory in controlling *S. zeamais* showing an efficiency of 10.00% in the contact test and an efficiency of 56% in the treated corn kernels.

Keywords: aqueos extracts, biological control, insecticide, maize.

Nazaré Alves Souza, J., Roberto Potenza, M., Martins De Araujo, M., Icassatti Saud Romano, J., & Arthur, V. (2023). Plant extracts for the control of *Sitophilus zeamais* (Motschulsky, 1895) (Coleoptera: Curculionidae). *Journal of the Entomological Research Society*, 25(3), 467-480.

Received: September 28 2022 *Accepted: June 21, 2023*

INTRODUCTION

Corn is one of the three most cultivated cereals in the world, of which Brazil is the third producer and second world exporter (Coêlho, 2021). However, a reasonable portion is retained in the rural areas where it is used for food and another part is lost by the action of insects and rodents. It is estimated that losses reach 30 to 40% of yields due to problems with drying, storage, transport and pests (Garcia, Ferreira, Biagginon, & Almeida, 2000). According to Manandhar, Milind, & Shah (2018) the quantitative loss is 15% in the field, 13 to 20% during processing and 15 to 25% during storage.

Insect control methods in stored grains can be divided into: physical (artificial cooling, temperature, radiation, humidity, modified atmosphere or diatomaceous earth), biological (natural enemies) and chemical (insecticides). In Brazil, the most used method is fumigation with aluminium and magnesium phosphide, in addition to the use of protective insecticides such as pyrethroids and organophosphates (Silva, Silva, Souza, Lima, & Santos, 2020).

For these reasons, new management strategies are needed in order to meet the growing demand for food, not just produce more and better, but also provide a means for the preservation of crops, avoiding or mitigating the damage caused by pests during storage. Grain purge, a phytosanitary measure used to eliminate pests in seeds and stored grains using gas (Lorini, Kryzanowski, França-Neto, Henning, & Henning, 2015). Although it offers satisfactory coverage, there is a tendency that new treatment alternatives are necessary, due to the identification of phosphine-resistant populations of *Sitophilus zeamais* Motschulsky (Pimentel, Faroni, Guedes, Souza, & Tótola, 2009).

Considering the physiological and biochemical aspects, the main mechanisms involved in insect resistance to control chemical products are: the reduction of insecticide penetration through the insect cuticle; the detoxification or metabolization of the insecticide by enzymes and the reduction of sensitivity at the site of action of the insecticide in the nervous system (Hemingway, 2000).

Phosphine resistance in stored grain insects occurs due to lack of proper operation, which mainly includes insufficient exposure time and temperature (Athanassiou, Rumbos, Sakka, & Sotiroudas, 2016). Normally, eggs and pupae are more tolerant to phosphine than adults and larvae, due to their metabolic activity, which can be influenced by low temperatures, postponing the time of exposure to total insect mortality (Wang, Hou, Huang, & Lyu, 2020). Pimentel et al (2009) when studying 22 Brazilian populations of *Sitophilus zeamais*, observed that populations with lower respiratory rates showed higher levels of phosphine resistance, suggesting that the lower respiratory rate is associated with the physiological basis of phosphine resistance due to reduced absorption of fumigants. Phosphine resistance was also recorded and quantified in Greece by Agrafioti, Athanassiou, & Nayak (2019), who, when analyzing 53 populations from different regions belonging to the species *Rhyzopertha dominica*, *Sitophilus oryzae*, *Sitophilus granarius*, *Cryptolestes ferrugineus*, *Tribolium confusum*, *Tribolium castaneum* and *Oryzaephilus surinamensis*, observed a resistance frequency of 81%.

Since 2008, Brazil has been the largest consumer of pesticides in the world (Rigotto, Vasconcelos, & Rocha, 2014). According to the Notifiable Diseases Information System (Sinan), which collects data generated by the National Epidemiological Surveillance System (SNVE), between 2007 and 2017, 41.6 million cases of intoxication by pesticides for agricultural use occurred, on an increasing scale, considering all cases, 88% refer to acute poisoning and 42% were due to occupational exposure (Valadares, Alves, & Galiza, 2020). These impacts are associated with our current development model, which primarily focused on the production of primary goods for export (Carneiro, Rigotto, Augusto, Friedrich, & Búrigo, 2015). In 2015, Brazil sprayed approximately 899 million liters of pesticides, with soy, corn, and sugar cane accounting for 82% of the entire volume used in the country (Pignati et al, 2017). The use of chemical products guarantees protection to the means of production, but with the consumption of approximately 1 million tons per year it causes damage to man and the environment, such as diseases to human health and destruction of soils and rivers (Demartelaere et al, 2021).

Vendramim & Castiglioni (2000) reported that the use of toxic compounds of vegetable origin is not a recent technique since their use in pest control was common in tropical countries before the advent of synthetic insecticides. The use of insecticidal plants stands out among the alternative methods to conventional chemical control, due to its safety and preservation of the balance of agro ecosystems. Moreover, the flora is very rich in plant species that contain chemicals with insecticidal activity (Ferracini et al, 1990). Tropical plants constitute a reservoir of substances that are originally employed in the defense of the plants themselves against herbivores that serve them. Research on plants with insecticidal activity are quite frequent in the literature, but are very scarce in Brazil (Vilela, 1990).

Jacobson (1958, 1975) conducted two literature reviews on the plant species whose extracts showed insecticidal action on insects (*Annona spp*, *Berberis thunbergii*, *Cordia* spp, *Anthemis* spp, and other *Ocimum* spp.). Craveiro et al, (1981) reported several plants of northeastern Brazil with insecticidal properties (*Lippia* sp., *Croton compressus* Lamarck, *Anacardium occidentale* Linnaeus, *Eucalyptus alba* Von Blume, *Eugenia jambolana* Lamarck). Guerra (1985) reported that 974 plants with insecticidal properties were identified (*Calendula officinalis* Linnaeus, *Allamanda nobilis* T. Moore, *Anona squamosa* Linnaeus, *Anthemis* spp., *Capsicum annuum* Linnaeus, *Croton* spp. and others). Aguiar, Monteiro, & Calafiori (1994) evaluated the black pepper (*Piper nigrum* L.), soybean oil and garlic against *Sitophilus zeamais* in stored rice, and concluded that soybean oil had higher efficiency, with 96-100% on pest control, followed by black pepper, but with low efficiency from 14 days after treatment.

Rajapakse & Van-Emden (1997) evaluated the possibility of using powder Botanical *Vigna unguiculate* (Linnaeus) Walpers, *Vigna radiata* (Linnaeus) Wilczek, *Vigna angularis* (Willdenow) Ohwi & Ohashi, *Cymbopogon citratus* Stapf, *Ciannamomum camphora* Linnaeus, *Monodora myristica* (Gartner) Dunal, *Zingiber spectabile* Griffith and vegetable oils *Helianthus annuus* Linnaeus, *Sesamum indicum* Linnaeus, *Zea mays* Linnaeus and *Arachis hypogaea* Linnaeus in control of *Callosobruchus chinesis*

Linnaeus, *C. maculatus* Fabricius and *C. rhodesianus* Pic and observed that all vegetable oils tested significantly reduced oviposition of three species and also reduced the longevity of adults of *C. maculatus* and *C. chinensis*. Only oils *Z. mays* and *H. annuus* caused a significant reduction in the longevity of *C. rhodesianus* at 10 ml/kg. The number of eggs laid by three pest species tested was significantly reduced in treated powder Botanical *C. citratus, C. camphora, M. myristica, Z. spectabile.*

Park, Kim, & Ahn (2003) evaluated the insecticidal activity of *Acorus gramineus* Aiton (derived from a rhizome with phenylpropenes and asarones) against adults of *Sitophilus oryzae* Linnaeus*, C. chinensis* and *Lasioderma serricorne* Fabricius being examined using direct contact application and fumigation method, and verified in the test of the filter paper a mortality of 70% and 90% in adults of *S. oryzae* respectively at 4 days after treatment and 100% mortality after 7 days from the treatment. Against adults of *C. chinensis*, phenylpropenes and asarones caused 100% mortality at 3 and 7 days after treatment, respectively. Against adult *L. serricorne*, had 90% and 83% mortality after 7 days, respectively, after treatment, also indicating that the toxicity can be qualified against the adults of the three tested species.

This study evaluates the effect of some insecticidal plant extracts, seeking alternative products to conventional chemical control, which do not degrade the environment or harm human health, in addition to providing a lower final cost to the farmer.

MATERIAL AND METHODS

The study was carried out on the premises of Arthropod Protection and Plant Clinic laboratories at the Biological Institute of São Paulo. The adults of *S. zeamais* used in this study were obtained from breeding, kept in glass containers with approximately 23 cm high x 11 cm in diameter, with corn (grain) previously placed in an oven for disposal of unwanted infestations. The containers were covered with fine mesh fabric, secured by elastic bands. The insects were maintained in a room with temperature of 25±2°C and relative humidity of 70±5% (Fig. 1). We opted for the use of the corn itself in the rearing of *S. zeamais* due to the fact that there are changes in the susceptibility of insects to a certain toxic product when changing the food substrate, according the work of Teotia & Prasad (1974).

Figure 1. Creation of *Sitophilus zeamais* do Biological Institute of São Paulo.

To obtain the aqueous extracts, the method adopted by Robert (1976) was used. The fresh parts of the adult plant (150 g) (Table 1 and Table 2) were collected at the Botanical Institute and Biological Institute of São Paulo and crushed with 450 ml of distilled water, separating the plant mass with a strainer and obtaining the aqueous extract. This was stored in plastic vials and frozen for approximately 24 hours for use in assays.

Table 1. List of plants used in Experiment 1 and 3: botanical species, family, and the popular name used.

Species botanical	**Family**	**Part used**
Mirabilis jalapa Linnaeus	Nyctaginaceae	Leaves
Ricinus communis Linnaeus	Euphorbiaceae	Leaves
Euphorbia pulcherrima Willdenow ex Klotzch	Euphorbiaceae	Leaves
Pilea microphylla Linnaeus	Urticaceae	Leaves
Pteridium aquilinum (Linnaeus) Kuhn	Dennstaedtiaceae	Leaves
Sansevieria trifasciata Prain	Asparagaceae	Leaves
Dahlia pinnata Cavanilles	Asteraceae	Leaves

Control (water)

Table 2. List of plants used in Experiments 2 and 4: botanical species, family, and the popular name used.

Species botanical	**Family**	**Part used**
Codiaeum variegatum Linnaeus	Euphorbiaceae	Leaves
Agave sp.	Amaryllidaceae	Leaves
Ruta graveolens Linnaeus	Rutaceae	Leaves
Malvaviscus arboreus Cavanilles	Malvaceae	Leaves
Santolina chamaecyparissus Linnaeus	Asteraceae	Leaves
Impatiens walleriana Hooker	Balsaminaceae	Leaves
Dieffenbachia brasiliensis Veitch	Araceae	Leaves
Allamanda cathartica Linnaeus	Apocynaceae	Leaves

Control (water)

The methodology used in the execution of the experiments was adapted by Takematsu (1983). Initially, two types of tests were carried out: impregnation technique, filter paper / contact contamination and grain treatment (Fig. 2). For the impregnations of filter paper, papers with a diameter of 9 cm treated with plant extracts were used. Each paper received 1 ml of the extracts that were applied with an automatic pipette to 50 g of corn. To prevent loss of products through contact, the paper disks were supported on the ends of the pins 3, resting on a small stopper, thus reducing to a minimum the contact of the treated paper with a given surface.

Figure 2. a) Natural aqueous extracts used in research, b) impregnation of filter paper in petri dishes, c) contamination by ingestion, application of the extract.

After drying, the paper disks thus treated were transferred to plastic Petri dishes measuring 9.0 cm in diameter by 1.0 cm and left to rest for 24 hours. To contain the insects, bringing them into direct contact with the surfaces treated with insecticides, glass rings with a diameter of 4.5 cm x 2.5 cm were used, previously treated with pure talc, which prevents weevils from climbing the walls of the rings. To prevent escapes through flight, the rings were covered with a thin transparent mesh fabric and held together with elastic band.

To test the treatment of grains, 50 g of corn were placed in each glass of approximately 14 cm height x 7 cm in diameter with 10 insects, and 1 ml of extract was applied with an automatic pipette, closing the glass with a screen. In all, fifteen plant species were evaluated in each test. Each repeat was composed of 10 adult insects.

To assess the physiological criterion, insect mortality evaluations at 24 hours and 15 days after treatment were adopted, counting the number of dead insects. Mortality was checked using a heat source provided by a 100-watt lamp approximately 10 cm from the insects, for a few seconds, as the weevil (*S. zeamais*) has the habit of remaining motionless when disturbed (Takematsu, 1983). The efficiency of control (EC, %) was obtained using the equation of Abbott (1925):

$$EC=\frac{T-Tr}{T}x100$$

where: T - number of live insects in the control; and Tr - number of live insects in the treatment.

The statistical design used was completely randomized, and each treatment had 4 replications. For the purposes of statistical analysis, the data were transformed into (square root + 0.5), comparing the means using the F test and Tukey at 5% significance.

RESULTS

Contact test (Technical impregnating filter paper): In Experiment 1 (Table 3) only the extract of *E. pulcherrima* did not differ statistically from the control, and satisfactory control was not obtained with the treatments. In the second experiment, the best result was obtained with the extract of *Agave* sp., showing 20.00% efficiency (Table 4). Extracts of *I. walleriana* and *A. cathartica* did not differ statistically from the control, and satisfactory control was not obtained with the treatments.

In Experiment 3, using grain treatment, the best results were obtained with the extracts of *D. pinnata, S. trifasciata* and *R. communis*, with efficiency of 56.00%, 25.00% and 20.00%, respectively (Table 5). Extracts of *P. microphylla* and *M. jalapa* did not differ statistically from the control.

In Experiment 4, the best results were obtained with the extracts of *R. graveolens, M. arboreus* and *Agave* sp., showing efficiency of 27.50%, 22.50% and 20.00%, respectively (Table 6). Extracts of *S. chamaecyparissus, D. brasiliensis* and *A. cathartica* did not differ statistically from the control.

Table 3. Experiment 1. Evaluation of the efficacy of plant extracts in the control of *S. zeamais* by the filter paper impregnation method. Number of dead adult beetles (n.) per plot (original mean) and percentage efficiency (% Efic) of some plant extracts.

Plants	**nº**	**% Efic.***
Mirabilis jalapa	2.75a	27.50
Pteridium aquilinum	1.20b	12.00
Ricinus communis	1.20b	12.00
Sansevieria trifasciata	1.00bc	10.00
Dahlia pinnata	1.00bc	10.00
Pilea microphylla	0.50c	5.00
Euphorbia pulcherrima	0.00d	-
Control (water)	0.00d	-
C.V. (%)	13.15	

*Calculated by the formula of Abbott (1925).

Means followed by the same letter do not differ by Tukey test at 5% probability.

Table 4. Experiment 2. Evaluation of the efficacy of plant extracts in the control of *S. zeamais* by the filter paper impregnation method. Number of dead adults (n.) per plot (original mean) and percentage efficiency (% Efic.) of some plant extracts.

Plants	**nº**	**% Efic.***
Agave sp.	2.00a	20.00
Malvaviscus arboreus	1.50ab	15.00
Dieffenbachia brasiliensis	1.25ab	12.00
Ruta graveolens	1.00 bc	10.00
Santolina chamaecyparissus	1.00 bc	10.00
Codiaem variegatum	0.50cd	5.00
Impatiens walleriana	0.00d	-
Allamanda cathartica	0.00d	-
Control (water)	0.00d	-
C.V. (%)	12.03	

*Calculated by the formula of Abbott (1925).

Means followed by the same letter do not differ by Tukey test at 5% probability

Table 5. Experiment 3. Evaluation of the efficacy of plant extracts in the treatment of grain for *S. zeamais*. Number of dead adult beetles (n.) per plot (original mean) and percentage efficiency (% Efic.) of some plant extracts.

Plants	**nº**	**% Efic.***
Dahlia pinnata	5.60a	56.00
Sansevieria trifasciata	2.50b	25.00
Ricinus communis	2.00bc	20.00
Euphorbia pulcherrima	1.00cd	10.00
Pteridium aquilinum	0.75de	7.50
Pilea microphylla	0.50de	5.00
Mirabilis jalapa	0.50de	500
Control (water)	0.00e	-
C.V. (%)	14.16	

*Calculated by the formula of Abbott (1925).

Means followed by the same letter do not differ by Tukey test at 5% probability.

Table 6. Experiment 4. Evaluation of the efficacy of plant extracts in the treatment of grain for *S. zeamais.* Number of dead adults (n.) per plot (original mean) and percentage efficiency (% Efic.) of some plant extracts.

Plants	**nº**	**% Efic.***
Ruta graveolens	2.75a	27.50
Malvaviscus arboreus	2.25ab	22.50
Agave sp.	2.00ab	20.00
Codiaeum variegatum	1.50abc	15.00
Impatiens walleriana	1.00bcd	10.00
Santolina chamaecyparissus	0.75cde	7.50
Dieffenbachia brasiliensis	0.50de	5.00
Allamanda cathartica	0.25de	2.50
Control (water)	0.00e	-
C.V. (%)	14.40	

*Calculated by the formula of Abbott (1925).

Means followed by the same letter do not differ by Tukey test at 5% probability.

DISCUSSION

Plant extracts have substances in their composition that show different effects, including ovicide, repellent, phage-inhibitor, anti-nutritional and entomotoxic effects, which are generated through strategies either by contact, ingestion or by fumigant action. It is known that plants produce a wide variety of primary (protease and lectin inhibitors) and secondary (terpenes, phenolic compounds, and nitrogen-containing components) metabolites that often have no determined or relevant role in the physiological or biochemical processes of the plant, but which are increasingly cited as important mediators of interactions between plants and other organisms (Cespedes et al, 2013; Borges et al, 2019).

Diniz, Astarita, & Santarém (2007) report that depending on the temperature and drying process used to obtain plant extracts, there may be changes in the concentrations of their metabolites, as when evaluating samples of the herbal medicine Hypericum perforatum they found a decrease in the amount of flavonoids, glycosylates and hypericin a -20°C temperature. Demirgül, Divriklioğlu-Kundaki, & Sağdıç (2022), when comparing the secondary metabolites synthesized from fresh and dried plants of *Polygonum cognatum* Meissn with frozen ones (-30ºC) for a period of 6 months, observed that there was a significant decrease in the total phenolic compounds over the period of storage. This fact demonstrates that each plant species must have a standardized extraction method, in order to obtain maximum results.

With regard to secondary metabolites, Lima et al (2020) observed that the presence of tannins, saponins and phenolic compounds in the trunk bark extract of *Genipa americana* L. was able to inhibit the feeding of *Tribolium castaneum* adults, and its extract caused mortality of 73% of the insects and induced a decrease in the hatching rate (40–96%) of larvae.

Among the researches using plants with insecticidal action, we can mention the work of Procópio, Vendramim, Ribeiro Júnior, & Santos (2003), where they evaluated the insecticidal effect of powders of *Azadirachta indica* A. Jussieu, *Capsicum frutescens* Linnaeus, *Chenopodium ambrosioides* Linnaeus, *Eucalyptus citriodora* Hooker, *Melia azedarach* Linnaeus and *R. communis* on the maize weevil (*S. zeamais*) and found that *E. citriodora* had a repellent effect while the dust of *C. ambrosioides* affected the weevil survival causing mortality without any emergence of adults.

The efficiency of *Agave* sp. is presented in research by Potenza, Takematsu, Sivieri, Sato, & Passerotti (1999) carried out experiments with various plant extracts and obtained satisfactory control of *T. urticae* with extracts from *Annona* sp., *Agave* sp., *R. graveolens* and *D. brasiliensis*, with efficiency greater than 80%. Other plants showed acaricidal activity such as *Melia azedarach*, *S. oleraceus*, *Nicotiana tabacum*, *Hevea brasiliensis*, *Spondias* sp., *P. purpureum*, *C. variegatum* and *I. walleriana*, with efficacy between 51.3 and 77.3%.

Schiavon et al (2015) performed a systematic review showing that the Asteraceae family, of *D. pinnata* popularly known for its antibacterial, antifungal, larvicidal, insecticide, antiparasitic, nematicidal, antihyperglycemic, antitumor, biocidal and antioxidant activity. As well as Soberón & Francisco (2020) evaluated the anti-inflammatory effect of the methanolic extract of *D. pinnata* leaves in rats and as a result the methanolic extract of *D. pinnata* showed an anti-inflammatory effect in rats, data confirmed by Test Duncan, DMS, compared with the control group ($p<0.05$), where in thin layer chromatography, the FR of the standard was: 0.80, methanolic extract 0.79. What broadens the understanding of the tests carried out in experiment 3 presented efficiency of 56.00%, with the extracts of *D. pinnata*.

The extracts with *Sansevieira trifasciata* were also evaluated by Corassa, Machiner, Valladao, Andrighetti, & Weberling (2022) who tested the insecticidal potential of the leaves of the plants *Chrysanthemum morifolium* (Asterales: Asteraceae), *Dieffenbachia picta* (Alismatales: Araceae), *S. trifasciata* (Asparagales: Asparagaceae) and *Tagetes erecta* (Asterales : Asteraceae) on the mortality of *Spodoptera frugiperda* caterpillars and concluded that despite the low mortality with the extracts, the plants *C. morifolium* and *S. trifasciata* showed higher mortality than the others.

The extracts of *R. communis* were also investigated by Behling (2011) who evaluated the repellent activity of plant extracts from weeds on adults of *Sitophilus* spp. in rice seeds, under laboratory conditions. The experiment consisted of two tests to evaluate the effect of aqueous extracts at 10% w/v of leaves and branches of Macaé Herb (*Leonurus sibiricus* L.), Picão Preto (*Bidens pilosa* L.), Maria Mole (*Senecio brasiliensis* Less.) and Castor bean fruits (*Ricinus communis* L.). Both in the first test and in the second test, the black beggartick extract showed the best insect repellency results. Therefore, from the results presented in the two tests, it was verified that in the laboratory the extract of Picão Preto presented better repellent activity to *Sitophilus* spp. Extracts from *L. sibiricus* and *R. communis* also showed promise as natural repellents against weevil in rice.

Ruta graveolens extracts were also tested by Potenza, Junior, & Alves (2006), who analyzed the essential oil of *R. graveolens* leaves by hydrodistillation to assess its repellent and toxic activities against the rice weevil, *Sitophilus oryzae*, and the study revealed that the essential oil present in the leaves of *R. graveolens* has very high potential as a repellent, contact toxicant and a potent fumigant in the control of *S. oryzae* in pest management programs.

Malvaviscus arboreus Cavanilles were also researched by Da Costa Rocha, Bonnlaender, Sievers, Pischel, & Heinrich (2014) who shows the expansion in research with hibiscus extracts, *M. arboreus*, due to its performance against microorganisms, studies show the existence of metabolites of high pharmacological interest, mainly alkaloids and flavonoids, which can be responsible for the pharmacological action attributed to the hibiscus plant. The two compounds detected, 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one and DL-Proline-5-oxomethyl ester, potential antimutagenic, antitumor and anticancer agents, make *M. arboreus* a promising species for the manufacture of new drugs because it has cytotoxic activity (it can be used as an insecticide, analgesic and anti-tumor among others.

Almeida, Da Silva, De Melo, Gomes, & Da Silva, (2013) used a hydroalcoholic extract of the fruits of chili pepper (*Capsicum baccatum* Linnaeus) with concentration of 70% was observed a mortality of 100% of *S. zeamais* at a dose of 6 mL in 48 h, and the dust of the pepper resulted in a 94% repellency of this insect. According to Gullan & Cranston (2014), repellency is a reaction of the insect's sensory system when exposed to undesirable substances. Insects have chemoreceptors located in different parts of the body and are responsible for assessing the conditions of the environment where the insect is, fleeing if conditions are not favorable.

Regarding essential oils, Ko, Juntarajumnong, & Chandrapatya, (2009) evaluated their potential use of *Melaleuca cajuputi* Powell leaves, in the repellency and control of *S. zeamais* through fumigation and contact, and found repellency in treated grains exposed for 2 and 3 hours at a concentration of 0.47 $\mu g\ cm^{-2}$, repelling 80 and 90% of insects respectively. Through fumigation, the LD95 was 408.54 $\mu L\ L^{-1}$ and upon contact with the LD95 was 0.111 $\mu L\ insect^{-1}$. The chromatographic analysis showed a higher amount of terpineolene (29.77%) and γ–terpinene (25.25%). However, Jairoce et al (2016) used clove essential oil (Syzygium aromaticum (Linnaeus) Merril & Perry) to control *S. zeamais* and *Acanthoscelides obtectus* Say under laboratory conditions and observed that at concentrations of 17.9 and 35 $\mu L\ g^{-1}$ there was 100% mortality for both species 48 hours after treatment, and the main compound found was eugenol (62.72%).

The various substances from plants demonstrate the importance of analyzing the phytochemical characteristics and taxonomically and chemically identifying their compounds for human use and benefit (Ortiz-Rojas & Chaves-Bedoya, 2017).

In the present study, the extract that presented the highest efficiency in the treatment of grains was that of *D. pinnata* with 56.00%, Wang et al (2015) determined the chemical composition of the essential oil of *D. pinnata*, to understand its insecticidal activity against *S. zeamais* and *S. oryzae*, identifying three constituents that have greater toxicity and repellency against insects were D-limonene, 4-terpineol and α-terpineol.

This insecticidal activity occurs because, according to De Oliveira, Riveiro-Pinto, & Paumgartten (1997) these monoterpenes inhibit the enzyme acetylcholinesterase and cytochrome P450.

Potenza et al (2005) report that the types of solvents (water, ethanol, and hexane) involved in the extraction of compounds interfere with the efficiency of natural products, as they extract different chemical substances according to polarity. According to Andreo & Jorge (2006) water efficiently extracts phenolic compounds in a shorter time due to its high polarity. In addition, several factors can influence the content of secondary metabolites in plants, such as seasonality, circadian rhythm, developmental stage and age, temperature, water availability, UV radiation, soil nutrients, altitude, atmospheric composition, and tissue damage (Gobbo Neto & Lopes, 2007).

Thus, the use of plant extracts and/or essential oils is promising in controlling insect pests, standing out as an alternative method, which should be used in an integrated pest management.

CONCLUSIONS

Among the aqueous extracts of plants used in this experiment, only the *D. pinnata* extract can be considered satisfactory for use in the control of the weevil *S. zeamais*, as it presented 56.00% of control.

Other forms of extraction must be carried out in order to better evaluate the plant extracts used in this study for more appropriate indications and recommendations for their use as “insecticidal plants.” There is also a need to evaluate all possible forms of control or use of the extracts (ingestion, contact, repellency, attractiveness), which can be seen with the *D. pinnata* extract showing 10.00% efficiency in the contact test and 56.00% efficiency in the grain test treatment.

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J. Entomol. Res. Soc., 25(3): 481-490, 2023 *Research Article*
Doi: 10.51963/jers.v25i3.2390 *Online ISSN:2651-3579*

Field Bioefficacy and Residue Dynamics of Most Effective Insecticides Against Aphids in Taro (*Colocasia esculenta* (L) Scott, 1832)

Gouri Shankar GIRI[1*] Ashish NARAYAN[2] Sanjay Kumar SAHOO[3]
Vinod Kumar DUBEY[4] Badavath SUJATHA[5]

[1,2]Tirhut College of Agriculture-843121, Dholi, Muzaffarpur, Dr. Rajendra Prasad Central Agricultural University-848125, Pusa, Samastipur, Bihar, INDIA

[3,4,5] Post Graduate College of Agriculture, Dr. Rajendra Prasad Central Agricultural University-848125, Pusa, Samastipur, Bihar, INDIA

e-mails: [1]gsgiri@rpcau.ac.in, [2]2003303003@rpcau.ac.in, [3]sksahoo@rpcau.ac.in, [4]vinodkumardubey42@gmail.com, [5]b.sujatha47@gmail.com

ORCID IDs: [1]0000-0003-0177-8634, [2]0000-0003-2891-5977, [3]0000-0002-9337-5041, [4]0000-0003-4175-0468, [5]0000-0003-3979-7793

ABSTRACT

Tuber crops including aroids fulfil ample demands of global food supply and utilized as feed of animals along with processed products for human consumption. Aphid (*Aphis gossypii*), an oligophagous pest, known to cause considerable qualitative and quantitative losses to the cultivation of aroids in general and taro in particular. This study reports the field bio-efficacy of some insecticides of plant and chemical origin against aphid as well as residue dynamics of most effective insecticides in leaves and tubers of taro. For residue analysis QuEChERS (Quick, Easy, Cheap, Effective, Rugged, Safe) methodology was standardized for extraction and clean up followed by estimation through UHPLC (Ultra High-Performance Liquid Chromatography). Among different insecticides evaluated, single spray of imidacloprid both at single and double doses was found most effective in managing the aphid population with a remarkable higher tuber yield. The initial deposit of imidacloprid in the leaves of taro was 0.65 and 1.32 mg kg $^{-1}$ with half-life value of 1.04 and 1.41 days at single and double dose, respectively. The dietary exposure of the measured residues was found lower than the maximum permissible intake (MPI) of 0.33 mg person^{-1}day^{-1} on all the sampling days at both the doses. However, considering the default Maximum Residue Limit (MRL) of imidacloprid as 0.01 mg kg^{-1} on taro leaves and safety of the consumers the pre harvest intervals of 7 days may be suggested for effective dose following good agricultural practices.

Keywords: Neonicotinoid, sucking pest, management, phytotoxicity, dissipation, safety evaluation.

Giri, G. S., Narayan, A., Sahoo, S. K., Dubey, V. K., & Sujatha, B. (2023). Field Bioefficacy and Residue Dynamics of Most Effective Insecticides Against Aphids in Taro (*Colocasia esculenta* (L) Scott, 1832). *Journal of the Entomological Research Society*, 25(3), 481-490.

Received: December 24 2022 *Accepted: August 19, 2023*

INTRODUCTION

Taro, (*Colocasia esculenta* (L.) Scott) is a popular tuber crop commonly grown in India and other parts of South Asia, Africa, South Europe, South America and many oceanic island (Rao, 2010). Globally it ranked fourteen among the staple vegetable and occupies an area of about 10.8 million hectare in which about 1.5 million hectare is shared by Asia (FAO, 2010). It is primarily cultivated in eastern and north eastern states of India such as Bihar, Chhattisgarh, Odisha, West Bengal, Assam, & Uttar Pradesh. Bihar, owing to its geographical and ecological conditions, remains in folded condition during the month of May to July in almost every year. Because of this, there is always a deficit of vegetable production during this period of the year. At the same time, being tolerant to water logging conditions, taro act as a substitute for other vegetables. In, Bihar, it occupies an area of about 1367 hectare with production of 18864 metric tonnes annually and productivity of 13.80 metric tonnes per hectare (Annonymous, 2019). Both leaves and roots are edible and considered to be an important source of nutrients, rich in carbohydrate, proteins, and dietary minerals (Onwueme, 1978; Temesgen & Retta, 2015). It is also an important source of micronutrient like iron (Fe), cupper (Cu), magnesium (Mg), potassium (K), and zinc (Zn) (Gupta, Kumar, Tomer, Kumar, & Saini, 2019). Being a rich source of dietary fibre, it is used for the treatment of various diseases like high blood sugar, cancer, obesity, and problems related to alimentary canal (Saldanha, 1995). The leaves of taro are rich source of protein including minerals such as calcium, phosphorous, irons, and vitamins like vitamin C and B complex (www.ndsu.edu). The leaves of taro are used for preparation of curries as well as for making some crispy snacks.

Though, the crop is known to provide shelters to a number of insect visitors, aphid (*Aphis gossypii)* and Tobacco caterpillar (*Spodoptera litura*) causes considerable economic losses to it (Bhattacharyya & Mandai, 2006). Aphid causes damage to taro plant directly by sucking the cell sap from under surface of leaves and indirectly by enhancing the development of black sooty mould fungus. Though, aphid causes significant reduction in quality of leaves that are consumed by people, there was always a negligence of management of this pest or some time it is managed by application of synthetic organic insecticides. Presently, insecticide of organophosphate and synthetic pyrethroid groups were greatly used by farmers (Simaremare, 2020). Use of these harmful chemicals in a non-judicious way has led to the problem of insecticide resistance, residue, and pest resurgence. Hence, use of insecticides having systemic action offer huge scope in controlling the insect pest as these are considered to be relatively safe to non-target organisms because of high vertebrate selectivity ratio.

Imidacloprid and thiamethoxam are the selective insecticides, and belong to the neonicotinoid group, a systemic class of insecticide known to provide excellent control of aphid, whitefly, thrips, jassids, and other sucking pest (Elbert, Haas, Springer, Thielert, & Nauen, 2008; Jeschke, Nauen, Schindler, & Elbert, 2011) due to its competitive binding to the neonicotinic acetylcholine receptors at post synaptic membrane (Casida & Durkin, 2013). Till date, there is not a single insecticide recommended for management of insect pest in taro. As imidacloprid and thiamethoxam are very much effective in controlling sucking pest of different crops and are already recommended for use against aphid in other crops, the present study evaluated the efficacy of both the insecticides for management of aphid in taro. Since the leaves of taro are mainly used as vegetable and the foliar application may leads to the problem of residues and no scientific publication is available on taro, hence it is essential to study the residue dynamics at different intervals to suggest suitable waiting period for safe consumption.

MATERIALS AND METHODS

Experimental site and raising of a crop

The trial was undertaken at the field of All India Co-ordinated Research Project on Tuber Crops (AICRP), Dr. Rajendra Prasad Central Agricultural University-848125, Pusa, Samastipur, Bihar, India for the period of three consecutive seasons *i.e.* during 2018-19, 2019-20, and 2020-21. The experimental site was located at latitude of 25.98^0 N, longitude of 85.61^0E and an altitude of 52.12 m above MSL with medium slope. The soil type was typically calcareous having pH 8.3 with loamy to sandy loam in texture and good water holding capacity. The available N, P_2O_5 and K_2O in the experimental field were 195, 32 and 125 $kgha^{-1}$, respectively. The average maximum temperature was 30. 2^0C, 30.65^0C and 29.5 ^{0}C whereas average minimum temperature was 18.8^0C, 19.3^0C and 18.3^0C during the three years (crop period) of study, respectively. The average relative humidity was 74% during the crop period 2018-19, 74% during the crop period 2019-20 and 80% during the crop period 2020-21. The field was prepared and levelled by ploughing with disc harrow followed by rotavator and cultivator Arvi (Variety-Rajendra Arvi 1) was planted during the second week of February in plot of size 5m×4 m having planting geometry of 50-30 cm. Except pest management practices, all other agronomical practices were followed to raise the healthy crops.

Experimental design and treatments imposition

The experiment was laid out in Randomised Block Design (RBD) having seven treatments and three replications. The insecticidal treatments for management of aphid consists of two doses of Imidacloprid 17.8 SL @ 25 and 50 g $a.iha^{-1}$, two doses of Thiamethoxam 25 WG @ 37.5 and 75 g $a.iha^{-1}$ along with two botanicals (Neem oil and Cassava Leaf Extract @ 0.5 %) and an untreated control. The spraying of chemicals was done during the afternoon hour of a sunny day with little or no wind by using knapsack sprayer. The volume of the water used for spraying was 500 Lha^{-1}. The infestation of aphid was noticed during the last week of May which was increased with time and crossed the Economic Threshold Level (ETL) during second week of June when the crop was of 112 days old. At the time of spraying, the crop was in vegetative stage and formation of tuber was also started.

Bio-efficacy against aphid in taro

The observation on aphid population was taken on one day prior to the treatments and 1, 3, 5, 7, and 10 days after the treatments. For that ten plants were selected randomly and population was counted from three leaves per each plant. At the end, the per cent reduction of pest over control was calculated as per following formula.

Per cent Reduction= [(control count-treatment count/control count) × 100]

Treatment wise corm and cormel yield were recorded after harvesting and were pooled, expressed in tonnes per ha.

Statistical Analysis

The statistical analyses were carried out by using *Statistical Package for the Social Sciences* (SPSS version 16.0). The data were pooled over the years and subjected to Analysis of Variance (ANOVA) as there were no significant interaction found among the treatments and years. The effects of the years were also non-significant. When ANOVA was significant, comparisons of significant means were made using least significant difference (LSD) at the probability level of 0.05.

Residue dynamic studies

Taro leaves samples (500 g) were collected from 9 different sites of each treated plot separately, at 0 (after 2 hours), 1, 3, 5, 7, and 10 days after treatment. As aphid is a pest of vegetative stages and the tubers were not formed properly at the time of treatments, tuber samples were collected 30 days after the treatment. Quick, Easy, Cheap, Effective, Rugged, Safe (QuEChERS) techniques with slight modification is used for processing of taro leaves and tuber samples for residue analysis (Anastassiades, Lehotay, Stajnbaher, & Schenck, 2003). The methodology was standardised by conducting linearity and recovery studies only. A macerated taro leaves and tuber sample (10 g) was transferred to 50 mL polypropylene centrifugal tube later kept it overnight in refrigeration. Samples were taken from refrigerator and 20 mL of acetonitrile (C_2H_3N) (HPLC grade) was added to each tubes. To each centrifuge tubes, NaCl (10 ± 0.1 g) was added and shaken for 10 min at 50 rpm on rotospin (Tarson®). Samples were centrifuged for 3 min at 2500 rpm. Moisture if any was removed from aliquot of acetonitrile by anhydrous sodium sulfate (Na_2SO_4) followed by cleanup through "dispersive solid phase extraction (DSPE)". For this, a polypropylene tube constituting "0.15 ± 0.01 g PSA sorbent, 0.90 ± 0.01 g anhydrous (MgSO4) and 0.05 ± 0.01 g graphitic carbon black" was prepared for an aliquot of 6 mL which was mixed thoroughly by vortex spinix (Tarson®). Again it was centrifuged for 3 min at 2500 rpm and finally a 3 mL aliquot was taken for residue analysis.

Ultra High-Performance Liquid Chromatography (UHPLC) was used for residue determination at wavelength 271 nm. The choice of mobile phase is very important for the separation of parent pesticide from co-extractives. Elution was performed in the gradient mode with the ratio 70:30 (HPLC grade acetonitrile: HPLC grade water) with the flow rate of 0.30 mL min^{-1}. 20ul samples were injected at ambient column temperature.

Risk evaluation through consumption of taro leaves was done by comparing the dietary exposure, *i.e.* theoretical maximum residue concentration (TMRC) vis-a-vis the maximum permissible intake (MPI). The values of the dietary exposure were calculated by multiplying the residue levels in each sample (mg kg^{-1}) with an average per capita consumption of taro leaves in Bihar which is about 0.023 kg $person^{-1}$ day^{-1}.

RESULTS

Bio-efficacy of imidacloprid against aphid in taro

The mean number of nymph and adult of aphid per plant before and after the treatment of insecticide were presented in Table 1. The aphid population prior to treatment varies non-significantly ($F_{cal\ (6,\ 36)} < F_{tab(6,\ 36)}$, Table 3.) from 24.93 ± 0.69 to 27.53 ± 1.16 in different experimental plots. After 10 days of treatment, significant reduction of aphid population was observed in plot treated with imidacloprid @ 50 g a.i.ha^{-1} (4.91 ± 0.19 aphid/plant) followed by plot treated with imidacloprid @ 25 g a.i.ha^{-1} (5.78 ± 0.68 aphid/plant). Thiamethoxam @ 75 and 37.5 g a. i. ha^{-1} was also found very effective in reducing the aphid population below Economic Threshold Level (ETL) i.e. 6.07 ± 0.47 and 6.67 ± 12 aphid/plant, respectively. Maximum reduction of aphid population was observed on 5th days after treatment, thereafter there was slight rise in population in all the plots but was found below the ETL. Though all the chemical insecticides were found statistically at par in controlling the aphid population, imidacloprid @ 50 g a.i.ha^{-1} showed maximum reduction of aphid population. On the other hand, botanicals were found ineffective in suppressing the pest population below the ETL. However, in control plot, continuous rise in pest population was observed. Thus, one spray of imidacloprid at both the doses was considered sufficient to suppress the aphid population below ETL in taro.

Table 1. Effect of insecticidal treatments on aphid population in taro (pooled data of 3 years of experiment) .

Treatments	Dose (a.i/ha)	Aphids population/three leaves						PROC
		1 DBT	1 DAT	3 DAT	5 DAT	7 DAT	10 DAT	
Imidacloprid 17. 8 SL	25	*26.34 ± 1.57a	16.00 ± 0.18 e	10.33 ± 0.40c	3.98 ± 0.37 c	4.53 ± 0.23 de	5.78 ± 0.68 c	80.49
Imidacloprid 17.8 SL	50	27.04 ± 1.33 a	15.22 ± 0.34 e	9.18 ± 0.57c	2.78 ± 0.23 c	3.84 ± 0.04 e	4.91 ± 0.19 c	83.43
Thiamethoxam 25 WG	37.5	27.53 ± 1.16a	17.67 ± 0.54cd	11.36 ±0.52 c	5.40 ± 0.08 c	6.04 ± 0.24 c	6.67 ± 0.12 c	77.49
Thiamethoxam 25 WG	75	25.20 ± 1.68a	16.51 ± 0.29de	10.76 ±0.35c	4.67 ± 0.14c	5.71 ± 0.23 cd	6.07 ± 0.47 c	79.52
Cassava Leaf Extract	0.5%	26.31 ± 1.49a	20.04 ±0.17 b	15.31 ± 0.71b	12.69 ± 0.50 b	13.47 ± 0.37b	15.42 ± 0.49 b	47.97
Neem Oil	0.5%	24.93 ± 0.69a	18.89 ±0.72 bc	15.42 ± 1.34 b	11.73 ± 0.97 b	12.40 ± 0.74 bc	14.78 ± 0.87 b	50.13
Control	-	24.93 ± 0.79a	25.29 ± 0.83a	27.82 ± 0.71 a	28.67 ± 0.76 a	28.66 ± 1.25 a	29.64 ± 1.19 a	-
SE (m)±	-	1.17	0.49	0.80	0.90	0.51	0.69	-
CV(%)	-	7.77	4.62	9.71	15.54	8.20	10.10	-

Values followed by different letters in columns are significantly different at p = 0.05 by LSD

*Mean of three replication

DBT: Days before treatment

DAT: Days after treatment

PR: Per cent Reduction over control

Among the treatments, plot treated with imidacloprid @ 50 g a.i.ha^{-1} recorded maximum cormel yield of 15.58 ± 0.27 t ha^{-1} which was found statistically at par with plot treated with imidacloprid @ 25 g a.i.ha^{-1} (15.14 ± 0.10 t ha^{-1}). Similarly maximum total (corm + cormel) yield was recorded in the plot treated with imidacloprid @ 50 g a.i.ha^{-1} (26.41 ± 0.70 t ha^{-1}) followed by plot treated with imidacloprid @ 25 g a.i.ha^{-1} (25.41 ± 0.22 t ha^{-1}) and thiamethoxam @ 75 (25. 16 ± 0.35 t ha^{-1}) and 37.5 g a. i.ha^{-1} (24.85 ± 0.40 t ha^{-1}) (Table 2).

Table 2. Effect of insecticidal treatments on yield and yield attributes in taro (pooled data of 3 years of experiment).

Treatment	Dose (a.i/ha)	Cormel Yield (tha^{-1})	Corm Yield (tha^{-1})	Total Yield (tha^{-1})
Imidacloprid 17. 8 SL	25	15.14 ± 0.10 a	10.27 ± 0.31 ab	25.41 ± 0.22 ab
Imidacloprid 17.8 SL	50	15.58 ± 0.27a	10.83 ± 0.43 a	26.41 ± 0.70 a
Thiamethoxam 25 WG	37.5	14.89 ± 0.30 a	9.96 ± 0.33 b	24.85 ± 0.40 ab
Thiamethoxam 25 WG	75	14.98 ± 0.17 a	10.18 ± 0.24 ab	25.16 ± 0.35 ab
Cassava Leaf Extract	0.5%	12.52 ± 0.44 b	8.60 ± 0.13 c	21.13 ± 0.38 c
Neem Oil	0.5%	12.78 ± 0.31 b	8.60 ± 0.07 c	21.38 ± 0.34c
Control	-	10.50 ± 0.34 c	7.19 ± 0.23 d	18.19 ± 0.54d
Standard error of the mean (±)	-	0.31	0.27	0.43
CV (%)	-	3.89	4.92	3.22

Values followed by different letters in columns are significantly different at p = 0.05 by LSD

Table 3. ANOVA Table for polled analysis.

Source	DF	Ftab (P = 0.05)	Fcal (P = 0.05)						Cormel Yield	Corm Yield	Total Yield
			1 DBT	1 DAT	3 DAT	5 DAT	7 DAT	10 DAT			
Year	2	3.259	2.692	2.786	1.609	0.031	1.062	1.159	1.171	1.036	1.746
Replication	2	2.364	1.484	0.449	0.331	0.553	1.827	0.530	0.702	0.288	0.433
Treatment	6	2.364	2.288	144.020	191.927	299.786	814.342	476.560	110.311	53.422	139.625
Year ×Treatment	6	2.033	0.688	1.080	1.179	0.955	1.892	0.198	0.484	0.297	0.489
Pooled Error	12	-	-	-	-	-	-	-	-	-	
Total	36	-	-	-	-	-	-	-	-	-	

$F_{cal\,(6,\,36)} > F_{tab(6,\,36)}$) : Significant otherwise Non-Significant

DBT: Days before treatment

DAT: Days after treatment

DF: Degree of Freedom

Persistence dissipation and safety evaluation of imidacloprid in taro

The correlation coefficient (r^2) for the linearity curve prepared by injecting different concentration of imidacloprid standards i.e 0.01, 0.05, 0.1, 0.5 and 1.0 in solvent and matrix matched was more than 0.99. By considering the signal to noise ratio of 3, the Limit of Detection (LOD) was calculated as 0.004. The Limit of Quantification (LOQ) was calculated by dividing the nanogram of standard injected giving 10 per cent full scale deflection by the milligram of sample injected giving no interference are the retention time of standard eluted and found to be 0.01 mg kg^{-1}, respectively. The per cent recoveries at LOQ, 5 X LOQ and 10 X LOQ were more than 80 per cent with relative standard deviation (RSD) within 20 per cent and hence the results are presented as such without any correction factor.

The extractable residues of imidacloprid 17.8 SL in the leaves of taro are presented in Table 4. The represented chromatograms of standard, control taro leaves sample are presented in Fig. 1. The mean initial deposit of imidacloprid was 0.65 and 1.32 mg kg^{-1} at single and double dose, respectively. More than 45 per cent of the initial deposit was dissipated after 1 day of spraying at both the doses. The residue of imidacloprid dissipated to below the LOQ of 0.01 mg kg^{-1} after 5 days of application in single doses whereas that of in double doses dissipated after 7 days of application. Taro tuber samples collected 30 days after treatment did not showed the presence of imidacloprid residues above LOQ of 0.01 mg kg^{-1}.

Table 4. Residues (mg kg-1) of imidacloprid in taro leave samples collected at different intervals.

Days after treatment	Residues following application @ 25 g a.i. ha^{-1} (mean ± sd)	Per cent dissipation	Residues following application @ 50 g a.i. ha^{-1} (mean ± sd)	Per cent dissipation
0 (2 hours after treatment)	0.65 ± 0.13	-	1.32 ± 0.06	-
1	0.35 ± 0.07	46.15	0.72 ± 0.10	45.45
3	0.09 ± 0.03	86.15	0.20 ± 0.07	84.85
5	< 0.01	-	0.12 ± 0.03	90.91
7	< 0.01	-	< 0.01	-
10	< 0.01	-	< 0.01	-
Taro				
(30 days after treatment)	< 0.01	-	< 0.01	-

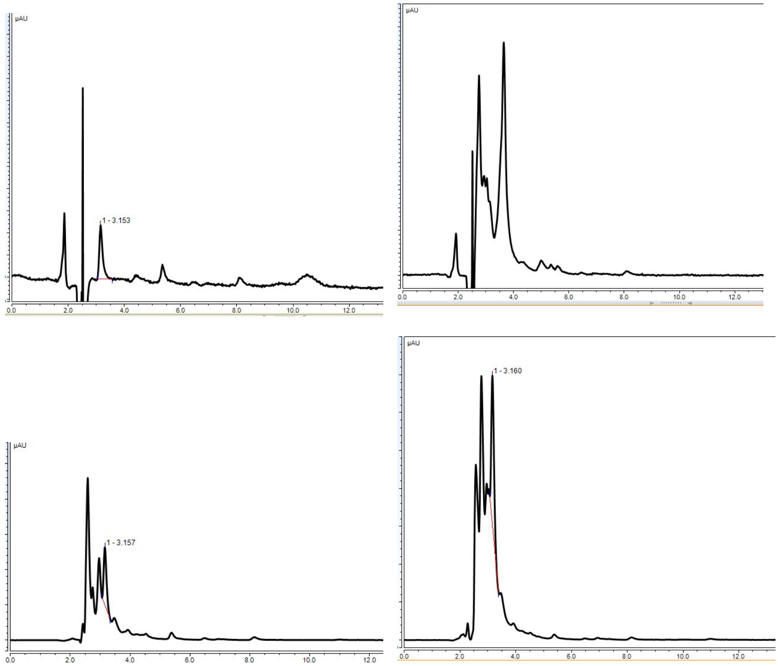

Fig. 1. Representative chromatograms a. standard imidacloprid @ 0.01 mg L^{-1} ; b. control taro leaves; c. single dose 0 day sample d. double dose 0 day sample

The degradation kinetics of the imidacloprid in leaves of taro was determined by plotting total residue concentration against time, and the maximum squares of correlation coefficients found was used to determine the equations of best fit curves. Confirmation of the first order kinetics was further made graphically from the linearity of the plots of log C against time (C= residues × 100). Half life ($t_{1/2}$) of imidacloprid was calculated (In 2/b value from the equation) as per formula given by (Hoskins, 1961). The dissipation parameters of imidacloprid in taro leaves are presented in Table 5. Fifty per cent of the initial deposits of imidacloprid dissipated within 1.05 (single dose) and 1.41 (double dose) days of field application. The day wise residue data had excellent fit to the 1st order models giving r^2 value of > 0.96. The Pre Harvest Intervals (PHIs) of imidacloprid were 6.33 and 9.71 days for single and double dose, respectively.

Table 5. Dissipation parameters of imidacloprid in taro leaves.

Parameters	Default MRL 0.01 (mg kg $^{-1}$)	
	Imidacloprid @ 25g a.i. ha^{-1}	Imidacloprid @ 50g a.i. ha^{-1}
r2	0.99	0.964
a (mg kg $^{-1}$)	1.819	2.069
B	-0.287	-0.213
DT50 (Days)	1.049	1.413
PHI (Days)	6.337	9.713
Y	1.819 – 0.287x	2.069 – 0.213x

b = Slope of regression line

a = Initial deposit obtained as in the regression equation

DT50 = Residual half-life (in days)

PHI (Pre Harvest Interval) = Time (in days) required for the pesticide residue to reach below the maximum residue limit of 0.01 mg kg-1

r2= Correlation Coefficient

In the state of Bihar, there is always short supply of vegetables during the month of June to August as on an average 7.18 per cent of total geographical area of the state was under flood or water logged situation affecting more than 7 lakhs people. During this time, taro act as a substitute for vegetable because of it's relatively tolerance to waterlogged conditions. Hence it is important to assess the safety associated with the use of imidacloprid. The risk evaluation of imidacloprid in taro leaves was evaluated as the MRL value is not available. For the risk evaluation, maximum permissible intake (MPI) was calculated as 0.33 mg person^{-1}day^{-1} by multiplying the Acceptable Daily Intake (ADI) (0.006 mg kg^{-1} body weight day^{-1}) value of imidacloprid with the average body weight of adult Indian (55 kg). The MPI value was compared with the TMRC. For calculation of TMRC, the maximum residues data of each day was multiplied with the daily consumption of taro leaves (0.023 kg) and found less that MPI on all the sampling days for single as well as the double dose (Table 6). The MRL for imidacloprid in taro leaves is not available, hence following the default MRL value of 0.01 mg kg^{-1}, PHI of 6.33 and 9.71 days for single and double dose, respectively may be suggested though TMRC were well below MPI for all sampling days. For taro tubers, PHI of 30 days may be suggested for safe consumption by following good agricultural practices.

Table 1. Effect of insecticidal treatments on aphid population in taro (pooled data of 3 years of experiment).

Interval (days)	Imidacloprid				
	MPIa for 55 kg person (mg person^{-1} d^{-1})	25 g a.i. ha^{-1}		50 g a.i. ha^{-1}	
		Maximum residues (mg kg^{-1})	TMRCb (mg person^{-1} d^{-1})	Maximum residues (mg kg^{-1})	TMRC(mg person^{-1} d^{-1})
0	0.33	0.79	0.0182	1.40	0.0322
1	0.33	0.44	0.0101	0.82	0.0189
3	0.33	0.13	0.0030	0.29	0.0067
5	0.33	<LOQ	-	0.16	0.0037
7	0.33	<LOQc	-	<LOQ	-

aADI = Acceptable Daily Intake

bTMRC = Theoretical maximum residue contribution

cLOQ= Limit of quantification (0.01 mg kg-1)

DISCUSSION

Since no published report on management of aphids in taro is available, the findings were not compared. However, the finding of (Khedakar, Bharpda, Patel, & Patel, 2012) was inconformity with present finding, who found that imidacloprid and thiamethoxam were most effective in controlling the mustard aphid whereas excellent control of wheat aphid by imidaclporid was also observed by (Joshi & Sharma, 2009) under field conditions. Since, no published work on residues of imidacloprid on taro leaves and tubers was found, hence the data observed were not compared with other published scientific reports. However (Yu et al, 2007) reported that no significant residues of imidacloprid was detected in leaves and stem of rice as well as in the expanded new leaf after 7 days of treatment agreeing our observation.

ACKNOWLEDGEMENTS

We are thankful to the Director, ICAR- Central Tuber Crop Research Institute (Thriuvanathapuram) 695614 Kerala, India for providing the fund for conducting the research trial and Head, Entomology, RPCAU, Pusa (Samastipur) 848125 Bihar, India for providing necessary facilities for estimating the residue of imidacloprid in leaves and tuber of taro.

Funding: All India Co-ordinated Research Project on Tuber Crops (Other than Potato)

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J. Entomol. Res. Soc., 25(3): 491-505, 2023 *Research Article*
Doi: 10.51963/jers.v25i3.2401 *Online ISSN:2651-3579*

Toxic and Behavioral Effect of Pesticides on Aphidophagus Predator, *Coccinella septempunctata* (Linnaeus, 1758) (Coleoptera: Coccinellidae) Under Laboratory Conditions

Razia RASHEED[1*] Abdul A. BUHROO[1]

[1]Entomology Research Unit, Department of Zoology, University of Kashmir, Hazratbal Srinagar –190006, Jammu and Kashmir, INDIA

e-mails: [1*]raziarasheed123@gmail.com, [1]abuhroo@yahoo.com

ORCID IDs: [1*]0000-0002-6161-7544, [1]0000-0002-9576-1165

ABSTRACT

Eco-friendly natural enemies have received increasing attention in recent years due to their contribution in reducing the use of chemical pesticides. However, the deleterious effects of pesticides on these natural enemies remain to be understood. In the present study, toxic and behavioral effect of eight most commonly used pesticides in different orchards of Kashmir were tested against aphidophagus predator, *Coccinella septempunctata* Linnaeus, 1758 under the laboratory conditions. According to IOBC classification, alphamethrin was moderately harmful to the adults of *C. septempunctata* whereas, other pesticides were slightly harmful under direct exposure of pesticides. However, all the pesticides resulted harmful to the immature stages of *C. septempunctata*. Under the residual effect of pesticides on adults of *C. septempunctata*, thiamethoxam and dimethoate caused highest mortality. All the insecticides were observed to be toxic to the grubs of *C. septempunctata*, whereas, fungicides were moderately harmful to the grubs of *C. septempunctata*. The present results also showed that alphamethrin altered the behavior of the predator and reduced its predation rate, while as fungicide had minor behavioral effects.

Keywords: Environment, Pest control, natural enemies, pesticides, toxicity, behavior.

Rasheed, R. & Buhroo, A.A. (2023). Toxic and behavioral effect of pesticides on aphidophagus predator, *Coccinella septempunctata* (Linnaeus, 1758) (Coleoptera: Coccinellidae) under laboratory conditions. *Journal of the Entomological Research Society*, 25(3), 491-505.

Received: January 11 2023 *Accepted: October 10, 2023*

INTRODUCTION

Natural enemies are vital components of agroecosystem and are consistently used as biological control agents. A wide variety of insect pest infests fruits in the Kashmir valley and cause damage to the fruit, reducing its yield and quality. Among these insect pests, scales, aphids and mites are most destructive sucking sap from the bark, foliage and fruit. They are the most recognized pests of fruit crop in Kashmir valley. In order to control the pest infestation, farmers have adopted the use of chemical pesticides and synthetic fertilizers (Bhanti & Taneja, 2007). The improvement in crop yield which is fostered by pesticides and fertilizers application is sometimes associated with the occurrence and persistence of pesticide and nutrient residues in the soil and water (Ware & Whitacre, 2004). The increasing use of these pesticides in agricultural areas have polluted aquatic environment through direct run off, discharge, careless dumping of empty containers and washing equipment directly from the rivers (Milidas, 1994). The use of pesticides is the most common, economical and easiest available method. However, the problems associated with these pesticides such as bioaccumulation, pollution and resistance are usually ignored. Also, one of the major concerns is the development of pesticide resistance. In recent years, pesticides are being used in a multipronged strategy to control insect pests (Azimizadeh, Ahmadi, Imani, Takalloozadeh, & Sarafrazi, 2012; Sánchez-Bayo, 2021). One of the important challenges of pest control with pesticides is to kill the target pests and minimizing the mortality of beneficial insects at the same time. Biological control agents such as insect predators, mite predators and hymenopterous parasitoids are usually more sensitive to pesticides than the target pests (Theiling & Croft, 1988). The effect of pesticides on natural enemies are associated with either direct effect (mortality) or indirect effects (Stapel, Cortesero, & Lewis, 2000; Elzen, 1990; Haseeb, Liu, & Jones, 2004; Borgemeister, Poehling, Dinter, & Holler, 1993). Indirect effects may be more chronic that inhibit the ability of beneficial insects to establish populations, suppress the predation and decrease reproduction (Croft, 1990; Van de Veire & Tirry, 2003; Grafton-Cardwell, Lee, Stewart, & Olsen, 2006). Biological control occupies a central position in integrated pest management (IPM) programmes, because biological control agents for pests have enormous and unique advantage, it is safe, permanent and economical. Among different insect predators Coccinellidae is the largest family of order Coleoptera and its members are commonly known as ladybird beetles, ladybugs or Coccinellid beetles. *Coccinella septempunctata* Linnaeus, 1758 is one of the most important beetle belonging to this family. The Coccinellidae are an important group of beetles from both economic point of view as their use in biological control and in their diversity and adaptation to a number of differing habitats. *C. septempunctata* is extremely diverse in their habitats and play important role as biological control attacking different pests such as aphids, coccids and other soft bodied insect pests. Thus, the aim of the present investigation was to study the lethal and behavioral effects of the most commonly used pesticides in the Kashmir valley, on predatory lady bird beetle *C. septempunctata.*

MATERIALS AND METHODS

Experimental Setup

The experiment was conducted in the entomology laboratory University of Kashmir. The adults and immature stages of *C. septempunctata* used in the experiment were laboratory reared under controlled conditions (28.55 ± 2.83˚C and 75.15 ± 6.06% relative humidity). Experiment was initiated by collecting adults of *C. septempunctata* from fields. Mating pairs were kept in glass jars covered with muslin cloth. They were provided with abundant supply of food in the form of infested twigs of cabbage aphids, *Brevicoryne brassicae* (Linnaeus, 1758) until oviposition. Dry twigs were replaced with fresh ones after every 24 hrs in order to avoid contamination. The newly emerged adults and immature stages of *C. septempunctata* were used for further experimentations.

Pesticides

Pesticides tested in this study include six insecticides such as alphamethrin, chlorpyrifos + cypermethrin, dimethoate, quinalphos, thiamethoxam, dichlorvos and two fungicides such as myclobutanil and flusilazole. These pesticides are locally used by the farmers against different pests in various crops and their use has been increased during recent years in Kashmir, India (Table 1). These insecticides were tested at a single rate of application, corresponding to their maximum recommended label rate which is generally used by the farmers (Handbook, 2015, 2017 and 2018). Adult and immature stages of *C. septempunctata* were exposed to pesticide through double exposure method: directly exposed to spray droplets and residually through walking on sprayed leaf. A double exposure method signifies the best condition that insects are likely to experience in the field.

Table 1. List of pesticides used in the present study. All pesticides were used at their highest label rates against predators.

Active ingredient	Commercial name	Mode of Action	Target Pests
Alphamethrin 10% EC	Stop 10 EC	Contact and Stomach Action. It acts on Nervous system.	Aphids
Chlorpyrifos50% +Cypermethrin 5%	Cyclone	It is non-systematic insecticide of organophosphorus and synthetic pyrethroids group with contact and stomach action.	Chewing and sucking insect pests
Dimethoate 30%EC	Rogor	Contact and ingestion type of systematic insecticide. It is Acetylcholinesterase inhibitor.	Mites, hoppers, bugs, borers, thrips, white flies and aphids.
Thiamethoxam 25% WG	Tara	Systematic insecticide having Contact and stomach action and belonging to Neonicotinoid group.	Borers, aphids, thrips, hoppers, caterpillars
Quinalphos 25% EC	Krush	Non-Systematic insecticide. Contact and stomach action belonging to Organothiophosphate group.	Paddy pests, wooly aphids, red gram pests.
Dichlorvos 76%	Nuvan	Organophosphate insecticide Acetylcholinesterase inhibitor.	Army worm, leaf eating pests and caterpillars.
Myclobutanil 10% WP	Index	Triazole group of Systematic fungicide. Steroid demethylation inhibitor.	Used against Ascomycetes, powdery mild dew, dollar spot, brown patch and scab.
Flusilazole 40% EC	Governor	Triazole group of Systematic fungicide. It is an organosilicon compound.	Powdery mild dew and scab.

Source: PubChem Compound Database (https://pubchem.ncbi.nlm.nih.gov/ 49833, 3082, 5821911, 26124, 3039, 6336, 73675, 2730, 2912).

Direct exposure of pesticides

In this experiment, adult individuals of *C. septempunctata* were placed in petri dish (9 cm diameter) in a group of 10. The top of petri dish was covered with fine muslin cloth tightened with rubber band and were maintained at 28.55 ± 2.83˚C and 75.15 ± 6.06% relative humidity. They were also provided with food (cabbage aphids, *Brevicoryne brassicae).* Distilled water was used as control. The spray volume of pesticide solution and distilled water per application was 1 ml, using small calibrated hand sprayer (1liter capacity) equipped with a nozzle suited to low volume spray application (Martinou, Seraphides, & Stavrinides, 2014) (Table 2). The experiment was replicated thrice. The mortality was recorded after 24 hr, 48 hr, 72 hr and 96 hr. Moribund individuals were classified as dead. Similar method was applied to first and fourth immature stages of *C. septempunctata*.

Table 2. Direct exposure of Pesticide treatments used against the predator *Coccinella septempunctata* using petri plate assay under laboratory conditions (28.55 ± 2.83◦ C and 75.15 ± 6.06% relative humidity).

Sample No.	Group*	No. of Petri dishes in each group/ Individual	Treatments	Spray Volume (ml/Petri dish)
1	G1	3/30	Alphamethrin	1
2	G2	3/30	Chlorpyrifos + Cypermethrin	1
3	G3	3/30	Dimethoate	1
4	G4	3/30	Quinalphos	1
5	G5	3/30	Thiamethoxam	1
6	G6	3/30	Dichlorvos	1
7	G7	3/30	Flusilazole	1
8	G8	3/30	Myclobutanil	1
9	G9	3/30	Distilled water	1

*Each group represents three petri dishes with 10 individuals subjected to 9 treatments.

Residual effect of pesticides

In this study, fresh kale leaf discs (90 mm in diameter) sprayed with pesticide solution and distilled water as control was used (three replicates). The spray volume per application was 1 ml for each pesticide solution and distilled water, using small calibrated hand sprayer (1 litre capacity). Treated leaves were dried at room temperature for 2 hr and then placed on the bottom of clean petri dishes (9 cm diameter). In each petri dish 10 adult individuals of *C. septempunctata* were released using camel- hair brush. Cabbage aphids, *Brevicoryne brassicae* were provided as food and the top of petri dish was covered with fine muslin cloth. The petri dishes were maintained at 28.55 ± 2.83˚C and 75.15 ± 6.06% relative humidity. Moribund individuals were classified as dead (Martinou et al, 2014). Mortality was recorded after 24 hr, 48 hr, 72 hr and 96 hr (Table 3). Similar method was applied to first and fourth immature stages of *C. septempunctata*.

Behavioral effect of pesticides

In a second assay, behavioral effect of pesticides, that showed highest and lowest toxicity in previous experiment were evaluated. For this experiment young kale plant, *Brassica oleracea* var. *acephala,* Linnaeus with aphid infestation (five-week-old, height 15 cm, 5 leaves /plant) was sprayed with pesticide with the help of calibrated handheld sprayer and distilled water for control. Treated plant was dried at room temperature for 2 hours, after that adult *C. septempunctata* was placed at the bottom of the main stem of plant with the help of camel-hair brush. The adult predator was observed for 11 min with two magnifying glass held at 45° angle and at 30 cm distance. The duration for each of the following behaviors were recorded: feeding, preening, walking and resting. The experiment was carried out in 7 days between 10.00 am to 14.00 pm with three replicates of each treatment per day.

Table 3. Residual effect of pesticide treatments used against the predator *Coccinella septempunctata* using petri plate assay under laboratory conditions (28.55 ± 2.83◦ C and 75.15 ± 6.06% relative humidity).

Sample No.	Group*	No. of Petri dishes with treated leaf in each group/ Individual	Treatments
1	G1	3/30	Alphamethrin
2	G2	3/30	Chlorpyrifos + Cypermethrin
3	G3	3/30	Dimethoate
4	G4	3/30	Quinalphos
5	G5	3/30	Thiamethoxam
6	G6	3/30	Dichlorvos
7	G7	3/30	Flusilazole
8	G8	3/30	Myclobutanil
9	G9	3/30	Distilled water

*Each group represents three petri dishes with 10 individuals subjected to 9 treatments.

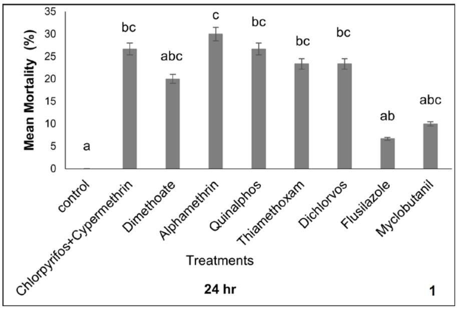
Data Analysis

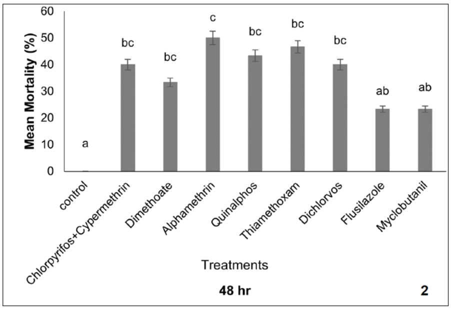
The mortality results obtained after 24, 48,72 and 96 hours were adjusted for mortality that occurred in control (water) using the Abbot's formula (Abbot, 1925). The corrected mortalities of adults and immature stages were evaluated by the IOBC toxicity rating scale for pesticides under laboratory conditions: 1, Harmless (<30% Mortality), 2, Slightly Harmful (30-79% Mortality), 3, Moderately Harmful (80-99% Mortality) and 4, Harmful (>99% Mortality) (Hassan et al., 1994). For statistical comparison of the mortality of adults, the data was subjected to one-way analysis of variance (ANOVA). Post hoc Tukey HSD tests (Tukey HSD) were used for multiple pair wise comparison. The significance difference between the means was determined at $P \leq 0.05$. Behavioral data were expressed as time in "minutes" allocated to each different activity during the 11 minutes observation period.

RESULTS

Direct exposure of pesticides on adults of *C. septempunctata*

We observed differential degree of toxicity for the pesticides used against adults of *C. septempunctata*. After 24 hr exposure to the insecticides, the mortality caused by alphamethrin, quinalphos and chlorpyrifos+ cypermethrin was 30%, 26.66% and 26.66%, respectively. Mortality of *C. septempunctata* due to thiamethoxam and dichlorvos was found to be in the same range of 23.33%. Mortality caused by the treatments flusilazole and myclobutanil was found 6.66% and 10%, respectively. One-way ANOVA results showed no significant difference in mean percentage mortality among alphamethrin, quinalphos, thiamethoxam, dichlorvos, chlorpyrifos + cypermethrin, dimethoate and myclobutanil treatments (Fig. 1). Moreover, no mortality was observed in control treatment. Flusilazole and myclobutanil caused lowest mortality (23.33%) of *C. septempunctata* after 48 hr duration. However, 50% mortality was observed with treatment alphamethrin. Thiamethoxam and dimethoate caused second and third highest mortality. Alphamethrin showed significant difference from flusilazole and myclobutanil mortality (Fig. 2). Again, control was found with no mortality.





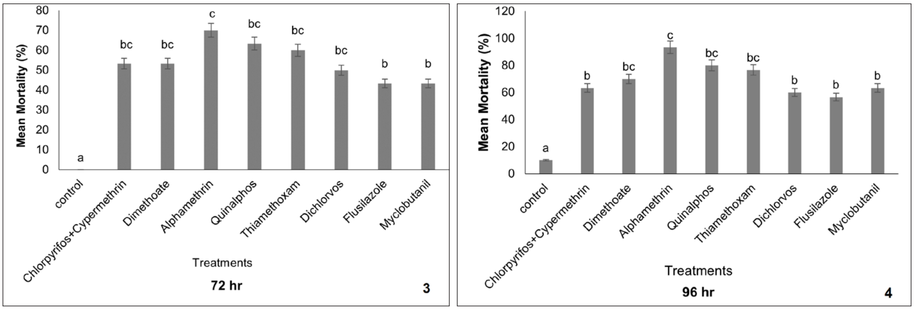
Figures 1-2. Mean percentage mortality of *Coccinella septempunctata* after 24 hr and 48 hr by direct exposure to pesticides. Percentage error is added on bars and different letters on bars indicate statistically significant differences at $p \le 0.05$, in one-way ANOVA followed by the Tukey's multiple comparison test.

After 72 hr duration, alphamethrin again showed highest mortality of 70% in *C. septempunctata* followed by quinalphos 63.33%. ANOVA results showed that mortality caused due to alphamethrin differ significantly at $p \le 0.05$ from control, flusilazole and myclobutanil mortality. However, there was no significant difference between the mortality caused by flusilazole and myclobutanil. Similar results were shown by quinalphos, thiamethoxam, dimethoate, dichlorvos and chlorpyrifos + cypermethrin (Fig. 3). After 96 hr of exposure, highest mortality was observed in alphamethrin (93.33%) followed by Quinalphos (80%) and Thiamethoxam (76.66%) against *C. septempunctata*. We observed 10% mortality of *C. septempunctata* in the control group. Mortality produced by alphamethrin was significantly ($p \le 0.05$) higher than control, chlorpyrifos+cypermethrin, dimethoate, flusilazole and myclobutanil (Fig. 4). The corrected mortality of *C. septempunctata* under different treatments after 96 hr is shown in table 4. The corrected mortalities of adult *C. septempunctata* was evaluated by IOBC toxicity rating scale for pesticide under laboratory conditions. According to IOBC rating for laboratory assays, alphamethrin was observed moderately harmful to *C. septempunctata* after 96 hr treatment while others are slightly harmful during the present study.

Table 4. Mean and Corrected Mortality of *Coccinella septempunctata* after 96 hr direct exposure to Pesticides and toxicity rating.

Pesticide Group	Treatment	Mean Mortality (±SD)	Corrected Mortality (%)	IOBC* Rating
Insecticides	Alphamethrin	9.33±0.57	92.58	3
	Quinalphos	8.00±1.00	77.77	2
	Thiamethoxam	7.66±0.57	74.06	2
	Dimethoate	7.00±1.00	66.66	2
	Chlorpyrifos +Cypermethrin	6.33±0.57	59.25	2
	Dichlorvos	6.00±0.00	55.55	2
Fungicides	Myclobutanil	6.33±1.15	59.25	2
	Flusilazole	5.66±1.52	51.84	2

*1, Harmless (<30% Mortality), 2, Slightly Harmful (30-79 % Mortality), 3, Moderately Harmful (80-99% Mortality) and 4, Harmful (>99% Mortality).



Figures 3-4. Mean percentage mortality of *Coccinella septempunctata* after 72 hr and 96 hr by direct exposure to pesticides. Percentage error is added on bars and different letters on bars indicate statistically significant differences at p≤0.05, in one-way ANOVA followed by the Tukey's multiple comparison test.

Direct exposure of pesticides on immature stages of *C. septempunctata*

The newly hatched first instar grubs and fourth instar grubs of *C. septempunctata* were used in this experiment. The results showed 100% mortality after 24 hr exposure to the treatments alphamethrin, thiamethoxam, dimethoate, dichlorvos, quinalphos and chlorpyrifos+ cypermethrin in first instar grubs of *C. septempunctata*. Whereas, myclobutanil and flusilazole caused 96.66% and 93.33% mortality, respectively. However, after 48hr 100% mortality was observed due to fungicides. On the other hand, 6.66% and 16.66% mortality were observed in control after 72 and 96 hr respectively (Table 5).

Similarly, in fourth instar grubs of *C. septempunctata*, 100% mortality was observed after 24 hr exposure to the treatments alphamethrin, thiamethoxam, quinalphos and chlorpyrifos +cypermethrin. Dimethoate and dichlorvos caused 93.33% and 96.66% mortality after 24 hr, respectively. On the other hand, both treatments caused 100% mortality after 72 hr. The mortality of fourth instar grubs due to treatments myclobutanil and flusilazole were 90% and 86% respectively after 24 hr and 100% after 72 hr in both the treatments. Moreover, in control 3.33% and 6.66% mortality was observed after 72 hr and 96 hr respectively during the present observations (Table 6). All the pesticides were found harmful to both the grubs of *C. septempunctata* according to IOBC rating for laboratory assay (Table 5 and 6).

Table 5. Mean Mortality of First instar grubs of *Coccinella septempunctata* after direct treatments of Pesticides and toxicity rating.

Pesticide Group	Treatment	Mean Mortality (±SD)				IOBC* Rating
		24hr →	48hr →	72hr →	96hr	
Insecticides	Alphamethrin	100 ±0.00	100 ±0.00	100 ±0.00	100 ±0.00	4
	Quinalphos	100 ±0.00	100 ±0.00	100 ±0.00	100 ±0.00	4
	Thiamethoxam	100 ±0.00	100 ±0.00	100 ±0.00	100 ±0.00	4
	Dimethoate	100 ±0.00	100 ±0.00	100 ±0.00	100 ±0.00	4
	Chlorpyrifos +Cypermethrin	100 ±0.00	100 ±0.00	100 ±0.00	100 ±0.00	4
	Dichlorvos	100 ±0.00	100 ±0.00	100 ±0.00	100 ±0.00	4
Fungicides	Myclobutanil	100 ±0.00	100 ±0.00	100 ±0.00	100 ±0.00	4
	Flusilazole	100 ±0.00	100 ±0.00	100 ±0.00	100 ±0.00	4
Control	Distilled water	0.00±0.00	0.00±0.00	6.66±0.00	16.66±0.00	1

*1, Harmless (<30% Mortality), 2, Slightly Harmful (30-79 % Mortality), 3, Moderately Harmful (80-99% Mortality) and 4, Harmful (>99% Mortality).

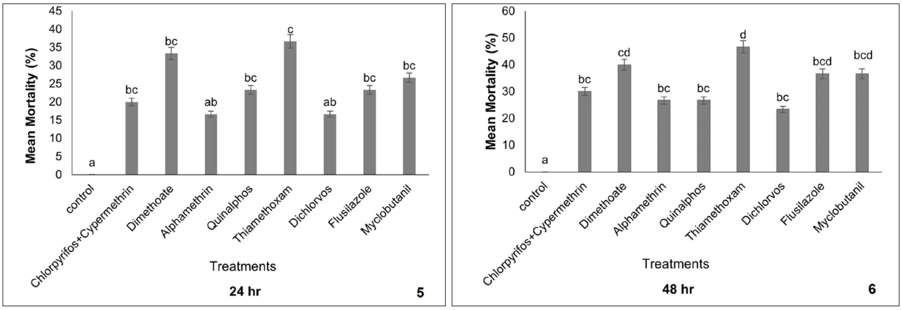
Table 6. Mean Mortality of Fourth instar grubs of *Coccinella septempunctata* after direct treatments of Pesticides and toxicity rating.

Pesticide Group	Treatment	Mean Mortality (±SD)				IOBC* Rating
		24hr	48hr →	72hr	96hr	
Insecticides	Alphamethrin	100 ±0.00	100 ±0.00	100 ±0.00	100 ±0.00	4
	Quinalphos	100 ±0.00	100 ±0.00	100 ±0.00	100 ±0.00	4
	Thiamethoxam	100 ±0.00	100 ±0.00	100 ±0.00	100 ±0.00	4
	Dimethoate	93.33±0.00	100 ±0.00	100 ±0.00	100 ±0.00	4
	Chlorpyrifos +Cypermethrin	100 ±0.00	100 ±0.00	100 ±0.00	100 ±0.00	4
	Dichlorvos	96.66 ±0.00	100 ±0.00	100 ±0.00	100 ±0.00	4
Fungicides	Myclobutanil	90.00 ±0.00	93.33 ±0.00	100 ±0.00	100 ±0.00	4
	Flusilazole	86.66 ±0.00	90.00 ±0.00	100 ±0.00	100 ±0.00	4
Control	Distilled water	0.00±0.00	0.00±0.00	3.33±0.00	6.66±0.00	1

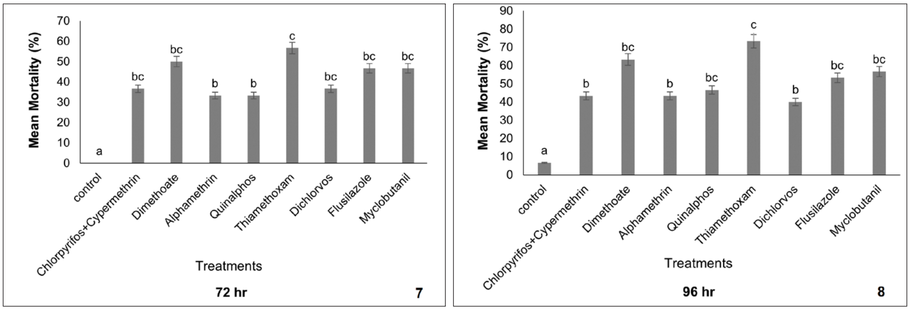
*1, Harmless (<30% Mortality), 2, Slightly Harmful (30-79 % Mortality), 3, Moderately Harmful (80-99% Mortality) and 4, Harmful (>99% Mortality).

Residual effect of pesticides on adults of *C. septempunctata*

Under the residual effect after 24 hr duration, insecticide thiamethoxam and dimethoate was found to cause highest mortality of adult *C. septempunctata*. One-way ANOVA results showed that thiamethoxam caused significantly high mortality (36.66%) at $P \leq 0.05$ than other treatments. There was no significant difference at $p<0.05$ between the percentage mortalities caused by chlorpyrifos+cypermethrin, dimethoate, quinalphos, flusilazole and myclobutanil (Fig. 5). After 48 hr duration, the observed mortality of 46.66% was caused by thiamethoxam, 40% with dimethoate, 36.66% with flusilazole and myclobutanil and 30% with chlorpyrifos +cypermethrin against adult *C. septempunctata*. ANOVA results showed no significant difference at $p \leq 0.05$ between the mortality caused by chlorpyrifos+cypermethrin, alphamethrin, dimethoate, quinalphos, flusilazole and myclobutanil (Fig. 6). After 72 hr duration, the mortality of 56.66% and 50% was observed by thiamethoxam and dimethoate respectively against *C. septempunctata*. There was no significant difference amongst the mortality caused by flusilazole, myclobutanil, dimethoate, chlorpyrifos +cypermethrin and dichlorvos. Till 72 hr, no mortality was observed in control (Fig. 7). After 96 hr, the mortality of *C. septempunctata* reached to a maximum of 73.33% with treatment thiamethoxam, 63.33% with dimethoate and 56.66% in case of myclobutanil. The mean percent mortality of *C. septempuctata* in control was observed 6% during the present study. There was no significant difference at $p \leq 0.05$ between the mortalities caused by alphamethrin, dichlorvos and chlorpyrifos+cypermethrin (Fig. 8). The corrected mortality of *C. septempunctata* after 96 hr exposure to different treatments is represented in Table 7. The corrected mortalities of adults *C. septempunctata* were evaluated by IOBC toxicity rating scale for pesticide under laboratory conditions. All the treatments were found slightly harmful to the adults of *C. septempunctata* at 96 hr according to IOBC rating for laboratory assays.



Figures 5-6. Mean percentage mortality of *Coccinella septempunctata* after 24 hr and 48 hr residual exposure to pesticides. Percentage error is added on bars and different letters on bars indicate statistically significant differences at p≤0.05, in one-way ANOVA followed by the Tukey's multiple comparison test.



Figures 7-8. Mean percentage mortality of *Coccinella septempunctata* after 72 hr and 96 hr residual exposure to pesticides. Percentage error is added on bars and different letters on bars indicate statistically significant differences at p≤0.05, in one-way ANOVA followed by the Tukey's multiple comparison test.

Table 7. Mean and Corrected Mortality of *Coccinella septempunctata* after 96 hr exposure to Pesticides under residual effect and toxicity rating.

Pesticide Group	Treatment	Mean Mortality (±SD)	Corrected Mortality (%)	IOBC* Rating
Insecticides	Alphamethrin	4.33 ±1.15	34.61	2
	Quinalphos	4.66 ±0.57	38.45	2
	Thiamethoxam	7.33 ±0.57	69.22	2
	Dimethoate	6.33 ±1.15	57.69	2
	Chlorpyrifos +Cypermethrin	4.33 ±1.52	43.33	2
	Dichlorvos	4.00 ±1.00	30.77	2
Fungicides	Myclobutanil	5.66 ±0.57	49.99	2
	Flusilazole	5.33 ±0.52	46.15	2

*1, Harmless (<30% Mortality), 2, Slightly Harmful (30-79 % Mortality), 3, Moderately Harmful (80-99% Mortality) and 4, Harmful (>99% Mortality).

Residual effect of pesticides on immature stages of *C. septempunctata*

Like in above experiment newly hatched first instar grubs and fourth instar grubs were used for this experiment. We observed 96.66% and 100% mortality of first instar grubs in the treatment alphamethrin after 24 and 72 hr respectively. However, treatments with thiamethoxam and quinalphos were found to cause 100% mortality in first instar grubs after 24 hr. Whereas, other treatments were found to cause 100%

mortality after 48 hr. Myclobutanil and flusilazole were also observed to cause 100% mortality in first instar grubs after 72 hr. Besides this, control showed 3.33% and 10% mortality after 72 and 96 hr respectively. All the eight pesticides were found harmful to first instar grubs according to IOBC rating scale for pesticides under laboratory conditions (Table 8).

Table 8. Mean Mortality of first instar grubs of *Coccinella septempunctata* after residual treatments of Pesticides and toxicity rating.

Pesticide Group	Treatment	Mean Mortality (±SD)				IOBC*Rating
		24hr	48hr →	72hr	96hr	
Insecticides	Alphamethrin	96.66 ±0.00	100 ±0.00	100 ±0.00	100 ±0.00	4
	Quinalphos	100 ±0.00	100 ±0.00	100 ±0.00	100 ±0.00	4
	Thiamethoxam	100 ±0.00	100 ±0.00	100 ±0.00	100 ±0.00	4
	Dimethoate	93.33±0.00	100 ±0.00	100 ±0.00	100 ±0.00	4
	Chlorpyrifos +Cypermethrin	86.66 ±0.00	100 ±0.00	100 ±0.00	100 ±0.00	4
	Dichlorvos	93.33 ±0.00	100 ±0.00	100 ±0.00	100 ±0.00	4
Fungicides	Myclobutanil	90.00 ±0.00	96.66±0.00	100 ±0.00	100 ±0.00	4
	Flusilazole	86.66 ±0.00	93.33 ±0.00	100 ±0.00	100 ±0.00	4
Control	Distilled water	0.00±0.00	0.00±0.00	3.33±0.00	10.00±0.00	1

*1, Harmless (<30% Mortality), 2, Slightly Harmful (30-79 % Mortality), 3, Moderately Harmful (80-99% Mortality) and 4, Harmful (>99% Mortality).

In the fourth instar grubs of *C. septempunctata* treatment alphamethrin showed 93.33% mortality at 24 hr and 100% mortality was perceived after 72 hr. However, in treatments like quinalphos and thiamethoxam 100% mortality was observed after 48 hr. 100% mortality was also observed in fourth instar grubs after 96 hr in treatments dichlorvos and chlorpyrifos +cypermethrin. Fungicides, myclobutanil and flusilazole were found to cause 96.66% and 86.66% mortality respectively. According to IOBC rating scale for pesticides under laboratory conditions, all the insecticides were found to be harmful to the fourth instar grubs except fungicide flusilazole and myclobutanil that are moderately harmful. Moreover, in control 6.66% mortality of fourth instar grubs was observed (Table 9).

Table 9. Mean Mortality of Fourth instar grubs of *Coccinella septempunctata* after residual treatments of Pesticides and toxicity rating.

Pesticide Group	Treatment	Mean Mortality (±SD)				IOBC* Rating
		24 hr	48 hr →	72 hr	96 hr	
Insecticides	Alphamethrin	93.33±0.57	96.66 ±0.57	100 ±0.00	100 ±0.00	4
	Quinalphos	96.66 ±0.57	100 ±0.00	100 ±0.00	100 ±0.00	4
	Thiamethoxam	96.66 ±0.57	100 ±0.00	100 ±0.00	100 ±0.00	4
	Dimethoate	90.00±0.1	96.66 ±0.57	100 ±0.00	100 ±0.00	4
	Chlorpyrifos +Cypermethrin	83.33 ±0.57	93.33 ±0.57	96.66 ±0.57	100 ±0.00	4
	Dichlorvos	86.66 ±0.57	90.00 ±0.1	96.66 ±0.57	100 ±0.00	4
Fungicides	Mycobutanil	73.33 ±0.57	76.66 ±0.57	86.66 ±0.00	96.66±0.57	3
	Flusilazole	66.66 ±0.57	73.33 ±0.57	80.00 ±0.57	86.66±0.57	3
Control	Distilled water	0.00±0.00	0.00±0.00	0.00±0.00	6.66±0.57	1

*1, Harmless (<30% Mortality), 2, Slightly Harmful (30-79 % Mortality), 3, Moderately Harmful (80-99% Mortality) and 4, Harmful (>99% Mortality).

Behavioral effect of pesticides on *C. septempunctata*

Behavioral effect of pesticides on predator *C. septempunctata* was carried out under laboratory conditions (28.55 ± 2.83° C and 75.15 ± 6.06% relative humidity).

Young kale plant sprayed with two insecticides, alphamethrin and thiamethoxam that caused highest mortality and one fungicide (myclobutanil) with lowest mortality in the previous experiment were used for recording behavioral activities. Distilled water was used as control. Four main behavioral activities; resting, walking, preening and feeding were observed. The present observations showed that *C. septempunctata* spent more time in resting when subjected to insecticide alphamethrin and thiamethoxam as compared to myclobutanil and control (Fig. 9a). Similarly, *C. septempunctata* on control plants spent lower amount of time in preening compared to plants sprayed with alphamethrin (Fig. 9b). Regarding the behavioral activity of feeding, *C. septempunctata* on control plants spent significantly longer time in feeding. In case of the plants sprayed with alphamethrin, *C. septempunctata* spent less time in feeding. Moreover, *C. septempunctata* also spent longer time in feeding on plants sprayed with myclobutanil as compared to thiamethoxam (Fig. 9c). Total walking time of *C. septempunctata* did not differ much among the plants subjected to pesticides and Control (Fig. 9d).

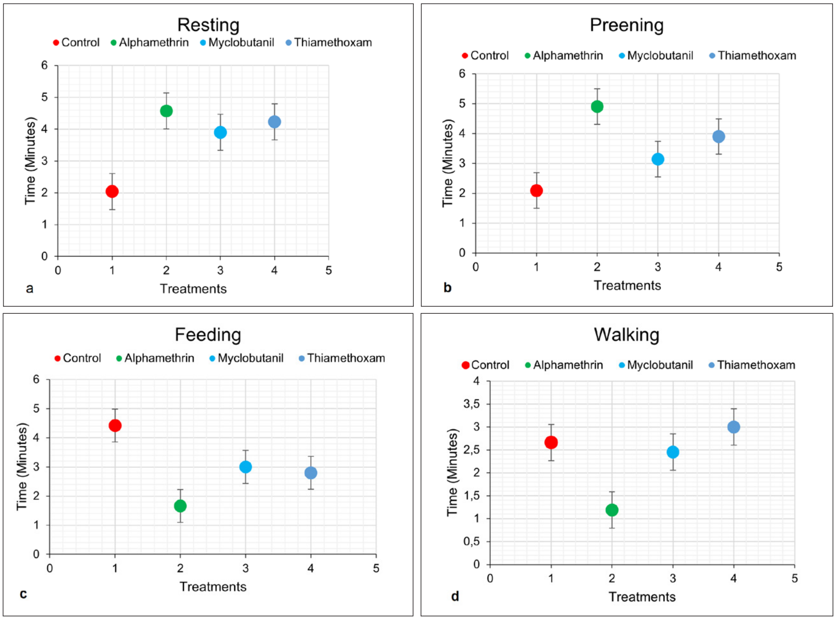


Figure 9. Time in minutes allocated to (a) resting (b) preening (c) feeding and (d) walking by adults of *Coccinella septempunctata* on plants treated with different pesticides and distilled water (control). Duration of the observation period was 11 minutes. Different points with standard error bars showing behavior among different treatments.

DISCUSSION

The laboratory contact toxicity assay conducted on immature stages of *coccinella septempunctata* with different insecticides showed highest mortality rates. Results obtained in the present study revealed that in particular alphamethrin, quinalphos and thiamethoxam caused highest mortality on all the tested ladybird beetles. The results attained with different insecticides is in agreement with others reported in the literature, according to which the compounds belonging to chemical group organophosphate, organochlorides, neonicotinoids pyrethroids and thiazole insecticides are highly harmful to natural enemies (Hassan et al, 1994; Hirai,1993; Gorri et al, 2015; Martinou et al, 2014; He, Zhao, Zheng, Desneux, & Wu, 2012; Lanka, Ottea, Davis, Hernandez, & Stout, 2013 and Liu & Zhang, 2012). Similar study was observed by Mollah, Rahman, & Alam (2013) in which they evaluate the effect of some insecticides on the abundance and mortality of predacious ladybird beetles in bean ecosystem. Their results showed the highest number of dead ladybird beetles after treatment with insecticide Curtap followed by Esfenvalerate, Deltrametrin, Cypermethrin, Fenitrothion, Fenvelarate and Emamectin benzoate. Also, Shah & Khan (2014) studied the coccinellid biodiversity under pesticide pressure in horticulture ecosystem of Kashmir valley. The observations are in agreement with the present results that pesticide treated ecosystem support less number of ladybird beetle species as compared to untreated horticulture ecosystem. Ujjan et al, (2017) worked on toxicity of insecticides and biopesticides against predatory beetle, *Menochilus sexmeculatus* Fabricius. The insecticides Curacron and Novastar showed severe toxicity to the egg and adults of *M. sexmaculatus*. On Contrary, the influence of 7 pesticides (6 insecticides & 1 acaricide) on different stages (adults, larvae, eggs) of *C. septempunctata* and adults of *Adalia bipunctata* were evaluated under laboratory conditions by Olszak, Ceryngier, & Warabieda (2004). Their results showed that aphids contaminated with chemicals such as pirimicarb, novaluron, pyriproxyfen and fenpyroximate did not cause serious effects on the *C. septempunctata* and *A. bipunctata*.

In second assay under residual effect of pesticides higher percentage of mortalities were shown by immature grubs of *C. septempunctata*. Similarly, all the pesticides when tested on adults of *C. septempunctata* showed reduced survival rate. Serious effect of thiamethoxam and dimethoate on the adults of *C. septempunctata* were observed. Indirect effect of pesticides is related to the residues remaining after foliar application that indirectly affect natural enemies by inhibiting adult emergence (Teodoro, Pallini, & Oliveira, 2009; Rill, Grafton-Cardwell, & Morse, 2008). Also some natural enemies are indirectly affected by consuming contaminated food or prey. Besides toxic effects, the behavioral effect of pesticides on adults of *C. septempunctata* was observed. Some behavioral activities of adults of *C. septempunctata* were carefully observed on pesticide treated plants. The present results showed that the adults of *C. septempunctata* changed behavior on pesticide treated plants like less feeding and walking. They show lower activities and learning response. The sub lethal dose does not cause adults death but inference in biological traits that may reduce the population. Earlier studies indicated that behavioral effect of insecticides may result in a rapid disruption of predatory behavior and a potential decrease in the ability of coccinellids to trace and capture their prey (Singh, Walters, & Port, 2001; Singh, Walters, Port, & Northing, 2004; Stark, Banks, & Acheampongl, 2004; Stark, Vargas, & Bank, 2007). The behavioural responses may also alter the predator’s hunt pattern (Thornham, 2005; Thornham, Blackwell, Evans, Wakefield, & Walters, 2008). Dent (2000) documented that non-lethal effects of pesticides include weakening of the predatory insects, changing their behaviour and lengthening the development period of the immature stages which will lead to the reduced prey consumption and reproductive ability.

It is evident from the present results, that all the pesticides tested were observed slightly harmful against adults of *C. septempunctata*. This proves helpful in deciding the most suitable pesticide to be used according to target predator. However, the major concern is about immature stages of *C. septempunctata*, as all the pesticides tested proved harmful against them. Thus, we suggest that the use of pesticides against serious pests should be in accordance with the compatibility of predators and pesticides. Different aspects such as class of pesticide applied, type of natural enemy, pesticide preparation, concentration to which natural enemies are exposed to, contact time, timing of application and developmental life stage exposed to pesticide should be considered. The outcome of the laboratory assay highlights the importance of recommending the selective pesticides for different pest management.

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J. Entomol. Res. Soc., 25(3): 507-519, 2023
Doi: 10.51963/jers.v25i3.2404

Research Article
Online ISSN:2651-3579

Life Table and Reproductive Parameters of Ladybird Beetle, *Coccinella undecimpunctata* (Linnaeus) (Coleoptera: Coccinellidae) on Aphids, *Myzus persicae* (Sulzer) and *Brevicoryne brassicae* (Linnaeus) (Hemiptera: Aphididae)

Zahida RAMZAN[1a] Sheikh KHURSHEED[2a*] Mohd Ayoub MANTO[1b]
Hamidullah ITOO[2b] Nuzhat NASEEM[3] Farooq Ahmad BHAT[4]
Gh Hassan RATHER[2c] Zahoor Ahmad BHAT[2d] Mohd Amin MIR[2e]
Fahim Jeelani WANI[5] Shabir Ahmad GANIE[6]

[1]Division of Entomology, Faculty of Agriculture, Wadura, Sher-e- Kashmir University of Agricultural Sciences and Technology of Kashmir, 193201, INDIA

[2]Ambri Apple Research Centre, Shopian, Faculty of Horticulture, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir-192303, INDIA

[3]Division of Genetics and Plant Breeding, Wadura, Sher-e- Kashmir University of Agricultural Sciences and Technology of Kashmir, 193201, INDIA

[4]Division of Pathology, Faculty of Agriculture, Wadura, Sher-e- Kashmir University of Agricultural Sciences and Technology of Kashmir, 193201, INDIA

[5]Division of Agricultural Economics & Statistics, Faculty of Agriculture, Wadura, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, 193201, INDIA

[6]Division of Basic Sciences and Humanities, Faculty of Agriculture, Wadura, Sher-e- Kashmir University of Agricultural Sciences and Technology of Kashmir, 193201, INDIA

e-mails: [1a]zahidaramzan07@gmail.com, [2a]sheikhento@gmail.com, [1b]mantooskuastk@gmail.com, [2b]hamidbhu@gmail.com, [3]tantraynuzhat46@gmail.com, [4]farooqbhat@skuastkashmir.ac.in, [2c]drghassan71@gmail.com, [2d]zahoornano@gmail.com, [2e]mohdaminnan@gmail.com, [5]faheemwani@skuastkashmir.ac.in, [6]shabir.muntazir82@gmail.com)

ORCID IDs: [1a]0000-0003-1836-0082, [2a]0000-0002-3252-7419, [1b]0000-0001-8851-1945, [2b]0000-0002-3221-5036, [3]0000-0001-7392-1044, [4]0000-0001-5951-3327, [2c]0000-0003-1514-0199, [2d]0000-0001-8024-5283, [2e]0000-0002-9515-033X, [5]0000-0002-4427-3540, [6]0000-0002-4668-8248
*Corresponding Author

Ramzan, Z., Khursheed, S., Manto, M. A, Itoo, H., Naseem, N., Bhat, F. A., Rather, G.H., Bhat, Z. A., Mir, M., Wani, F. J., & Ganie, S. A. (2023). Life table and reproductive parameters of ladybird beetle, *Coccinella undecimpunctata* (Linnaeus) (Coleoptera: Coccinellidae) on aphids, *Myzus persicae* (Sulzer) and *Brevicoryne brassicae* (Linnaeus) (Hemiptera: Aphididae). *Journal of the Entomological Research Society*, 25(3), 507-519.

Received: January 18 2023 *Accepted: September 19, 2023*

ABSTRACT

The study aimed to determine the effect of two aphid species; *Myzus persicae* (Sulzer) and *Brevicoryne brassicae* (Linnaeus) (Hemiptera: Aphididae) on the life table and reproductive parameters of ladybird beetle, *Coccinella undecimpunctata* (Linnaeus) (Coleoptera: Coccinellidae). The results revealed that the total developmental period from egg to adult emergence was significantly shorter on *Myzus persicae* (18.6 ± 0.42 days) and longer on *Brevicoryne brassicae* (27.4±0.50 days). The adult longevity of males and females was noted significantly higher on *M. persicae* than *B. brassicae*. The reproductive parameters such as oviposition period (37±1.00 days), post-oviposition period (4.4±0.41 days), and fecundity (315.09±0.34 eggs) were found higher on *M. persicae* than *B. brassicae* (31.2±1.09 days, 3.3±0.35 days, 189.54±0.43 eggs, respectively). Among the life table parameters, net reproductive rate and intrinsic rate of increase were significantly higher on *M. persicae* (132.18 females/female and 0.19/day, respectively) and lower on *B. brassicae* (80.77 females/female and 0.12 /day, respectively). Our results showed that *C. undecimpunctata* can be easily reared on *M. persicae* and is a more efficient biological control agent for *M. persicae* than *B. brassicae*.

Keywords: aphids, population, coccinellids, biology, performance.

INTRODUCTION

Insect pests are the prime reason for biotic stresses that cause significant damage to crops worldwide. Among the insect pests, aphids are the major global group of pests causing serious economic damage to almost all crops. There are about 4000 aphid species described feeding over 250 agricultural and horticultural crops throughout the world (Ali & Rizvi, 2007). For the last few years, the population of aphids has been increasing and attaining the status of the alarming pest in Kashmir. The aphids cause damage both directly and indirectly to almost all cultivated crops. In direct damage, aphids suck the cell sap which results in the drying of shoots, wilting, and distortion of the plants, and secrete honeydew which results in sooty mold formation on leaves and shoots. While in indirect damage aphids serve as vectors of various viral plant diseases (Elwaki & Mossler, 2016). For instance, cabbage aphid is a vector of 20 plant viral diseases in a wide range of plants (Lashkari, Sahragard, & Ghadamyari, 2007). Similarly, green peach aphid, *Myzus persicae* (Hemiptera: Aphididae) spreads more than 100 viruses (Umina, Edwards, Carson, Van Rooyen, & Anderson, 2014) & is the most economically important aphid pest worldwide (van Emden & Harrington, 2007). There are several factors that have intensified the status of aphid species as a pest, including its distribution, host range, mechanisms of plant damage, life cycle, capacity to disperse, and ability to evolve resistance to insecticides. *M. persicae* is an extremely cosmopolitan species with a worldwide distribution and is highly polyphagous, with a host range of more than 400 species in 40 different plant families, including many economically important crop plants (Blackman & Eastop, 2000). Synthetic pesticides have made important impacts on aphid control, but it has limitations due to the negative impacts on the environment and the development of resistance in aphids (Peris & Kiptoo, 2017). The exhibition of frequent resistance to insecticides has made aphids serious global pests in agriculture. For instance, green peach aphid has developed resistance to at least 70 different chemical compounds and different insecticide resistance mechanisms have been reported worldwide (Silva et al, 2012).

Biological control of aphids is an environmentally friendly alternative to very hazardous and toxic insecticides that are frequently applied to protect plants (Bellows, 2001). Among the natural enemies of aphids, the ladybird beetles are the best known beneficial predatory insects. There are about 6000 ladybird beetle species in the Coccinellidae family described in the world (Che et al, 2021; Szawaryn & Czerwinski, 2022). The majorities of coccinellid species are beneficial predators and have played a significant role in the development of biological control strategies (Berthiaume, Hebert, & Cloutier, 2007). Ladybird beetles are of great economic importance in agricultural production and have been used successfully for the biological control of spider mites, aphids, coccids, and other soft-bodied insects (Ullah, Haq, Ahmad, Inayatullah, & Saeed, 2012).

Coccinella undecimpunctata L. (Coleoptera: Coccinellidae) is an old-world aphid predator that is native to central Asia, North Africa, Iceland, and much of Europe (Smyth, Allee, & Losey, 2013). It is a euriphagous predator, which prefers to feed on aphids (Hodek & Honek, 1996). It is now a widespread predator of sucking pests that have been established successfully in many countries. It has also been considered to be an important and successful predator of many sucking pests attacking cotton, sunflower, citrus (Naveed, Salam, & Saleem, 2007), wheat, and vegetable crops (El-Heneidy, Rezk, Abdel-Megeed, Salwa, & Abdel-Samad, 2004). The existence of *C. undecimpunctata* has been observed in various agroecosystems of Kashmir valley (Khursheed et al, 2021).

Life parameters of any biological control agent such as development rate and reproductive parameters depend on various biotic and abiotic factors (Jervis, Copland, & Harvey, 2005). Among the biotic factors, the quality and abundance of food are very important, influencing directly the growth and development of the predator (Dixon, 2005). Life tables are used to measure mortality, survivorship, and the life expectancy of a population at varying ages. There are several types of life tables. A generation or cohort life table is a life history of the mortality experiences of an actual cohort of individuals. Life table parameters are an important tool used by researchers for the selection of the most suitable biocontrol agent (Messenger, 1964). The intrinsic rate of increase is of great importance among other parameters for selecting candidate species as biocontrol agents. The intrinsic rate of population increase of the predator should be equal to, or greater than, that of its prey. A life table analysis provides the basis for elucidating the fitness components of an organism and how they fluctuate with abiotic and biotic factors. Tabulating the survivorship and fecundity schedules of individuals from birth to death is fundamental for cohort life tables. This enables the calculation of several parameters that allow the prediction of an insect's performance, as well as an investigation of mortality and reproduction patterns. A common estimate of population fitness is the intrinsic rate of increase, assuming a closed population that displays constant birth and death rates. In addition, the generation time ratio of prey to predator is often used as an indicator of prey population regulation (Borges, Soares, & Hemptinne, 2006). The successful mass rearing of the coccinellids in a biological control program, and assessment of their reproductive characteristics such as growth rate, fecundity, and predation rate are very important for the development of an efficient biological control strategy (Yu, Chi, & Chen, 2013). Further, the provision of an ample and nutritional diet is a major concern in the predation potential and biology of coccinellids (De Clercq, Bonte, Van Speybroeck, Bolckmans, & Deforce, 2005) and food quality can influence the development, survival, and reproduction of predators (Omkar, Kumar, & Sahu, 2009). The present investigation is undertaken to compare the population dynamics including development, survival rate and fecundity of the *C. undecimpunctata* reared on two major aphids, *M. persicae,* and *B. brassicae*. The results provided basic information for biological control programs by using *C. undecimpunctata*.

MATERIALS AND METHODS

Maintenance of stock culture

Aphid cultures

The seedlings of kale and capsicum were obtained from field populations and transplanted to plastic pots containing a mixture of soil and FYM and were grown under polyhouse conditions. The nymphs and adults of cabbage aphid, *B. brassicae* from kale plants and green peach aphids, *M. persicae* from capsicum plants were collected directly from the field, released on the respective host plants, and the cultures were maintained for further use. The aphids were identified on the basis of available key (Opfer & McGrath, 2013). *B. brassicae* siphunculi short and dark, cauda broad and triangularand body colour green covered with white-greyish waxy powder. Whereas, *M. persicae* has siphunculi and cauda light green, long convergent, siphunculi with dark tip, body colour light green, sometimes pink or red and sometimes with dark green longitudinal bands.

Ladybird beetle cultures

The initial culture of *C. undecimpunctata* was initiated by collecting adults from the field and maintained in plastic jars (20 cm length and 15 cm diameter) with a sufficient supply of aphid species. The culture was maintained pairly on each aphid species separately. The aphid supply was replenished every 24 h. The jars were monitored daily and eggs laid were collected, transferred with the help of a fine camel hair brush to clean Petri dishes lined with moistened filter paper, and allowed to hatch. Newly emerged larvae of *C. undecimpunctata* from the stock were used in the experiments. The culture was maintained under the laboratory as well as polyhouse conditions till the experiments were completed.

Development and survival

To study the development and survival, one hundred eggs of *C. undecimpunctata* were obtained from the adults reared on the two different aphid species and were transferred separately in clean Petri plates. The incubation period and the number of eggs hatched were recorded at 12 h intervals. The first and second larval instars of *C. undecimpunctata* were provided by 1st and 2nd nymphal instars of each aphid species and later developmental instars were provided by all the instars of each host aphid species. The experiment was replicated ten times; the five larvae in each beaker constituted a replicate. The observations were recorded at 12 h intervals for the below mentioned biological parameters (Omkar et al, 2009; Arshad et al, 2020).

Reproduction

Ten pairs of newly emerged adults of *C. undecimpunctata* (n = 10) fed on *B. brassicae* and *M. persicae* separately were isolated and kept in plastic jars containing 1-2 branches of each host plant. Each plastic jar represented one replication. Similarly, the adult pairs were supplied daily with *B. brassicae* and *M. persicae* separately. The laid eggs were collected daily for each couple. Data were recorded daily to determine the below mentioned reproductive parameters:

Pre-mating period and mating period

Pre-oviposition period

Oviposition period

Post-oviposition period

Reproductive rate

Life table parameters

Life table parameters for this ladybird when reared on each of the two aphid species were calculated by following Birch (1948) and Southwood (1978) methods:

Net reproductive rate (Ro) = ΣlxMx.

Mean generation time (Tc) = Σ lxMx/Ro (where, x = pivotal age).

Where lx = number of females surviving in a given population (n = 10).

Mx = net fecundity of emerging female.

The intrinsic rate of increase (rm) = ln Ro/Tc.

The finite rate of increase (λ_m) = antilog e^{-rm} (where e = 2.718228).

Generation time (GT) = ln Ro/rm.

Doubling time (DT) = ln 2/rm.

Individual female fitness

The measurement of individual fitness (R) was calculated from life history data by using the following equation given by McGraw and Caswell (1996):

R = {ln (mV)}/ D

Where, m = survival (1 or 0).

V = potential fecundity.

D = total development time.

Statistical analyses

The bootstrap techniques were used for the estimation of population parameters (Efron & Tibshirani, 1993). Turkey-HSD test was performed to compare the differences among the treatments (Dunnett, 1980). All the statistical analysis was carried out using R, version 4.1.0 (R Core Team, 2021).

RESULTS

Developmental period

The data presented in Table 1 revealed that the duration of the incubation period of *C. undecimpunctata*, when fed on *M. persicae*, was shorter than those fed on *B. brassicae* (t = 3.94, df = 18, p = 0.002). Similarly, the 1st, 2nd, 3rd, and 4th larval instars showed significantly faster development when reared on *M. persicae* and slow development on *B. brassicae* (t = 3.97, df = 18, p = 0.001) (t = 3.90, df = 18, p = 0.000), (t = 3.05, df = 18, p = 0.006), (t = 2.74, df = 18, p = 0.013). The total larval developmental period was significantly shorter on *M. persicae* than on *B. brassicae* (t = 8.70, df = 18, p = 0.000). The duration of pre-pupal and pupal stages was also observed shorter on *M. persicae* than that of *B. brassicae* (t = 3.85, df = 18, p = 0.001; t = 3.84, df = 18, p = 0.001). The longevity of both females and males of *C. undecimpunctata* was recorded significantly longer when fed on *M. persicae* than *B. brassicae* (t = 5.80, df = 18, p = 0.000; t = 2.90, df = 18, p = 0.009). The longevity of females was higher than males on both *M. persicae* and *B. brassicae*. The results of the present investigation indicated that the aphid species *M. persicae* was a more suitable prey species for the development of *C. undecimpunctata*.

Table 1. Mean duration of different developmental stages of *Coccinella undecimpunctata* on *Myzus persicae* and *Brevicoryne brassicae.*

Observations	Mean duration (Days ± S.E)		t value	df	p value
	Brevicoryne brassicae	*Myzus persicae*			
Egg incubation	3.73 ± 0.30	2.31 ± 1.63	3.94	18	0.002
1st instar	3.61 ± 0.30	2.24 ± 0.20	3.97	18	0.001
2nd instar	3.64 ± 0.22	2.56 ± 0.16	3.90	18	0.000
3rd instar	4.25 ± 0.44	2.72 ± 0.21	3.05	18	0.006
4th instar	3.74 ± 0.30	2.60 ± 0.26	2.74	18	0.013
Total larval period	15.24 ± 0.45	10.12 ± 0.36	8.70	18	0.000
Pre pupa	2.52 ± 0.24	1.60 ± 0.23	3.85	18	0.001
Pupa	5.84 ± 0.32	4.34 ± 0.21	3.84	18	0.001
Total development period	27.33 ± 0.50	18.37 ± 0.42	12.47	18	0.000
Adult longevity (Female)	41.43 ± 0.81	51.12 ± 0.63	5.80	18	0.000
Adult longevity (Male)	35.65 ± 0.73	39.35 ± 0.80	2.90	18	0.009

Survival rate

The results presented in Table 2 indicated that the percent pupation was significantly higher when larvae fed on *M. persicae* than on *B. brassicae* (t = 4.02, df = 18, p = 0.000). A similar trend was recorded as far as the percent immature survival was concerned. The percent immature survival was also observed significantly higher on *M. persicae* than on *B. brassicae* (t = 4.24, df = 18, p = 0.000). Similarly, the growth index was significantly higher on *M. persicae* than on *B. brassicae* (t = 9.0, df = 18, p = 0.000). However, there was no significant difference in the sex ratio of

C. undecimpunctata when fed on *M. persicae* and *B. brassicae* separately (t = 2.5, df = 18, p = 0.017).

Table 2. Mean survival rate and sex ratio of *Coccinella undecimpunctata* on *Myzus persicae* and *Brevicoryne brassicae*.

Observations	*Brevicoryne brassicae*	*Myzus persicae*	t value	df	p value
Percent pupation (No. of pupae/No. of 1st instars)	73 ± 3.01	84 ± 3.20	4.02	18	0.000
Percent immature survival (No. of adults emerged/No. of 1st instars*100)	68 ± 2.32	81 ± 3.33	4.24	18	0.000
Growth index (% pupation/mean larvel duration)	3.3 ± 0.22	6.8 ± 0.32	9.0	18	0.000
Sex ratio (No. of females/total adult emergence)	0.58 ± 0.03	0.69 ± 0.02	2.5	18	0.017

Reproduction

The data on reproductive parameters of *C. undecimpunctata* are enumerated in Table 3, which revealed that the duration of the pre-mating period was significantly found shorter in adults when fed on *M. persicae* than those that were fed on *B. brassicae* (t = 2.94, df = 18, p = 0.008). There was no significant difference in the mating period of *C. undecimpunctata* when fed on *M. persicae* and *B. brassicae* separately (t = 1.55, df = 18, p = 0.138). The predator showed a significantly longer pre-ovipositional period on *B. brassicae* and shorter on *M. persicae* (t = 5.6, df = 18, p = 0.000). The oviposition and post-oviposition periods were recorded significantly longer when fed on *M. persicae* than those fed on *B. brassicae* (t = 5.6, df = 18, p = 0.000; t = 3.5, df = 18, p = 0.002). Maximum egg-laying capacity was recorded in females that fed on *M. persicae*, which was significantly higher than those that fed on *B. brassicae* (t = 3.73, df = 18, p = 0.000). The reproductive rate of *C. undecimpunctata* was also significantly higher when females fed on *M. persicae* than that of *B. brassicae* ($t = 4.18$, df = 18, p = 0.000). Peak oviposition age of predator also differed, occurring significantly earlier on *M. persicae* and later on *B. brassicae* (($t = 10.32$, df = 18, p = 0.000).

Table 3. Mean duration of different reproductive parameters of *Coccinella undecimpunctata* on *Myzus persicae* and *Brevicoryne brassicae.*

Observations	*Brevicoryne brassicae*	*Myzus persicae*	t value	df	p value
Pre-mating period (days)	5 ± 0.32	3.61 ± 0.20	2.94	18	0.008
Mating period (minutes)	46.5 ± 0.41	38.58 ± 0.34	1.55	18	0.138
Pre-ovipositional period (days)	9.47 ± 0.33	6.53 ± 0.28	5.6	18	0.000
Oviposition period (days)	31.24 ± 1.09	37 ± 1.00	3.5	18	0.002
Fecundity (eggs)	189.54 ± 0.58	315.09 ± 0.76	3.73	18	0.000
Post -oviposition period (days)	3.34 ± 0.35	4.43 ± 0.41	3.52	18	0.000
Reproductive rate (eggs/day)	10.52 ± 0.09	17.31 ± 0.07	4.18	18	0.000
Day of peak oviposition (days)	29.67 ± 0.76	19.54 ± 0.89	10.32	18	0.000

Life table parameters

The data pertaining to life table parameters of *C. undecimpunctata* in Table 4 revealed that net reproductive potential (Ro), intrinsic rate of increase, and finite rate of increase were higher when fed on *M. persicae* (132.18 females/female, 0.19/day and 1.18/day) than that those fed on *B. brassicae* (80.77 females/female, 0.12/day and 1.10/day). Mean generation time (T) varied from 42.06 days on *B. brassicae* to 35.11 on *M. persicae*. Doubling time was shorter on *M. persicae* (4.34 days) than on *B. brassicae* (5.93 days). Individual female fitness was higher for those females reared on *M. persicae* (0.54) than those reared on *B. brassicae* (0.22).

Table 4. Life table parameters of *Coccinella undecimpunctata* on *Brevicoryne brassicae* and *Myzus persicae*.

Observations	*Brevicoryne brassicae*	*Myzus persicae*
Net reproductive potential (Ro) (Females/Female)	80.77	132.18
Intrinsic rate of increase (rm) (Individual/ Day)	0.12	0.19
The finite rate of increase (λm) (Individual/ Day)	1.10	1.18
Generation time (Days)	42.06	35.11
Doubling time (Days)	5.93	4.34
Individual female fitness	0.22	0.54

DISCUSSION

The egg incubation period was shorter in the females fed on *M. persicae* than those fed on *B. brassicae*. The findings are also similar to Arshad et al (2020) who reported that the incubation period of the coccinellid, *H. convergens* fluctuated when feeding on different aphid species. The nature and quality of prey have a significant influence on the duration of egg incubation and larval instars of *C. septempunctata* (Majerus et al, 1989). This perhaps indicates the role of the adult diet in the rate of development of embryos. Previous studies also revealed same results for *C. sexmaculata* (Fabricius) (Chaudhary, David, & Singh,1983) and *Brumoides suturalis* (Fabricius) (Gautam, 1990). The developmental period of all four larval instars was shorter when they fed on *M. persicae* than on *B. brassicae.* Our results are similar to Arshad et al (2020) who reported that the developmental period of all four larval instars of *H. convergens* was shorter when they fed on *L. erysimi* than *on A. gossypi*i. The increased growth and development of *C. undecimpunctata* when fed on *M. persicae* may be attributed to the presence of higher protein levels in this species, and their higher consumption, which could be due to higher palatability (Atwal & Sethi, 1963). In the present study, aphid species (*B. brassicae*) that were consumed in lesser amounts resulted in a longer larval development period of *C. undecimpunctata*. The developmental rate of ladybird instars was thus found to be proportionate to the amount of food eaten by them. The reduction in consumption rate probably leads to reduced nutritional levels, thus having a substantial effect on larval development. Blackman (1967) also noticed the difference in larval development of *C. septempunctata* after being fed on different aphid species like *M. persicae*, *A. fabae* (Theobald), *A. pisum* (Harris), *Megoura viciae* (Buckton), *B. brassicae* and *A. sambuci* (Linnaeus). The less consumption of some aphids by predatory beetles has been ascribed to the presence of certain alkaloids or unsuitable chemicals, not suitable to the constitution and metabolism of the ladybirds (Okamoto, 1966). The toxicity of certain aphid species needs to be confirmed by chemical analysis of body contents.

The shortest pre-oviposition period of C. *undecimpunctata* was observed after feeding on *M. persicae*, and this suggests that the increased quantity of high quality food decreased the length of the pre-oviposition period. This is also supported by the findings of Rhamhalinghan (1985). The decreased consumption of less suitable foods and/or the presence of alkaloids probably affect pre-adult development, resulting in slower sexual maturation and a longer pre-oviposition period on less suitable aphids (*B. brassicae*). This view is supported by the findings for *H. axyridis* (Hukusima & Kamei 1970) and *Propylea japonica* (Thunberg) (Kawauchi, 1981). The quality of the host has been reported to influence the survival, fecundity, and longevity of predators (Moghaddam et al, 2016). The longer oviposition period of *C. undecimpunctata* when fed on *M. persicae* is due to the high consumption of suitable food (along with the suitable nutrients) helps in early ovariole maturation and provides energy to sustain a longer oviposition period, while the reduced consumption of less suitable food affects and probably slows down ovariole development. This lends support to the hypothesis that a certain amount of food is necessary for maturation of ovarioles in *C. undecimpunctata*. A similar finding, that quality and quantity of food affects the oviposition period, was also recorded for *M. sexmaculatus* (Agarwala & Choudhuri, 1995). Thus, when the ladybirds were fed on suitable aphids, the pre-reproductive period was shortened, while the consumption of less suitable food increased the non-reproductive phase. The effect of quality as well as quantity of food was also seen on fecundity and longevity. There was a linear relationship between food consumption with the longevity and fecundity of the ladybird. A linear relationship between longevity and fecundity suggested that longevity may be an important determinant of fecundity. These findings are in conformity with those recorded for *C. septempunctata* (Rhamhalinghan, 1987; Kawauchi, 1991). Maximum fecundity was recorded in females who fed on *M. persicae* and minimum fecundity in females who fed on *B. brassicae*, again reflecting the nutritive value and palatability of the former species. The low fecundity recorded when fed on *B. brassicae* is most likely due o the prey's effect on egg maturation. Coccinellids fed on the prey of high quality have more ovarioles and mature them earlier than those fed on poor quality prey (Rhamalinghan, 1986). In the case of *Brachinus lateralis*, Juliano (1985) reported that the consumption of high quality prey resulted in faster development, larger food reserves, and larger body size.

Life table parameters are strongly influenced by many factors, including the nature of the prey species, the host plant, and laboratory conditions. We observed that the net reproductive rate (Ro) of *C. undecimpunctata* varied from 80.77 offspring on *B. brassicae* to 132.18 offspring on *M. persicae*. The mean generation time (T) was lower on *M. persicae* than on *B. brassicae*. The highest value of the intrinsic rate of increase (r) and finite rate of increase was recorded on *M. persicae* than *on B. brassicae*. Kontodimas et al (2008) estimated that the total fecundity, net reproductive rate, and intrinsic rate of increase of *C. septempunctata* on *A. fabae* increased with an increase in adult longevity. However, species with the highest fecundities are not necessarily the most successful in decreasing pest populations because individual longevity and fecundity may be correlated. Solano, Delgado, Morales, & Vasquez (2016) reported that *Cycloneda sanguinea* (Linnaeus) reared on *A. craccivora* had very high values of reproductive rate (Ro), intrinsic rate of increase (r), and finite rate of increase (λ) which are consistent with the present study. The intrinsic rate of increase (r) is the most valuable life table factor for comparing population growth potential under different treatments (Southwood, 1966). Lewontin (1965) reported that predators with short pre-ovipositional periods are expected to have high intrinsic rates of increase. Ali and Rizvi (2010) calculated values of R0, T, r, λ, and doubling time (DT) for *C. septempunctata* reared on *L. erysimi*, which are comparable with our results. The highest R0 and rm values were recorded on a diet of *M. persicae*, suggesting this aphid is suitable for mass rearing of *C. undecimpunctata*.

CONCLUSION

It can be concluded from the results of this study that the ladybird, *C. undecimpunctata* could complete its development on both aphid species (*M. persicae* and *B. brassicae*). However, larval development was faster and reproductive potential was maximum on *M. persicae* than on *B. brassicae*. *M. persicae* can be used as a natural host for the mass rearing of *C. undecimpunctata* and the development of a biological control strategy.

ACKNOWLEDGEMENTS

Authors are thankful to Director Research of SKUAST-Kashmir has provided the required funds to conduct this study. Authors are also thankful to University authority for providing all the facilities to conduct the research and also to supporting staff of AARC for their help to maintain the cultures of insects.

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J. Entomol. Res. Soc., 25(3): 521-527, 2023
Doi: 10.51963/jers.v25i3.2441

Research Article
Online ISSN:2651-3579

Coleopteran Pests Infesting *Astragalus* Plants on Karacadağ Mountain with a New Species for the Turkish Fauna

Adil TONĞA[1*] Vladimir SAKALIAN[2]

[1] Diyarbakır Plant Protection Research Institute, Entomology Department 21110 Diyarbakır, TÜRKİYE
[2] Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, 1 Tzar Osvoboditel Blvd., 1000 Sofia, BULGARIA
e-mails: adton21@gmail.com, vladimir.sakalian@gmail.com
ORCID IDs: [1]0000-0001-7485-8411, [2]0009-0002-4172-2555
*Corresponding author

ABSTRACT

Karacadağ Mountain, the shield volcano in Southeastern Türkiye, has been a treasure of biodiversity in terms of flora and fauna that serve a diverse number of agricultural practices and forestry. The floral composition is mainly composed and represented by the shrub plant, *Astragalus* spp. which is under threat by insect pests. This study aims to report the coleopteran woodboring species infesting *Astragalus* spp. along with their density and infestation rate on different altitude levels (lowest=1450-1550 m, middle=1650-1750 m, and upper=1850-1950 m) in 2016. Laboratory incubation of the specimens yielded six woodboring species: *Agapanthia coeruleipennis* Frivaldszky, 1878 and *Xylotrechus sieversi* (Ganglbauer, 1890) (Coleoptera: Cerambycidae), *Sphenoptera* (*s.str.*) *anthracina* Jakovlev, 1887, *Sphenoptera* (*s.str.*) *coracina* (Steven, 1829), *Sphenoptera* (*s.str.*) *tragacanthae* (Klug, 1829), and *Anthaxia* (*s.str.*) *truncata* Abeille de Perrin, 1900 (Coleoptera: Buprestidae). Among these species, *S. anthracina* is recorded for the first time in Turkish fauna. The infestation rate did not differ between altitudes while the number of woodborer pests per plant was highest at the highest level of altitude which did not differ between lowest and middle altitude levels.

Keywords: Altitude-dependent density, Mountain fauna, new record, woodboring pests, shrub plants, Southeastern Türkiye.

Tonğa, A. & Sakalian, V. (2023). Coleopteran pests infesting *Astragalus* plants on Karacadağ Mountain with a new species for the Turkish fauna. *Journal of the Entomological Research Society*, 25(3), 521-527.

Received: April 05 2023 *Accepted: October 04, 2023*

INTRODUCTION

The shield volcano structured Karacadağ Mountain is located in Southeastern Türkiye and connects agriculturally important provinces, Diyarbakır, and Şanlıurfa and partly, Mardin. The last eruptions of the mountain are addressed to 100.000 years ago. The mountain, since then, has become a great source of many products having economic importance for people. For example, a previous study revealed that cultivation of einkorn cereals started around the mountain, and ancestors of many kinds of cereals still grow as wild cereals on the slopes of the mountain (Heun et al, 1997). Livestock remains one of the main activities of natives and one of the most important livestock practices, bee-keeping largely depends on the flora of the mountain.

The flora of the mountain is mainly represented by shrub plants. Among the shrub plants, milkvetch, *Astragalus* spp. (Fabaceae) is the most abundant and prevalent plant genus on the mountain (Ertekin, 2002). The *Astragalus* spp. provide a perfect wintering shelter for insect pests of crops such as *Eurygaster* spp. (Hemiptera: Scutelleridae), *Aelia* spp., *Dolycoris* spp. (Hemiptera: Pentatomidae) as well as many other agriculturally important pests and beneficial insects. In addition, *Astragalus* spp. stands among the best feeding sources for pollinator insect species, especially, honeybees, *Apis mellifera* Linnaeus, 1761 (Hymenoptera: Apidae) (Richards, 1987).

Astragalus species could be either in herbaceous or shrub forms. The genus *Astragalus* is native to the Palearctic and Nearctic regions and is present in Middle East countries (PFAF, 2023). One of the most common milkvetch species on the Karacadağ Mountain is Gum tragacanth milkvetch, *Astragalus gummifer* and its distribution commences from 1300m-1400m altitude to the highest points, above 1950 m of altitude (Ertekin, 2002). *Astragalus gummifer* is a thorny plant having small branches and exudes a demulcent gum, which cannot be used internally because of its deficient dissolution (Grieve, 1971).

During the winter surveys for *Eurygaster integriceps*, the most worldwide distributed wheat pest, on February – April of 2016 on Karacadağ Mountain, several broken milkvetch branches and stems having larval feces of woodboring pests were encountered. Dissecting broken branches and some seemingly healthy plants having small holes, many larvae belonging to families, Cerambycidae and Buprestidae (Coleoptera) were located. Many studies have addressed the trophic levels between herbaceous milkvetch species and insect communities. For example, the reproduction of *A. lusitanicus*, a perennial legume, is highly suppressed by a lepidopteran pest, *Tomares ballus* (Lepidoptera: Lycaenidae) in Southern Spain due to its voracious feeding activity on leaves and seeds (Jordano et al, 1990). Seed predation by other insect groups is also possible among milkvetch species such that seed - feeding beetles, *Acanthoscelides* fraterculus, and *A. pullus* (Bruchidae), infests *A. filipes*, a commercially farmed milkvetch species in the United States of America (Cane et al, 2013). Previous studies have focused on the possible association between herbivorous insects and herbaceous *Astragalus* plant species. Thus, our knowledge of insect pests, especially wood-boring insects, of *Astragalus* plants in shrub forms remains scarce and requires attention.

This study aims to report coleopteran woodboring pests and their altitude-dependent density on Karacadağ Mountain. The findings are discussed in terms of the threat to Karacadağ shrub flora which is composed of Coleopteran pests.

MATERIALS AND METHODS

Study area and surveys

The mountain divides Diyarbakır and Şanlıurfa provinces in the northeast-southwest direction and its slopes lie inside the borders of Mardin province in the south as well. The mountain has a steppe climate. The long period summer months are warm while the mountain has a cold winter period. The highest point on Karacadağ Mountain is 1952 m altitude. The soil covering the mountain mostly represents basaltic characters.

Distribution of *Astragalus* spp. on the mountain commences from 1300 – 1400 meters till the top. The density of *Astragalus* spp. plants increase with altitude since the up-rooting practices by villagers are performed in lower altitudes. In total, nine locations were selected as study sites on Karacadağ Mountain (37^{o}60` N, 39$^{o}$83` E). Each location consisted of three sampling sites. Every three sites constituted a different altitude range. The sampling sites were located on different altitude levels as lowest=1450-1550 m; middle=1650-1750 m; highest=1850-1950 on Karacadağ Mountain.

Surveys and samplings were performed in February – April 2016. At each study site, 40, 30, and 20 plants were systematically sampled at highest, middle, and lowest altitudes, respectively. These plants were then randomly inspected to assess the presence and abundance of Coleopteran wood borers. All plants having broken branches/stems or holes were up-rooted and dissected while those that did not have similar symptoms were not harmed. The number of larvae and pupae of species belonging to Buprestidae and Cerambycidae was recorded. The specimens were incubated under laboratory conditions (25 ± 2 °C, 60 ± 10 RH, 16 h light). Emerged adults were preserved in 70% ethanol for identification.

Statistical analysis

Several generalized linear models were assessed to reveal whether the density and infestation rate of the coleopteran woodboring pests varied between different altitude ranges (Faraway, 2016; Dunn & Smyth, 2018). The number of coleopteran pests was the response variable and the altitude range was the explanatory variable in the models. The models were compared based on AIC and dispersion parameters (Faraway, 2016; Dunn & Smyth, 2018). Tukey's multiple post hoc tests were employed for comparison. All statistical analyses were performed using R statistical software and the integrated development environment, RStudio (R Core Team, 2022)

RESULTS

The surveys on Karacadağ Mountain shrub plants, *Astragalus* spp. pests yielded two Cerambycid and four Buprestid species (Table 1). The count data representing the number of coleopteran pests infesting *Astragalus* spp. were subjected to several generalized linear models (Table 2). Zero-inflated negative binomial was considered the best-fit model as it had the lowest Akaike information criterion (ACI) and a dispersion parameter very close to 1. Even though the negative binomial model had a closer value to 1 when compared with the zero-inflated negative binomial model, it had a greater AIC value. However, multiple comparison tests of both models yielded similar results, indicating the number of coleopteran pests was higher at the highest altitude level when compared with the lowest and middle altitudes. There were no differences between the number of coleopteran pests infesting *Astragalus* spp. at lowest and middle altitude levels (Fig. 1). The binomial distribution model for the infestation rate of *Astragalus* plants by the coleopteran pests yielded under dispersion (0.061) and thus was confirmed by a quasibinomial model (Table 2). In both cases, the altitude effect on the infestation rate of *Astragalus* species by coleopteran wood borers was not statistically significant (Table 2, Fig. 1).

Table 1. Coleopteran insect pests infesting *Astragalus* spp. on Karacadağ Mountain in Southeastern Türkiye.

Family	Coleopteran woodboring species infesting *Astragalus* spp.
Cerambycidae	*Xylotrechus sieversi* (Ganglbauer, 1890)
	Agapanthia coeruleipennis Frivaldszky, 1878
Buprestidae	*Sphenoptera (s.str.) anthracina* Jakovlev, 1887
	Sphenoptera (s.str.) coracina (Steven, 1829)
	Sphenoptera (s.str.) tragacanthae (Klug, 1829)
	Anthaxia (s.str.) truncata Abeille de Perrin, 1900

Table 2. The statistical importance and evaluation parameters of the generalized linear models tested for the density and infestation rate of Coleopteran woodboring pests infesting *Astragalus* spp. on Karacadağ Mountain.

Model	χ^2 (df=2)	P	AIC*	Dispersion**
No. coleopteran wood borers				
Poisson model	137.53	<0.001	2719.30	7.82
Negative binomial	20.06	<0.001	1346.70	0.97
Zero-inflated	51.92	<0.001	1607.83	1.90
Zero-inflated negative binomial	28.45	<0.001	1342.61	1.07
Hurdle	91.92	<0.001	1607.83	1.90
The infestation rate				
Binomial distribution	0.15	0.929	18.73	0.061
Quasibinomial distribution	2.38	0.305	NA	0.061

*AIC=Akaike information criterion; The lower the AIC value is, the better the model fits data.
** Dispersion parameter (DP): if DP>1.

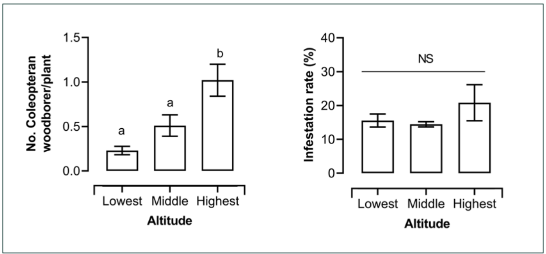

Figure 1. The mean density (left) and infestation rate (right) of coleopteran woodboring species infesting *Astragalus* spp. on different levels of altitude (Lowest=1450-1550m; Middle=1650-1750m; Highest=1850-1950m) on Karacadağ Mountain.

DISCUSSION

This study was performed to document the severe damage by coleopteran woodboring pests infesting *Astragalus* spp., the shrub plants on Karacadağ Mountain located in Southeastern Türkiye. The coleopteran woodboring community consisted of six species i.e., two cerambycids and four buprestids. The number of all larvae and pupal specimens was calculated as the total number of coleopteran wood borers. Therefore, the species-dependent relative abundances of wood borers are undefined as the preimaginal stages of species belonging to each family were quite identical. However, the majority of wood-boring beetles were represented by the cerambycids especially *X. sieversi* when compared with other species (personal observation).

The number of coleopteran wood borers infesting *Astragalus* spp. differed between altitude levels. For example, the density of the pests was highest at the highest altitude levels and did not differ between the lowest and middle altitude levels. The recorded pests together with their host plants were present at higher levels of altitudes with cold winter and calm summer climatic conditions. The percent number of infested plants was between ~14-21% and did not differ between altitude levels. Therefore, the presence of the pests in different levels could be more dependent on the presence of their host plants rather than adapting to specific climatic conditions as the host is a higher altitude specific plant.

The surveys targeted the infestation of *Astragalus* spp. by coleopteran wood borers on Karacadağ Mountain and it is well-known that *Astragalus* plants having shrub form are native to western Asia countries including middle east countries such as Iraq, Iran, Lebanon, Syria, and Türkiye. They are also widely distributed to several close countries like Afghanistan, Pakistan, and Russia (Verbeken, Dierckx, & Dewettinck, 2003). This study was unable to extend the range of the study area to other mountains of Türkiye and surrounding countries. The coleopteran wood-borers, especially *X. sieversi* and *A. coeruleipennis* infesting shrub plants have been reported in Türkiye for a long time (Özdikmen, 2013; Rapuzzi & Sama, 2018). Among the buprestids, only *S. coracina* was reported in Diyarbakır (Kısmalı et al, 1995) while the other species have never been reported in Southeastern Türkiye. The presence of *A. truncate* was reported in Erzurum province with a single female being collected on high elevation levels i.e., 1750m with no further details (Tozlu & Özbek, 2000). Furthermore, no study was located reporting the presence of *S. anthracina* in Türkiye while S. *tragacanthae* is also reported in Türkiye (Kubáň et al, 2016). The presence of the pest was reported in neighboring countries such as Azerbaijan, Armenia, Iran, and also in Turkmenistan (Kubáň et al, 2016). Therefore, this study is the first to report the presence of *S. anthracina* in Türkiye. The studies aiming to report the presence of such pests have been faunistic studies that are highly dependent on random and irregular samplings and generally overlook the tropic interactions including host, pest, and natural enemies such as parasitoids. Since the identification of species being reported in this study relies on the incubated samples, the herbivory of the host plants by the coleopteran wood borers is now confirmed. Incubation of *Astragalus* plants infested by coleopteran wood borers yielded several Braconid and Ichneumonid specimens, however, we could not identify the samples due to overloaded work. Therefore, a detailed sampling procedure should be devoted to the identification of natural enemies of the coleopteran wood borers on the mountain.

The infestation of *Astragalus* species on the mountain by wood borer pests seemingly compromises a threat to the protection of the mountain soil structure and consequently may trigger erosion that can negatively affect the agricultural, apicultural, and livestock practices as well as the composition of the flora and fauna. Further studies targeting to collect data on the bioecological life traits of the pests that could benefit future control measures are required.

ACKNOWLEDGMENT

The authors thank Prof. Georgi Georgiev (Sofia, Bulgaria) for the identification of Cerambycid samples and Vit Kubáň (Brno, Czechia) for the identification of *Sphenoptera* (*s.str.*) *anthracina*.

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J. Entomol. Res. Soc., 25(3): 529-544, 2023 *Research Article*
Doi: 10.51963/jers.v25i3.2445 *Online ISSN:2651-3579*

Deep Learning Based Classification for Hoverflies (Diptera: Syrphidae)

Anıl UTKU[1] Zafer AYAZ[2] Derya ÇİFTÇİ[3*] M. Ali AKCAYOL[4]

[1]Faculty of Engineering, Department of Computer Engineering, Munzur University, Tunceli, TÜRKİYE

[2]Department of Management Information Systems, Gazi University, Ankara, TÜRKİYE

[3]Veysel Karani District, University Housing Complex 3E Block No:8, Siirt, TÜRKİYE

[4]Faculty of Engineering, Department of Computer Engineering, Gazi University, Ankara, TÜRKİYE

e-mails: [1]anilutku@munzur.edu.tr, [2]zafer@gazi.edu.tr, [3*]dcanpolat@gmail.com [4]akcayol@gazi.edu.tr

ORCID IDs: [1]0000-0002-7240-8713, [2]0000-0001-7667-7921, [3]0000-0002-7670-4392 [4]0000-0002-6615-1237

*Corresponding author

ABSTRACT

Syrphidae is essential in pollinating many flowering plants and cereals and is a family with high species diversity in the order Diptera. These family species are also used in biodiversity and conservation studies. This study proposes an image-based CNN model for easy, fast, and accurate identification of Syrphidae species. Seven hundred twenty-seven hoverfly images were used to train and test the developed deep-learning model. Four hundred seventy-nine of these images were allocated to the training set and two hundred forty-eight to the test dataset. There are a total of 15 species in the dataset. With the CNN-based deep learning model developed in this study, accuracy 0.96, precision 0.97, recall 0.96, and f-measure 0.96 values were obtained for the dataset. The experimental results showed that the proposed CNN-based deep learning model had a high success rate in distinguishing the Syrphidae species.

Keywords: Convolutional neural network, image classification, automatic species identification

Utku, A., Ayaz, A., Çiftçi, D., & Akcayol, M.A (2023). Deep Learning Based Classification for Hoverflies (Diptera: Sryphidae). *Journal of the Entomological Research Society*, 25(3), 529-544.

Received: April 09 2023 *Accepted: August 21, 2023*

INTRODUCTION

Insects are a class within the arthropods and have an exoskeleton and a characteristic body structure consisting of 3 parts (head, thorax, and abdomen) and three pairs of legs, compound eyes, and a pair of antennae (Martineau et al, 2017; Xia, Chen, Wang, Zhang, & Xie, 2018; Hassan, Rahman, Htike, & Win, 2014). Arthropods show more biodiversity than other groups of living things. Arthropods are important indicators of ecosystem function. It was used to determine the quality of many habitats (agriculture, forest, and meadow) and the fauna richness and diversity of habitats. It is also used to determine the extent of arthropod diversity, habitat fragmentation, and degradation (Martineau et al, 2017; Xia et al, 2018; Hassan et al, 2014).

Traditionally, insect species identification is based on morphological identification. Taxonomists and trained technicians identify using taxa-specific identification keys because species identification requires skills gained through training and experience. Although technicians can identify taxa using identification keys in some cases, some insect taxa also require experts. Furthermore, the need for sufficient experts and technicians in some insect groups delays the insect identification stage. Consequently, alternative and accurate identification methods are required, which at least non-experts can use (Martineau et al, 2017; Xia et al, 2018).

In biodiversity studies, there are other difficulties, in addition to the difficulty of finding an expert insect taxonomist. Some of these are the lack of up-to-date identification keys and catalogues, the scattered family or species-specific sources, the lack of arrangement of synonymous species names, the difficulty of identifying many taxa, and the collection of large numbers of specimens in field studies (Gaston and Neil, 2004; Gaston and May, 1992; Mound and Gaston, 1993).

These difficulties in taxonomy and identification have led to the development of identification methods in the last 30 years (Gaston and Neil, 2004). One of these solutions is the automatic identification process. Image-based insect recognition is widely used, especially in agriculture, ecology, and biodiversity (Hassan et al, 2014; Martineau et al, 2017; Karar, Alsunaydi, Albusaymi, & Alotaibi, 2021).

Fedor, Vaňhara, Havel, Malenovský, & Spellerberg (2009) identified 18 economically important common European species of Thysanoptera with 97% accuracy using the artificial neural network (ANN) model.

Yang, Ma, Wen, Zhan, & Wang (2015) developed a program to identify species with 90-98% accuracy using the wings of Neuroptera. Faria et al (2014) developed an identification method with more than 98% accuracy for Anastrepha fruit pest species, widely distributed in the American tropics and subtropics, using a multimodal fusion approach. The automatic bee identification system (ABIS) made species distinction with Support Vector Machine (SVM) using the front wing photograph of the bee (Arbuckle, Schröder, Steinhage, & Wittmann, 2001). O'Neill (2008) developed a successful invertebrate identification system with (the digital automated identification system) DAISY based on eigen-images recognition.

Zhu et al (2017) presented a study that explores the application of hybrid deep-learning techniques for the automated classification of lepidopteran insect images. The research focuses on leveraging the strengths of various deep learning architectures, combining them in a hybrid approach to achieve improved accuracy in insect classification. By combining Convolutional Neural Networks (CNN) and Recurrent Neural Networks (RNN), the authors create a robust framework capable of capturing spatial and sequential features in insect images. The study demonstrates the efficacy of this hybrid model in accurately classifying lepidopteran insects, offering a promising avenue for automating the identification process in entomology and pest management applications. The research contributes to advancing the field of insect image classification by proposing an innovative approach that effectively addresses the complexities associated with recognizing and categorizing diverse insect species.

Thenmozhi and Reddy (2019) proposed a novel approach for accurately classifying crop pests by utilizing advanced techniques in deep learning. The study employs CNN and transfer learning to enhance the classification accuracy for various crop pest species. By leveraging pre-trained models and fine-tuning them on a specialized dataset of crop pest images, the authors achieve significant improvements in classification performance. Transfer learning enables the network to learn intricate features from the data, resulting in robust and effective pest classification. The research outcomes demonstrate the potential of combining deep learning techniques and transfer learning in addressing complex agricultural challenges such as pest identification and management.

Li et al (2021) presented a comprehensive analysis of research endeavours centered around utilizing deep learning techniques for insect classification and detection in field images, specifically emphasizing applications in intelligent pest management systems. The study surveys a broad range of approaches, methodologies, and advancements in this domain through a systematic review. The authors identify common trends and challenges, highlighting the effectiveness of deep learning models in accurately identifying and categorizing insects from images captured in real-world agricultural environments. The review underscores the potential impact of such methods on improving pest management strategies by enabling timely and targeted interventions. By synthesizing the findings of multiple studies, this paper offers valuable insights into the state-of-the-art techniques and future directions in intelligent pest management using deep learning technologies.

Tiwari et al (2021) present an innovative study focusing on developing and implementing customized deep-learning models for real-time classification of insect pests in soybean crops. The research addresses the challenges the agricultural setting poses, where rapid and accurate insect identification is crucial for effective pest management. The authors create models capable of real-time insect classification by tailoring deep learning architectures to the specific features of soybean crop images and insect pests. This approach demonstrates remarkable accuracy and potentially significantly enhances pest detection and control strategies in soybean farming. The study showcases the importance of adapting deep learning methods to suit the demands of real-world agricultural applications, providing a valuable contribution to intelligent farming and pest management.

Kasinathan, Singaraju, & Uyyala (2020) comprehensively explore the application of contemporary machine-learning methods for insect classification and detection within field crops. The study delves into utilizing state-of-the-art machine learning techniques, such as CNN and SVM, to address the intricate challenge of identifying and detecting insects in complex agricultural environments. By harnessing the power of these advanced techniques, the authors develop effective models capable of accurate insect classification and timely pest detection. The research underscores the potential of modern machine learning approaches to revolutionize pest management strategies by providing timely insights and enabling proactive interventions in field crop protection.

Butterfly species, which are of great ecological importance, are one of the orders that show species richness and are used as indicators in biodiversity studies (Beccaloni and Gaston, 1995; Dennis et al, 2008; Dover, Sparks, Clarke, Gobbett, & Glossop, 2000; Zamora, Verdu, & Galante, 2007; Zupan, Bužan, Grubar, & Jugovic, 2020). Morphological features such as wing shape, structure, and color are used to identify butterfly species. Several automatic machine viewings were designed to make morphological identification accurate, easy, and faster (Kaya & Kaycı, 2014; Wang, Ji, Liang, & Decheng, 2011; Qing et al, 2012; Wen, Guyer, & Li, 2009).

Many methods were used in the literature to classify insects and insect pests (Martineau et al, 2017; Xia et al, 2018; Kasinathan et al, 2020). Classification methods used in the literature are divided into monolithic, combinations, and instance-based groups. Monolithic classification methods are divided into two levels discriminative and generative. The combinations method is divided into two basic levels: boosting and bagging. Instance-based methods use the k-nearest neighbours (k-NN) algorithm.

In discriminative methods, least-squares approximation (Wen et al, 2009; Wen and Zhu, 2010) and fisher linear discriminant (Dietrich and Pooley, 1994; Dietrich, Emigh, & Deitz, 1991; Zayas and Flinn, 1998; Tofiski, 2004; Francoy et al, 2008) were used in the literature to classify different insect species. However, in cases when the data could not be separated linearly, the support vector machine (Qing et al, 2012; Yang et al, 2015; Silva et al, 2015; Wang et al, 2012) was applied using the polynomial kernel or the radial basis function with standard deviation. Neural network architectures were also used as parser classifiers for different insect species by using nonlinear activation functions in the literature (Do, Harp, & Norris, 1999; Al-Saqer and Hassan, 2011; Wang, Lin, Ji, & Liang, 2012; Wen, Wu, Hu, & Pan, 2015; Leow, Chew, Chong, & Dhillon, 2015; Silva, Grassi Sella, Francoy, & Costa, 2015; Xia et al, 2018). Decision tree-based classifiers were also used to classify insect species (Mayo and Watson, 2007; Larios et al, 2008; Silva et al, 2015). However, there are no studies to classify hoverflies using deep learning.

The family Syrphidae of the order Diptera are commonly known as “hoverflies” or “flower flies.” This family is among the insects that visit flowering plants the most. Hoverflies are important pollinators of many plants and crops (Klecka, 2018). The fact that most syrphids are effective natural enemies of different insect groups, especially aphids, allows them to be used in biological control studies (Van Driesche, Hoddle, & Enter, 2008). Adults of all known syrphid species feed almost exclusively on pollen, nectar, or honeydew (Rotheray and Gilbert, 2011) and are usually considered general visitors to the flowers (Klecka, 2018). The fact that they are involved in biodiversity conservation studies, that they are widely distributed, and that various ecological conditions are required for their larvae support the use of this fly family as a bioindicator (Sommaggio, 1999; Sommaggio and Burgio, 2014).

Morphological characters such as abdomen pattern, antenna segment colour, and length, leg colour are used in species identification, but male genitalia should be examined for definitive identification. (Speight, 2018, 2020). In recent years, diagnostic studies with molecular methods have been conducted, especially in problematic groups (Vujić et al, 2015, 2017, 2020; Likov et al, 2020; Kočiš et al, 2018). The identification with both classical methods and molecular methods takes a long time.

In recent years, there have been expanded and detailed identification keys for identifying Syrphidae species distributed in Europe. However, more than these identification keys are needed for the species distributed in Turkey (Speight, 2012, 2018, 2020).

This study aims to develop a habitus images-based deep learning model for faster and more accurate identification of some ecologically important adult Syrphidae (Diptera) species. In the literature, there are artificial intelligence-based studies for classifying and detecting plant and insect species. However, these studies generally focus on identifying some specific species. The datasets used are generally publicly accessible. Unlike the studies in the literature, an original dataset was used in this study. In the dataset used, there are images of 15 syrphid species. The detailed study, which will be carried out in the future, covering the syrphid species distributed in Turkey and neighbouring countries, will be preliminary.

DATASET AND MATERIAL

In this study, different images were used for the dipteran family Syrphidae both in the laboratory and natural environments. To this end, the photographs of some identified syrphid species deposited in Metin Aktaş Zoology Museum of Gazi University (MAZMGU, Ankara, Turkey) were taken for syrphid images stored in the museum environment. Furthermore, the photographs of these syrphid species photographed in their natural environment were taken from the ZMGU database. The number of photographs of the specimens with a sting in the museum and those taken from the database was insufficient for the training set. Therefore, more images of these species were collected from the Internet. The authors collected images using the Google search engine. Care was taken to use the photographs of the species mentioned in Table 1 from different angles. Seven hundred twenty-seven hoverfly images were used to train and test the deep learning model developed in our study. There are a total of 15 syrphid species in the dataset. Figure 1 provides specimen photographs for some species of hoverflies in the dataset.

Figure 1. Some photos of the hoverfly species used to train or test the convolutional neural network (from left to right: *Eristalis tenax, Chrysotoxum vernale, C. bicinctum*).

Four hundred seventy-nine images from the dataset were used for training, and two hundred forty-eight images were used for testing. Table 1 gives the names of each species used in the dataset and the number of images used in the training and test sets. 2/3 of the images in the dataset were allocated to the training set and the remaining 1/3 to the test set.

Table 1. Species names in the dataset and the number of images in the training and test set.

Species Names	Number of images in the training set	Number of images in the test set
Anasimyia contracta Claussen & Torp, 1980	37	18
Anasimyia interpuncta (Harris, 1776)	31	15
Anasimyia lineata (Fabricius, 1787)	29	14
Baccha elongata (Fabricius, 1775)	31	16
Callicera aurata (Rossi, 1790)	33	17
Ceriana conopsoides (Linnaeus, 1758)	22	11
Chrysotoxum arcuatum (Linnaeus, 1758)	33	17
Chrysotoxum bicinctum (Linnaeus, 1758)	31	15
Chrysotoxum vernale Loew, 1841	32	16
Dasysyrphus albostriatus (Fallen, 1817)	32	16
Epistrophe eligans (Harris, 1780)	31	16
Episyrphus balteatus (De Geer, 1776)	37	19
Eristalis interrupta (Poda, 1761)	34	17
Eristalis similis (Fallen, 1817)	33	16
Eristalis tenax (Linnaeus, 1758)	39	20

The total number of samples is 728. 485 samples were used for training and 243 for testing. Samples from the dataset were randomly selected. It was aimed to prevent over-learning by using 10-fold cross-validation. Cross-validation is a technique used to assess the performance of a machine-learning model. In this method, the dataset is divided into 10 equal-sized subsets. The model is then evaluated 10 times, where each time, one of the subsets is used as the test set, and the remaining 9 subsets are used as the training set. This process is repeated for each subset so that each subset can be the test set once. The results from the 10 evaluations are typically averaged to provide an overall performance metric for the model. This approach helps obtain a more robust estimate of the model's performance by reducing the impact of the specific data points in a single train-test split.

DESIGNED DEEP LEARNING MODEL

In this study, a deep-learning model was developed for hoverfly classification. After the developed deep learning model was trained, it was aimed to predict the species of an adult syrphid image entered from outside. To this end, different deep learning architectures were tried, and prediction rates were compared. In this study, a CNN-based deep learning model was developed. The detailed architecture of the developed model is shown in Figure 2.

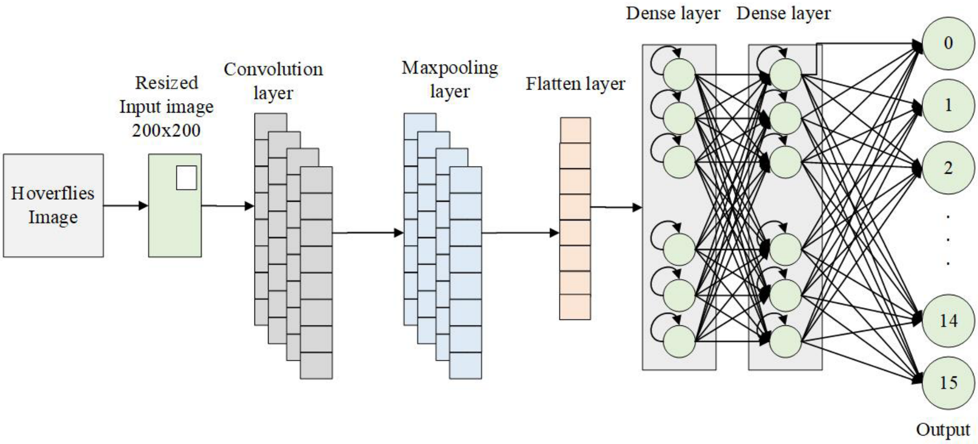

Figure 2. Designed deep learning model.

In the developed model, feature extraction was done with convolution and pooling layers, and classification values were calculated with a fully connected artificial neural network. The probability distribution of the classification categories was determined with the last softmax layer.

X nodes were created in the input layer of the developed model. One input node was used for each pixel value. Since the images have three channels as Red, Green, Blue (RGB), three input layers were used for each image. The developed deep-learning model has 15 nodes in the output layer. An output node was used for each species in the dataset. During the training, the one-hot-encoding method took the node output as 1, representing the species of the entered image, and the others as 0.

In the developed model, values of 0 for padding and 1 for stride were taken in the convolution layers. 3x3 filters were used in the convolution layer. The kernel matrix used as a filter is shown in Figure 3.

-1	-1	1
0	1	-1
0	1	1

Figure 3. The kernel used in the convolution layer.

The same kernel was used for all three channels. In the pooling layer, the window size was taken as 3x3, and the stride value was selected as one. The rectifier linear unit (ReLU) was used as the activation function in the nodes.

EXPERIMENTAL RESULTS

For experimental results, an application was developed in the Google Colabs environment using Python programming language and TensorFlow and Keras deep learning libraries. For the software interface, ReactJS was used on the frontend and a web service created with Python on the backend.

The training of the developed deep learning model was completed after 50 epochs. The categorical cross-entropy (CCE) function, which is widely used in multi-class classification problems, was used to calculate the loss values.

$$CCE = -\frac{1}{N} + \sum_{i=1}^{N} \sum_{c=1}^{C} (p_{ic} \log(y_{ic})) \qquad (1)$$

Here (1), N is the total number of observations, C is the total number of species, p_{ic} is the target (correct result) for the i^{th} observation of species c, and y_{ic} is the predicted probability distribution. The change in the loss value during the training of the model is shown in Figure 4.

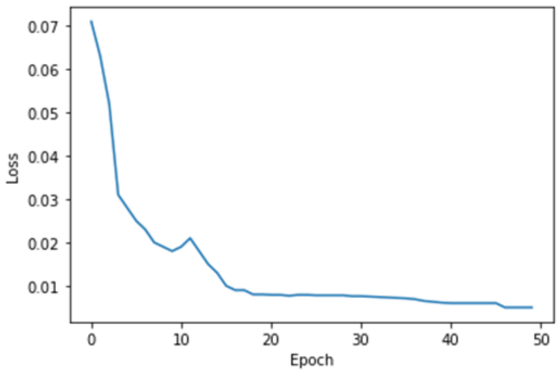

Figure 4. Change of loss values according to epoch.

As seen in Figure 4, the loss value decreases rapidly in the initial epochs, then continues to decrease slowly and reaches the lowest value at the 50th epoch, becoming stable and ending the training. The change in the loss value shows that the developed model was designed successfully. The change in the accuracy value during the training of the model is shown in Figure 5.

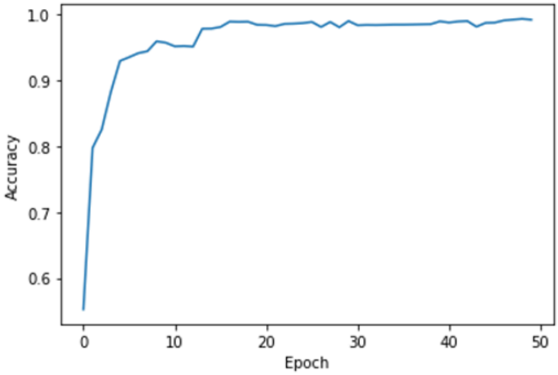

Figure 5. Accuracy value change.

As seen in Figure 5, the classification accuracy rate increases rapidly in the initial epochs, the rate of increase decreases after the 5th epoch, and the increase decreases considerably after the 20th epoch. At the 50th epoch, it reaches its highest value and becomes stable. The change in the accuracy value shows that the developed model is successful.

The true positive (TP), true negative (TN), false positive (FP) and false negative (FN) values for the experimental results obtained for 15 syrphid species are given in Table 2.

Table 2. TP, TN, FP, and FN values for species in the dataset.

Species names	TP	TN	FP	FN
Anasimyia contracta	19	227	2	-
Anasimyia interpuncta	15	232	-	1
Anasimyia lineata	14	233	-	1
Baccha elongata	14	231	1	2
Callicera aurata	17	231	-	-
Ceriana conopsoides	10	236	1	1
Chrysotoxum arcuatum	16	230	1	1
Chrysotoxum bicinctum	15	231	1	1
Chrysotoxum vernale	16	232	-	-
Dasysyrphus albostriatus	16	231	1	-
Epistrophe eligans	16	232	-	-
Episyrphus balteatus	19	229	-	-
Eristalis interrupta	17	231	-	-
Eristalis similis	16	231	-	1
Eristalis tenax	20	228	-	-

The confusion matrix obtained for 15 species is presented in Table 3.

Table 3. Confusion matrix.

		True Class														
		Anasimyia contracta	*Anasimyia interpuncta*	*Anasimyia lineata*	*Baccha elongata*	*Callicera aurata*	*Ceriana conopsoides*	*Chrysotoxum arcuatum*	*Chrysotoxum bicinctum*	*Chrysotoxum vernale*	*Dasysyrphus albostriatus*	*Epistrophe eligans*	*Episyrphus balteatus*	*Eristalis interrupta*	*Eristalis similis*	*Eristalis tenax*
Predicted Class	*Anasimyia contracta*	19	1	-	1	-	-	-	-	-	-	-	-	-	-	-
	Anasimyia interpuncta	-	15	-	-	-	-	-	-	-	-	-	-	-	-	-
	Anasimyia lineata	-	-	14	-	-	-	-	-	-	-	-	-	-	-	-
	Baccha elongata	-	-	-	14	-	1	-	-	-	-	-	-	-	-	-
	Callicera aurata	-	-	-	-	17	-	-	-	-	-	-	-	-	-	-
	Ceriana conopsoides	-	-	-	1	-	10	-	-	-	-	-	-	-	-	-
	Chrysotoxum arcuatum	-	-	1	-	-	-	16	1	-	-	-	-	-	-	-
	Chrysotoxum bicinctum	-	-	-	-	-	-	1	15	-	-	-	-	-	-	-
	Chrysotoxum vernale	-	-	-	-	-	-	-	-	16	-	-	-	-	-	-
	Dasysyrphus albostriatus	-	-	-	-	-	-	-	-	-	16	-	-	-	1	-
	Epistrophe eligans	-	-	-	-	-	-	-	-	-	-	16	-	-	-	-
	Episyrphus balteatus	-	-	-	-	-	-	-	-	-	-	-	19	-	-	-
	Eristalis interrupta	-	-	-	-	-	-	-	-	-	-	-	-	17	-	-
	Eristalis similis	-	-	-	-	-	-	-	-	-	-	-	-	-	16	-
	Eristalis tenax	-	-	-	-	-	-	-	-	-	-	-	-	-	-	20

The total number of TP = 240 and FP = 8. The accuracy value was obtained as 8/248 = 0.9677. The accuracy, recall, precision, and f-measure values for each category are shown in Table 4.

Table 4. Accuracy, precision, recall, and f-measure values for each species.

Class	Accuracy	Precision	Recall	F-Measure
Anasimyia contracta	0.99	0.90	1	0.94
Anasimyia interpuncta	0.99	1	0.93	0.96
Anasimyia lineata	0.99	1	0.93	0.96
Baccha elongata	0.98	0.93	0.87	0.89
Callicera aurata	1	1	1	1
Ceriana conopsoides	0.99	0.90	0.90	0.90
Chrysotoxum arcuatum	0.99	0.94	0.94	0.94
Chrysotoxum bicinctum	0.99	0.93	0.93	0.93
Chrysotoxum vernale	1	1	1	1
Dasysyrphus albostriatus	0.99	0.94	1	0.96
Epistrophe eligans	1	1	1	1
Episyrphus balteatus	1	1	1	1
Eristalis interrupta	1	1	1	1
Eristalis similis	0.99	1	0.94	0.96
Eristalis tenax	1	1	1	1

With the developed model, the accuracy, recall, precision, and f-measure values obtained for each species were very high. The lowest accuracy value was 0.98 in *Baccha elongata*. All others were calculated as 0.99 and 1. The lowest precision value was 0.90 in *Anasimyia contracta* and *Ceriana conopsoides* species. It was found to be 1 in 9 out of 15 species. The recall value was the lowest in *Baccha elongata* with 0.87. It was found to be 1 in 8 out of 15 species. The lowest f-measure value was 0.89 in *Baccha elongata*. It was found to be 1 in 6 out of 15 species. The graph of the obtained accuracy, precision, recall, and f-measure values by species is given in Figure 6.

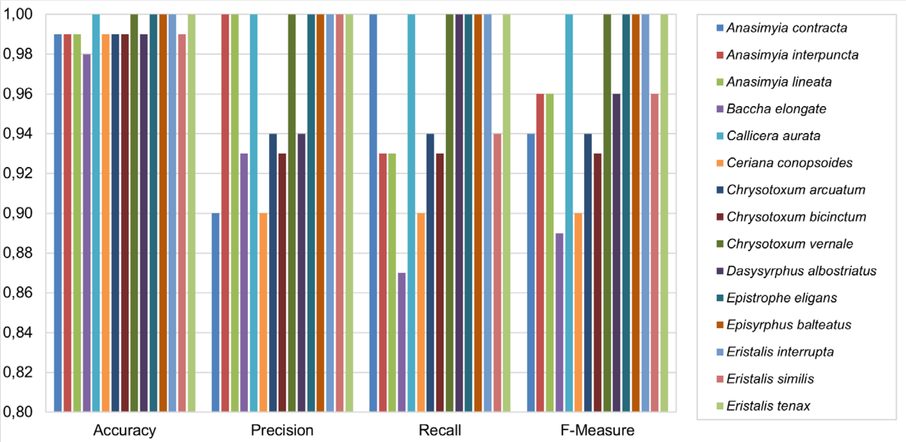

Figure 6. Accuracy, precision, recall, and f-measure values.

The graph of accuracy, precision, recall, and f-measure values for each species is given in Figure 7.

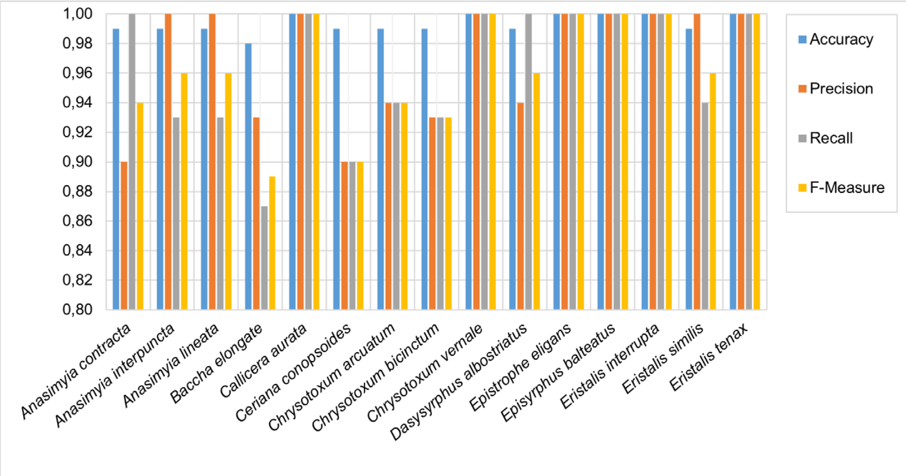

Figure 7. Accuracy, precision, recall, and f-measure values for each class.

The accuracy, precision, recall, and f-measure values obtained for all species are given in Table 5.

Table 5. Average values of accuracy, precision, recall, and f-measure for all species.

	Accuracy	Precision	Recall	F-measure
Average	0.96	0.97	0.96	0.96

As seen in Table 5, the model developed for classification has very high accuracy, precision, recall, and f-measure values for each species. The average values calculated for all classes of the developed deep learning-based classifier were also relatively high. In classification problems, the recall value used in the measurement of the FN value, in other words, the entries that could not be assigned to the correct class targeted in the classification, was relatively high as 0.87 even in the *Baccha elongate* species, in which it was the lowest. The obtained experimental results show that the developed deep-learning model successfully classifies hoverflies.

CONCLUSIONS

In this study, there are 728 samples in the dataset used. 2/3 of these samples were used for training and 1/3 for testing. Samples from the dataset were randomly selected. It was aimed to prevent over-learning by using 10-fold cross-validation. Three input layers, Red, Green, and Blue, are used for each image. 3x3 filters are used in the convolution layer of the developed model. There are 15 nodes in the output layer of the model.

The experimental results showed that the CNN-based deep learning model successfully classified hoverflies. Fifteen species from the family Syrphidae are successfully classified using the developed image-based recognition system. As with other taxonomic groups, the Syrphidae usually has taxonomic characters showing subtle differences in genus and species distinction. Although the characters at the microscopic level are used in the distinction of Syrphidae species (eye bristles, antennal segment lengths, thorax bristles), the macroscopic level is also used in the characters (color of the legs (femura, tibiae, tarsomers), abdomen coloration and pattern) (Speight and Sarthou, 2012). The high success rate in the image-based recognition system developed in our study is that body coloration and patterning in the abdomen tergites are the dominant characters in the distinction at the species level. Although we could not find a consistent error for species in this study, this program can misidentify species that are morphologically very similar and have similar patterns and coloration because the microscopic character is used together with expert opinion to distinguish such species.

This study showed that CNN is a suitable application for distinguishing Syrphidae species. Moreover, image-based recognition systems can be developed to identify more Syrphidae species and species belonging to similar dipteran families.

DISCUSSIONS

This study developed a CNN-based model to classify Syrphidae species, which play an essential role in pollinating flowering plants and grains. Syrphidae is a family with high species diversity in the order Diptera and is used in biodiversity and conservation studies. This is the first study on the classification of Syrphidae species in the literature. There are studies on the classification of insect species in the literature. However, these studies generally focus on classifying certain types using public datasets. An original data set was used in this study.

Experimental results show that the developed model has a very successful classification performance. Increasing the sample size used in the study and the image quality of the used samples will increase the model's success. The quality of the images used in this study is high. Expanding the dataset with new images will contribute to the model's training process. In addition, a more successful classification performance can be achieved with hybrid deep learning models to be developed.

ACKNOWLEDGMENTS

We thank Prof. Dr. Abdullah HASBENLI (Ankara, TURKEY) for allowing the use of syrphid photographs from the database of the MAZMGU and the taking of photographs of syrphid specimens.

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J. Entomol. Res. Soc., 25(3): 545-561, 2023
Doi: 10.51963/jers.v25i3.2452

Review Article
Online ISSN:2651-3579

Review of Scolytinae (Coleoptera, Curculionidae) of Serbia

Čedomir Marković[1*] Jovan Dobrosavljević[2]

University of Belgrade, Faculty of Forestry, Kneza Višeslava 1, 11030 Belgrade, Serbia
e-mails: [1*]cedomir.markovic@sfb.bg.ac.rs, [2]jovan.dobrosavljevic@sfb.bg.ac.rs
ORCID IDs: [1*]0000-0002-9268-8521, [2]0000-0002-6155-5654
*Corresponding author

ABSTRACT

The list of Scolytinae of Serbia was made based on the data that has been published so far and the unpublished data collected in the period from 1991 to 2023. In it, 96 species are listed in total. The Scolytinae fauna has not been sufficiently studied in Serbia. It is presumed that there are about 20 more species present in Serbian fauna.

Keywords: Fauna, xylophagous, bark beetles, ambrosia beetles.

Marković, Č. & Dobrosavljević, J. (2023). Review of Scolytinae (Coleoptera, Curculionidae) of Serbia. *Journal of the Entomological Research Society*, 25(3), 545-561.

Received: April 24 2023 *Accepted: August 21, 2023*

INTRODUCTION

Scolytinae are small insects (0,5-12 mm). They usually develop under the bark or in the wood of various trees and shrubs, while some tropical species can develop in seeds (Iku et al, 2018). They usually inhabit physiologically weakened and freshly felled material (Marković & Stojanović, 2001, 2003). However, certain species attack completely healthy trees during outbreaks. This consequently causes their dieback (Grégoire et al., 2015; Fernandez-Carrillo et al, 2020). Some Scolytinae species can also cause indirect damage to forests as they serve as vectors of some pathogens (Marković & Stojanović, 2003, 2011; Santini & Faccoli, 2015). Due to their importance, they have always attracted the attention of foresters and entomologists.

So far, about 5,800 species of Scolytinae have been described in the world (Wood, 2007). The first written information about their fauna in Serbia was given by Langhoffer (1915). From then until the end of the Second World War, they were not researched extensively in Serbia when Professor Dr. Svetislav Živojinović started studying them (Marković, 2013). After his death in 1966, Professor Dr. Dragić Tomić and forestry engineer Miroslav Stevanović continued the research on the Scolytinae fauna of Serbia. Their research ended in the 1990s, and was continued by Dr. Čedomir Marković and agricultural engineer Aleksandar Stojanović to this day.

The first faunistic list of Scolytinae of Serbia was made in 1997 (Marković & Stojanović, 1997a). How 25 years have passed since then and many new data on their fauna have been collected (Marković & Stojanović, 1997b, c, 1999, 2000a, b, c, 2001, 2003, 2004, 2005, 2010, 2011, 2012, 2014, 2015, 2019, 2020; Marković, Stojanović, & Milenković, 1997; Manojlović, 1998; Stevanović, 1998; Marković, 1999, 2005, 2011, 2012, 2013; Manojlović et al, 2000a, b, 2001; 2003; Stojanović & Marković, 2001, 2007; Milošević, 2003; Roganović, 2003; Marković, Jančić, & Milanović, 2004; Tabaković-Tošić & Milosavljević, 2015, 2016, 2018a, b; Milosavljević et al, 2021a, b, 2022; Češljar et al, 2022; Vujić & Vesović, 2022) there was a need to create a new list of species and to determine: 1. which species of Scolytinae have been reported in Serbia so far; 2. how well are the Scolytinae investigated in Serbia. That new list of species can be found in this paper.

MATERIAL AND METHODS

The species list presented in this paper was compiled based on the data on the Scolytinae fauna that have been published in Serbia so far and unpublished data collected by Čedomir Marković and Aleksandar Stojanović in the period from 1992 to 2023 at 46 sites (Table 1) in Serbia. The trees inhabited by Scolytinae were sought at the localities where the research was conducted. If the Scolytinae adults were found on them, they were collected in glass vials and killed by ether. However, if their larvae or pupae were found, 30-50 cm long pieces of wood were cut from the inhabited parts of the trees. They were then brought to the laboratory of the Faculty of Forestry, the University of Belgrade where they were placed in photoeclectors to obtain adults. At the time of their appearance, the photoeclectors were inspected daily. The emerged adults were collected, killed by ether, prepared, and identified.

The identification of the collected material was carried out by Čedomir Marković using the published works of Balachowsky (1949), Stark (1952), Nunberg (1954), Karaman (1971), and Pfeffer (1989). The data obtained this way and the already published data were entered into a database from which a list of the species that have been recorded in Serbia so far was derived. The names of the species used in it are coordinated with the names given by Alonso-Zarazaga (2013) for this group of insects. The localities mentioned in the database are grouped according to the geographic entities (mountains) or administrative units (municipalities, urban areas) to which they belong.

Table 1. List of investigated localities.

Locality	Coordinates		Average altitude (m)
	N	E	
Aleksinac			
Brdjanka	43°32'0"	21°42'42"	185
Paniče	43°33'57"	21°42'45"	245
Rujevica	43°32'46"	21°43'6"	235
Arandjelovac			
Banja	44°17'15"	20°37'30"	190
Belgrade			
Bojčin Forest	44°44'19"	20°8'52"	90
Košutnjak	44°46'50"	20°25'38"	130
Lipovica Forest	44°38'51"	20°24'15"	285
Stepin gaj	44°44'59"	20°32'2"	260
Trešnja	44°37'29"	20°34'21"	190
Ušće	44°49'30"	20°25'18"	80
Brus			
Milentija	43°25'48"	20°58'37"	620
Ćuprija			
Mijatovac	43°57'15"	21°19'50"	120
Deliblato Sands			
Čardak	44°51'50"	21°3'43"	135
Kučevo			
Majdan Kučajna	44°26'50"	21°36'56"	355
Loznica			
Gornja Koviljača	44°29'2"	19°8'22"	170
Grnčara	44°31'15"	9°17'35"	220
Majdanpek			
Debeli Lug	44°20'19"	21°53'52"	430
Mt. Bukovik	43°41'15"	21°40'13"	530
Mt. Goč			
Brezna	43°34'1"	20°40'44"	650
Brezjak	43°33'52"	20°43'23"	870
Ravnine	43°34'13"	20°40'9"	830
Mt. Avala	44°41'25"	0°30'29"	375
Mt. Kamenički vis			
Gradac	43°22'36"	21°59'59"	590

Locality	Coordinates		Average altitude (m)
	N	E	
Mt. Kopaonik			
Barska river	43°17'36"	20°45'37"	1385
Hajdučka voda	43°18'9"	20°47'7"	1600
Jankova bara	43°19'12"	20°46'27"	1480
Kadijevac	43°19'3"	20°45'46"	1450
Karaman	43°17'11"	20°49'31"	1885
Saborište	43°17'1"	20°48'19"	1730
Samokovska river	43°20'31"	20°44'50"	1240
Mt. Povlen			
Vujinovača	44°10'34"	19°43'2"	780
Smiljevo polje	44°7'35"	19°39'32"	975
Mt. Tara			
Kaludjerske Bare	43°53'42"	19°33'18"	1120
Predov Krst	43°56'24"	19°18'37"	1085
Studenac, Klade	43°53'28"	19°19'33"	1225
Negotin	44°13'58"	22°31'46"	45
Obrenovac			
Draževac	44°34'51"	44°34'51"	95
Pirot	43°9'22"	22°34'59"	370
Pristina			
Lipljan, Lipovica hunting area	42°32'59"	21°0'8.04"	630
Raška			
Gnjilica	43°15'4"	20°38'4"	520
Zimovnik, Breze, Vilje kolo	43°19'2"	20°43'54"	1270
Ražanj			
Ražanj	43°40'22"	21°32'55"	280
Praskovče	43°37'19"	21°31'41"	215
Ruma			
Karakuša	44°48'11"	19°45'24"	85
Sjenica			
Pape	43°16'24"	20°1'27"	1025
Vlasotince			
Donja Lopušnja	42°55'27"	22°10'33"	815

RESULTS

Based on the data obtained 96 species of Scolytinae from 17 tribes and 34 genera were identified (Table 2). During our research, the findings of 25 species from 12 tribes and 16 genera were confirmed.

Table 2. Scolytinae recorded in Serbia*.

	Species	Reference/New finding
	Tribus Corthylini	
1	*Pityophthorus balcanicus* Pfeffer, 1940	Karaman, 1971
2	*P. glabratus* Eichhoff, 1878	Reference: Mamontov, 1937; Živojinović, 1954a, b; Maksimović& Milanović, 1964; Živojinović & Živojinović, 1969; Radonjić & Tomić, 1995; Marković, 2013
3	*P. lichtensteinii* (Ratzeburg, 1837)	Reference: Tomić, 1954; Živojinović, 1954a, 1960; Stevanović, 1987; Radonjić & Tomić, 1995; Roganović, 2003; Marković, 2013
4	*P. micrographus* (Linnaeus, 1758)	Reference: Živojinović, 1948, 1950b, 1954a, 1961; Janković, 1949; Tomić, 1954; Maksimović & Barlov, 1961; Maksimović & Milanović, 1961, 1964

	Species	Reference/New finding
5	*P. pityographus* (Ratzeburg, 1837)	New finding: Aleksinac, Brdjanka 11.9.1993. on *Pinus nigra* Arn.; Belgrade: Košutnjak 9.12.1992. on *P. omorica* (Pančić) Purk., 18.2.1993. on *Cedrus* sp., 3.2.1996. on *P. nigra*, 2.2.1996. on *P. silvestris* L., Stepin gaj 7.3.1993. on *P. nigra*; Mt. Avala 25.7.1995. on *Picea abies* (L.) Karst.; Mt. Tara, Studenac, Klade 27.7.1993. on *P. omorica*. Reference: Živojinović, 1960, 1961; Janković, 1972; Stevanović, 1987; Adamović, 1990; Marković & Stojanović, 2000a; Roganović, 2003; Marković, 2013
	Tribus Cryphalini	
6	*Cryphalus abietis* (Ratzeburg, 1837)	Reference: Živojinović, 1950b; Radonjić & Tomić, 1995; Marković, 2013
7	*C. piceae* (Ratzeburg, 1837)	New finding: Mt. Goč, Brezjak 20.7.2005. on *P. abies*. Reference: Idrizović, 1947; Živojinović, 1948, 1950b, 1954a, 1961; Janković, 1949; Tomić, 1954, 1957; Adamović, 1990; Marković & Stojanović, 2000a; Roganović, 2003; Marković, 2013
8	*C. saltuarius* Weise 1891	Reference: Živojinović, 1950b
9	*Ernoporicus caucasicus* (Lindemann, 1876)	Reference: Marković & Stojanović 2000c, 2015; Marković, 2013
10	*E. fagi* (Fabricius, 1798)	Reference: Živojinović, 1954a; Radonjić & Tomić, 1995; Marković & Stojanović, 2000a; 2015
11	*Ernoporus tiliae* (Panzer, 1793)	Reference: Živojinović & Živojinović, 1969; Radonjić & Tomić, 1995; Marković, 2013; Marković & Stojanović, 2015
12	*Trypophloeus asperatus* (Gyllenhal, 1813)	Reference: Langhoffer, 1915; Živojinović & Živojinović, 1969
13	*T. granulatus* (Ratzeburg, 1837)	Reference: Langhoffer, 1915
	Tribus Crypturgini	
14	*Crypturgus cinereus* (Herbst, 1793)	Reference: Živojinović, 1950b, 1954a, 1961; Živojinović & Živojinović, 1969; Karaman, 1971; Stevanović, 1987; Marković & Stojanović, 2000a, b; Marković, 2013
15	*C. cribellus* Reitter, 1894	Reference: Stevanović, 1987; Marković & Stojanović, 2000a, b; Roganović, 2003; Marković, 2013
16	*C. pusillus* (Gyllenhal, 1813)	Reference: Janković, 1949; Žojinović, 1950b, 1954a; Tomić, 1954; Stevanović, 1987; Adamović, 1990; Marković & Stojanović, 2000a, b; Marković, 2013
	Tribus Dryocoetini	
17	*Dryocoetes autographus* (Ratzeburg, 1837)	Reference: Živojinović, 1950b; Tomić, 1954, 1957; Janković, 1972; Stevanović, 1987; Tomić et al, 1992; Radonjić & Tomić, 1995; Roganović, 2003; Marković, 2013
18	*D. hectographus* Reitter, 1913	Reference: Marković, 2013
19	*D. villosus* (Fabricius, 1792)	Reference: Živojinović & Živojinović, 1969
20	*Lymantor aceris* (Lindemann, 1875)	Reference: Karaman, 1971
21	*L. coryli* (Perris, 1853)	Reference: Langhoffer, 1915
22	*Xylocleptes bispinus* (Duftschmid, 1825)	Reference: Langhoffer, 1915; Karaman, 1971; Radonjić & Tomić, 1995; Marković & Stojanović, 2015
	Tribus Hylastini	
23	*Hylastes angustatus* (Herbst, 1793)	New finding: Ćuprija, Mijatovac 1.9.1993. on *P. nigra*; Pirot 1.12.1992. on *P. nigra*. Reference: Mamontov, 1937; Živojinović & Živojinović, 1969; Kovačević, 1982; Stevanović, 1987; Radonjić & Tomić, 1995; Marković & Stojanović, 2000a; Marković, 2013
24	*H. ater* (Paykull, 1800)	Reference: Tomić, 1954; Živojinović, 1960; Živojinović & Živojinović, 1969; Kovačević, 1982; Stevanović, 1987; Roganović, 2003; Marković, 2013
25	*H. attenuatus* Erichson, 1836	Reference: Živojinović & Živojinović, 1969
26	*H. cunicularius* Erichson, 1836	Reference: Živojinović, 1950b; Tomić, 1957; Kovačević, 1982; Roganović, 2003; Marković, 2013
27	*H. linearis* Erichson, 1836	Reference: Živojinović, 1960; Marković & Stojanović, 2000a
28	*H. opacus* Erichson, 1836	Reference: Marković & Stojanović, 2000a
29	*Hylurgops palliatus* (Gyllenhal, 1813)	New finding: Majdanpek, Debeli Lug 27.5.1992.on *P. nigra*; Mt. Goč, Ravnine 20.7.2005. on *P. nigra*; Mt. Tara, Predov Krst 19.9.2005. on *P. abies*. Reference: Živojinović, 1950b, 1960, 1961; Tomić, 1954, 1957; Janković, 1972; Stevanović, 1987; Adamović, 1990; Radonjić & Tomić, 1995; Marković & Stojanović, 2000a; Roganović, 2003; Marković, 2013

	Species	Reference/New finding
	Tribus Hylesinini	
30	*Hylastinus obscurus* (Marsham, 1802)	Reference: Langhoffer, 1915
31	*Hylesinus crenatus* (Fabricius, 1787)	Reference: Živojinović, 1950a, 1961; Živojinović & Živojinović, 1969; Karaman, 1971; Radonjić & Tomić, 1995; Marković, 2013
32	*H. fraxini* (Panzer, 1779)	Reference: Živojinović, 1950a, 1954a, 1961; Tunguz, 1951; Ogrizek, 1955; Grujić, 1956; Živojinović & Živojinović, 1969; Tomić et al, 1992; Radonjić & Tomić, 1995; Marković & Stojanović, 2000a, 2015; Marković, 2013
33	*H. toranio* (Danthoine, 1788)	Reference: Langhoffer, 1915; Živojinović, 1954a; Ogrizek, 1955; Grujić, 1956; Živojinović & Živojinović, 1969; Marković & Stojanović, 2000b, 2015; Marković, 2013
34	*H. wachtli* Reitter, 1887	Reference: Živojinović, 1954a; Živojinović & Živojinović, 1969
35	*Kissophagus hederae* (Schmitt, 1843)	Reference: Marković & Stojanović, 2014
36	*Pteleobius kraatzi* (Eichhoff, 1864)	Reference: Langhoffer, 1915; Manojlović, 1973; Maksimović, 1979; Manojlović et al, 2000a, 2003; Marković, 2013
37	*P. vittatus* (Fabricius, 1787)	Reference: Langhoffer, 1915; Živojinović & Živojinović, 1969; Karaman, 1971; Marković & Stojanović, 2000b, 2012, 2015, 2020; Stojanović & Marković, 2007; Marković, 2013
	Tribus Hypoborini	
38	*Hypoborus ficus* Erichson, 1836	Reference: Langhoffer, 1915; Vujić & Vesović, 2022
	Tribus Ipini	
39	*Ips acuminatus* (Gyllenhal, 1827)	Reference: Živojinović, 1948, 1954a, b; Tomić, 1954; Maksimović & Milanović, 1964; Živojinović & Živojinović, 1969; Kovačević, 1982; Stevanović, 1987; Adamović, 1990; Marković, 2013, Tabaković-Tošić & Milosavljević, 2015
40	*I. amitinus* (Eichhoff, 1871)	Reference: Idrizović, 1947; Živojinović, 1948, 1950b, 1960, 1961
41	*I. mansfveldi* (Wachtl, 1879)	Reference: Živojinović, 1954a; Živojinović & Živojinović, 1969; Stevanović, 1987; Adamović, 1990; Radonjić & Tomić, 1995; Marković, 2013
42	*I. sexdentatus* (Börner, 1776)	New finding: Aleksinac, Brdjanka 11.9.1993. on *P. nigra*; Belgrade: Ušće 25.4.1993. on *P. nigra*, Stepin gaj 9.6.1997 on *P. nigra*; Mt. Avala 27.5.1995, 21.2.1998. on *P. nigra*; Mt. Bukovik 5.8.2015. on *P. nigra*; Mt. Kamenički vis, Gradac 4.7.2005. on *P. nigra*; Negotin 24.9.1993. on *P. nigra*; Raška, Zimovnik, Breze, Vilje kolo 16.4.1994. on *Pinus* sp. Reference: Idrizović, 1947; Živojinović, 1948, 1954a, 1960; Živojinović & Živojinović, 1969; Stevanović, 1987; Adamović, 1990; Radonjić & Tomić, 1995; Marković & Stojanović, 2000a; Roganović, 2003; Marković, 2013
43	*I. typographus* (Linnaeus, 1758)	New finding: Mt. Kopaonik: Barska river 16.7.2008. on *P. abies*, Hajdučka voda 30.5.2001. on *P. abies*, Jankova bara 1.8.2006. on *P. abies*, Kadijevac 30.5.2001. on *P. abies*, Karaman 30.5.2001. on *P. abies*, Marine vode 16.7.2008. on *P. abies*, Samokovska river 1.8.2006. on *P. abies*; Mt. Tara: Šljivovica 17.5.2001. on *P. abies*, Predov Krst 19.9.2005. on *P. abies*. Reference: Mamontov, 1937; Idrizović, 1947; Živojinović, 1948, 1950b, 1960, 1961; Tomić, 1954, 1957; Živojinović & Petrović, 1955; Damnjanović, 1955; Maksimović & Milanović, 1961, 1964; Maksimović & Bošković, 1962; Janković, 1972; Vasić et al, 1982; Radonjić & Tomić, 1995; Marković & Stojanović, 2000a, 2010; Marković et al, 2004; Marković, 2012, 2013; Roganović, 2003; Tabaković-Tošić & Milosavljević, 2015, 2016, 2018; MIlosavljević et al, 2021a, 2021b, 2022; Češljar et al, 2022
44	*Orthotomicus erosus* (Wollaston, 1857)	New finding: Aleksinac: Brdjanka 3.1.1994., 28.1.1994., 18.5.1996. on *P. nigra*, Paniče 28.1.1994. on *P. nigra*; Belgrade: Košutnjak 18.2.1992., 11.2.1993., 14.3.1996. on *P. nigra*, 2.2.1996. on *P. silvestris*, Lipovica Forest 20.2.1992. on *P. nigra,* Trešnja 2.2.1993. on *P. nigra*; Ćuprija, Mijatovac 30.11.1992. on *P. nigra*; Deliblato Sands, Čardak 8.7.2005. on *P. silvestris*; Mt. Avala 8.6.2005. on *P. nigra*; Mt. Kamenički vis, Gradac 4.7.2005. on *P. nigra*. Reference: Mamontov, 1937; Tunguz, 1951; Živojinović, 1954a; Kovačević, 1982; Stevanović, 1987; Radonjić & Tomić, 1995; Marković & Stojanović, 2000a; Marković, 2013
45	*O. laricis* (Fabricius, 1792)	New finding: Aleksinac, Brdjanka 11.9.1993. *P. nigra*. Reference: Živojinović, 1950b; Kovačević, 1982; Stevanović, 1987; Radonjić & Tomić, 1995; Marković & Stojanović, 2000a; Marković, 2013
46	*O. longicollis* (Gyllenhal, 1827)	Reference: Marković & Stojanović, 2000a; Marković, 2013
47	*O. proximus* (Eichhoff, 1867)	Reference: Grujić, 1956; Živojinović & Živojinović, 1969; Kovačević, 1982; Adamović, 1990; Marković, 2013

	Species	Reference/New finding
48	*O. suturalis* (Gyllenhal, 1827)	New finding: Vlasotince, Donja Lopušnja 17.9.1993. on *P. nigra*. Reference: Tomić, 1954; Živojinović, 1954a; Živojinović & Živojinović, 1969; Kovačević, 1982; Stevanović, 1987; Marković & Stojanović, 2000a, b; Marković, 2013
49	*Pityogenes bidentatus* (Herbst, 1784)	New finding: Aleksinac, Paniče 28.1.1994. on P. nigra; Ćuprija, Mijatovac 1.9.1993. on *P. nigra*; Raška, Zimovnik, Breze, Vilje kolo 16.4.1994. on *Pinus* sp.; Ražanj, Praskovče 20.5.1996. on *P. nigra*. Reference: Živojinović, 1954a, b, 1961; Živojinović & Živojinović, 1969; Kovačević, 1982; Stevanović, 1987; Adamović, 1990; Marković, 2013
50	*P. bistridentatus* (Eichhoff, 1878)	New finding: Aleksinac: Brdjanka 11.9.1993., 28.1.1994, 18.5.1996. on *P. nigra*, Paniče 28.1.1994. on *P. nigra*; Belgrade: Košutnjak 11.2.1993., 18.2.1993., 14.3.1996. on *P. nigra*, Lipovica Forest 20.2.1993. on *P. nigra*, Trešnja 2.2.1993. on *P. nigra*, Stepin gaj 7.3.1993. on *P. nigra*; Brus, Milentija 10.9.1993. on *P. nigra*; Ćuprija, Mijatovac 1.9.1993. on *P. nigra*; Deliblato Sands, Čardak 8.7.2005. on *P. silvestris*; Kučevo, Majdan Kučajna 23.6.2005. on *P. nigra*; Loznica, Grnčara 16.9.1992. on *P. strobus*; Mt. Avala 8.6.2005. on *P. nigra*; Mt. Goč, Ravnine 20.7.2005. on *P. nigra*; Mt. Kamenički vis, Gradac 4.7.2005. on *P. nigra*; Mt. Povlen: Smiljevo Polje 1.8.2005. on *P. nigra*, Vujinovača 1.8.2005., 16.9.2005. on *P. nigra*; Pirot 1.12.1992. on *P. nigra*; Raška, Gnjilica 2.10.1992. on *P. strobus*; Sjenica, Pape 27.5.1992. in flight. Reference: Mamontov, 1937; Živojinović, 1948, 1954a, b, 1960, 1961; Živojinović & Živojinović, 1969; Kovačević, 1982; Stevanović, 1987; Adamović, 1990; Radonjić & Tomić, 1995; Marković & Stojanović, 2000a; Roganović, 2003; Marković, 2013
51	*P. chalcographus* (Linnaeus, 1761)	New finding: Mt. Kopaonik, Samokovska river 16.7.2008. on P. abies; Mt. Tara, Kaludjerske Bare 19.9.2014. on P. abies; Sjenica, Pape 27.5.1992 in flight. Reference: Mamontov, 1937; Idrizović, 1947; Živojinović, 1948, 1950b, 1954a, 1960, 1961; Tomić, 1954, 1957; Živojinović & Petrović, 1955; Maksimović & Milanović, 1961; Maksimović & Barlov, 1961; Maksimović & Bošković, 1962; Vasić et al, 1982; Stevanović, 1987; Marković & Stojanović, 2000a, 2010; Marković et al, 2004; Roganović, 2003; Marković, 2012, 2013; Tabaković-Tošić & Milosavljević, 2016; Češljar et al, 2022
52	*P. quadridens* (Hartig, 1834)	Reference: Živojinović, 1954a, 1960, 1961; Maksimović & Milanović, 1964; Živojinović & Živojinović, 1969; Kovačević, 1982; Stevanović, 1987; Adamović, 1990; Marković & Stojanović, 2000a; Marković, 2013
53	*P. trepanatus* (Nordlinger, 1848)	Reference: Živojinović, 1960, 1961; Marković, 2013
54	*P. monacensis* Fuchs, 1911	Reference: Živojinović, 1961
55	*Pityokteines curvidens* (Germar, 1824)	Reference: Idrizović, 1947; Živojinović, 1948, 1950b, 1954a, 1961; Janković, 1949; Grujić, 1956; Maksimović & Milanović, 1964; Adamović, 1990; Marković & Stojanović, 2000a; Roganović, 2003; Marković, 2013
56	*P. spinidens* (Reitter, 1894)	Reference: Janković, 1949; Živojinović, 1950b, 1954a, 1961; Tomić, 1954; Adamović, 1990; Marković & Stojanović, 2000a; Roganović, 2003; Marković, 2013
57	*P. vorontzovi* (Jakobson, 1895)	Reference: Živojinović, 1948, 1950b, 1954a, 1961; Janković, 1949; Tomić, 1954, 1957; Adamović, 1990; Radonjić & Tomić, 1995; Marković & Stojanović, 2000a; Marković, 2013
	Tribus Phloeosinini	
58	*Phloeosinus aubei* (Perris, 1855)	Reference: Živojinović, 1961; Kovačević, 1982; Tomić et al, 1992; Marković & Stojanović, 2000a, b, 2004; Marković, 2013
59	*Ph. thujae* (Perris, 1855)	New finding: Aleksinac, Brdjanka 30.10.1993. on *Thuja occidentalis* L.; Arandjelovac, Banja 29.4.1992. in flight; Belgrade, Košutnjak 17.6.2005. on *Juniperus* sp.; Mt. Kopaonik, Saborište 30.5.2001. on *Juniperus communis* L.; Mt. Tara, Kaludjerske Bare 16.9.2005. on *Chamaecyparis lawsoniana* (A. Murray) Parlatore; Obrenovac, Draževac 3.6.1993. by Malaise trap; Ražanj 19.9.1993. on *Th. Occidentalis*. Reference: Živojinović, 1954a; Živojinović & Živojinović, 1969; Tomić et al, 1992.; Milijašević et al, 1994; Marković & Stojanović, 2000a, 2004; Marković, 2013
	Tribus Phloeotribini	
60	*Phloeotribus brevicollis* (Kolenati, 1846)	Reference: Marković & Stojanović, 1999
61	*Ph.caucasicus* Reitter, 1891	Reference: Marković & Stojanović, 1997b
	Tribus Polygraphini	
62	*Carphoborus minimus* (Fabricius, 1798)	New finding: Raška, Zimovnik, Breze, Vilje kolo 16.4.1994. on *Pinus* sp. Reference: Živojinović, 1954a, 1961; Maksimović & Milanović, 1964; Živojinović & Živojinović, 1969; Marković, 2013
63	*C. perrisi* (Chapuis, 1869)	Reference: Živojinović & Živojinović, 1969
64	*Polygraphus grandiclava* Thomson, 1886	Reference: Živojinović, 1961

	Species	Reference/New finding
65	*P. polygraphus* (Linnaeus, 1758)	Reference: Živojinović, 1950b; Tomić, 1954, 1957; Živojinović, 1961; Roganović, 2003; Marković, 2013
66	*P. subopacus* Thomson, 1871	Reference: Živojinović, 1961
	Tribus Scolytini	
67	*Scolytus carpini* (Ratzeburg, 1837)	Reference: Živojinović, 1954a; Radonjić & Tomić, 1995; Marković & Stojanović, 2015
68	*S. ensifer* Eichhoff, 1881	New finding: Aleksinac, Rujevica 15.4.2023. on *Ulmus* sp. Reference: Manojlović, 1973; Marković et al, 1997; Marković & Stojanović, 2000a, b, 2012, 2015; Manojlović et al, 2000a, b, 2003; Stojanović & Marković, 2007; Marković, 2011
69	*S. intricatus* (Ratzeburg, 1837)	New finding: Aleksinac, Rujevica 15.4.2023. on *Quercus cerris* L.; Loznica, Gornja Koviljača 12.4.2023. on *Q. cerris* and *Q. frainetto* Ten.; Mt. Kamenički vis, Gradac 18.8.2013. on *Q. cerris*; Ruma, Karakuša 12.4.2023. on *Q. robur* L. Reference: Živojinović, 1954a; Grujić, 1956; Živojinović & Živojinović, 1969; Marković, 1995, 1999, 2005, 2013; Marković & Stojanović, 1996, 2000a, 2001, 2003, 2005, 2011, 2015; Milošević, 2003
70	*S. kirschi* Skalitzky, 1876	Reference: Grujić, 1956; Marković et al, 1997., Marković & Stojanović, 2000b, 2012, 2015; Stojanović & Marković, 2007; Marković, 2011, 2013
71	*S. laevis* Chapuis, 1869	Reference: Marković & Stojanović, 1997a, 2000a 2012; Stojanović & Marković, 2007; Marković, 2011
72	*S. mali* (Bechstein, 1805)	Reference: Langhoffer, 1915; Radosavljević, 1938; Živojinović, 1954a; Grujić, 1956; Radonjić & Tomić, 1995; Marković, 2013; Marković & Stojanović, 2015
73	*S. multistriatus* (Marsham, 1802)	Reference: Langhoffer, 1915; Tunguz, 1951; Grujić, 1956; Živojinović, 1961; Živojinović & Živojinović, 1969; Manojlović, 1973, 1986a, b; Maksimović, 1979; Karaman, 1971; Vrkić, 1989; Radonjić & Tomić, 1995; Manojlović et al, 2000a, b, 2001, 2003; Marković & Stojanović, 2000a, 2012, 2015; Stojanović & Marković, 2007; Marković, 2011, 2013
74	*S. pygmaeus* (Fabricius, 1787)	New finding: Aleksinac, Rujevica 15.4.2023. on *Ulmus* sp. Reference: Živojinović & Živojinović, 1969; Karaman, 1971; Manojlović, 1973, 1986b, 1998; Maksimović, 1979; Marković et al, 1997; Marković & Stojanović, 2000a, b, 2012, 2015; Manojlović et al, 2000a, b, 2001, 2003; Stojanović & Marković, 2007; Marković, 2011, 2013
75	*S. ratzeburgi* Janson, 1856	Reference: Živojinović, 1954a; Tomić, 1954; Živojinović & Živojinović, 1969; Radonjić & Tomić, 1995; Marković, 2013; Marković & Stojanović, 2015
76	*S. rugulosus* (Muller, 1818)	New finding: Mt. Goč, Brezjak 20.5.1997. on *Prunus avium* L. Reference: Langhoffer, 1915; Živojinović & Živojinović, 1969; Vrkić, 1989; Radonjić & Tomić, 1995; Mihajlović et al, 1994; Marković & Stojanović, 2000a, 2015; Stojanović & Marković, 2001; Marković, 2013
77	*S. scolytus* (Fabricius, 1775)	Reference: Langhoffer, 1915; Ogrizek, 1955; Grujić, 1956; Živojinović, 1961; Živojinović & Živojinović, 1969; Karaman, 1971; Manojlović, 1973, 1986b; Maksimović, 1979; Vrkić, 1989; Manojlović & Sivčev, 1995a, b; Radonjić & Tomić, 1995; Manojlović et al, 2000a, b, 2001, 2003; Stojanović & Marković, 2007; Marković & Stojanović, 2012, 2015; Marković, 2011, 2013
	Tribus Taphrorychini	
78	*Taphrorychus bicolo*r (Herbst, 1793)	Reference: Mamontov, 1937; Živojinović, 1950a, 1954a, 1961; Tomić, 1954, 1957; Radonjić & Tomić, 1995; Marković & Stojanović, 2000a, 2015; Marković, 2013
79	*T. villifrons* (Dufour, 1843)	New finding: Loznica, Gornja Koviljača 12.4.2023. on *Q. cerris*. Reference: Radonjić & Tomić, 1995; Milošević, 2003; Marković & Stojanović, 2011, 2015, 2019
	Tribus Thamnurgini	
80	*Thamnurgus varipes* Eichhoff, 1878	New finding: Belgrade, Lipovica Forest 23.7.1995. on *Euphorbia dendroides* L., Progar, Bojčin Forest 2.5.1995. on *Euphorbia* sp.; Pristina, Lipljan, Lipovica hunting area 9.5.1995. on *E. dendroides*. Reference: Stevanović, 1998; Marković & Stojanović, 2000b
	Tribus Tomicini	
81	*Dendroctonus micans* (Kugelann, 1794)	Reference: Živojinović, 1961; Roganović, 2003
82	*Hylurgus ligniperda* (Fabricius, 1787)	Reference: Živojinović & Živojinović, 1969; Stevanović, 1987; Marković & Stojanović, 2000b; Marković, 2013

	Species	Reference/New finding
83	*Tomicus minor* (Hartig, 1834)	New finding: Belgrade, Stepin gaj 17.6.2005., 26.6.2006. on *P. nigra*; Kučevo, Majdan Kučajna 23.6.2005. on *P. nigra;* Mt. Avala 8.6.2005., 30.7.2005., 26.6.2006. on *P. nigra*; Mt. Bukovik 5.8.2015. on *P. nigra;* Mt. Goč: Brezna 23.6.2008. on *P. nigra*, Ravnine 20.7.2005. on *P. nigra*; Mt. Povlen: Smiljevo Polje 1.8.2005. on *P. nigra*, Vujinovača 1.8.2005., 16.9.2005. on *P. nigra*; Mt. Tara, Kaludjerske Bare 14.7.2006., 26.6.2008. on *P. nigra*; Raška, Zimovnik, Breze, Vilje kolo 16.4.1994. on *Pinus* sp. Reference: Živojinović, 1954a, b, 1961; Živojinović & Živojinović, 1969; Stevanović, 1987; Adamović, 1990; Radonjić & Tomić, 1995; Marković & Stojanović, 2000a; Roganović, 2003; Marković, 2013
84	*T. piniperda* (Linnaeus, 1758)	New finding: Mt. Avala 30.7.2005. on *P. nigra*. Reference: Mamontov, 1937; Idrizović, 1947; Tunguz, 1951; Tomić, 1954; Živojinović, 1954a, 1961; Grujić, 1956; Maksimović & Milanović, 1964; Živojinović & Živojinović, 1969; Stevanović, 1987; Adamović, 1990; Radonjić & Tomić, 1995; Marković & Stojanović, 2000a; Marković, 2013
85	*Xylechinus pilosus* (Ratzeburg, 1837)	Reference: Marković, 2013
	Tribus Xyleborini	
86	Xyleborinus alni Niijima, 1909	Reference: Marković & Stojanović, 2011; Marković, 2013
87	*X. saxesenii* (Ratzeburg, 1837)	New finding: Belgrade: Bojčin Forest 22.4.1996 in flight, Košutnjak 9.3.1994., 4.3.1995., 6.3.1995., 19.3.1995. on *Carpinus betulus* L. Reference: Živojinović, 1950a; Radonjić & Tomić, 1995; Marković & Stojanović, 2015
88	*Xyleborus dispar* (Fabricius, 1792)	Reference: Langhoffer, 1915; Živojinović, 1950a; Maksimović, 1959, 1964; Karaman, 1971; Marković & Stojanović, 2011, 2015, 2019; Marković, 2013
89	*X. cryptographus* (Ratzeburg, 1837)	Reference: Langhoffer, 1915; Živojinović et al, 1962
90	*X. dryographus* (Ratzeburg, 1837)	New finding: Obrenovac, Draževac 2.8.1993. by Malaise trap. Reference: Langhoffer, 1915; Mamontov, 1937; Živojinović, 1950a; Karaman, 1971; Marković & Stojanović, 2015
91	*X. eurygraphus* (Ratzeburg, 1837)	Reference: Karaman, 1971; Marković, 2013
92	*X. monographus* (Fabricius, 1792)	Reference: Živojinović, 1954a; Maksimović, 1959, 1964; Marković, 2013
93	*X. pfeilii* (Ratzeburg, 1837)	Reference: Živojinović, 1950a
	Tribus Xyloterini	
94	*Trypodendron domesticus* (Linnaeus, 1758)	Reference: Mamontov, 1937; Tomić, 1954, 1957; Živojinović, 1954a; Marković, 2013
95	*T. lineatum* (Olivier, 1795)	New finding: Mt. Kopaonik: Karaman 30.5.2001. on *P. abies*, Hajdučka voda 30.5.2001. on *P. abies*. Reference: Živojinović, 1948, 1950b, 1954a, 1961; Janković, 1949; Tunguz, 1951; Tomić, 1954; Damnjanović, 1955; Maksimović & Barlov, 1961; Maksimović & Milanović, 1961; Stevanović, 1987; Adamović, 1990; Radonjić & Tomić, 1995; Marković & Stojanović, 2000a; Marković et al, 2004; Roganović, 2003; Marković, 2013
96	*T. signatum (Fabricius, 1787)*	Reference: Karaman, 1971; Marković & Stojanović, 2015

* The tribes, genera and species in the table are listed according to Alonso-Zarazaga (2013)

DISCUSSION

With around 150 species, Scolytinae do not represent a very large group of insects in Europe. As they are economically very important, they have been studied intensively in Serbia. In the list of species that Marković & Stojanović (1997a) made 25 years ago for Serbia, 85 species are listed. In this paper, 11 more species are mentioned. However, if these two lists are compared, it can be seen that the new list contains only 9 more species since two species from it were synonyms in the previous list. The species *C. cribellus* was listed in the previous list as a synonym of the species *C. pusillus* and the species *P. micrographus* as a synonym of the species *P. pityographus*. In the species list listed in this paper, these four species are separated.

Most of the species listed in this paper are widely distributed in Europe (Pfeffer, 1989). The findings of some species were not confirmed in the last 50 years or more in Serbia. In the case of species from the genus *Trypophloeus* Fairmaire, 1868, the reason for this is the difficulty in identification. They were found in several locations in Serbia, but due to that problem, their new findings are not listed in this paper. In the case of other species, the finding was not confirmed, probably because some of them are rare, some are less researched, and some may have been erroneously identified.

If the list of Scolytinae in Serbia is compared with similar lists that exist in other European countries (Nunberg, 1954; Pfeffer, 1989; Balaschowsky, 1949; Karaman, 1971; Mokrzycki et al, 2011), it is realistic to expect about 115 to 125 species of Scolytinae species in Serbia. Since 96 species have been found so far, more will certainly be found in the future. Most of the identified species were found in the area of western and central Serbia. The reason for this is that those areas are rich in coniferous forests, and most Scolytinae are associated with coniferous trees. The number of species found in other parts of Serbia is significantly lower. In Northern Serbia, a large number of species is not expected, since just a small percentage of its area is covered by forests. Eastern and southern Serbia is much more forested, so those parts of Serbia should be explored more in the future. The insect fauna of that part of Serbia is very rich (Živojinović, 1950a; Marković, 2022; Marković & Stojanović, 2019; Stojanović et al, 2018; Dobrosavljević et al, 2018a, 2018b) and as coniferous forests are present in some parts, new species of Scolytinae will most certainly be found there. It is likely that the invasive species *I. duplicatus* (Sahlberg, 1836), which is already present in neighboring Romania and Hungary (Wermelinger et al, 2020), will soon be found there, and throughout Serbia.

As an economically very important group of insects, Scolytinae are studied extensively on the Balkan Peninsula (Stanivuković & Vasiljević, 2018; Belilov et al, 2021; Milosavljević et al, 2021a, b, 2022; Georgieva et al, 2022). Unfortunately, the studies focused on their fauna in recent years are very few. Generally speaking, in this part of Europe, they have so far been investigated in detail only in Macedonia (Karaman, 1971). The list of species mentioned in this paper will help to complete the picture of their fauna on the Balkan Peninsula.

Taking into account all of the above, it can be concluded that with 96 species found so far, Serbia represents an area in which Scolytinae have not yet been sufficiently studied. To improve the knowledge of the Scolytinae fauna in the future, it is necessary to research the area of eastern and southern Serbia in more detail. Since the diversity of Scolytinae increases from the north to the south of Europe (Karaman, 1971), it is likely that at the time when these areas are researched, the total number of species in Serbia will be higher than the number of species identified in the countries of Central and Northern Europe (Nunberg, 1954; Pfeffer, 1989; Balaschowsky, 1949; Mokrzycki et al, 2011). This will show that the diversity of Scolytinae, as well as other xylophagous insects (Marković & Stojanović, 2011, 2012, 2019, 2020), is quite high in Serbia, even though it is a small country. The richness of its insect fauna can be seen in the research conducted by other authors (Živojinović, 1950; Petrović, 1998; Jerinić-Prodanović, 2010;

Ilić & Ćurčić, 2015; Dobrosavljević et al, 2017, 2023; Cebeci et al, 2018; Marković et al, 2018, 2021a, b; Jakšić, 2022).

ACKNOWLEDGMENTS

We would like to thank the conservator of the Museum of Natural History in Belgrade, Aleksandar Stojanović, for his help in collecting new data on the Scolytinae fauna of Serbia.

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J. Entomol. Res. Soc., 25(3): 563-578, 2023 *Research Article*
Doi: 10.51963/jers.v25i3.2455 *Online ISSN:2651-3579*

Effects of Environmental Variables and Role of Food Attractants for Management of *Bactrocera zonata* (Saunders, 1842) and *Bactrocera dorsalis* (Hendel, 1912) (Diptera: Tephritidae) in Mango Orchard

Muhammad SARWAR[1, 3a] Bilal RASOOL[2*] Syed Muhammad Masoom SHAH[3b]
Nazir AHMAD[3c]

[1]National Institute for Biotechnology and Genetic Engineering (NIBGE), Faisalabad 38000, Punjab, PAKISTAN

[2]Department of Zoology, Faculty of life sciences, Government College University, Faisalabad, (GCUF) 38000, Punjab, PAKISTAN

[3]Nuclear Institute of Agriculture (NIA), Tando Jam-70060, Sindh, PAKISTAN

e-mails: [1,3a]drmsarwar64@gmail.com, [2*]bilalrasool@gcuf.edu.pk, [3b]masummsha@gmail.com, [3c]nazahmad1957@gmail.com
ORCID IDs: [1,3a]0000-0001-6585-4072, [2]0000-0002-4465-1169, [3b]0000-0001-5680-5252, [3c]0000-0001-9340-0799
*Corresponding author

ABSTRACT

The exploitation of food attractants for tephritid fruit flies (Diptera: Tephritidae), is the key element widely used for pest management. Population dynamics and the relative attractiveness of five commercially available chemicals in different concentrations were studied for the suppression of both sexes of *Bactrocera* species in mango orchards. The fruit flies, *Bactrocera zonata* (Saunders) and *Bactrocera dorsalis* (Hendel) exhibited maximum reduced population intensity from August to February and an increased population from March to July. The peak population of adult flies emerged in June depending on the host fruit maturity and meteorological influences. Observable differences in attractiveness between the tested products were confirmed at the experimental site of host institute. Resultantly, the attractions of female and male fruit flies of both species in Ammonium acetate, Trimethyl amine and Putrecine mixture were significantly more efficient than the male populations. Both male and female sexes exhibited an enhanced response to Torula yeast and Boric acid with the rise in their concentrations. Expressively, higher flies were collected in a combination of Torula yeast and Boric acid with 7:3 ratio. Concludingly, both *Bactrocera* species constantly revealed a substantial positive response to the odor of proteinaceous food attractants for their management.

Keywords: Fruit flies, food sprays, baits, mango pests, insects control.

Sarwar, M., Rasool, B., Shah, S.M.M., & Ahmad, N. (2023). Effects of environmental variables and role of food attractants for management of *Bactrocera zonata* (Saunders, 1842) and *Bactrocera dorsalis* (Hendel, 1912) (Diptera: Tephritidae) in mango orchard. *Journal of the Entomological Research Society*, 25(3), 563-578.

Received: April 26 2023 *Accepted: September 18, 2023*

INTRODUCTION

Fruit flies (Diptera) belong to the maximum numbers of primarily vital pest species due to their direct economic impacts. The Dacini tribe, genus *Bactrocera* in the family Tephritidae, is of great significance (San Jose et al, 2018). Among the *Bactrocera* genus, *B. dorsalis* (Hendel), *B. correcta* (Bezzi), *B. zonata* (Saunders) and *B. cucurbitae* (Coquillett) are economically significant pests. The first three *Bactrocera* species mostly damage to the fruit crops, whereas *B. cucurbitae* damages diverse species of cucurbits (Verghese, Madhura, Jayanthi, & Stonehouse, 2004). The fruit flies, *B. zonata* and *B. dorsalis* have a wide range of geographical distribution and are deliberated important quarantine insect pests in various countries (Zeng et al, 2019; Zingore et al, 2020).

Mango (*Mangifera indica*), peach (*Prunus persica*), guava (*Psidium guajava*) and other fruit crops serve as hosts for various pests (Sarwar, 2006, 2023). Both studied flies seriously harm a variety of economically important fruit crops. Infected regions suffer high protection costs due to additional efforts and resources employed for eliminating the pest (Alzubaidy, 2000). The damage caused by *B. dorsalis* in mango is assessed from 40 to 90% depending on the varieties, geographical locations and the seasons (Vayssières et al, 2009; Nankinga et al, 2014; Badii et al, 2015). The average fruit loss due to tephritid populations in mango orchards varied from 12% at the beginning of April to 50% in June (Vayssieres, Goergen, Lokossou, & Akponon, 2005).

Pakistani mango and other horticultural crops are facing severe damage from both *B. zonata* and *B. dorsalis* as major fruit pests. Both pests have influential effects on local and export markets (Sarwar et al, 2013). *Bactrocera dorsalis* is one of the most damaging horticultural insects in the Asia-Pacific (Huang & Chi, 2014) and may harm up to 250 fruits and vegetables host species (Schutze et al, 2017). *Bactrocera zonata* is famous for damaging over 50 known fruit plants and vegetables (El-Akhdar & Afia, 2009). Fruit flies are a major threat to the fruits especially mango, peach, apricot, fig, apple, guava and vegetables including cucurbits, squash, tomato, capsicum, cucumber and eggplant are their favorite hosts. However, plants belonging to the family Cucurbitaceae are most preferred (El-Akhdar & Afia, 2009; Sarwar, 2014a; 2014b; 2016; 2020a). Therefore, a complementary study is necessary to confirm the prevalence of the genus *Bactrocera* and to conduct a better inventory of the various food attractants, and meteorological information.

Producers organize expensive commodity for pest protection arrangements to meet trade necessities at the global level; however, inevitable increases in chemical uses can adversely impact the environment (Shah, Ahmad, Sarwar, & Tofique, 2014; Sarwar, Ahmad, Rashid, & Shah, 2015). The prevailing fruit flies control strategies are exclusively depending on chemical control, which has serious persuasions and negative impacts on the ecosystem and populations of beneficial organisms. Therefore, eco-friendly pest control approaches remain needful over time (Roessler, 1989). Various chemicals have been empirically identified as attractive to females of certain *Bactrocera* species. Field studies conducted by Oliver et al, 2004, Ros et al, 2005 and Shivayya, Kumar, & Jayappa, 2008 on the management of *Bactrocera* flies using different food attractants were found to be promising in reducing the incidence of pest flies and showed very good results. Methyl eugenol, protein hydrolysate bait and malathion are often used to trap the male populations of both *B. dorsalis* and *B. zonata* in the field (Agarwal, Pramod, & Vinod, 1999). Management of *B. zonata* and *B. dorsalis* relies on the choice of appropriate attractants, which can be used in traps and spot treatments. The utilization of 'methyl eugenol' in traps for trapping tephritid fruit flies is quite popular in many localities and has been established to be effective both for surveillance and control (Sarwar, 2015a; 2015b). Even though methyl eugenol has considerably established better effectiveness in discovering and or management of the *Bactrocera* flies (Jang & Light, 1996), but its attraction is restricted merely to males control.

The significance of methyl eugenol as a male lure has accelerated positive determinations to advance female lures of comparable attraction. Female attractants for tephritid fruit flies are needed to complement currently used male attractants. These would help to eradicate the future progeny and improve surveillance programs (Jang, 1997). So, significant work on other food-type attractants, such as protein products and synthetic chemicals is needed to focus on the female flies in the field environment. An improved female trapping system would be important for finding early populations and then eradication plan (Miranda, Alonso, & Alemany, 2001). Recently various efforts were appreciated in improving trapping methods aimed to capture female populations. The combinations of food-based attractants have been emerged as synergistic (Heath et al, 1997). Recently, these female attractants have already been tried effectively in several countries (Epsky et al, 1999). Different researchers have been endeavoring to identify further attractive chemicals within certain compounds to enhance the development of such products (Sarwar, 2020b).

Therefore, the present research was commenced to evaluate different chemicals. The diverse concentrations and combinations were exploited to improve fruit flies attraction technique for the management of *Bactrocera* species in mango orchards.

MATERIALS AND METHODS

Study site

Research and experiments to study the fruit fly populations build-up to construct a link between trap catches and environmental variables (temperature, humidity rain and cloudiness) and comparative efficacy of food attractants on *Bactrocera* fruit flies infesting mango orchard were conducted at the Nuclear Institute of Agriculture, Tando Jam, Pakistan. For this study, the mango orchard of late variety, "Began Pali" was selected, wherein the study site is an important area of exportable horticultural products.

Population dynamics of *B. zonata* and *B. dorsalis*

Fluctuations in tephritid populations in mango orchards and the levels of mango fruit fly infestations due to tephritid species were studied. Fruit flies were captured in orchards using para pheromone traps baited with methyl eugenol (85%), sugar solution (10%) and Endosulfan (5%) retained on cotton wool sticks placed in Steiner plastic trap with small openings at either end to facilitate the access of flies laid out @ 5 traps per acre. The fruit specimens were collected from the ground or randomly detached from the trees. These treatments were replicated five times. Traps were commonly retained in fruit trees at about 2 m above ground level in mango orchards during flowering up to fruit harvesting season and emptied regularly at weekly intervals. Traps were placed in mango branches at the lower third of the foliage to avoid long exposure to the sun rays. Traps were checked once a week and trapped flies were counted. Insecticide, lure and cotton wool were changed and filled at monthly intervals. Once a week, the infested fruits were collected and retained within plastic containers in the laboratory under standardized laboratory conditions of 25 ^{O}C and 65% RH until the full decay of the host fruits. Different life stages including larvae and pupae of fruit flies were observed once a week and collected in separate vials/ petri dishes until the complete emergence of adult flies.

To characterize fruit fly species involved in mango infestations, pest species that emerged as adults from fruits were identified by following Vreysen et al, (2007). Meteorological observations during the study periods were obtained from Regional Agro Met Center, Tandojam.

Evaluation of different chemicals to attract the female or male of *B. zonata* and *B. dorsalis*

The aim of this field experiment was to develop improved attractants for attracting females of *B. zonata* and *B. dorsalis* in commercial mango orchards. A bait attractant trial was conducted at the research station in the months of March to July. This study compared different products from different groups such as Torula Yeast plus Boric Acid in different formulations (1:1, 2:3, 3:2, 3:7 & 7:3), as well as Ammonium Acetate, Trimethyl amine plus Putrescine and Ammonium Acetate, Trimethyl amine plus Boric acid in similar formulations (1:1:1). The formulation of every treatment was organized by liquefying the respective product of each mixture in 200 ml of water.

The chemicals were tested separately to catch adult female or male populations placed inside Plastic Cylindrical Traps having a 2.5-liter capacity. The mixture of 200 ml of 10 percent attractant was utilized for baiting purposes in each trap. The traps were placed in mango plants at a height of 2.0 m from the ground level. The trapping system comprised the installation of approximately 5 traps per acre in each replicate. The traps were re-baited with the lure at one weak interval. The installation scheme of traps carried out was identical for all treatments. Traps were checked twice a week, trapped flies collected in tubes using flexible grips and the flies caught, counted, sexed and recorded. All traps were cleaned at one-week intervals and changed after two-month intervals. These treatments were replicated five times. Comparative data on the effectiveness of synthetic lures among different treatments were evaluated by comparing the detection, monitoring and counting of the captured flies.

STATISTICAL ANALYSIS

Data generated from these studies were analyzed using the computer package Statistix 8.1 software, and Analysis of Variance (ANOVA) was performed at $p < 0.05$. Further, for the fruit flies, correlation between environmental factors and population abundance were calculated through multiple linear regression and Pearson correlation coefficient (Sarwar & Rasool 2022a; 2022 b; Schober, Boer, & Schwarte, 2018).

RESULTS

From the initiation of experiments to onward, *B. zonata* and *B. dorsalis* were found the only the most abundant and frequent fruit fly species in mango orchards. During the rainy season (abiotic factor) and in accordance with the phenological maturing stage of the mango fruit (biotic factor), these were the most prevalent pests.

Population dynamics and environmental variables of *B. zonata* and *B. dorsalis*

The population dynamics of fruit flies followed the tendency of mango fruit's development from formation towards the maturity stages and occurrence of prevailing climatic factors. Samples of infested fruits and fly trap catches in mango habitats taken from treated and untreated areas during each week, investigated that both *B. zonata* and *B. dorsalis* were the most prevalent species during field testing. Following the figure 1 trend, it was noted that during the trapping periods in the mango habitat, flytrap catches were not regular (Fig. 1). However, data coincided with the time of fruiting of its major mango host and meteorological factors (Fig. 2).

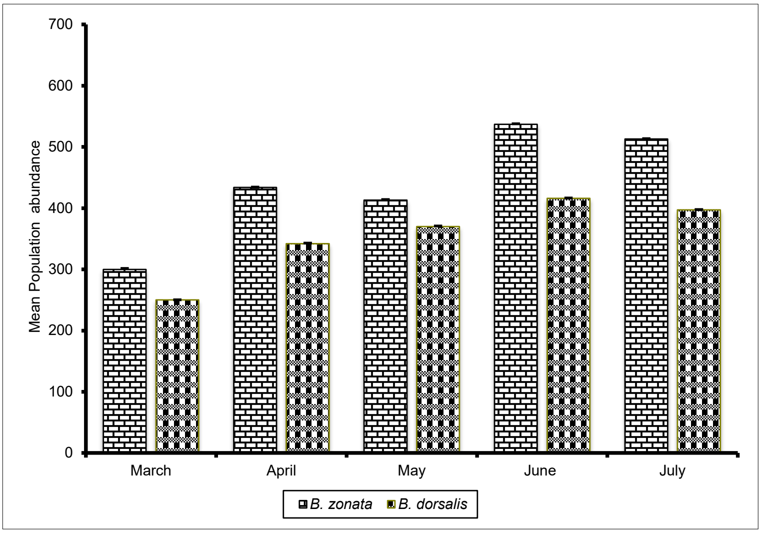

Figure 1. Mean populations of *B. zonata* and *B. dorsalis* during the study months.

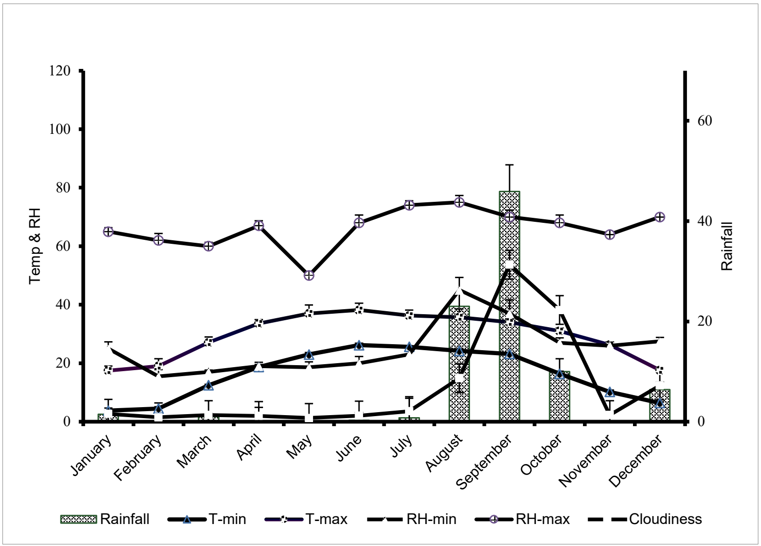

Figure 2. Mean meteorological observations noted at the Regional Agro Met Center, Tandojam, during the year (2018-2020), Temperature (°C), Relative Humidity (%), Rainfall (millimeter) and cloudiness (octas).

The population build up in traps was started from March. It was fair during April and May and attained the peak during June and July. The population build-up in traps was observed during March, while April and May had a fair number of catches, but June and July revealed the peak populations density. The environmental factors (rainfall, temperature, relative humidity and cloudiness) seemed to have effects on the populations buildup of both *B. zonata* and *B. dorsalis*. At the start of the rainy season, it was observed that the populations of *B. zonata* and *B. dorsalis* were enhanced by the rain (0.03-0.8 mm), temperature (minimum 26.2-25.6 °C, maximum 38.2-36.3 °C), relative humidity (68-74%) and cloudiness (2.1-3.6 octas) in June and July, respectively. There was a positive link of populations of fruit flies with maximum rain, temperature, relative humidity and cloudiness. This jump over was attenuated by the important rainfall recorded in June and July making it possible to reach the peak population intensity. Environmental variables (temperature, humidity, rainfall and cloudiness) during the research period and arena are expressed in Figure 2. Monthly average minimum and maximum temperatures were expressed in the range of (3.8 and 26.2 °C) and (17.5 and 38.2 °C), respectively. Minimum relative humidity ranged from 15.5 to 45.0% and the maximum from 50.0 to 75.0%. The mean higher rainfall was observed in September (45.9 mm) followed by August (23.0 mm) and December (6.4 mm). High populations of fruit flies were observed during the warmer months and lower density in cooler months. The Pearson correlation coefficient and multiple linear regression analysis exhibited a significant correlation between density abundance and mean monthly temperature, humidity and rainfall (Table 1). A weak negative correlation was observed in *B. zonata* (- 0.118), and a moderate negative correlation with *B.*

dorsalis (- 0.450) for the overall mean monthly maximum temperature. Further, the overall mean monthly minimum temperature presented a weak negative correlation in *B. zonata* (- 0.032) and *B. dorsalis* (- 0.270). The moderate positive correlation in *B. zonata* (0.425), and *B. dorsalis* (0.544) was calculated for monthly average maximum relative humidity. The monthly average minimum relative humidity exhibited a weak correlation in *B. zonata* (0.102) and *B. dorsalis* (0.217). A significant strong positive correlation was recorded between rainfall values in *B. zonata* (0.720) and moderate in *B. dorsalis* (0.677). The weak positive correlation was recorded between cloudiness values in *B. zonata* (0.167) and in *B. dorsalis* (0.225). The populations of *B. zonata* and *B. dorsalis* showed a negative link with sunshine hours, wind speed and wind direction (wind-blown from south to west direction). In orchard habitations, populations reduced towards the completion of fruiting seasons, but the opposite trend of low population captures was observed during cooler months, flowering, fruit setting and after fruit harvesting phases. Thus, the reductions in populations were resulted between August to February. Fruit sampling results throughout the experiment indicated that in the control orchard, both males and females were found from the first week of samplings.

Table 1. Fruit flies abundance and monthly average environmental variables (temperature, relative humidity, rainfall and cloudiness) exhibited through Pearson coefficient correlation (r-values) and multiple linear regressions (p-values) from January 2018 to December 2020.

Environmental variables	Fruit flies			
	B. zonata		*B. dorsalis*	
	r-value	p-value	r-value	p-value
Maximum Temperature (◦C)	− 0.118	<0.000	− 0.450	<0.030
Minimum Temperature (◦C)	− 0.032	<0.000	− 0.270	<0.025
Maximum Relative Humidity (%)	0.425	<0.023	0.544	<0.010
Minimum Relative Humidity (%)	0.102	<0.020	0.217	<0.012
Rainfall (mm)	0.720	<0.020	0.677	<0.032
Cloudiness (octas)	0.167	<0.019	0.225	<0.017

r = 1.0-0.9 (very strong correlation), r = 0.89-0.7 (strong correlation), r = 0.69-0.4 (moderate correlation), r = 0.39-0.1 (weak correlation), p < 0.05 (significant).

Evaluation of different chemicals to attract the female or male of *B. zonata* and *B. dorsalis*

The attraction of female fruit flies to all the tested chemicals was considerably more than the males. Three mixtures (ammonium acetate, trimethyl amine and putrescine) exhibited significantly the highest attraction as compared to the other compounds in both *B. zonata* and *B. dorsalis* for either male or female sexes. Torula yeast and Boric acid improved the attraction gradually with the increase in concentrations in response to both sexes. A comparatively higher number of flies were significantly collected from torula yeast and Boric acid combination with the ratio of 7:3. The results further revealed that both *B. zonata* and *B. dorsalis* constantly showed a substantial positive response to the odor of proteinaceous food attractants (Tables 2- 3). Therefore, efforts will be continued to further standardize the female attracting system for *B. zonata* and *B. dorsalis*. Clearly, Ammonium acetate, trimethyl amine and putrescine showed a better potential for attracting the females of *B. zonata* and *B. dorsalis*. Hence, these three chemicals are needed to be evaluated in still different concentrations to standardize the attracting system for both sexes of fruit flies. Experimental results indicated that all female food attractant lures were highly choosy for females, which represented an average of higher number than male captures, thus more females attracted than the number of males caught by the chemical attractants.

Table 2. Evaluation of different chemicals as female attracting system for *B. zonata.*

Treatment	Catches of fruit flies per trap per week during different months					
	May		June		July	
	Female	Male	Female	Male	Female	Male
Torula yeast + Boric acid (7:3)	15.5 b	13.0 b	21.0 b	16.5 b	25.2 b	22.7 b
Torula yeast + Boric acid (3:2)	10.7 cd	9.5 c	12.5 c	13.7 bc	15.5 c	14.5 c
Torula yeast + Boric acid (1:1)	10.2 cd	7.5 cd	13.0 c	13.7 bc	14.7 c	15.2 c
Torula yeast + Boric acid (2:3)	11.7 c	7.2 d	12.7 c	13.5 bc	15.7 c	16.0 c
Torula yeast + Boric acid (3:7)	9.2 d e	7.7 cd	12.7 c	14.2 bc	14.2 c	14.2 c
Ammonium acetate + Trimethyl amine + Putrecine (1:1:1)	25.0 a	15.7 a	45.2 a	22.0 a	65.2 a	38.7 a
Torula yeast + Ammonium acetate + Boric acid (1:1:1)	8.7 cd	6.7 d	10.7 c	13.2 c	13.7 c	14.2 c

Means followed by different letters within treatments are significantly different at $p < 0.05$.

Table 3. Evaluation of different chemicals as female attracting system for *B. dorsalis.*

Treatment	Catches of fruit flies per trap per week during different months					
	May		June		July	
	Female	Male	Female	Male	Female	Male
Torula yeast + Boric acid (7:3)	13.5 c	11.0 c	19.0 b	14.5 c	23.0 b	20.2 b
Torula yeast + Boric acid (3:2)	7.7 cd	8.5 cd	11.5 c	10.3 c	13.2 c	12.1 c
Torula yeast + Boric acid (1:1)	8.2 cd	7.0 cd	12.5 c	11.5 c	12.4 c	13.0 c
Torula yeast + Boric acid (2:3)	10.2 c	5.2 d	9.7 c	10.2 c	12.2 c	12.0 c
Torula yeast + Boric acid (3:7)	7.2 cd	6.7 cd	10.2 c	11.2 c	11.0 c	12.0 c
Ammonium acetate + Trimethyl amine + Putrecine (1:1:1)	21.0 b	14.7 a	40.2 a	20.0 b	57.2 a	32.5 a
Torula yeast + Ammonium acetate + Boric acid (1:1:1)	8.2 cd	6.0 d	8.5 cd	11.2 c	13.0 c	11.7 c

Means followed by different letters within treatments are significantly different at $p < 0.05$.

DISCUSSION

The results presented on the population dynamics and fruit fly control experiments were convincingly successful and significant. The results indicated that a low pest population level was detected in early fruiting (March, April and May), whereas there was a higher density near to fruit ripening stage (June and July). A similar trend was obtained for female and male captures in all treatments. Such dissimilar patterns of pests population were certainly due to the phenological stage of the mango fruit and environmental deviations from month to month. This discrepancy of trap catches trends exhibited that during the experiment, odor emission from the traps and lures were not uniform as induced by the variable climatic variations of temperature and precipitation, which encouraged or discouraged flies activity.

Previous research has also reported a significant role of environmental features in the catches of *Bactrocera* fly species. Miranda et al, (2001) explained that rain and temperature are important environmental variables that could have shown important raise or fall in trap captures. The temperature and humidity levels had direct and indirect effects on species demography, availability of host plants and the existence of natural enemies (Vayssieres, 2004). Ndiaye, Elhadji, & Gilles (2008) mentioned that the development of fly populations had been related to rainfall. The current findings may be due to the effects of the cooler months because at that period flies were at very low-intensity levels, therefore incapable to be identified at the ratio of attractants utilized in experiments. So, the opinion may be extracted that the development of eggs present in fruits was delayed during the lower winter temperatures. Nevertheless, the sensitivity of attractants to discover the pest in low population levels and unfavorable environmental variables need time. This is for the reason that it is a vital aspect of early population management approaches. Hence, trapping methodologies as necessary elements of monitoring, surveillance and control may deliver information on existing species and seasonal distribution, and helpful to build an arena with freedom from fruit fly species. The population build-up until May was inadequate, which might be due to the limitation of environmental variables and or very low survived overwintering adult populations during cooler months. Fruit infestation rate was identified in tree fruits and fallen ground fruits. The early fruit infestation resulted from the least adult appearance, whereas late infestation had high captures in traps because the fruit was then available or mainly due to optimum temperatures. A small peak between March and April was observed, which was most probably due to the early mango fruiting period. This peak was commonly exaggerated between May and June when the investigational arena practiced one of the best mangoes spells.

The present research displays a tendency for greater fruit fly density at the end of the mango season. This kind of build-up of fruit fly populations may be due to the continuous accessibility of enormous attractive fruit. Thus, host fruit availability is another important contributor to the seasonal abundance of fruit flies, which is in agreement with other workers such as Drew, Zalucki, & Hooper (1984). According to Mahmood & Mishkatullah (2007), the availability of host fruits was an essential factor affecting population fluctuations. In agreement with this, other workers Gupta & Bhatia (2000), discovered the maximum fly catch corresponded with the maturity period of fruits in a mango plantation. The mango plants are damaged in Central Punjab in July-August and 35% of the fruiting bodies were spoiled by *B. dorsalis* and *B. zonata* (Mohyuddin & Mahmood, 1993). Fifteen species of *Bactrocera* fruit flies were captured in total during the survey and the populations were not of high density every year, and few fruit flies were captured from September to March (Kawashita, Rajapakse, & Tsuruta, 2004). The population dynamics of three important *Bactrocera* species (*zonata, cucurbitae* and *dorsalis*) exhibited a low density from November to February and higher from March to August. The highest population was observed in July and August, and the maximum reduction perceived in October depending on the host fruit maturity and environmental variables (Mahmood & Mishkatullah, 2007). Consequently, the availability of host fruits and meteorological parameters are essential factors affecting fruit flies population fluctuations.

Of all attractants tried for the attraction of both sexes of fruit flies, Ammonium acetate, Trimethyl amine and Putrecine composite appeared to be the most effective, while Torula yeast and Boric acid at an increased concentration showed promising results. Torula yeast, Ammonium acetate and Boric acid compound were reasonably effective, but their efficacy still needs to be improved further. In concurrence with this, a number of scientific publications dealing with technology for controlling of tephritid pests had stated the successful implication of attractants for the attraction of female and male fruit flies. Earlier research revealed that the mixture of molasses with ethyl acetate and ethyl butyrate at the ratio of 5:5 had 54.7% higher attraction effects on flies. Furthermore, various mix ratios with molasses displayed a high attraction of 60.5% of female populations (Liu & Hwang, 2000). During the research when *Bactrocera* fruit flies were fed with an attractant-bait mixture comprising boric acid-borax (3:1) and protein hydrolysate (4%), resulted in 40-98.3% population reduction with diverse (1-12%) formulations (Sunandita & Gupta, 2001). Duyck, Quilici, Fabre, & Ryckewaert (2004) studied the relative attractiveness of six commercially available protein hydrolysates and the influences of their concentrations on adult *Bactrocera* flies. Clear differences in attractiveness between the tested products were demonstrated, within the range of 0.5-10%, and a general tendency for an increase in effectiveness with increasing concentration was shown. Alkalinization of the bait solution appeared to increase the attractiveness to the flies. Rousse, Duyck, Quilici, & Ryckewaert (2004) evaluated the relative attractiveness of yeasts to the *Bactrocera* flies. The addition of higher rates of acid or alkali decreased attractiveness. Olivero, Garcia, Wong, & Ros (2004) proved that plastic McPhail and the Tephri-Trap with the Nu Lure hydrolyzed protein were the best treatments for capturing of female *Bactrocera* individuals. Saafan (2005) conducted experiments to evaluate the efficiency of some different attractants for adults of the peach fruit fly *B. zonata*, in mango orchards. All attractants attracted peach fruit flies, but Diammonium phosphate 2% was the most effective in attracting of adults, followed by Diammonium phosphate 3%. Lu et al, (2006) reported that whenever the sex attractant was offered in the trapping pots with 1/3 or 1/4 each time in a surveillance arena, it could calculate precise male population density records.

Because of the noticeable variance in the male population densities between diverse positions inside and outside plants, the surveillance arena should include both the plants and the adjacent places. The findings also discovered that annual population dynamics records of the male could be found by surveillance of huge and varied orchard arenas. Countering to this, disproportionate observations were drawn by Khattak, Shahzad, & Ghulam (2006), who evaluated the repellent and growth-inhibiting effects of certain extracts on the settling and growth of fruit flies. The adults emergence was considerably less in all the extracts as compared to the control. In the current field studies, findings were able to demonstrate that relative trapping efficiency was variable among the different attractants of the *B. zonata* and *B. dorsalis* females. The average number of female fruit flies trapped during the investigation time in all the tested chemicals was considerably more than the males. In this sense, these results are similar to previous reports of female fruit fly attraction to certain chemicals.

According to Jang & Light (1996), and Cornelius, Duan, Messing, & Cornelius (2000), the food-type attractants, such as hydrolyzed protein products and synthetic chemical blends were reasonably attractive to both males and females of many tephritid species. Siderhurst & Jang (2006) reported a similar attraction of both male and female fruit flies due to certain extracts. An uncommon non-target host plant and ovipositional attractants for females have also been described in previous studies as well (Jang & Light, 1996). The current findings have practical implications in fly trapping techniques suggesting that female attraction sensitivity could be improved with a further detailed study. Moreover, the present results would be suitable for observing females at high or lower pest density levels. Investigational results further pointed out that responses of females and males to Torula yeast and Boric acid were improved and considerably higher flies have been trapped in Torula Yeast and Boric acid treatment, though all the treatments captured more females compared to males. Additionally, all attractants tried showed negligible or less non-targeted insect detection, which is an important aspect that should be kept in mind, while evaluating the lure's consequence. This may propose that these chemicals determine a different odor emission rate, and thus may play an important role in insect attractiveness and detection.

In the present work, Torula yeast and Boric acid treatments increased the number of female captures, so, all female attractant treatments were more selective for females, which represented an average of about 25% higher number of female captures, thus many times more female trapped than the number of males captured by the female attractants. Furthermore, the present results may suggest that there were also discerning effects of attractants to attract females, which could be an important way of improving sterility assessment. Furthermore, the present results among synthetic lures proved more effective to attract females than males *B. zonata* and *B. dorsalis,* consistently exhibiting a significant positive response of females to the odor of proteinaceous food attractants. In concord with this, other research had led to the confidence that males and females have some basically diverse behaviors, which are a result of physiological alterations in insects. Females have a more multifarious attributable repertoire, which comprises the need for food required in nutrition and ovarian growth, mating, oviposition, host finding and egg-laying (Sarwar, 2015c; 2020b). Whereas, male flies do not have to betroth, oviposition and egg-laying attributes (Jang, 1997). Both sexes require protein for their normal growth and development. However, females are fascinated more by protein bait sprays in greater numbers than males due to their need for protein to develop their eggs and oviposition (Vickers, 1997). In addition, the collection for large numbers of specimens of major flies pest species using traps baited with male cue lure and methyl eugenol, provided valuable data and evidenced that these lure traps will become important tools for both fruit fly pests monitoring and field pests management. These traps also attracted large numbers of mature female fruit flies, thus adding value to this new technology (Maula et al, 2023). Additionally, the life cycle study of fruit fly revealed that when their maggots emerge inside the fruit and change to an adult form, they must feed regularly on carbohydrates and water. In contrast, female flies require proteinaceous base food for the development of their sexual organs and survival (Khan, Hussain, & Jehangir, 2023). These facts insight into how female behavior might be measured in these species and other economically important tephritid fruit flies, which may be key directions toward the development of female lures. And such approaches should be one of the primary deliberations in search of better and qualitative female attractants. Further attempts to improve and identify the chemicals and novel compounds responsible for the attractiveness of both fruit flies sexes should be continued.

CONCLUSIONS

Qualitative and efficient female attractants will advance the surveillance, monitoring and management of fruit flies, and supplement existing male lures. On the basis of the present findings all food attractants applied in diverse formulated combinations were efficient in fruit flies management. Pests destruction intensities were carried to large using the formulation of Ammonium acetate, Trimethyl amine and Putrecine compounds where the attraction of female fruit flies was significantly more than the males for the period of the trials. This may develop qualitative consideration of attractants to identify the pest in low density, which is the mainly significant trait of initial population control approaches. The anticipatory positive results accomplished with these attractants can be replicated using the new formulations and their regular applications are essential. It is expected that long shelf life preparations of these products may establish both spray and bait appropriate for use in traps. The findings of the present investigation revealed that different environmental factors have striking effects on the development of *B. zonata* and *B. dorsalis*. Environmental variables are substantial factors affecting the overall fruit flies abundance in the research arena. The present achievement of this endeavor and the solicitation of similar tools in other arenas will consequently be dependent on economics, awareness of the farmers and appreciation of product value for efficient fruit fly management.

ACKNOWLEDGMENTS

The authors acknowledge the staff members for sampling and data collection

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J. Entomol. Res. Soc., 25(3): 579-599, 2023
Doi: 10.51963/jers.v25i3.2472
Research Article
Online ISSN:2651-3579

Studies on Effect of Gamma Radiation on Biological Parameters and Male Sterility in Parental and F1 Generation of *Spodoptera frugiperda* (J.E. Smith)

Mohammad RAFI[1a] Gonchikara Siddanaa PANDURANGA[1*]

Tamminana RAJASEKHARAM[2] Karnam V. HARI PRASAD[1b]

[1]Department of Entomology, S. V. Agricultural College, ANGRAU, Tirupati - 517 502, INDIA
[2]Department of Pathology, Citrus Research Station, YSR Horticultural University, Tirupati - 517 502, INDIA

e-mails: [1a]rafimohammad.2314@gmail.com, [1*]gs.panduranga@angrau.ac.in, [2]trajasekharam@gmail.com, [1b]kv.hariprasad@angrau.ac.in

ORCID IDs: [1]0009-0008-8260-6765, [1*]0000-0001-5713-2010, [2]0009-0007-6165-1797, [1b]0000-0002-9680-0736

ABSTRACT

The male pupae (7 days old) of fall armyworm, *Spodoptera frugiperda* were exposed to eight different doses of gamma radiation (25, 50, 75, 100, 125, 150, 175 and 200 Gy) using Co-60 source. Among the doses tested, a radiation dose of 100 Gy had induced >80.00% of sterility with least negative effects on adult emergence (71.00%), deformation (9.00%), adult longevity (6 days) and survival under food stress (59.00%) in parental generation. In F1 generation, larval duration was 23.67 days at 100 Gy compared to 20.33 days at unirradiated control. More than 50% of pupae (51.33%) were recovered with pupal weight of 1.51 g/10 pupae at 100 Gy. Emergence of F1 adult at 100 Gy was 66.23% with least percentage of deformation (12.60%) and F1 adults lived up to 5.55 days with 52.00% of survival under food stress. Biological parameters of F1 generation were severely affected at radiation doses ranging from 125 to 200 Gy. Percentage of male sterility was increased with increase in radiation doses. A radiation dose, 100 Gy has induced male sterility of 81.89% and 86.23% in parental and F1 generation with no deleterious effects on adult emergence, longevity, survival and other quality parameters of *Spodoptera frugiperda*.

Key words: Fall armyworm, gamma radiation, parental generation, F1 generation, biological parameter, male sterility.

Rafi, M., Panduranga, G.S., Rajasekharam, T., & Hari Prasad, K V. (2023). Studies on Effect of Gamma Radiation on Biological Parameters and Male Sterility in Parental and F1 Generation of *Spodoptera frugiperda* (J.E. Smith). *Journal of the Entomological Research Society*, 25(3), 579-599.

Received: May 24 2023 *Accepted: October 02, 2023*

INTRODUCTION

Maize *(Zea mays L.)* is the third most important cereal crop next to rice and wheat sharing about 2% of world's maize production. In India, the area under cultivation of maize is 9.9 million ha with an annual production of 23.10 million tonnes (Anonymous, 2022-23) contributing nearly 10% to the national food basket. Maize is used as a basic raw material in thousands of industrial products including starch, oil, protein, food sweeteners, cosmetics and pharmaceuticals.

Insect pests *viz.,* shoot fly, *Antherigona soccata* Rondani; corn aphid, *Rhopalosiphum maidis* (Fitch); stem borer, *Chilo partellus* (Swinhoe) and corn earworm, *Helicoverpa zea* (Boddie) are the most common pests in maize ecosystem. Recently In India, Fall armyworm (FAW), *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) was first reported on maize in the Shivamogga district of Karnataka during May-June, 2018 by Sharanabasappa et al (2018) and most devastating invasive pest has become a major threat to maize cultivation in India (Suby et al, 2020).

Fall armyworm (FAW) is a serious pest of maize native to tropical and subtropical regions of America. It is also known to feed on more than 350 plant species belonging to cereals, millets, cotton and vegetables (Wan et al, 2021). Later in the year 2019, FAW was reported in Andhra Pradesh, Telangana, Maharastra, Tamil Nadu, Bihar, Chhattisgarh, Gujarat, Odisha, West Bengal and Madhya Pradesh (Vishwakarma, Pragya, Patidar, Das, & Nema, 2020). Its invasiveness is due to its wide host range, high dispersal ability, no diapause and high fecundity (Montezano et al, 2018). Warm and humid growing seasons with heavy rainfall favour its survival, population build up and insects cannot develop at temperature below 10°c (Stokstad, 2017). The female moth lays more than 1000 eggs in single or multiple clusters under the surface of leaf or on the stem. The eggs of *S. frugiperda* are creamy white and egg mass covered with an anal tuft of hairs. Sometimes female adult lays egg mass without hair covers. There are six instars with duration of 15 -19 days and extends up to 30 days during winter. The presence of white inverted "Y" shaped marking on the head region of mature larvae and the presence of four black dots in a square shape on the last abdominal segment are the distinctive features of FAW (Prasanna, Huesing, Eddy, & Peschke, 2018, Sharanabasappa et al, 2018b).

Fall armyworm attacks maize plants at all phases of crop development, but it is most common during the whorl stage, which lasts up to 45 days after sowing. Early instars feed by scraping and skeletonising the upper epidermis of leaves resulting in short pin holes (window pane) on leaves. The damage by the late instars (4^{th} instar onwards) results in extensive defoliation of leaves and the presence of faecal pellets in whorls. Due to this pest, maize production in India reduced by 5-10% equivalent to 0.04 to 0.075million tonnes (Suby et al, 2020).

Farmers and commercial growers depend predominately on the use of synthetic insecticides for controlling this insect pest. The use of insecticides as a sole tool in the management of insect pests has potential drawbacks such as the development of insecticide resistance, persistence of pesticide residues on crop produce, outbreak of secondary insect pests and pest resurgence (Togbe, Zannou, Gbehounou, Kossou & Van Huis, 2014). Hence, it is necessary to explore other alternative methods that are ecologically safe.

SIT (Sterile Insect Technique) is a species specific, non-polluting and environmental friendly method of insect control that relies on mass production and sterilization of target insects by radiation and systematic release of sterile males into the target environment to induce sterility in a wild population (Knipling, 1979). It is a method of pest control that uses radiation to generate mutations or chromosomal abnormalities in germ cells, resulting in reproductively sterile adult insects (Reichard, 2002).This technique is successfully used against several insect pests including Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) in Chile and Peru regions during 1995; Melon fly, *Bactrocera cucurbitae* (Coquilett) in Japan during 1993; Mexican fruit fly, *Anastrepha ludens* (Loew) in southern California and northern Mexico during 1964; Pink bollworm,*Pectinophora gossypiella* (Saunders) in south western USA and northwestern Mexico; Codling moth,*Cydia pomonella* (Linnaeus) in Columbia and Tsetse fly,*Glossina austeni* Newstead in Zanzibar during 1998 (Hendrichs,Vreysen, Enkerlin, & Cayol, 2005).

Determination of the optimum dose of gamma radiation for male sterilization without compromising the quality of adults in terms of adult emergence, adult longevity and survival of sterile males is crucial in the successful application of SIT. Because, high doses of radiation increase the sterility but decrease the mating competitiveness of sterile males. Whereas lower doses may not induce enough sterility (Calkins and Parker, 2005; Collins, Weldon, Banos, & Taylor, 2008). To induce the desired level of sterility with the least negative impacts on sexual performance of sterile insects, the radiation dose must be optimized. An optimum dose of gamma radiation should not interfere with the ability of sterile males to compete with wild males for their wild female mates in the released Environment (Dyck, Hendrichs, & Robinson, 2005). Hence the quality of sterile males must be assessed by studying the effects of gamma radiation on quality parameters *viz.,* adult emergence, adult longevity and survival under food stress which primarily reflects the male’s ability to survive, interact with its environment, locate, mate and fertilize females of target wild populations (Collins, Weldon, Banos, & Taylor, 2009).

MATERIALS AND METHODS

The studies on the effect of different doses of gamma radiation on biological parameters of Fall armyworm, *Spodoptera frugiperda* were conducted during 2021-22 at Insectary, Department of Entomology, Sri Venkateswara Agricultural College, Acharya N.G. Ranga Agricultural University, Andhra Pradesh, India.

Maintenance of Fall armyworm culture

The initial culture of FAW was obtained by collecting egg masses and early instar larvae from farmer’s fields and the College farm of S.V. Agricultural College, Tirupati, Andhra Pradesh. Hatched neonates and early instars of FAW were inoculated on artificial diet (Barreto, Loguercio, Valicente & Paiva, 1999) and accessed to feed on artificial diet up to second instar stage. The third instar larvae were reared individually in a separate plastic cell of diet tray to avoid cannibalism. Fresh diet was provided as and when required for the developing caterpillars of FAW till pupation. Pupae were collected and transferred to adult rearing cages (30×30×30 cm) for adult emergence. Male and female adult moths were collected and confined to cages (30×30×30 cm) provided with 10% honey solution (cotton swabs) as an adult diet and water-soaked cotton swabs in 100 ml conical flasks as a source of water. Potted corn plants of 4-5 leaf stage were provided as oviposition substrate for collection of egg mass. Egg masses were collected and placed on clean and disinfected corn leaves for hatching. Hatched neonates were transferred to artificial diet.

Exposure of pupae to gamma radiation

Pupae required for irradiation were obtained by rearing the larvae on artificial diet. Male and female pupae were differentiated by observing the distance between the genital opening and anal slit (Figure 1. a, b). The distance between genital opening and anal slit is more in females. The male pupae of 7-days old were taken in plastic petri plates provided with blotting paper at the base and the inner sides were lined with non-absorbent cotton to avoid damage to the pupae. Petri plates containing male pupae (50 pupae for each replication and each treatment replicated thrice) were exposed to gamma radiation (Cobalt-60 source) at 25, 50, 75, 100, 125, 150, 175 and 200 Gy using a gamma chamber (Table 1) at IIHR, Bengaluru, Karnataka, India (Figure 1. c, d).

Table 1. Specifications of gamma chamber used for irradiation of fall armyworm (*Spodoptera frugiperda*) pupae.

S. No.	Specifications	Capacity
1	Model	GC-5000, BRIT and AERB
2	Maximum Co-60 source capacity	518 TBq (14000 ci)
3	Discharge rate	9 KGy/hr
4	Irradiation volume	5000cc
5	Size of sample chamber	17.2cm (dia) 20.5cm(ht)
6	Shielding material	Lead and stainless steel
7	Weight of the unit	5600kg
8	Size of the unit	17.2cm(dia) 106.5cm(w) 150cm(ht)
9	Timer range	6 seconds onwards

Figure 1. a) Female pupa, b) Male pupa, c) Pupae sampled for irradiation, d) Gamma chamber used for irradiation (as. anal slit, gp. genital pore)

Recording of quality parameters of parental generation

Male pupae after exposure to different doses of gamma radiation were brought to the Insectary and confined to adult rearing cages. The quality parameter tests viz., adult emergence (%), deformation (%), survival under food stress (%) and adult longevity (days) and of parental generation were recorded.

Adult emergence (%): Fifty irradiated male pupae from each dose were taken in a glass Petri dish and kept in an adult emergence cage provided with 10% honey solution. After the complete ceasing of adult emergence (up to 5 days from the day of emergence), the number of adults emerged out of 50 pupae were worked out and expressed in percentage. Fifty unirradiated male pupae were also maintained as control and a similar procedure was followed.

$$\text{Adult emergence (\%)} \frac{\text{Number of adults emerged}}{\text{Total number of pupae kept for adult emergence}} \text{X100}$$

Deformation (%): After the complete ceasing of adult emergence, the number of deformed pupae, partially emerged adults and deformed moths with defective wings and other appendages were recorded and expressed as per cent deformation.

Adult longevity of irradiated males (Days): It was conducted by confining freshly emerged moths to adult rearing cages provided with an adult diet. Cages were checked for adult mortality and the average longevity of adult moths was worked out and expressed in days.

Survival under food stress (%): This experiment was conducted by confining 10 freshly emerged adult male moths from irradiated pupae into separate adult rearing cages without adult diet and water. The number of moths that were survived after 48 hrs of commencement of test was recorded and expressed as per cent survival under food stress.

Recording of quality parameters of F1 generation

Freshly emerged irradiated males (IM) are confined with unirradiated females (UF) in 1:1 ratio (10UF×10IM) into cages (30×30×30 cm) provided with 10% honey solution soaked cotton swabs. After 2 days, potted corn plants (4-5 leaf stage) were provided for oviposition. Egg masses from each cross were collected daily and hatched neonates were inoculated onto the artificial diet. Quality parameters of F1 generation viz., larval duration (days), pupal recovery (%), pupal weight (gm/10 pupae), adult emergence (%), sex ratio, adult longevity (days) and survival under food stress (%) of F1 generation were recorded as follows;

Larval duration (Days): Eggs laid by females that were mated with irradiated males were allowed for hatching. After hatching the neonates were inoculated on larval artificial diet. Larval duration represents the period between egg hatching and initiation of pupation.

Pupal recovery (%): Pupal recovery was calculated by dividing the number of pupae produced by the total number of neonate larvae inoculated onto the artificial diet and expressed in per cent.

Pupal weight: It is the weight of 10 pupae resulted from the cross between irradiated males and unirradiated females.

Sex ratio: It is measured as number of males to females. Sexing was done during pupal stage based on distance between genital pore and anal slit. In females, the distance between genital pore and anal slit is more compared to males.

Other parameters viz., adult emergence, deformation, adult longevity and survival under food stress of F_1 generation were recorded by following the procedure followed for recording the quality parameters of parental generation.

Male sterility in parental and F1 generation of Fall armyworm

Male pupae of 7 days old (48 hrs. before adult emergence) were exposed to gamma radiation at 25, 50, 75, 100, 125, 150, 175 and 200 Gy using gamma chamber at IIHR, Bengaluru, Karnataka, India.

Irradiated males(IM) from each dose were confined with an equal number of unirradiated females(UF) (10UF×10IM) of the same age into cages (30×30×30cm) provided with potted corn plants for oviposition. Eggs from each cage were sampled and inoculated on corn leaves held in Petri dishes. After 5 days of inoculation, the number of unhatched eggs and hatched larvae were counted. Similar procedure was followed for control using unirradiated males (UM) and unirradiated females (UF) of same age (10UF×10UM). The percentage of sterility was calculated by using the following formula (Toppozada, Abdallah, & Eldefrawi, 1966);

$$\text{Male sterility}(\%)=100-\left(\frac{a\times b}{A\times B}\right)\times 100$$

Where,

a = Number of eggs/female in treatment

b = Percent egg hatch in treatment

A= Number of eggs/female in control

B= Per cent egg hatch in control

(Where; treatment refers to mating between unirradiated females and irradiated males. Control refers to mating between unirradiated females and unirradiated males).

Corrected Sterility was computed according to the formula given by Seth and Reynolds (1993).

$$\%\ \text{Corrected sterility} = \frac{F_c - F_t}{F_t}\times 100$$

Where, F_c = % egg hatch in control; F_t = % egg hatch in treatment.

Statistical Analysis

Data collected on quality parameters and male sterility were subjected to ANOVA using Honestly Significant Differences (HSD) values calculated as Tukey Statistics at $\alpha = 0.05$ using SPSS Software version 20 available at Department of Statistics and Computer Applications, S.V. Agricultural College, Tirupati, Acharya N.G. Ranga

Agricultural University, Andhra Pradesh, India. The mean of three replications for each parameter was separated by using HSD -Tukey test (α = 0.05) SPSS Software version 20.

RESULTS

The significant differences among the different doses of gamma radiation on quality parameters of parental generation of Fall armyworm, *Spodoptera frugiperda* are presented in table 2. The percentage of adult emergence from the irradiated pupae was decreased with an increase in gamma radiation doses. Emergence of *Spodoptera frugiperda* adult at different doses (25 Gy to 200 Gy) ranged from 83.13% to 20.30%. The percentage of adult emergence at 25 Gy (83.13%) was statistically on par with the control (88.00%). Adult emergence at 50 Gy and 75 Gy are 79.46% and 75.90 %, respectively and were significantly different from control. 71.00% of adults were emerged from pupae irradiated at 100 Gy. But further increase in radiation dose (at 100 Gy onwards) has significantly declined the adult emergence to 58.70%, 36.30%, 27.00% and 20.30% at 125, 150, 175 and 200Gy, respectively (Table 2).

Table 2. Effect of gamma radiation on biological parameters of parental generation of fall armyworm, *Spodoptera frugiperda*.

Radiation dose (Gy)	Adult Emergence (%)*	Deformed adults (%)*	Adult longevity (Days)**	Survival under food stress (%)*
0 (Control)	88.00^{a} (69.74±1.02)	3.00^{e} (9.87±0.98)	9.00^{a} (3.16±0.09)	76.20^{a} (60.78±0.38)
25	83.13^{ab} (65.74±0.79)	4.10^{e} (11.53±1.32)	8.67^{a} (3.10±0.05)	72.33^{b} (58.24±0.21)
50	79.46^{bc} (63.05±1.07)	5.00^{de} (12.87±0.76)	8.33^{a} (3.05±0.04)	67.50c (55.26±1.63)
75	75.90^{cd} (60.59±1.02)	7.50^{de} (15.78±1.35)	7.50a (2.90±0.13)	61.00^{d} (51.34±0.34)
100	71.00^{d} (57.41±1.09)	9.00^{d} (17.43±0.57)	6.00^{b} (2.64±0.10)	59.00^{d} (50.16±0.37)
125	58.70^{e} (50.00±1.53)	13.80^{c} (21.73±1.29)	5.00^{bc} (2.44±0.00)	47.00^{e} (43.26±0.66)
150	36.30^{f} (36.99±1.99)	17.00^{c} (24.29±1.18)	4.33^{c} (2.30±0.15)	41.00^{f} (39.80±0.67)
175	27.00^{g} (31.25±1.49)	27.20^{b} (31.39±1.29)	3.67^{cd} (2.15±0.07)	26.80^{g} (31.15±0.93)
200	20.30^{h} (26.71±1.46)	35.90^{a} (36.77±1.52)	2.50^{d} (1.86±0.07)	21.50^{h} (27.61±0.54)
ANOVA	F=144.28; df=8,18; C.D.= 3.96; P<0.01	F=60.98; df=8,18; C.D.=3.54; P<0.01	F=24.84; df=8,18; C.D.=0.29; P<0.01	F=242.02; df=8,18; C.D.= 2.26; P<0.01

Within a column, means followed by the same letter are not significantly different (α = 0.05), (Honestly significant difference (HSD) Tukey's statistics at α = 0.05 using SPSS software version 20). Figures in the parenthesis are transformed values ± standard errors. *Arc sine transformation; ** Square root transformation.

Deformation includes deformed pupae/adults, partially emerged adults, adults with defective wings, legs and other appendages as a result of exposure to gamma radiation (Fig. 2). The formation of deformed pupae/adults ranged from 4.10 to 9.00% at radiation doses ranging from 25 to 100 Gy. Percentages of deformation at 25, 50 and 75 Gy were statistically on par with control. Deformation at 100 Gy (9.00%) was on par with radiation doses ranging from 25 to 75 Gy. The percentage of deformation was increased with increase in gamma radiation doses at 125 (13.80%), 150 (17.00%), 175 Gy (27.20%) and 200 Gy (35.90%), respectively.

Figure 2. Deformation of fall armyworm due to irradiation of male pupae at 125, 150, 175 and 200 Gy.

Longevity is the duration of irradiated males survived provided with an adult diet and water. Adult longevity of unirradiated males was 9 days. The longevity period decreased with an increase in radiation dose. The longevity of adult moths from the pupae irradiated at 25, 50 and 75 Gy was 8.67, 8.33 and 7.5 days respectively and on par with control. The longevity of irradiated males at 100 and 125 Gy was 6.00 and 5.00 days, respectively, which are on par with each other. Whereas males irradiated at 150, 175 and 200 Gy lived up to 4.33, 3.67 and 2.50 days respectively. Adult longevity of male moths was reduced by 50% at higher doses of 150 Gy onwards.

Males exposed to gamma radiation showed an inverse relationship between the percentage of survival under food stress and radiation doses. At 25 and 50 Gy, survival rates were 72.33% and 67.50%, respectively and were significantly different from the control (76.20%). No significant difference was found at 75Gy and 100 Gy with survival rates of 61% and 59%, respectively. Percentage of survival of male moths from the pupae irradiated at 125, 150, 175 and 200 Gy significantly reduced to 47.00%, 41.00%, 26.80% and 21.50% respectively.

It was found that radiation doses (up to 100 Gy) had least negative impacts on quality parameters of male moths. Whereas at radiation doses ranging from 125 to 200 Gy, the Quality parameters such as adult emergence, deformation, adult longevity and survival under food stress were severely affected.

Effect of gamma radiation on quality parameters in F1 generation of *Spodoptera frugiperda* are presented in table 3. Larval period had a positive relationship with the increasing doses of gamma radiation. Larval duration at 25, 50 and 75 Gy was 20.67, 21.33 and 22.50 days respectively, which are statistically on par with the control (20.33 days). Larval duration at 100(23.67 days) and 125 Gy (25.17 days) were statistically on par with each other. Larval duration significantly increased from 26.50 to 28.33 days at radiation doses ranging from 150 to 200 Gy, when compared to remaining doses (25-75 Gy). Larval duration was prolonged to 5-8 days at higher doses from 125 Gy onwards compared to control (Table 3).

The percentage of pupal recovery from larvae resulted from cross between unirradiated females and irradiated males decreased with increasing doses of gamma radiation. Pupal recovery at 25 Gy was 73.30% and on par with control (81.52%). No significant difference was found at 50 and 75 Gy with pupal recovery of 67.42% and 58.53%, respectively. Pupal recovery at 100 Gy was 51.33%. Significant reduction in pupal recovery was recorded at 125, 150, 175 and 200 Gy with 39.27%, 30%, 23.33 and 15.27% respectively.

Pupal weight decreased with increasing doses of gamma radiation. Pupal weight at 25 Gy was 2.03 gm and statistically on par with control (2.11 gm). At gamma radiation doses of 50 and 75 Gy, pupal weight was 1.90 and 1.86 gm and statistically on par and significantly different from control. Pupal weight at 100 Gy was 1.72 gm which slightly decreased from 75 Gy. A drastic decreasing trend was recorded at 125, 150, 175 and 200 Gy with 1.51, 1.25, 1.02 and 0.89 gm respectively and was statistically different.

Table 3. Effect of gamma radiation on biological parameters of F1 generation of fall armyworm, *Spodoptera frugiperda.*

Radiation dose (Gy)	Larval duration (days)**	Pupal recovery (%)*	Pupal weight (gm/10 pupae)**	Adult emergence (%)*	Deformation (%)*	Adult longevity (days)**	Survival under food stress (%)*	Sex ratio (female: male)
0 (Control)	20.33[e] (4.62±0.09)	81.52[a] (64.57±1.46)	2.11[a] (1.76±0.003)	84.35[a] (66.72±1.27)	3.87[g] (11.32±0.53)	8.50[a] (3.08±0.00)	80.00[a] (63.41±0.00)	1:0.85
25	20.67[e] (4.65±0.13)	73.30[ab] (58.93±1.88)	2.03[a] (1.74±0.01)	80.47[a] (63.81±1.53)	7.65[f] (16.04±0.37)	7.67[ab] (2.94±0.07)	70.00[b] (56.82±1.81)	1:0.88
50	21.33[e] (4.72±0.09)	67.42[bc] (55.18±0.66)	1.90[b] (1.70±0.01)	78.94[a] (62.68±0.79)	7.79[f] (16.17±0.67)	7.17[b] (2.85±0.07)	63.33[bc] (52.75±2.00)	1:0.96
75	22.50[de] (4.84±0.07)	58.53[cd] (49.90±0.95)	1.86[b] (1.69±0.01)	72.87[b] (58.65±1.98)	10.12[ef] (18.54±0.29)	6.83[b] (2.79±0.07)	60.00[c] (50.80±2.90)	1:1.04
100	23.67[cd] (4.97±0.03)	51.33[d] (45.74±4.71)	1.72[c] (1.64±0.009)	66.23[c] (54.47±1.28)	12.60[e] (20.74±1.02)	5.55[c] (2.55±0.00)	52.00[d] (46.13±0.66)	1:1.12
125	25.17[bc] (5.11±0.05)	39.27[e] (38.73±2.53)	1.51[d] (1.58±0.007)	48.17[d] (43.93±0.56)	23.22[d] (28.76±1.19)	4.16[d] (2.27±0.03)	38.89[e] (38.56±0.32)	1:1.13
150	26.50[ab] (5.24±0.02)	30.00[ef] (33.19±0.72)	1.25[e] (1.50±0.01)	40.26[e] (39.36±0.44)	30.55[c] (33.53±0.95)	3.83[de] (2.19±0.03)	33.33[e] (35.25±0.50)	1:1.16
175	27.67[a] (5.35±0.03)	23.33f[g] (28.77±2.21)	1.02[f] (1.42±0.01)	27.67[f] (31.67±1.68)	45.67[b] (42.49±0.38)	3.00[e] (1.99±0.07)	18.00[f] (25.07±0.86)	1:1.18
200	28.33[a] (5.41±0.08)	15.27[g] (22.88±1.85)	0.89[g] (1.37±0.02)	16.67[g] (24.07±0.68)	50.00[a] (44.98±0.00)	2.00[f] (1.73±0.00)	10.67[g] (18.91±1.80)	1:1.21
ANOVA	F=15.66; df=8,18; C.D.=0.23; P<0.01	F=80.39; df=8,18; C.D.= 4.76; P<0.01	F=115.39; f=8,18; C.D.=0.04; P<0.01	F=151.40; df=8,18; C.D.=3.73; P<0.01	F=298.16; df=8,18; C.D.=2.17; P<0.01	F=75.83; df=8,18; C.D.=0.16; P<0.01	F=96.05; df=8,18; C.D.=4.52; P<0.01	–

Within a column, means followed by the same letter are not significantly different (α = 0.05) using Honestly significant difference (HSD) Tukey's statistics at α = 0.05 using SPSS software version 20. Figures in the parenthesis are transformed values ± standard errors. *Arc sine transformation; ** Square root transformation.

Production of females in F1 generation were more (1:0.85) in unirradiated control (UF×UM). Similar trend was observed at 25 and 50 Gy with sex ratio (female to male) of 1:0.88 and 1:0.96. As the radiation dose increased from 75 to 200 Gy, the sex ratio was slightly shifted in favour of males.

The percentage of adult emergence of *Spodoptera frugiperda* from pupae resulted from the cross between unirradiated females and irradiated males decreased with an increase in gamma radiation doses. These values were recorded as 80.47% and 78.94% at 25 and 50 Gy, respectively and were statistically on par with the control (84.35%). But, the adult emergence at 75 Gy (72.87%) and 100 Gy (66.23%) were statistically different from control. Further increase in radiation doses, adult emergence was declined to 48.17% (125 Gy), 40.26% (150 Gy), 27.67% (175 Gy) and 16.67% (200 Gy), respectively. Adult emergence was severely affected and decreased by <50% at 150 Gy onwards, when compared to control.

The percentage of deformation ranged between 7.65-10.12% at doses ranging from 25-75 Gy, which were statistically on par with control. Deformation at 100 Gy was 12.60 and statistically different from the control. The percentage of emergence of defective adults with deformation was significantly high at 125, 150, 175 and 200 Gy and was recorded as 23.22, 30.55, 45.67 and 50.00%, respectively compared to the remaining doses.

The longevity of adults from the pupae irradiated at 25 Gy was 7.67 days and on par with control (8.5 days). Radiation doses; 50 and 75 Gy showed no significant differences in adult longevity with 7.17 and 6.83 days, respectively. The longevity of irradiated males at 100 Gy was 5.55 days. Adult longevity period of male moths reduced to 4.16 and 3.83 days at 125 and 150 Gy, respectively. Adult longevity decreased significantly at 175 and 200 Gy with 3.00 and 2.00 days, respectively.

There was an inverse relationship between the percentage of survival and radiation doses. Survival of unirradiated males was 80%. Percentage of adult survival at 50 Gy (63.33%) was on par with 25 Gy (70%). No significant difference was observed in survival rate at 75 Gy (60.00%), which is statistically on par with 50Gy (63.33%). At 100 Gy, more than 50% of adults survived (52.00%) without an adult diet and water. A sudden decreasing trend of adult survival under food stress was recorded at 125 and 150 Gy with 38.89% and 33.33%, respectively. Adult survival was severely affected at 175 and 200 Gy with least survival rates of 18.00 and 10.67%, respectively.

Effect of gamma radiation on fecundity and male sterility in the parental generation of *Spodoptera frugiperda* among the different doses of gamma radiation were presented in table 4. Fecundity was decreased with increasing doses of gamma radiation. Radiation doses; 50, 75 and 100 Gy decreased the fecundity to 155.06, 131.50 and 118.67 eggs/female/day, respectively, and were significantly different from control (202.33 eggs/female/day). The fecundity of females that were mated with irradiated males of 125, 150, 175 and 200 Gy was significantly impaired with mean fecundity of 80.00, 53.67, 41.00 and 28.00 eggs/female/day, respectively (Table 4).

The percentage of male sterility had an inverse relationship with increasing doses of gamma radiation. The percentage sterility from the unirradiated females mated with irradiated males at 25 Gy was 14.25%. Further, increase in radiation doses to 50 and 75 Gy, sterility percentage was also increased to 36.51%and 64.89%, respectively and they were significantly different from 25 Gy. More than 80% sterility was obtained at 100 Gy (81.89%). Male sterility was higher when unirradiated females crossed with males irradiated at 125, 150,175 and 200 Gy with sterility percentages of 91.58%, 96.81%, 98.52% and 99.25% respectively. The percentage of sterility significantly increased at radiation doses ranging from 100-200 Gy (Table 4).

Table 4. Effect of gamma radiation on fecundity and male sterility of parental generation of fall armyworm, *Spodoptera frugiperda*.

Radiation dose (Gy)	Fecundity**	Egg hatch (%)*	Male Sterility (%)*	Corrected Sterility (%)*
0 (Control)	202.33^{a} (14.26±0.19)	89.33^{a} (70.98±1.28)	---	---
25	189.00^{b} (13.78±0.05)	82.00^{b} (64.88±0.74)	14.25^{g} (22.13±0.98)	8.20^{f} (16.57±1.14)
50	155.06^{c} (12.49±0.17)	74.00^{c} (59.33±0.75)	36.51^{f} (37.16±0.52)	17.16^{e} (24.43±0.98)
75	131.50^{d} (11.51±0.20)	60.33^{d} (50.94±0.52)	64.89^{e} (53.64±0.18)	32.46^{d} (34.71±0.61)
100	118.67^{e} (10.94±0.18)	46.67^{e} (43.06±1.71)	81.89^{d} (64.59±0.94)	47.76^{c} (43.69±1.90)
125	80.00^{f} (8.90±0.15)	31.67^{f} (34.22±0.74)	91.58^{c} (73.11±0.06)	64.89^{b} (53.65±0.88)
150	53.67^{g} (7.39±0.08)	27.00^{f} (31.29±0.37)	96.81^{b} (79.70±0.38)	69.77^{b} (56.62±0.40)
175	41.00^{h} (6.48±0.21)	16.33^{g} (23.72±1.84)	98.52^{ab} (83.02±0.54)	81.72^{a} (64.77±1.97)
200	28.00^{i} (5.37±0.27)	12.00^{g} (20.08±2.06)	99.25^{a} (85.05±0.57)	86.57^{a} (68.65±2.20)
ANOVA	F=335.30; df=8,18; C.D.=0.53; P<0.01	F=211.93; df=8,18; C.D.=3.77; P<0.01	F=1429.96; df=7,16; C.D.=1.84; P<0.01	F=179.26; df=7,16; C.D.=4.28; P<0.01

Within a column, means followed by the same letter are not significantly different using Honestly significant difference (HSD) Tukey's statistic at α = 0.05 using SPSS software version 20. Figures in the parenthesis are transformed values ± standard errors.*Arc sine transformation; ** Square root transformation. Fecundity refers to eggs/female/day, confined with 10 Unirradiated Females×10 Irradiated Males in case of Treatment and 10 Unirradiated Females×10 Unirradiated Males in Control).

The present study found that a radiation dose of 100 Gy had induced 81.89% sterility with the least negative effects on adult emergence (71.00%), deformation (9%), adult longevity(6 days) and survival under food stress (59.00%). Based on these observations and comparison with all other doses of gamma radiation, a radiation dose of 100 Gy was found as suitable dose to induce enough sterility in the wild female population without affecting the biological parameters of irradiated males.

Effect of gamma radiation on fecundity and male sterility in the F1 generation of *Spodoptera frugiperda* among the different doses of gamma radiation were presented in table 5. Egg laying abilities of unirradiated females mated with irradiated males from the pupae irradiated at 25, 50, 75 and 100 Gy were significantly decreased with mean fecundity of 180.67, 150.33, 128 and 109.67 eggs/female/day, respectively, when compared to control (191.67 eggs/female/day). Further increase in radiation doses to 125, 150, 175 and 200 Gy resulted in a significant reduction of fecundity to 67.67, 43.33, 29.00 and 16.00 eggs/female/day, respectively and were statistically different (Table 5).

Table 5. Effect of gamma radiation on fecundity and male sterility of F1 generation of fall armyworm, *Spodoptera frugiperda*.

Radiation dose (Gy)	Fecundity**	Egg hatch (%)*	Male Sterility (%)*	Corrected sterility (%)*
0 (Control)	191.67a (13.88±0.11)	87.00a (69.46±4.02)	---	---
25	180.67b (13.48±0.14)	73.00b (58.69±0.98)	20.62f (26.97±1.05)	16.09g (23.58±1.35)
50	150.33c (12.30±0.12)	64.00c (53.13±1.38)	42.19e (40.49±0.90)	26.44f (30.87±1.73)
75	128.00d (11.35±0.24)	53.33d (46.90±1.95)	67.19d (55.04±0.87)	38.70e (38.40±2.31)
100	109.67e (10.52±0.04)	35.00e (36.22±1.93)	86.23c (68.22±0.93)	59.77d (50.64±2.16)
125	67.67f (8.28±0.19)	19.67f (26.28±1.05)	95.21b (77.36±0.59)	77.39c (61.62±1.15)
150	43.33g (6.65±0.13)	9.33g (17.62±1.80)	99.03a (84.37±0.48)	89.27b (71.02±1.94)
175	29.00h (5.46±0.29)	2.67gh (9.26±1.13)	99.79a (87.45±0.48)	96.93a (80.02±1.22)
200	16.00i (4.09±0.36)	0.00h (0.00±0.00)	100a (90.00±0.00)	100a (90.00±0.00)
ANOVA	F=308.46; df=8,18; C.D.=0.61; P<0.01	F=156.76; df=8,18; C.D.=5.66; P<0.01	F=988.11; df=7,16; C.D.=2.24; P<0.01	F=213.98; df=7,16; C.D.=4.95; P<0.01

Within a column, means followed by the same letter are not significantly different using Honestly significant difference (HSD) Tukey's statistics at α = 0.05 using SPSS software version 20. Figures in the parenthesis are transformed values ± standard errors.*Arc sine transformation; **Square root transformation. Fecundity refers to eggs/female/day, confined with 10UF×10IM in case of Treatment and 10UF×10UM in Control

The percentage of inherited male sterility increased with increasing doses of gamma radiation. At a gamma radiation dose of 25 Gy, the percentage of male sterility was 20.62%. Further, increase in radiation doses to 50 and 75 Gy, percentage of sterility was also increased to 42.19% and 67.19%, respectively and they were significantly different from 25 Gy. At a gamma radiation dose of 100 Gy, the percentage sterility was recorded as 86.23%. Male sterility was higher when normal females crossed with males irradiated at 125, 150,175 and 200 Gy with sterility percentages of 95.21%, 99.03%, 99.79% and 100% respectively.

In the present study, it was found that inheritance of male sterility in F1 generation was more compared to the male sterility in parental generation. The gamma radiation dose of 100 Gy induced 86.23% sterility with the least negative effects on larval duration (23.67 days), pupal recovery (51.33%), pupal weight (1.72 gm/10 pupae), adult emergence (66.23%), deformation (12%), adult longevity (5.55 days) and survival under food stress (52.00%). Based on these observations and comparison with all other doses of gamma radiation, a radiation dose 100 Gy was found to be more desirable to induce enough sterility in the wild female population without affecting the biological parameters of irradiated males. It clearly indicated the increasing trends in inheritance of male sterility from parental generation to F1 generation through irradiated males of parental generation. Inheritance of male sterility from parental sterile males to F1 generation was more and increasing trend was observed with an incremental increase in gamma radiation dose by 25 Gy.

CONCLUSIONS AND DISCUSSIONS

Exposure of mature pupae of *Spodoptera frugiperda* to gamma radiation (Co-60 source) at doses ranging from 25 to 100 Gy had least negative impacts on adult emergence and deformation. Further increase in radiation doses ranged between 125-200 Gy significantly decreased the adult emergence with increased percentage of deformation. Percentage of adult emergence was 71.00% at 100 Gy with deformation of 9.00%. Whereas further increase in radiation dose from 100 to 125 Gy; adult emergence was drastically reduced to 58.70% with increased percentage of deformation (13.80%). The present results could be due to the residual effects of high doses of gamma radiation (125-200 Gy) in parental and F_1 generations of FAW. The residual effects of gamma radiation were magnified in F_1 generation compared to parental generation. It could be due to the increased damage to the somatic cells at higher doses of gamma radiation (125 Gy onwards). Similar results were reported by Ibrahim & El-Naggar (2001) that irradiation of six-day old male pupae of *Spodoptera littoralis* at doses >100 Gy significantly decreased the adult emergence and adult emergence was completely ceased at 200 Gy. Boshra & Mikhaiel (2006) irradiation of male pupae of *Ephestia calidella* at 200, 400, 600 and 800 Gy of gamma radiation decreased adult emergence to 85.9, 68.8, 40% and 11.2% respectively. Similarly, Dhoubi & Abderrahmane (2002) reported only 6% of adult emergence at 500 Gy in *Ectomylois ceratoniae*. In the present study, radiation doses at 125 Gy onwards significantly increased the percentage of deformation in *Spodoptera frugiperda.* These results are corrobarated with earlier reports by Hasaballa, Ahmed, & Rizk (1985) in *Spodoptera littoralis* and reported that increasing doses of gamma radiation increased the percentage of deformation and complete lethality was observed at 350 and 400 Gy. The current findings are inclined with Seth et al (2020) who found that adult emergence of *Maruca vitrata* was severely affected at 150 and 200 Gy. No emergence of adult at 250 Gy. They also reported that percentage of malformed adults increased at 150 Gy (24.50%) and 200 Gy (100%). The present research results on effect of radiation doses ranging from 25 to 75 Gy has showed least negative impact on adult emergence of *Spodoptera frugiperda*. The similar findings are also reported by Abass, Salem, Abd-E-Hamid, Gabarty, & Embaby (2017) that radiation doses 25, 50 and 75 Gy resulted in adult emergence of 93.33, 81.88 and 75.35% respectively. Similarly, Ramesh, Garg, & Seth (2002) also reported that, radiation dose of 70 Gy had no negative impact on adult emergence (75.50%) of *Spodoptera litura.* Nearly 80% of Adult emergence was recorded in current studies which are on par with the findings of Arthur et al (2016), who reported 81.00% of adult emergence at 50 Gy in *Spodoptera frugiperda*. They also found that radiation dose of 200 Gy drastically affected the adult emergence and reduced to 10.00%. Pransopon, Sutantawong, & Hormchan (2000) found no significant differences in adult emergence and deformation at radiation doses 50-100 Gy in *Helicoverpa armigera*.

In the current study, the percentage of deformation was increased with increase in radiation doses at 150 and 200 Gy. These results are in agreement with Arthur et al (2013) who irradiated the pupae of *Spodoptera frugiperda* at 50, 100, 200 and

300 Gy and found least adult emergence (10.00%) at 200 Gy. Whereas at 300 Gy, eclosions of adults were completely ceased and they found that percentage of adult emergence at 50 Gy (81.00%) was on par with unirradiated control (85.00%).

The longevity of adults decreased as radiation dose increased. The present results on effect of gamma radiation on adult longevity and survival of FAW are on par with Seth et al. (2020) who reported that, longevity of irradiated males was sharply declined with increase in radiation doses from 150 to 250 Gy in *Maruca vitrata.* Similarly, Pransopon, Sutantawong, & Hormchan (2000) also found that higher doses of gamma radiation (150 Gy) significantly reduced the longevity of irradiated males from 13.35 days (Control) to 9.45 days (150 Gy) in *H. armigera.* Recently, Osouli, Ahmadi, & Kalantarian (2021) studied the radiation biology in *H. armigera* and found that longevity of adults decreased from 17.28 days (control) to 12.21 days (at 250 Gy). Ali, Rizwana, Ahmad, Hassan, & Ali (2014) also reported that unirradiated adults of *Pectinophora gossypiella* lived up to 11 days, whereas at 65 Gy, longevity of irradiated males decreased to 6.93 days.

Developmental period of larvae resulted from cross between normal females and parental males irradiated at doses ranging from 25 to 100 Gy prolonged the larval duration to 3 days. Whereas at radiation doses ranging from 125 to 200 Gy increased the larval duration from 5 to 8 days more, compared to unirradiated control. These results are on par with reports of Abass, Salem, Abd-E-Hamid, Gabarty, & Embaby, (2017) who reported slight increase in larval period at doses from 25 Gy (24.33 days) to 75 Gy (26.5 days), indicating that radiation doses up to 100 Gy were not had much negative impacts on larval development period. Larval period was increased with increasing radiation doses from 20.80 days (unirradiated control) to 27.2 days (at 175 Gy) in *Spodoptera frugiperda* (Arthur & Aguiler, 2002). Sayed & El-Helay (2018) also reported that larval duration of *S. littoralis* increased to 15.10 days at 100 Gy, compared to unirradiated control (13.80 days), but further increase in radiation doses significantly increased the larval duration.

In the present study, gamma radiation doses showed significant variation in pupal recovery. At 100 Gy, percentage of pupal recovery was 51.33%, further increase in radiation doses from 100 to 125 Gy reduced the pupal recovery to 39.27%. Pupal recovery was drastically affected at radiation doses ranged between 150 Gy (30.00%) to 200 Gy (15.27%). The present findings were inclined with the reports of Seth *et al*. (2020), in *Maruca vitrata* who found that percentage of pupal formation was decreased to 14.70% compared to 68.00% at 100 Gy. Pupal recovery of unirradiated control was 74.00%. Larval and pupal mortality were increased at 150 Gy onwards in *S. littoralis* (Abass, Salem, Abd-E-Hamid, Gabarty, & Embaby, 2017). Arthur, Arthur, & Machi (2016) also found drastic reduction in pupal formation of *S. frugiperda* at 200 Gy (30.00%) compared to control (95.00%), Whereas at 100 Gy, pupal recovery was 70.00%. It clearly indicates that radiation doses higher than 100 Gy had much negative effects on pupal recovery due to increased percentage of larval mortality.

Pupal weight at unirradiated control was 2.11 g/10 pupae. There was a sharp decline in pupal weight with increase in radiation doses from 50 Gy (1.90 g) to 100 Gy

(1.72 g). Pupal weight was significantly decreased at 125 Gy onwards from 1.51g/10 pupae (125 Gy) to 0.89 g/10 pupae (200 Gy). The similar results were reported by El-Naggar, Megahed, Sallam, & Ibrahim (1984) reported that the pupal weight was significantly decreased at radiation doses higher than 100 Gy in F_1 generation and radiation doses higher than 50 Gy in F_2 generation of *Agrotis ipsilon*.

There was no significant difference in adult emergence of F_1 generation at 25 and 50 Gy, compared to unirradiated control. A sharp decline in adult emergence was recorded at 75 and 100 Gy. Percentage of adult emergence at 100 Gy was 66.23% with least percentage of deformation (12.60%). Adult emergence was drastically decreased from 48.17% (125 Gy) to 16.67% (200 Gy) with increased percentage of deformation (23.22-50.00%), indicating that radiation doses that are higher than 100 Gy had much negative impacts on emergence of F_1 adults. These results are in line with Arthur, Arthur, & Machi (2016) who reported least percentage emergence of F_1 adults (10.00%) of *S. frugiperda* at 200 Gy. The similar results reported by Pransopon, Sutantawong, & Hormchan (2000) in *H. armigera* that radiation doses higher than 100 Gy significantly increased the emergence of deformed adults with decreased emergence of F_1 active moths. The present findings are very close with the reports of Carpenter, Young, Knipling, & Sparks (1986) that 58.00% of F_1 adults of *S. frugiperda* were emerged at 100 Gy, further increase in radiation dose to 150 Gy, decreased the adult emergence to 20.00% with increased percentage of deformation and adult mortality. Recently, Osouli, Ahmadi, & Kalantarian (2021) also found that radiation doses; 150 and 200 Gy decreased the percentage of F_1 adult emergence in *H. armigera.* Seth, Khan, Rao, & Zarin (2016) reported significant decrease in adult emergence with high rates of defective adults at 130 Gy compared to 100 Gy in F_1 generation of *Spodoptera litura*.

Longevity of irradiated adults of F_1 generation at radiation doses from 25 (7.67 days) to 75 Gy (6.83 days) was not significantly different. Percentage of survival of irradiated adults under food stress at 25 to 75 Gy was vary from 60.00% to 70.00%. Percentage of survival of irradiated adults at 100 Gy was 52.00%. Further increase in radiation doses from 125 to 200 Gy was ranged between 38.89-10.67%. For success of any SIT programme, minimum of 50% of irradiated males should survive under food stress for at least 48 hours, which reflects the ability of adults to survive in released environment. Sterile males must live for at least 6 days to meet the pre-mating period 2 days and mating period of 4 days, which allows the sterile males to copulate with wild females to induce sterility in wild population of target insect. Current findings are on par with the reports of Seth et al (2020), in *Maruca vitrata* who found that radiation dose, 100 Gy had not negative impact on longevity of F_1 adults (7.28 days). But radiation dose, 200 Gy has reduced the longevity by 3.65 days compared to 100 Gy. Osouli, Ahmadi, & Kalantarian (2021) also reported significant decrease in longevity adults of F_1 generation from 17.28 days (control) to 12.21 days (250 Gy) in *Helicoverpa armigera* due to increased rates of adult mortality at higher doses. Salem, Fouda, Abas, Ali, & Gabarty (2014) also reported high percentage of pupal (59.90%) and adult mortality (49.30%) at 150 Gy in F_1 generation of *Agrotis ipsilon*.

Ali, Rizwana, Ahmad, Hassan, & Ali (2014) reported significant decrease in longevity of irradiated males of *Pectinophora gossypiella* (F_1 generation) with incremented increase in gamma radiation doses.

There was no much differences in sex ratio among the radiation doses. The proportion of females were high in unirradiated control (1:0.85 F:M). Radiation doses up to 75 Gy had not showed many differences in sex ratio of females to males. Comparatively high number of males were formed at radiation doses ranging from 100 to 200 Gy with female to male sex ratio of 1:1.12 - 1:1.21. These results were similar with the reports of Seth et al (2020) in *Maruca vitrata*, that female to male sex ratio at 100, 150 and 200 Gy was 0.79:1, 0.71:1 and 0.58:1, respectively. Whereas in unirradiated control female to male sex ratio was 1.03:1. Similar results were reported by Abass, Salem, Abd-E-Hamid, Gabarty, & Embaby (2017) who found high number of males (50) out of 100 pupae at 75 Gy compared to control (51 females and 49 males) in *Spodoptera littoralis*.

Lepidopteran species are more resistant to the sterilizing effects of radiation than insects of any other order (Lachance, 1985). As a consequence, the greater amount of radiation required to achieve full sterility in males may reduce their competitiveness and performance in the field (Suckling, Hackett, Barrington, & Daly. 2002). One approach to reducing these negative effects is the use of inherited sterility or F1 sterility (Carpenter, Bloem, & Bloem. 2001).

The present investigations were conducted on parental and F1 sterility of Fall armyworm and results are discussed here with appropriate literature on FAW and other related lepidopteran pests. Number of eggs laid by female and per cent egg hatch was significantly decreased as the gamma radiation dose was increased. At 100 Gy, the number of eggs laid by unirradiated females crossed with parental males emerged from irradiated pupae was 356 eggs/female and percentage of egg hatch was 46.67%. Whereas at 200 Gy the number of eggs laid by unirradiated females crossed with irradiated males was significantly reduced to 56.00 eggs and percentage of egg hatch was 12.00%. Therefore, the parental generation of *Spodoptera frugiperda* exhibited 81.89 and 99.25 percent sterility at 100 and 200 Gy, respectively. In the F_1 generation, at 100 Gy the total number of eggs and percentage of egg hatch was recorded as 329.00 eggs and 35.00% respectively. At 200 Gy total number of eggs laid and percentage of egg hatch was significantly reduced to 32.00 eggs and cent percent respectively. Therefore in F_1 generation, 86.23 and 100% sterility were recorded at 100 and 200 Gy, respectively. These results could be due to the increased damage to germ cells male gonads at higher doses of gamma radiation of 125 Gy onwards. Radiation induced germ cell damage could resulted in mutation in reproductive gonads of emerged male moths. These results are similar to Seth et al. (2020), who found that number of eggs laid /female and percent egg hatch in parental generation of *Maruca vitrata* was decreased as radiation dose increased. At 200 Gy the number of eggs laid/female was reduced to 99.3 eggs with egg hatch of 21.10%. They also found that 150 and 200 Gy as higher radiation doses in inducing male sterility. Further they found that radiation dose 200 Gy has induced 100 % sterility in F_1 generation.

In the present study; fecundity and fertility (egg hatch) were decreased with increase in radiation doses. Male sterility was increased with incremental increase in radiation doses. These trends were observed in both parental and F_1 generation of FAW. It was found that radiation doses higher than 100 Gy (*i.e.* 125, 150, 175 and 200 Gy) induced >90% of sterility both in parental and F_1 generation but these doses showed negative impacts on quality parameters of irradiated male moths of parental generation and also severely affected the biological parameters of F_1 generation.

In the current study, radiation dose 100 Gy was identified as optimum dose as it induced male sterility of 81.89% in parental and 86.23% in F_1 generation without any deleterious effects on adult emergence, adult longevity and survival which are directly related to mating ability of sterile males in inducing male sterility and its inheritance to subsequent generation. The progeny of irradiated males were more sterile than their male parents, this phenomenon has been accepted in several species of Lepidoptera. These results are in accordance with Abass, Salem, Abd-E-Hamid, Gabarty, & Embaby (2017) who found that radiation doses; 150, 200, 250 and 300 Gy had negative impacts on survival percentages of larvae, pupae and adults of F_1 generation of *Spodoptera littoralis.* They also found that 100 Gy has induced male sterility 64.30% without affecting the emergence of sterile males and their survival. Although male sterility of 90.11-94.60% was recorded at radiation doses 200-300 Gy, but they severely affected the adult emergence. Sallam, El-Shall, & Mohamed (2000) also reported that reproductive potential of irradiated males of *Earias insulana* decreased at 150 and 200 Gy. Salem, Fouda, Abas, Ali, & Gabarty (2014) tested the sub-sterilizing doses of 50, 100 and 150 Gy on full grown pupae of *Agrotis ipsilon* and found that percentage of larval and pupal mortality, larval and pupal durations increased at 150 Gy. These results are in line with Pransopon, Sutantawong, & Hormchan (2000) who studied the effect of radiation doses (50, 100, 150 and 200 Gy) on quality parameters and male sterility of *H. armigera* and found that percentage of male sterility increased with increase in radiation doses but radiation dose (150 and 200 Gy) significantly increased the percentage of deformation. F_1 progeny was more sterile than irradiated parental male in Diamondback moth, when male pupae irradiated at 50 to 250 Gy (Sutrisno, Hoedaya, Sutardji, & Rahaya, 1991). The present findings are on par with Arthur & Aguiler (2002) irradiated the male pupae of *Diatraea saccharalis* and *S. frugiperda* at gamma radiation doses of 50, 100, 125, 150 and 175 Gy. They found that percentage of male sterility in *D. saccharalis* at 100 Gy was 85.00%; whereas at 125 and 150 Gy, cent percent sterility was recorded. Percentage of inherited male sterility in F_1 generation was 95.70% at 100 Gy without affecting the longevity of sterile males. In *Spodoptera frugiperda* fecundity was decreased with increased doses of gamma radiation. Percentage of sterility was increased from 15.00% (50 Gy) to cent percent (200 Gy). Inheritance of male sterility at 100 Gy from parental males (sterile) to F_1 progeny was almost increased by 37.00% compared to parental generation. Inheritance of male sterility from F_1 to F_2 generation was decreased at all the radiation doses tested. They also reported 93-100% of male sterility at radiation doses 125-200 Gy in parental and F_1 generation of *Spodoptera frugiperda.* Arthur et al. (2013) also studied the effect of gamma radiation on quality parameters and male sterility of *S. frugiperda* at radiation doses vary from 50 to 300 Gy. Percentage of sterility was increased from 20% (at 50 Gy) to cent percent (at 200 and 300 Gy). 50% of male sterility was reported at 100 Gy without affecting the emergence of F_1 adults. Only 10% of adult emergence was recorded at 200 Gy. Further they concluded that radiation dose of 100 Gy was sterilizing dose to adults of Fall armyworm and 200- 300 Gy was the lethal dose and can be used for phytosanitary treatment of FAW. Osouli, Ahmadi & Kalantarian (2021) studied the radiation biology and inherited sterility in *H. armigera* and found that number of eggs laid per female and their hatchability declined with increase in radiation doses. Percentage of egg hatch was reduced from 90.70% (unirradiated control) to 49.50% (in parental generation) and 35.70% (in F_1 generation) at 100 Gy in *S.litura* (Seth, Khan, Rao, & Zarin, 2016).

The present findings are in line with Salem, Fouda, Abas, Ali, & Gabarty (2014) reported that fecundity and fertility were decreased with increase in radiation doses. At 100 Gy; 78% of male sterility was reported in parental generation of *Agrotis ipsilon.* Fecundity and egg hatch decreased from 106 eggs/female (control) to zero (65 Gy) and 80.57% to cent percent as the radiation doses increased from 35 Gy to 65 Gy in *Pectinophora gossypiella* (Ali, Rizwana, Ahmad, Hassan, & Ali, 2014). Recently, Sayed & El-Helay (2018) studied the effect of gamma radiation on *Spodoptera littoralis* at radiation doses of 40-100 Gy. Percentage of sterility was increased from 11.00% (unirradiated control) to 55.70% (100 Gy) and also suggested 100 Gy as suitable dose in inducing sterility. Larval and pupal duration were remain unaffected at 100 Gy. The present results with respect to inheritance of male sterility in *Spodoptera frugiperda* are in line with Rahman, Rahman, Islam, & Huque (2002) who found that sterility of F_1 generation of *Spilosoma obliqua* was increased compared to the parental generation. The sterility of F_1 generation was more i.e. 79% and 91% at 100 and 150 Gy respectively compared to 44 and 73% of parental sterility. Boshra & Mikhaiel (2006) also studied effect of gamma radiation on *Ephestia calidella* (Guenee) and found decrease in number of eggs/female from 256.5 eggs/female at 0 Gy to 66.6 eggs/female at 350 Gy. At a dose of 300 Gy, treated females were 100% sterile when mated with treated males. They also found that doses of 300 and 350 Gy led to the production of 83.9% and 90.2% sterile eggs, respectively.

The present research findings on radiation induced male sterility in parental and its inheritance to F_1 generation of *S. frugiperda*. Radiation dose; 100 Gy was the most appropriate dose in inducing 81.89% male sterility in parental and 86.23% of male sterility in F_1 generation without causing deleterious effects on quality of sterile male moths, which is very important for success of SIT programme.

ACKNOWLEDGEMENT

The first author is thankful to Acharya N.G. Ranga Agricultural University, Guntur for providing fellowship and ICAR-Indian Institute of Horticultural Research. Bengaluru for radiation facility during my post-graduation.

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J. Entomol. Res. Soc., 25(3): 601-614, 2023 *Research Article*
Doi: 10.51963/jers.v25i3.2493 *Online ISSN:2651-3579*

Evaluation of *Origanum majorana's* Toxicological Effects on Hemolymph and Some Biological Aspects of *Rhynchophorus ferrugineus* Larvae (Coleoptera: Curculionidae)

Heba Yehia MADY[1a] Heba Abdul Rauf MOHAMED[1b]

Tarek Afifi A. Hamed EL-SHEIKH[2] Maha Moustafa AHMED[1c*]

[1]Zoology Department, Women College, Ain Shams University, Cairo, EGYPT

[2]Plant Protection Research Institute, Agriculture Research Center, Dokki, Giza, EGYPT

e-mails:[1a]Heba.yehia@women.asu.edu.eg, [1b]dr.hbh2015@women.asu.edu.eg,

[2]tarekafifi71@gmail.com, [1c]maha.moustafa@women.asu.edu.eg

ORCID IDs: [1a]0000-0002-5990-3728, [1b]0000-0002-3236-4701, [2]0009-0007-8354-3391 [1c]0000-0001-9390-6618

[*]Corresponding author

ABSTRACT

Red palm weevil (RPW), *Rhynchophorus ferrugineus* is an invasive insect pest of date palm (*Phoenix dactylifera*) and several other palm species. RPW causes heigh damage in most date-producing areas of the world, particularly in the Middle East and North Africa. In this study, we discussed the drastic effects of *Origanum majorana* essential oil on different parameters, including total hemocyte counts (THCs), differential hemocyte counts (DHCs), total protein, lipid, and carbohydrates, as well as some enzyme activity like catalase (CAT), phenol oxidase, super oxide dismutase (SOD),α β-estrase, and acetyl-cholinesterase (AChE) in RPW larvae. Groups of five fourth-stage larvae were submerged for 60 seconds immediately in 9.8% (LC_{50}) of *O. majorana*. Compared to the control group, the THC levels are reduced after treatment with *O. majorana* essential oil. The estimated THCs were 27025 ±1356.54 cells/mm^3 and 23250 ±1035.82 cells/mm^3 for control and treated larvae, respectively. The DHCs showed that granulocytes (Gr) (26.47%) and plasmatocytes (Pl) (31%) were the two hemocyte types that predominated. Compared to the control group, the total protein, lipid, and carbohydrate were significantly lower. In comparison to control larvae, the treated larvae had significantly greater levels of CAT, SOD, and AChE activity. The activity levels of α, β-estrase and phenol oxidase were greatly decreased, but the phenol oxidase level was significantly increased in comparison to the control groups. Through the results obtained can be used *Origanum majorana* in the integrated control program especially in the period before harvest so that there are no residual pesticides in fruits when control palm after conducting some future studies infestation.

Keywords: red palm weevil, essential oil, biochemical studies, hemocytes, enzyme activity.

Mady, H. Y., Mohamed, H. A. R., El-Sheikh, T. A. A. H., & Ahmed, M. M. (2023). Evaluation of *Origanum majorana*'s Toxicological Effects on Hemolymph and Some Biological Aspects of *Rhynchophorus ferrugineus* Larvae (Coleoptera: Curculionidae). *Journal of the Entomological Research Society*, 25(3), 601-614.

Received: July 19 2023 *Accepted: September 18, 2023*

INTRODUCTION

The red palm weevil (RPW), *R. ferrugineus* (Olivier), is the most pest that seriously harms date palm trees. (Coleoptera: Curculionidae). RPW is recognized as Egypt's most destructive pest to date and in practically widespread all Egyptian governorates, causing immense damage to palm trees Because of the non-application of an internal quarantine and the non-implementation of an integrated control programme (Merghem, 2011; Merghem & Bibers, 2014).The widespread usage of these compounds has also resulted in issues with the economy and the environment, including threats to mammals, beneficial animals, and arthropods, as well as worldwide pollution (Gloria, Monroy, Gòmez, & Olguìn, 2008; El namaky, El sadawy, Al omari, & Bahareth, 2020). In response to the detrimental effects of the insecticides and the continued economic loss brought on by this borer, other tools that provide effective, safe, and economical control were urged to resolve these issues. It has been demonstrated that plant essential oils and their constituents have the potential to be developed into novel fumigants and that they may have advantages over them in terms of low mammalian toxicity, quick breakdown, and local availability. (Isman, 2008). One of the alternate measurements for the Lamiaceae family, which has 3500 species and 200 genera, is *Origanum* L. The genus contains around 44 species, several of which are oregano variants, sweet marjoram (*O. majorana* L.), and dittany of Crete (*O. dictamnus* L.). (Martins et al, 1999). They have strong antifungal, insecticidal effects antioxidant, and antimicrobial (Belhattab et al, 2005) (Khalfi, Sahraoui, Bentahar, & Boutekedjiret, 2008), (Mechergui et al, 2010:), Rodriguez-garcia et al, 2016).

Immunity plays an important role in the development of insects. Many insects belonging to significant orders have exhibited humoral and hemocyte-mediated immune reactions, including phagocytosis, nodulation, and encapsulation (Schmidt, Theopold, & Strand, 2001). Hemocytes that participate in hemocyte-mediated immunity have also been extensively discussed by several authors, along with all other types. The red palm weevil (RPW), *R. ferrugineus* (Olivier, 1790) (Coleoptera: Dryophthorinae), has been suggested as a useful model for studying some aspects of host-pathogen interactions and insect innate immunity (Cappa et al, 2020; Ibrahim et al, 2021). Understanding the immune systems of insects can help improve biological pesticides and increase our understanding of host-pathogen relationships. In this field, the common types of hemocytes discussed in this study include prohemocytes, granular cells (granulocytes), plasmatocytes, spherule cells (spherulocytes) and oenocytoid. These hemocyte forms have been recorded from numerous orders, including Lepidoptera, Diptera, Orthoptera, Blattaria, Coleoptera, Hymenoptera, Hemiptera, and Collembola (Hernandez et al, 1999; De silva, Dunphy, & Rau, 2000). However, changes in the activity of protective enzymes like glutathione-S-transferases (GSTs), - and α-/β-esterases (α-/β-ESTs), acetylcholinesterase (AChE), acid and alkaline phosphatases (ACP and ALP), and mixed-function oxidases (MFO), which aid organisms in the transformation and/or elimination of endogenous and exogenous compounds (Polson, Brogdon, Rawlins, & Chadeee, 2011; Koodalingam, Mullainadhan, & Arumugam, 2011). On the other hand, superoxide dismutase (SODs) are antioxidant components that can oxidase reactive oxygen species. In cells, superoxide, a reactive oxygen species, is dissimulated by them into hydrogen peroxide and oxygen. They are regarded as a crucial antioxidant line of defense against superoxide radicals (Bolter & Chefurka, 1990). Also, acetylcholine, a crucial neurotransmitter involved in the transmission of nerve impulses, is converted metabolically by the main enzyme, AChE, which is largely located in insects' synaptic clefts. AChE also serves as the target site for the majority of neurotoxic insecticides. It is sometimes thought that the structural change in AChE is the primary factor causing bugs to become resistant to synthetic chemical insecticides like organophosphates and carbamates (Chaudhari, Singh, Kedia, Das, & Dubey, 2021). This work aimed to investigate the activity of *O. majorana* essential oil against the last instar larvae of RPW. Furthermore, we studied the physiological modifications of enzymes and the amount of total protein, lipids, carbohydrates. Finally, the total and differential haemocyte counts of larvae were evaluated, from an immunological point of view.

MATERIAL AND METHODS

Insect

Red palm weevil larvae obtained from palm trees infestation in Abu Rawash, Egypt's Giza Governorate, RPW last instar larvae were found. The larvae were raised in a lab at a temperature of 25 °C while being fed sugar cane stems.

Essential oil

From a local market in Egypt, *O. majorana* essential oil was bought.

Immersion and contact assays

The study was conducted in the Research Laboratory of the women's college at Ain Shams University's Zoology Department.

Using five groups of fourth-stage larvae were submerged for 60 seconds immediately in 9.8% (LC_{50}) *O. majorana*. This concentration was determined based on previous research that we had conducted (Mady, Ahmed & El namaky, 2021) employing distilled water with 80% Tween at 1 ml/100 ml of emulsion. To increase homogeneity, a homogenizer was used on the generated emulsions. Treatment larvae were placed in plastic boxes (5 larvae per box) with filter paper for 24 hours before being moved to plastic containers with 50g of moist sugar cane sawdust and succulent sugarcane stem pieces under temperature 28°C incubated.

Tween-80 was present in both the treatment and control solutions at 0.02%. Five larvae replicated four times. After 48 hours, the larvae were inspected, and the dead ones were taken out.

Hematology assays

Cutting one of the prolegs of fourth-instar *R. ferrugineus* larvae using a pair of tiny scissors allowed for the examination of a drop of hemolymph. We counted the hemocytes in each individual larva. By using diluted solutions, total hemocyte counts per mm3 (THCs) were performed. The number of cells per μl of hemolymph was used to express THCs. By counting 100 cells and calculating the percentage differential of each type of hemocyte (DHC) found in the hemolymph, the DHCs were calculated. The total cells were counted, and DHCs were expressed as the mean of each hemocyte type. The total and differential hemocyte counts (THCs and DHCs of treated and untreated fourth instar *R. ferrugineus* were calculated. A one-way analysis of variance (ANOVA) was used to analyses the data from the THC and DHC ($P < 0.05$).

Biochemical studies

The Plant Protection Research Institute's Physiology Department conducted the entire biochemical analysis.

Protein assay

Using Coomassie Brilliant Blue G-250, a dye that binds to proteins, and bovine serum albumin as the standard, Bradford's method from 1976 was used to quantify the total amount of protein in the hemolymph.

Lipid assay

A technique adapted from van Handel (1985) and Waburg & Yuval (1996) was utilized for the total lipid analyses.

Total carbohydrates assay

Total carbohydrates were extracted, prepared for assay, and estimated in samples by the phenol-sulphuric acid reaction according to the procedure of Dubios, Gilles, Hamilton, Rebers, & Smith (1956) and Crompton & Birt (1967). Total carbohydrate is expressed as mg glucose/gm larval fresh weight.

Enzyme activity assays

Catalase activity was determined using a catalase assay kit purchased from Biodiagnostic, Co., according to the method described by Aebi (1984).

The activity of superoxide dismutase (SOD) was estimated by using a SOD assay kit purchased from Biodiagnostic, Co., according to the method described by Nishikimi, Roa, & Yogi (1972).

Phenoloxidase activity was determined according to a modification of the method described by Ishaaya (1971).

Acetylcholinesterase (AchE) activity was measured according to the method described by Simpson, Bulland, & Linquist (1964) using acetylcholine bromide (AchBr) as substrate and expressed as ug AchBr/min/g.b.wt.

Alpha esterases (α-EST) and beta esterases (β-EST) were determined according to Van Asperen (1962) using α-naphthyl acetate or β-naphthyl acetate as substrates, respectively. The activity of the EST was presented as ug α-nphthol/min/g.b.wt

RESULTS

Effect of *O. majorana* essential oil on the total and differential haemocyte counts (THCs and DHCs) of last instar larvae of RPW

When compared to the control group, the *O. majorana* essential oil after 48 hours had an impact on cell count. For the control and treatment groups, the estimated circulating hemocyte counts of the RPW, 4^{th} instar larvae, were 27025±1356.54 cells/ mm^3 and 23250±1035.82 cells/mm^3, respectively. The overall hemocyte counts are lower after using *O. majorana* essential oil compared to the control group.

After 48 hours, the effect of *O. majorana* essential oil on the various hemocyte counts in RPW, 4^{th} instar larvae was assessed. Differential counts showed that granulocytes (Gr) (26.47%) and plasmatocytes (Pl) (31%) were the two hemocyte types that predominated, while oenocytoides (Oe) (5.88%) had the lowest count. Spherulocytes (Sp), coagulocytes (Co), and prohemocytes (Pr) made up 14.71, 12.61, and 9.24% of the total, respectively (Table 1).

Table 1. Differential hemocyte counts of *Rhynchophorus ferrugineus* 4th larval instar (Mean ± S.E).

Haemocytes	Prohomocyte	Plasmocyte	Graneolocyte	Coagulocytes	Spherulocytes	Oenocyte
Control	7.50 ± 0.29 12.61%	18.50±0.29 31.09%	15.75±0.26 26.47%	8.75±0.48 14.71%	5.50±0.29 9.24%	3.50±0.29 5.88%
Larvae exposed to *Origanum majorana*	9.50 ±0.29 17.43%	11.50±0.33 21.10%	10.25±0.85 18.81%	12.00±0.65 22.02%	5.75±0.60 10.55%	5.50±0.20 10.09%
F	76.0	268.0	44.4	25.8	2.9	56.3

Values represent Mean ±SE of R. ferrugineus. P-values: P<0.05.
Total df = 11

Biochemical studies

The amount of total protein, lipid, and carbohydrates were determined in 4^{th} instar larvae of RPW 48h post treatment. In our study, the total protein, lipid, and carbohydrate contents of *R. ferrugineus* larvae treated with LC_{50} of *O. majorana* essential oil were significantly (P< 0.05) lower than those of the control group. (Fig. 1).

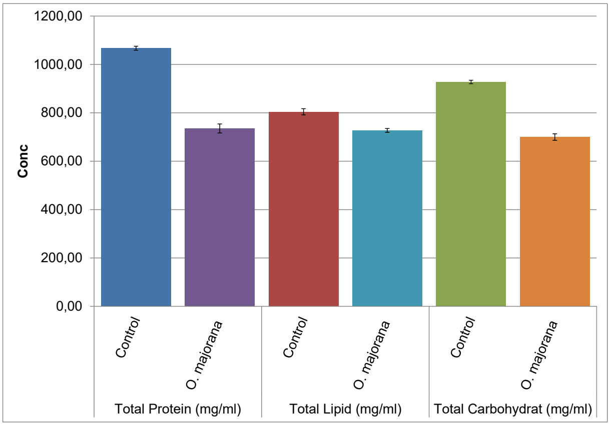

Figure 1. The total protein, lipid, and carbohydrate contents of *Rhynchophorus ferrugineus* 4^{th} larvae treated with LC_{50} of *Origanum majorana* essential oil.

In (Fig.2), it is shown how *O. majorana* essential oil affects the activity of the enzymes involved in detoxification in *R. ferrugineus* larvae. The activity level of the enzymes CAT, SOD, and AChE was significantly higher ($P< 0.05$) in the essential oil-treated 4th instar red palm weevil larvae than in the control larvae.

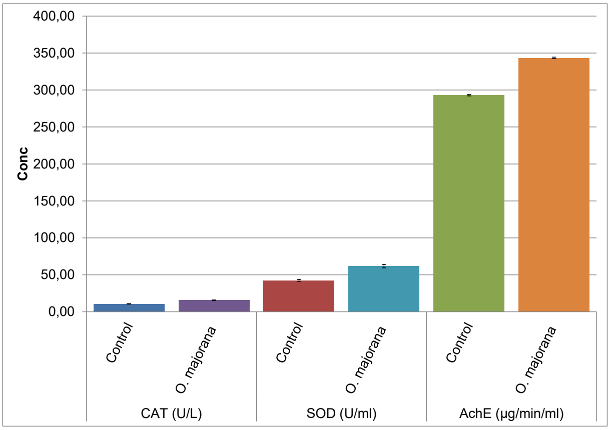

Figure 2. The activity level of the enzymes CAT, SOD, and AChE of *Rhynchophorus ferrugineus* 4th larvae treated with LC50 of *Origanum majorana* essential oil.

It was discovered by comparing the activity levels of α, β-estrase, and phenol oxidase that the level of both α, β-estrase were greatly decreased, but the phenol oxidase level was significantly increased in 4^{th} instar larvae in comparison to the control groups (Fig. 3).

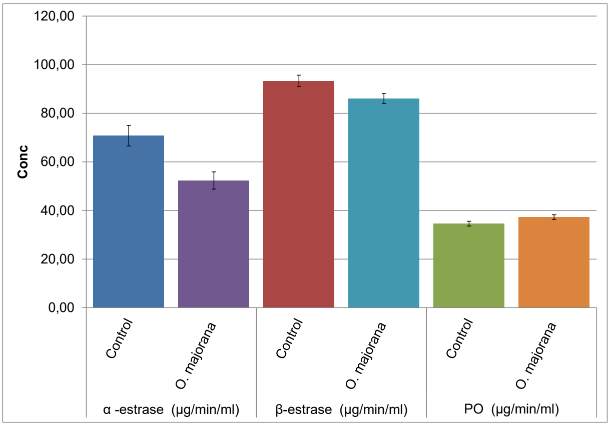

Figure 3. The activity levels of α, β -estrase, and phenol oxidase of *Rhynchophorus ferrugineus* 4th larvae treated with LC50 of *Origanum majorana* essential oil.

DISCUSSION

The results obtained from the present work show that after 48 hours of treatment with LC_{50} of *O. majorana*, the total Hemocyte count (THC) decreased when compared with untreated 4th instar larvae of *R. ferrugineus*. Many insects have shown reduced THC levels when exposed to various plant extracts or plant-derived materials, including the cotton bollworm *Helicoverpa armigera* when exposed to *Artemisia annua*, *A. conyzoides*, and *Azadirachta indica* oils (Padmaja & Rao, 2000); *S. litura* when exposed to *Acorus calamus* oils (Sharma, Sharma & Saxena, 2008), and the lemon butterfly *Papilio demoleus* when exposed by leaf extracts of *Eucalyptus globules, A. conzoides* and *Allium sativum* (Pandey, Pandey, & Tiwari, 2012).

THC levels were decreased in *E. integriceps* larvae after treatment with *A. annua* extract. (Zibaee & Bandani, 2010). The amount of *Ferula gummosa* oil greatly reduced the THC of the Mediterranean flour moth *Ephestia kuehniella*. (Ghasemi, Yazdib, Tavallaie, & Sendi, 2013). THC levels dropped after *Clerodendron inerme's* aqueous leaf extract was applied to *H. armigera* caterpillars in their sixth instar (Kalyani & Holihosur, 2015). Additionally, after being treated with an acetone extract of the *Calotropis procera* plant, individuals of the desert locust *Schistocerca gregaria* and the migratory locust *Locusta migratoria* showed notable decreases in THC in their hemolymph (*Kaidi, Amroun, Hocine, Doumandji, & Ghezali,* 2017). The amount of THC in *S. littoralis* larvae was substantially reduced after 48 hours of treatment with LC_{25} extracts of *C. procerae* and *Atriplex halimus* (Asiri, 2017).

The reduction in THC could be due to the suppression of larval hematopoietic cell proliferation and function (Zhu et al, 2012). Numerous authors (Sabri & Tariq, 2004; TiwariPandey, & Kumar, 2006; Pandey, Upadhyay,& Tiwari,2007; Sendi & Salehi, 2010; Zhu et al, 2012; Zibaee, Bandani, & Malagoli, 2012) have suggested that the decline in THC levels may be caused by the cytotoxicity of botanicals and the demise of pathologically deteriorated cells.

On the other hand, numerous reports have shown that various insects' THC levels have increased in reaction to botanical treatments. For instance, *azadirachtin* (Azt), *Margosan-O* (neem formulation), and some substances derived from urea waste and rice straw all caused a rise in THC in the hemolymph of the Egyptian cotton leafworm *Spodoptera littoralis* (*Hassan, Bakr, Abd el-bar, Nawar, & Elbanna,* 2013). After being exposed to the acetone solution of *Melia azedarach*, THC levels in the black cutworm *Agrotis ipsilon* larvae rose (El-Shiekh, 2002; Shaurub, & Sabbour, 2017). The non-mulberry silkworm *Antheraea assama* was treated with essential oils of *Ocimum sanctum*, *Ocimum gratissimum*, and *Ageratum conyzoides*, and early treatment results showed a THC rise (Khanikor & Bora, 2012).

The tested *O. majorana* essential oil obviously had an impact on the various hemocyte counts. The evaluated essential oil decreased the number of granulocytes (Gr) and plasmatocytes (Pl). Compared to the control, the percentage of (Gr) dramatically dropped from 26.47 to 18.81%. Additionally, after exposure, the proportion of (Pl) dropped from 31% in the control to 21%. Furthermore, when compared to control levels of 7.6 and 5%, respectively, the percentages of Oe and Sp significantly raised to 19.2 and 13.2%, respectively. These findings corroborate those of Giulianini, Bertolo, Battistella, & Amirante (2003). Granulocytes are the most prevalent phagocytes in the

RPW's hemolymph, just like in other insect species. (Chiang, Gupta, & Han, 1988; Carter & Green, 1987). Oenocytoids identified in the RPW are consistent with those observed in *Pseudomonas aeruginosa* and *Melolontha melolontha* (Devauchelle, 1971), (Giulianini et al, 2003).

The current findings were in agreement with those made by Abou-taleb, Zahran, & Gad (2015), who found that corpora allata (CA) activity and various haemoglobin counts decreased in *Spodoptera littoralis* larvae in their fourth instar after exposure to the insecticides lufenuron and chlorfluazuron. Additionally, Saxena & Tikku (1990) showed that plumbagin therapy damaged haemoglobin and prevented plasmatocytes and granulocytes from elongating their filopodia. (The types that are active in defence mechanisms). While oenocytoids had a high mitotic rate and a quick turnover, Arnold, & Hinks (1983) speculated that this was a method for releasing waste products from their metabolism into the hemolymph. Additionally, Sharma et al (2008) demonstrated that plasmatocytes (Pl) and granular hemoglobinocytes were the main targets of oil treatment. (Gr).

Our findings reveal that, compared to control larvae, the amount of total protein, lipid, and carbohydrates in *R. ferrugineus* 4th instar larvae treated with LC_{50} of *O. majorana* EO after 48 hours significantly decreased (Fig. 1).

Proteins are important biochemical building blocks required for an organism to grow, develop, and carry out its essential functions. Our findings reveal that, the total protein, lipid, and carbohydrates of the hemolymph of treated RPW larvae were significantly decreased after 48 hr. This decrease in protein concentration is likely the result of a decrease in protein synthesis or an increase in protein degradation, as well as a potential EO interaction with hormones that control protein synthesis (Vijayaraghavan, Sivakumar, Zadda kavitha, & Sivasub ramanian, 2010). Nasr, Sendi, Moharramipour, & Zibaee (2017) reported similar outcomes, reporting that the amount of total protein decreased following treatment with the LC_{50} dosage of *O. vulgare* essential oil.

Insect physiology is greatly influenced by carbohydrates, which serve as their primary source of energy. (Chapman, 1998; Kaufmann & Brown, 2008). Also, lipids are a crucial source of energy for ecdysis, cell maintenance, embryonic growth, and reproduction. In fatty bodies, they are conserved. (Chapman, 1998).

Sharma, Mohanm, Dua, & Srivastava (2011) observed a similar trend in the reduction of lipid levels in Anopheline and Culicine larvae following treatment with *A. annua* extract. Because of how plant EOs affect lipid metabolism and how stress-induced lipid reserves are used for energy production, there may be a reduction in lipid levels in plant EO-treated larvae. (Olga, Fevizi, & Ekrem, 2006).

Insect physiology is greatly influenced by carbohydrates, which serve as their primary source of energy. (Chapman, 1998; Kaufmann & Brown, 2008). Similar findings were made by Dris, Tine-djebbar, Bouabida, & Soltani (2017) who found that after treating *Cx. pipiens* larvae and pupae with *O. basilicum* essential oil, glucose levels were decreased. Insects treated for carbohydrate depletion are likely as a result of energy costs during stressful situations.

This outcome was consistent with Tarigan, Dadang, & Sakti harahap (2016) findings that total carbohydrate, protein, and fat contents in *T. castaneum* and *C. maculatus* considerably decreased in all treatments compared to controls.

According to Yazdani, Sendi, & Hajizadeh (2014) all concentrations of *T. vulgaris* and *O. vulgare* essential oils significantly reduced the total protein, fat, and carbohydrate content of larvae. Additionally, Bouguerra & Boukoucha (2021) showed that fourth-instar *Cx. pipiens* larvae fed with *O. glandulosum* (EO) had decreased protein, lipid, and carbohydrate contents.

The findings of our study evaluated the detoxifying activity of the enzymes CAT, SOD, and AChE on RPW larvae in their fourth instar after treatment with oil extract. Our research showed that after being treated with LC_{50}, *R. ferrugineus* fourth-instar larvae had considerably higher CAT, SOD, and AChE activity than the control larvae. In agreement with our findings, Chaudhari et al (2021) found a significant rise in the activity of the enzymatic antioxidant defense systems CAT and SOD which are crucial for preventing the accumulation of free radicals produced in cells in response to oxidative stress and other stresses like exposure to pesticides and detoxifying xenobiotics as well as for maintaining cellular redox homeostasis (Zhao et al, 2017).

Similar to this, treated larvae had significantly higher AChE and SOD activity than control larvae. These findings demonstrate that these enzymes are crucial for the process of detoxifying pollutants. (Gabr, Lemmons, & El-bokl, 2022). Additionally, Agliassa & Maffei (2018) confirmed that the treated larvae had significantly higher AChE and SOD activity than the control larvae. s. These findings show that these enzymes are crucial for the process of detoxifying poisons.

In a recent study, Upadhyay et al (2019) examined the insecticidal effect of Melissa officinalis EO against *T. castaneum* and noted an increase in ROS, SOD, and CAT levels as well as a drop in the GSH/GSSG ratio.

In their examination of the effects of *Carum carvi* EO against *T. castaneum*, Petrovi *et al* (2019) suggested changes to the first line of antioxidant defense (SOD and CAT) are the important parameters whose modification would be the main factor causing the mortality of the pests.

The results of this study demonstrated that RPW 4^{th} instar larvae treated for 48 hours with LC_{50} of *O. majorana* essential oil had significantly lower levels of, α, β -estrase and higher levels of phenol oxidase (PO) than controls. El-Aziz & El-Sayed (2009) showed that the activities of, α, β esterase, and GST of *Tribolium confusum* were significantly reduced in LD_{50} dose of basil, garlic, and sesame essential oils. This finding is consistent with our findings. The extract binds to the enzyme's active site and stops the enzyme from performing its detoxifying function. In most cases, detoxification enzymes in insects have been shown to be the enzymatic defense against foreign substances and to be crucial for the maintenance of their regular physiological processes (Mukanganyama, Figueroa, Hasler, & Niemeyer, 2003).

The concentration of detoxifying enzymes, such as esterase activities on *C. maculatus* and *S. oryzae*, were all considerably decreased in all sub-lethal concentrations of *A. monophylla* oil, according to similar studies. (Nattudurai et al, 2017). However, Magierowicz, Gòrska-drabik, & Sempruch (2019), observed that carvacrol increased CAT activity in *A. advenella* homogenates while *S. hortensis* essential oil induced polyphenol oxidase (PPO). Deterrent phenols and phenolic acids are detoxified by insect PPO and peroxidase (POX) into less harmful by-products (Urbańska, Tjallingii, Dixon, & Leszczyński, 1998). Accordingly, the findings imply that PPO, but not POX, may take part in the detoxification of carvacrol and/or other *S. hortensis* EO components in the tissues of *A. advenella*. The polyphenol oxidase studies demonstrated that essential oils may be more potent biopesticides than their individual active components (Bakkali, Averbeck, Averbeck, & Idaomar, 2008).

Additionally, it is thought that when an immunological challenge occurs, oenocytoids (OEs) play key roles in the phenoloxidase (PO) cascade. (Strand, 2008). Therefore, the substantial growth in their population may have resulted from the stimulation of the immune system that caused the oil-treated larvae to secrete PO. These findings indicate that the detoxification or metabolism of *O. majorana* essential oil depends on the activities of enzymes and esterases.

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J. Entomol. Res. Soc., 25(3): 615-622, 2023 *Research Article*
Doi: 10.51963/jers.v25i3.2504 *Online ISSN:2651-3579*

A New Species of Army Ant Genus *Aenictus* (Hymenoptera: Formicidae) from India

Bikash SAHOO[1*] Sahanashree RAMAKRISHNAIAH[2a]

Priyadarsanan DHARMA RAJAN[2b] Aniruddha DATTA-ROY[1]

[1]School of Biological Sciences, National Institute of Science Education and Research, An OCC of Homi Bhabha National Institute, Khordha, Jatni, 752050, Odisha, INDIA

[2]Insect Biosystematics and Conservation Laboratory, Ashoka Trust for Research in Ecology and the Environment, Royal Enclave, Srirampura, Jakkur Post, Bengaluru, 560064, Karnataka, INDIA

Emails: [1*]bikash.sahoo@niser.ac.in, [2a]sahana.shree@atree.org, [2b]priyan@atree.org, [1]datta.roy@niser.ac.in

ORCID IDs: [1*]0000-0002-3962-9581, [2a]0000-0002-3040-2102, [2b]0000-0001-8137-3404, [1]0000-0002-5365-3318

*Corresponding author

ABSTRACT

A new species, *Aenictus kadalarensis* sp. nov., belonging to the army ant genus *Aenictus* Shuckard 1840, is discovered from Kerala, India. The species is described based on the worker caste, and it belongs to the *Aenictus pachycerus* group. This paper provides a detailed discussion of its morphology and the characteristics that distinguish this species from its closely related species within the *A. pachycerus* group.

Keywords: Western Ghats, Kerala, *A. pachycerus* species group, *A. kadalarensis* sp. nov.

Sahoo, B., Ramakrishnaiah, S., Dharma Rajan, P., & Datta-Roy, A. (2023). A new species of army ant genus *Aenictus* (Hymenoptera: Formicidae) from India. *Journal of the Entomological Research Society*, 25(3), 615-622.

Received: August 21 2023 *Accepted: October 18, 2023*

INTRODUCTION

Aenictus Shuckard 1840, is the largest genus of the subfamily Dorylinae in the old world, with most species found in the Afrotropical and Southeast Asian regions, compared to the Australian region and the southern parts of the Palearctic region (Borowiec, 2016). Workers of *Aenictus* can be identified by 8-10 segmented antenna, elevated position of the propodeal spiracle, and binodal waist (Borowiec, 2016). Phylogenetically, this genus is sister to *Aenictogiton* and *Dorylus* (Borowiec, 2019).

There are 224 species known worldwide (Antwiki, 2023). From India, 35 species have been reported, of which 22 species have been described based on the worker caste and 12 based only on the male caste (Bharti, Guenard, Bharti, & Economo, 2016; Antony & Prasad, 2022; Dhadwal & Bharti, 2023; Antweb, 2023). This exemplifies 'dual taxonomy', where a new species is described based either on the worker caste or male caste, disregarding the corresponding castes (Borowiec, 2016). Due to the remarkable differences among the castes, identifying a particular caste of the same species becomes challenging, unless both castes from the same colony are collected simultaneously.

Aenictus species found in the eastern part of the Oriental, Indo-Australian, and Australasian regions are classified into 12 species groups namely *Aenictus ceylonicus* group, *Aenictus currax* group, *Aenictus hottai* group, *Aenictus inflatus* group, *Aenictus javanus* group, *Aenictus laeviceps* group, *Aenictus leptotyphlatta* group, *Aenictus minutulus* group, *Aenictus pachycerus* group, *Aenictus philippinensis* group, *Aenictus silvestri* group, and *Aenictus wroughtoni* group (Jaitrong & Yamane, 2011; Jaitrong & Hashimoto, 2012). In this paper, we describe a newly discovered species belonging to the *Aenictus pachycerus* group. This taxonomic group can be distinguished by several features, including long 10 segmented antennae, absence of typhlatta spot, convex anterior clypeal margin which is devoid of denticles, a triangular mandible with a prominent apical tooth followed by 4-12 smaller denticles, an indistinct metanotal groove, an angular propodeal junction, a concave declivity of the propodeum, and a weakly developed subpetiolar process (Jaitrong & Yamane, 2011).

It is worth noting that the *A. pachycerus* group exhibits a wide distribution across various biogeographic regions. Specifically, this group can be found in India, Sri Lanka, Southern China, the Malay Peninsula, Sumatra, Borneo, the Philippines, New Guinea, and Australia (Jaitrong & Yamane, 2011).

This newly described species adds to the growing body of knowledge regarding the biodiversity within the *Aenictus pachycerus* group and contributes to our understanding of its geographical distribution. Further research and exploration of this group in different regions may reveal additional species and shed light on their ecological significance and evolutionary history.

MATERIALS AND METHODS

The specimens were collected from the Kadalar estate near Munnar (Idukki district, Kerala). Fieldwork was conducted during daytime around 11 AM. Worker ants were seen on the ground, actively moving on a small rotten log. Upon lifting the log, more individuals were encountered. The specimens were collected using a plastic aspirator and stored in 70% ethanol at the collection site. GPS coordinates of the collection site were noted (Datum WGS 84). Taxonomic analysis was done using ZEISS Stemi 508 stereo microscope with an attached Axiocam 208 color camera in NISER, and photographs were taken using a Keyence VHX 6000 digital microscope in ATREE. The holotype and two paratypes are deposited in ATREE Insect Museum, Bengaluru (AIMB), and will be later transferred to the Zoological Survey of India, Calicut.

Measurements

Measurements and morphological terminology follow Zettel and Sorger, 2010 and Bharti, Wachkoo, & Kumar, 2012.

HL Head length, measured in full-face view along the midline from clypeus margin to occipital margin.

HW Head width. Maximum width of head, in full-face view.

SL Scape length. Length of antennal scape excluding basal constriction.

WL Weber’s length, measured from the point at which the pronotum meets the cervical shield to the posterior margin of the metapleuron in profile.

GL Gaster length in lateral view from the anteriormost point of the first gastral segment to the posteriormost point (excluding sting).

PL1 Petiole length. Maximum length of the petiole in dorsal view.

PL2 Postpetiole length. Maximum length of the postpetiole in dorsal view.

PHL Pronotal hair length. Length of longest hair on pronotum measured in a straight line from insertion to tip.

TL Total length. HL+WL+ PL1+ PL2+GL.

CI Cephalic index. HW/HL × 100.

SI Scape index. SL/HW × 100.

RESULTS

Description of new species

Aenictus kadalarensis **sp. nov.** (Figs. 1-5)

Material examined Holotype worker (AIMB/Hy/ Fr 25004). India, Kerala, Munnar, Idukki, Kadalar tea estate, 10.1330°N 76.9977°E, 1416 m, 19.v.2022, aspirator, coll. Bikash Sahoo.

64 paratype workers; same data as holotype.

Measurements of the holotype (in mm)**:** HL, 0.72; HW, 0.6; SL, 0.47; WL, 1; PL1, 0.24; PL2, 0.26; GL, 1; TL, 3.22; CI, 83.3; SI, 78; PHL, 0.20.

Measurements of paratype (n=10) (in mm): HL, 0.71-0.76; HW, 0.66-0.68; SL, 0.47-0.56; WL, 1- 1.1; PL1, 0.24-0.27; PL2, 0.22-0.27; GL, 0.95-1; TL, 3.20-3.36; CI, 88-94.3; SI- 70.1-84.8; PHL, 0.20-0.26.

Diagnosis Worker: *A. kadalarensis* sp. nov. is unique in the following combination of features: i) Head longer than broad with subparallel margins. ii) Masticatory margin with a broad apical tooth followed by seven denticles. iii) Dorsal side of propodeum and petiole microreticulate. iv) Declivity of propodeum concave. v) Subpetiolar process present, anteroposteriorly angulate.

Description

Worker: Monomorphic body.

Head: Subrectangular in frontal view, longer than broad, posterior margin concave; lateral sides subparallel. Antennae with 10 segments; scapes short, not reaching beyond the posterior region of head (Fig. 1). Anterior clypeal margin convex, without teeth; angularly produced in the middle (Fig. 2). Typhlatta spot absent. Parafrontal ridge present, extending 1 ⁄ 3 of the head length (ca 0.25 mm), with a small tooth between antennal sockets. Mandibles triangular, a large apical tooth followed by seven denticles of similar size (Fig. 3).

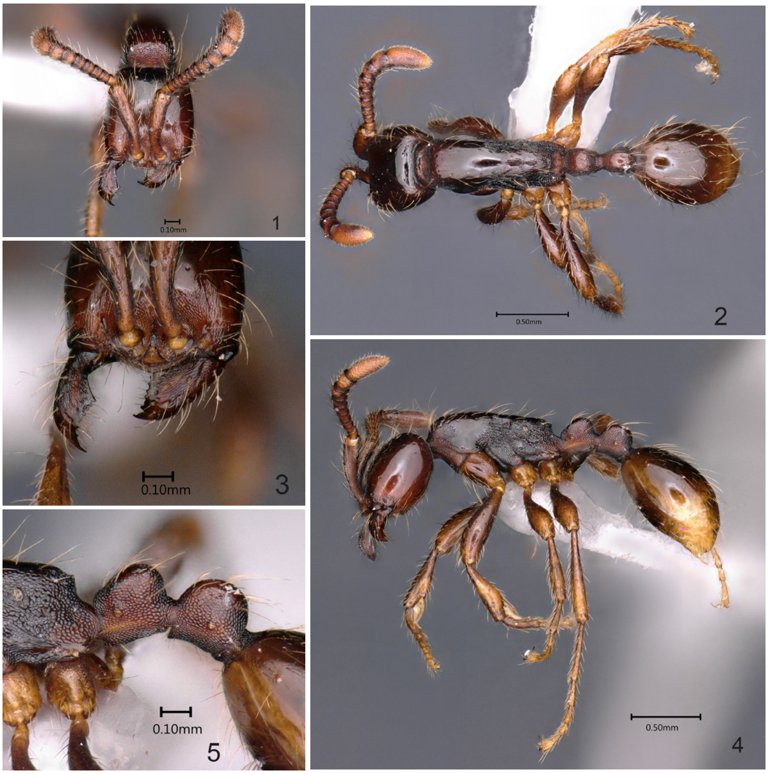
Mesosoma: Elongated**,** dorsal outline almost straight in profile view**,** pronotum moderately convex in lateral view, mesonotum straight; metanotum continuous; metanotal groove slightly impressed. Mesonotum continues with mesopleuron. Mesopleuron demarcated from metapleuron by a shallow impression. Propodeum acutely angulate. Dorsal outline of propodeum is almost straight but slightly downcurved at the most posterior part. Propodeal declivity shallowly concave, encircled by a thin rim (Fig. 2, Fig. 4).

Metasoma: Both petiole and postpetiole are almost the same length in profile; petiole slightly higher than postpetiole in width. Dorsal outline of petiole slightly tapered towards the posterior side. Postpetiole dorsally convex. Subpetiolar process low, quadrilateral shape, and anteroposteriorly angulate not developed as a process (Fig. 5). In lateral view, gaster oval with 4 tergites, sting distinct apically (Fig. 4).

Sculpture: Head smooth and shining except for hair pits in frontal view. The area between the parafrontal ridges micro-reticulate. Area beside parafrontal ridge reticulate with sparse rugae. Mandibles with longitudinal striation with scattered punctures and flagellomeres microreticulate with punctures. Reticulation on occiput is feeble (Fig. 1). Dorsally, areas behind occiput densely reticulate. Dorsum of pronotum and mesonotum smooth and shiny. Anterior most pronotum reticulate. Dorsal face of propodeum and declivity microreticulate. Dorsum of petiole microreticulate (Fig. 2). Subpetiolar process microreticulate. Dorsum of postpetiole smooth medially and feebly microreticulate. Gaster smooth and shiny except hair pits. In lateral view, pronotum smooth posteriorly and microreticulate anteriorly. In some individuals, lateral pronotum microreticulate posteriorly as well. Mesonotum, petiole, and postpetiole microreticulate in lateral view. Mesopleuron and metapleuron microreticulate along with moderate longitudinal striation. Legs feebly reticulate (Fig. 4).

Pilosity: Whole body with sparse, suberect long hairs. Long hairs present on scape, occiput, dorsum of petiole, postpetiole, and gaster. Short hairs present on lateral mesosoma, petiole, and postpetiole. Relatively denser and short hairs on funiculus of antenna, tibia, and tarsus of legs (Fig. 2, Fig. 4).

Color: Dark reddish brown to black. Head and antennae dark reddish brown. Occiput and mandible black. Mesosoma, petiole, and postpetiole reddish brown to black. Gaster is black except the apical part, which is yellowish. Legs light yellow to black (Figs. 1-5).



Figures 1-5. *Aenictus kadalarensis* sp. nov., worker (holotype). 1) Head in full-face view, 2) Body in dorsal view, 3) Mandibles, 4) Body in lateral view, 5) Petiole with subpetiolar process.

Male: Unknown

Queen: Unknown

Habitat and natural history: The specimens were collected from the ground where a few workers were seen on a rotten log and more individuals were present under the log. The collection was made in the month of May, prior to the monsoon. The type locality is a tea and cardamom plantation surrounded by evergreen forest. The forest vegetation is mostly comprised of *Cullenia exarillata, Mesua ferrea, Palaquium ellipticum* trees along with other tree species like *Diospyros sylvatica, Drypetes elata, Cinnamomum keralense, Syzygium gardneri, Dimocarpus longan, Aglain jainii*, and *Litsea oleoides* (Pascal, Ramesh, & De Franceschi, 2004). This area is part of the Southern Western Ghats biodiversity hotspot.

Etymology: The name of the species is derived from the type locality, Kadalar tea estate, Munnar, Kerala. The species is known only from the type locality.

Comparative notes

The *Aenictus pachycerus* species group comprises 17 species (Antwiki, 2023; Jaitrong, Yamane, & Tasen, 2012; Jaitrong & Wiwatwitaya, 2013; Jaitrong & Yamane, 2011; Yamane & Wang, 2015), of which 4 species are present in India; *A. aitkenii* (Forel, 1901), *A. dentatus* (Forel, 1911), *A. pachycerus* (Smith, 1858), and *A. punensis* (Forel, 1901) (status of *A. aratus* Forel, 1900 in India is dubious (Shattuck, 2008)) (Bharti et al., 2016). *A. kadalarensis* sp.nov. can be distinguished from these species by having a smooth pronotum (microreticulated in *A. aitkenii* and *A. dentatus*) and the lack of longitudinal rugulae on propodeum dorsum (present in *A. punensis*).

Aenictus kadalarensis sp. nov. is similar to *Aenictus carolianus* Zettel and Sorger, 2010, known from Cebu island (Philippines), in general morphology and body measurements. The new species can be differentiated from the latter by the following characters.

- Presence of subpetiolar process in *A. kadalarensis* sp. nov. (Fig. 6-1), which is absent in *A. carolianus* (Fig. 6-2).

- Dorsal propodeum and dorsal petiole are microreticulate in *A. kadalarensis* sp. nov. (Fig. 7-1), while smooth in the latter (Fig. 7-2).

- *A. kadalarensis* sp. nov. has a mandible with a large apical tooth followed by seven denticles, whereas *A. carolianus* has a mandible with a large apical tooth followed by only around five denticles.

Based on these distinct characters as well as the geographical separation from its closely resembling species, we can consider it a valid species.

Aenictus kadalarensis sp. nov. keys out at couplet 17 given by Bharti et al. 2012. Couplet 17 is edited, and a new couplet is added here.

17. Longitudinal rugulae present on propodeal dorsum; head shining except for microreticulate band of occiput……………………………..……..…*A. punensis* (Forel)

– Longitudinal rugulae lacking on propodeal dorsum; head partly shiny or completely microreticulate ………………..………………18

18. Head opaque and everywhere microreticulate………*A. pachycerus* (Fr. Smith)

– Head smooth and shining except the area close to parafrontal ridges.............
....*A. kadalarensis* sp. nov.

Figures 6. 1) *A. kadalarensis* sp.nov., subpetiolar process present, 2) *A. carolianus*, subpetiolar process absent (modified and taken from Zettel & Sorger, 2010).

Figures 7. 1) *A. kadalarensis* sp.nov., dorsal propodeum and dorsal petiole microreticulate, 2) *A. carolianus*, smooth dorsal propodeum and dorsal petiole (modified and taken from Zettel & Sorger, 2010).

ACKNOWLEDGMENTS

BS and ADR thank the Forest Department of Kerala for the field support and permission (File No. KFDHQ/4962/2020-CWW/WL10 dated 26.12.2020). BS acknowledges CSIR- UGC JRF and NISER for funding. BS thanks Avrajjal, Vivek, Sebinster, Augustin, and Rajah for their help during fieldwork. SR and PDR acknowledge the Department of Biotechnology, Government of India, for the financial assistance through a major research project on “Bio-resource and Sustainable livelihoods in North East India (BT/01/17/NE/TAX)” for the lab equipment, and Global Conservation, USA for financial support.

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J. Entomol. Res. Soc., 25(3): 623-644, 2023
Doi: 10.51963/jers.v25i3.2510

Research Article
Online ISSN:2651-3579

An Annotated Catalogue of the Superfamily Diaprioidea (Hymenoptera) in the Middle East

Neveen S. GADALLAH[1] Jan MACEK[2] Hassan GHAHARI[3]*

[1]Entomology Department, Faculty of Science, Cairo University, Giza, EGYPT

[2]Department of Entomology, National Museum /Nat.Hist./, CZ-193 00 Praha 9 - Horní Počernice, CZECH REPUBLIC

[3]Department of Plant Protection, Yadegar-e Imam Khomeini (RAH) Shahre Rey Branch, Islamic Azad University, Tehran, IRAN

e-mails: [1]n_gadallah@cu.edu.eg; [2]jan.macek@nm.cz; [3]*hghahari@yahoo.com

ORCID IDs: [1]0000-0002-4381-9599; [2]0000-0003-3104-325X; [3]0000-0001-6781-3776

*Corresponding author

ABSTRACT

A review of the genera and species of the superfamily Diaprioidea reported so far from the Middle East is provided with available host information. Data presented here is based on a review of existing literature by the authors, and reexamination of available specimens. Forty-eight species and 18 genera in two families (Diapriidae and Ismaridae) are recorded from the Middle East, with special reference to the Iranian fauna as being the more diverse country with this group of parasitic wasps. One species, *Psilomma dubia* Kieffer, 1908 (Diapriidae) is newly recorded for the Middle East. Among the 48 recorded species, 17 (35.4%) are found to be endemic or subendemic to the Middle East.

Keywords: Species diversity, checklist, new record, Diapriidae, Ismaridae.

Gadallah, N. S., Macek, J., & Ghahari, H. (2023). An Annotated Catalogue of the Superfamily Diaprioidea (Hymenoptera) in the Middle East. *Journal of the Entomological Research Society*, 25(3), 623-644.

Received: August 19 2023 *Accepted: September 14, 2023*

INTRODUCTION

Diaprioidea Haliday, 1833 is a hymenopteran superfamily containing five extant families, Austroniidae, Diapriidae, Ismaridae, Maamingidae, and Monomachidae. These families were formerly included in the superfamily Proctotrupoidea (Sharkey et al., 2012). It was first proposed a separate superfamily by Sharkey (2007) including Diapriidae, Maamingidae and Monomachidae. Currently, only two families Diapriidae and Ismaridae are reported from the Middle East.

Diapriidae Haliday, 1833 is a speciose family of small parasitoid wasps with 2100 described species distributed in 194 genera (Johnson, 1992; Belokobylskij & Lelej, 2017; Johnson, Musetti, & Cora, 2021), and three subfamilies: Diapriinae, Belytinae and Ambositrinae (Comério, Perioto, & Rosa Lara, 2016). In total, 800 species of Diapriidae within 90 genera have been recorded from the Palaearctic region so far (Belokobylskij & Lelej, 2017). Diapriids are tiny wasps with an average length of body between 2.0-4.0 mm, never exceeding 8.0 mm, mainly black and shiny, with antennae inserted on a shelf or at some distance above the level of the clypeus; the scape is distinctly elongate and at least 2.5 times as long as wide; the fore wing has one closed cell (radial) or none, or sometimes it is almost veinless (Belokobylskij & Lelej, 2017). Diapriids shows considerable diversity of forms, with aptery, fairly common, sometimes in both sexes. Nearly all species exhibit noticeable sexual dimorphism, with most notable differences in the antennae (Masner, 1993; Perichot & Nel, 2008). They typically attack larvae and pupae of wide range of insects, principally of dipterans, but a number of species are closely associated with ant nests (Loiácono, Margaría, & Acquino, 2013).

Among the Diapriidae, the subfamilies Belytinae and Diapriinae have been recorded from some of the Middle Eastern countries. Diapriinae is a cosmopolitan subfamily including about 1000 described species (Johnson, 1992). Most species of this subfamily are pupal or puparial endoparasitoids of Diptera or more rarely Coleoptera or Formicidae (Notton, 2014). Diapriinae are often a major component of the microhymenopteran fauna attacking Diptera in a range of habitats, but despite this they remain poorly known (Notton, 2014). On the other hand, Belytinae may be the most primitive subfamily based on morphology and hosts (Masner in Goulet & Huber, 1993). Species inhabit mostly moist places. They appear to be restricted to Mycetophilidae and Sciaridae (Diptera) as hosts (Masner in Goulet & Huber, 1993). The members of the subfamily Ambositrinae being distributed in Australia, New Zealand, Africa and South America, but the others have almost worldwide distributions (Belokobylskij & Lelej, 2017).

The family Ismaridae Thomson, 1858 is a monogeneric family within the superfamily Diaprioidea (Sharkey et al., 2012), with 57 described species worldwide in a single widespread extant genus, *Ismarus* Haliday, 1835 (Kim, Copeland, & Notton, 2018b). Palaearctic Ismaridae comprises 13 species (Johnson, 1992; Belokobylskij & Lelej, 2017). These are small to medium-sized (1.5-3.5 mm) parasitoids with a mainly dark body. Female antenna 15-merous, male antenna 14-merous; male antennomere 4 or rarely antennomeres 3 and 4 have tyloids; antennae inserted only slightly above clypeus; antennal shelf and notauli not developed; wing venation reduced, only a closed radial cell is developed; metasoma with one large basal tergite and 5 narrow segments beyond it (Belokobylskij & Lelej, 2017). It has been reported from all zoogeographical regions (Kim et al., 2018a), which some of them have wide distribution (Masner, 1976; Liu, Chen, & Xu, 2011). They prefer to inhabit higher elevations in wooded areas in warmer climatic zones, and at low elevations in cooler climatic zones (Kim et al., 2018a, b). From literature available, species of Ismaridae are hyperparasitoids of planthoppers (Hemiptera) via Dryinidae (Hymenoptera) (Chambers, 1955, 1981; Nixon, 1957; Wall, 1967; Kozlov, 1971; Masner, 1976; Jervis, 1979; Tussac & Tussac, 1991; Olmi, 2000). Ismaridae can also be parasitoids of cocoons of Dryinidae wasps, so Ismaridae may potentially be detrimental for biological control (Kim, Notton, Ødegaard, & Lee, 2018a).

The aim of this paper is to catalogue all the Diaprioidea (Diapriidae and Ismaridae) data of the Middle Eastern countries.

MATERIALS AND METHODS

The published data on the superfamily Diaprioidea (Hymenoptera) in the Middle East is summarized. The new specimens of this research were collected using Malaise traps placed at Chaharmahal & Bakhtiari, and Mazandaran provinces. The specimens were transferred to 75% ethanol for preserving, and studied by stereomicroscope; additionally, the specimens of Samin et al. (2018) which are deposited in her private collection were reexamined. The present checklist comprises the following data: 1/ the valid taxon name, 2/ published records for Iran with provincial distribution, 3/ distributional data (distribution in the Middle East and extralimital distribution), and 4/ host records. Classification and nomenclature are based on Johnson (1992) and Aguiar et al. (2013), and for distributional data, the related references are given. The provinces of Iran, and also the countries adjacent to Iran are represented in Figure 1.

Figure 1. Map of Iran with provincial boundaries, and adjacent countries (Ghahari, H., Gadallah, N.S., & Wahis, R. (2014)).

RESULTS

In this paper, a total of 45 diapriid species belonging to 18 genera and two subfamilies (Belytinae, and Diapriinae), and three ismarid species belonging to the genus *Ismarus* Haliday, 1835 are recorded from four Middle Eastern countries, Egypt, Iran, Syria, and Turkey. *Psilomma dubia* Kieffer, 1908 is recorded for the first time from the Middle East. An undetermined *Trichopria* sp. (of the *Trichopria keralensis* species group) was also first recorded for Saudi Arabia and Yemen by Kim, Notton, & Lee (2016).

Superfamily Diaprioidea Haliday, 1833

Family Diapriidae Haliday, 1833

Subfamily Belytinae Förster 1856

Genus *Acanopsilus* Kieffer, 1908

Acanopsilus Kieffer, 1908: 426. Type species: *Acanopsilus clavatus* Kieffer, 1908 *(= Belyta heterocera Haliday, 1857),* by monotypy.

Acanopsilus heterocera **(Haliday, 1857)**

Belyta heterocerus Haliday, 1857: 169, ♀.

Distribution in Iran: Golestan, Guilan, Mazandaran (Izadizadeh et al., 2023b).

Distribution in the Middle East: Iran (Izadizadeh et al., 2023b).

Extralimital distribution: Austria, Azerbaijan, China, Czech Republic, Georgia, Germany, Greece, Hungary, Italy, Russia, South Korea, Sweden, Turkmenistan, Ukraine, United Kingdom, Uzbekistan (Chemyreva & Kolyada, 2021).

Host records: Unknown.

Genus *Acanosema* Kieffer, 1908

Acanosema Kieffer, 1908: 407. Type species: *Acanosema rufum* Kieffer, 1908, by original designation.

Acanosema nervosum (Thomson, 1859)

Cinetus nervosus Thomson, 1859: 165, ♂.

Distribution in Iran: Golestan, Guilan, Mazandaran (Izadizadeh et al., 2023b).

Distribution in the Middle East: Iran (Izadizadeh et al., 2023b).

Extralimital distribution: Abkhazia, Azerbaijan, Czech Republic, Hercegovina, Hungary, Italy, Moldova, Norway, Poland, Russia, Slovakia, Sweden, Ukraine, United Kingdom (Hellén, 1964; Macek, 1990; Chemyreva & Kolyada, 2021).

Host records: Larvae of Sciaridae and Mycetophilidae (Diptera) living in rotten Wood (Nixon, 1957).

Genus *Belyta* Jurine, 1807

Belyta Jurine, 1807: 311. Type species: *Belyta bicolor* Jurine, 1807, by monotypy.

Belyta abrupta Thomson, 1859

Belyta abrupta Thomson, 1859: 168, ♂, ♀.

Distribution in Iran: Golestan, Guilan, Mazandaran (Izadizadeh et al., 2023a).

Distribution in the Middle East: Iran (Izadizadeh et al., 2023a).

Extralimital distribution: Austria, Czech Republic, Finland, France, Germany, Italy, Slovakia, Sweden, Switzerland (Izadizadeh et al., 2023a), Russia (Chemyreva in Belokobylskij & Lelej, 2017).

Host records: Unknown.

Belyta bicolor Jurine, 1807

Belyta bicolor Jurine, 1807: 311, plate 14, ♀.

Distribution in Iran: Guilan (Izadizadeh et al., 2023a).

Distribution in the Middle East: Iran (Izadizadeh et al., 2023a).

Extralimital distribution: Czech Republic, England, Germany, Hungary, Sweden, Switzerland (Macek, 1996).

Host records: Unknown.

Belyta depressa Thomson, 1859

Belyta depressa Thomson, 1859: 169, ♂, ♀.

Distribution in Iran: Alborz, Golestan, Guilan, Mazandaran, Qazvin (Izadizadeh et al., 2023a).

Distribution in the Middle East: Iran (Izadizadeh et al., 2023a).

Extralimital distribution: Austria, Belgium, Czech Republic, Finland, France, Germany, Italy, Malta, Poland, Slovakia, Sweden, United Kingdom (Nixon, 1957; Hellén, 1964; Wall, 1993; Macek, 1996; Notton & Mifsud, 2019), Russia (Chemyreva in Belokobylskij & Lelej, 2017).

Host records: Unknown.

Belyta elongata Thomson, 1859

Belyta elongata Thomson, 1859: 174, ♀.

Distribution in Iran: Mazandaran (Izadizadeh et al., 2023a).

Distribution in the Middle East: Iran (Izadizadeh et al., 2023a).

Extralimital distribution: Austria, Czech Republic, Finland, France, Germany, Ireland, Italy, Slovakia, Sweden, United Kingdom (Nixon, 1957; Hellén, 1964; Wall, 1993; Macek, 1996), Poland (Macek, 1996), Russia (Chemyreva in Belokobylskij & Lelej, 2017).

Host records: Unknown.

Belyta rugosicollis Kieffer, 1909

Belyta (*Belyta*) *rugosicollis* Kieffer, 1909: 490, ♀.

Distribution in Iran: Golestan, Guilan, Mazandaran (Izadizadeh et al., 2023a).

Distribution in the Middle East: Iran (Izadizadeh et al., 2023a).

Extralimital distribution: Austria, Czech Republic, France, Germany, Ireland, Sweden, Switzerland, United Kingdom (Nixon, 1957; Wall, 1967; Macek, 1996).

Host records: Unknown.

Belyta sanguinolenta Nees, 1834

Belyta sanguinolenta Nees, 1834: 431, ♂, type lost.

Distribution in Iran: Golestan, Guilan, Mazandaran (Izadizadeh et al., 2023a).

Distribution in the Middle East: Iran (Izadizadeh et al., 2023a).

Extralimital distribution: China, Czech Republic, Finland, France, Germany, Hungary, Japan, Malta, Norway, Poland, Romania, Russia, Slovakia, Sweden, Switzerland, United Kingdom (Nixon, 1957; Hellén, 1964; Wall, 1993; Macek 1996; Notton & Mifsud, 2019), Taiwan (Macek, 1996).

Host records: Unknown.

Belyta validicornis **Thomson, 1859**

Belyta validicornis Thomson, 1859: 168, ♂.

Distribution in Iran: Alborz, Golestan, Mazandaran (Izadizadeh et al., 2023a).

Distribution in the Middle East: Iran (Izadizadeh et al., 2023a).

Extralimital distribution: Austria, Czech Republic, Finland, Germany, Hungary, Italy, Slovakia, Sweden, Switzerland, United Kingdom (Nixon, 1957; Wall, 1993; Macek, 1996), Russia (Chemyreva in Belokobylskij & Lelej, 2017).

Host records: Unknown.

Genus *Diphora* Förster, 1856

Diphora Förster, 1856: 140. Type species: *Diphora westwoodi* Förster, 1856, by monotypy.

Diphora westwoodi **Förster, 1856**

Diphora westwoodi Förster, 1856: 141, ♀.

Distribution in Iran: Golestan, Guilan, Mazandaran (Izadizadeh et al., 2023b).

Distribution in the Middle East: Iran (Izadizadeh et al., 2023b).

Extralimital distribution: Austria, France, Germany, Romania, Switzerland, United Kingdom (Kieffer, 1916; Wall, 1967; Fabricius, 1980).

Host records: Unknown.

Genus *Pantolyta* Förster, 1856

Pantolyta Förster, 1856: 128. Type species: *Pantolyta atrata* Förster, 1861, by subsequent monotypy of Förster, 1861 .

Pantolyta nixoni **Macek, 1993**

Pantolyta nixoni Macek, 1993: 46, ♂, ♀.

Distribution in Iran: Mazandaran (Izadizadeh et al., 2021a).

Distribution in the Middle East: Iran (Izadizadeh et al., 2021a).

Extralimital distribution: Azerbaijan, Czech Republic, Germany, Hungary, Poland, Russia, Sweden (Macek 1993; Chemyreva & Kolyada, 2019a).

Host records: Unknown.

Pantolyta pallida **Kieffer, 1908**

Pantolyta pallida Kieffer, 1908: 430, ♂, ♀.

Distribution in Iran: Guilan (Izadizadeh et al., 2021a).

Distribution in the Middle East: Iran (Izadizadeh et al., 2021a).

Extralimital distribution: Armenia, Czech Republic, England, Georgia, Germany, Hungary, Japan, Kazakhstan, Mongolia, North Korea, Poland, Russia, South Korea, Sweden, Tajikistan, Turkmenistan, Ukraine (Macek, 1993; Chemyreva & Kolyada, 2019a).

Host records: Unknown.

Genus *Psilomma* Förster, 1856

Psilomma Förster, 1856: 132. Type species: *Psilomma fusciscapis* Förster, 1861, by subsequent monotypy of Förster, 1861: 43.

Psilomma dubia **Kieffer, 1908**

Psilomma dubia Kieffer, 1908: 426, ♂.

Distribution in Iran: Mazandaran province, Tonekabon, Jangal-e 3000, 2♂♂, leg. H. Ghahari, 14-16.viii.2011. New record for the fauna of Iran.

Distribution in the Middle East: Iran (new record).

Extralimital distribution: Czech Republic, England, France, Hungary, Ireland, Scotland, Sweden, Poland (Macek, 1990), Estonia, Poland, Russia, South Korea, Ukraine (Chemyreva & Kolyada, 2021).

Host records: Unknown.

Comments: This species is similar to *Psilomma fusciscapis* Förster, 1861, but differs from it by structure of antennae, produced base of macrosternite, homogenous basal striation of macrotergite and conspicuous pronotal shoulders (Macek, 1990).

Psilomma fusciscapis **Förster, 1861**

Psilomma fusciscapis Förter, 1861: 43, ♂.

Distribution in Iran: Golestan, Guilan, Mazandaran (Izadizadeh et al., 2023b).

Distribution in the Middle East: Iran (Izadizadeh et al., 2023b).

Extralimital distribution: Austria, Azerbaijan, Czech Republic, Hungary, Poland, Russia, Sweden (Macek 1990; Chemyreva & Kolyada, 2021).

Host records: Unknown.

Genus *Synacra* Förster, 1856

Synacra Förster, 1856: 134. Type species: *Diapria brachialis* Nees, 1834, designated by Ashmead (1893).

Synacra sociabilis **(Kieffer, 1904)**

Neoropria sociabilis Kieffer, 1904: 53, ♂.

Distribution in Iran: Guilan (Izadizadeh et al., 2023b).

Distribution in the Middle East: Iran (Izadizadeh et al., 2023b).

Extralimital distribution: Austria, Bulgaria, Czech Republic, Finland, France, Germany, Hungary, Luxembourg, Mongolia, Netherlands, Russia, Slovakia, Ukraine, United Kingdom (Macek, 1995; Chemyreva & Kolyada, 2019b).

Host records: This species is associated with nests of Ants (Hymenoptera: Formicidae) of the genera *Formica* and *Lasius* (e.g., *Lasius brunneus* (Latreille)). Some species were reared from sciarid flies larvae in a mushroom (Macek, 1995). Chemyreva & Kolyada, (2019b) collected two specimens from nests of *Formica rufa* L.

Subfamily Diapriinae Haliday, 1833

Genus *Aneuropria* Kieffer, 1905

Aneuropria Kieffer, 1905: 35. Type species: *Aneuropria clavata* Kieffer, 1911 (= *Polypeza foersteri* Kieffer, 1910), first included species.

Aneuropria foersteri (Kieffer, 1910)

Polypeza försteri Kieffer, 1910: 718, ♀ [♂].

Distribution in Iran: Golestan (Izadizadeh et al., 2020).

Distribution in the Middle East: Iran (Izadizadeh et al., 2020).

Extralimital distribution: Denmark, Germany, Finland, Ukraine, United Kingdom, Russia (Johnson, 2015).

Host records: Unknown.

Genus *Basalys* Westwood, 1833

Basalys Westwood, 1833: 343. Type species: *Basalys fumipennis* Westwood, 1833, by monotypy.

Basalys steueri (Kieffer, 1905)

Loxotropa Steueri Kieffer, 1905: 108, ♀.

Distribution in the Middle East: Egypt (Kieffer, 1905, 1916 both as *Loxotropa steueri*).

Extralimital distribution: Known only from Egypt.

Host records: Recorded by Kieffer (1916) in association with the following ant species: *Camponotus silvaticus* (Olivier), *Monomorium clavicorne* André, and *Pheidole sinaitica* Mayr.

Genus *Coptera* Say, 1836

Coptera Say, 1836: 281. Type species: *Coptera polita* Say, 1836, by monotypy.

Coptera depressa (Kieffer, 1911)

Galesus (*Schizogalesus*) *depressus* Kieffer, 1911a: 844, ♀.

Distribution in the Middle East: Syria (Chemyreva in Belokobylskij & Lelej, 2017).

Extralimital distribution: Europe, Russia (Chemyreva in Belokobylskij & Lelej, 2017).

Host records: Unknown.

Coptera inaequalifrons (Jansson, 1942)

Galesus (*Schizogalesus*) *inaequalifrons* Jansson, 1942: 211, ♂, ♀.

Distribution in Iran: Guilan (Samin et al., 2018).

Distribution in the Middle East: Iran (Samin et al., 2018), Turkey (Petrov & Beyarslan, 1996).

Extralimital distribution: Sweden, United Kingdom (Nixon 1980 as *Psilus inaequalifrons*).

Host records: Recorded by Nixon (1980 as *Psilus inaequalifrons*) as being a parasitoid of the lonchaeid *Lonchaea fugax* Becker (= *Lonchaea cariecola* Czerny).

***Coptera silvestrii* (Kieffer, 1913)**

Galseus (*Schizogalesus*) *silvestrii* Kieffer, 1913: 91, ♂, ♀.

Distribution in Iran: Southern Khorasan (Amini, Sadeghi, Lotfalizadeh, & Notton, 2014).

Distribution in the Middle East: Iran (Amini et al., 2014).

Extralimital distribution: Nigeria (Kieffer, 1913 as *Galesus* (*Schizogalesus*) silvestrii).

Host records: Recorded by Kieffer (1913 as *Galesus silvestrii*) as a parasitoid of the tephritids *Ceratitis anonae* (Graham), *Ceratitis nigerrima* Bezzi, and *Ceratitis giffardi* Bezzi. In Iran, it has been recorded as a parasitoid of *Carpomya vesuviana* Costa (Diptera: Tephritidae) (Amini et al., 2014).

Genus *Diapria* Latreille, 1796

Diapria Latreille, 1796: 110. Type species: *Ichneumon conicus* Fabricius, 1775, designated by Latreille (1810).

***Diapria conica* (Fabricius, 1775)**

Ichneumon conicus Fabricius, 1775: 343, sex not cited.

Distribution in Iran: Guilan, Mazandaran (Izadizadeh et al., 2020).

Distribution in the Middle East: Iran (Izadizadeh et al., 2020).

Extralimital distribution: Austria, Czech Republic, Denmark, Finland, France, Sweden, United Kingdom, North America (Kozlov, 1978; Johnson, 2015).

Host records: Recorded by Kieffer (1916) and Nixon (1980) as being a parasitoid of the syrphid *Eristalis tenax* (Linnaeus).

Genus *Entomacis* Förster, 1856

Entomacis Förster, 1856: 121. Type species: *Diapria* (*Glyphidopria*) *platyptera* Haliday, 1857, designated by Muesebeck & Walkley (1951).

***Entomacis perplexa* (Haliday, 1857)**

Diapria (*Glyphidopria*) *perplexa* Haliday, 1857: 172, ♂, ♀.

Distribution in Iran: Lorestan (Samin et al., 2018).

New material examined: Chaharmahal & Bakhtiari province, Lordegan (Deh-Chenar), 2♀, leg. M. Shahi, 16.vii.2010.

Distribution in the Middle East: Iran (Samin et al., 2018).

Extralimital distribution: Austria, Canada, Czech Republic, Germany, Hungary, Japan, Moldova, Poland, Russia, Slovakia, USA (Chemyreva, 2015), China (Chemyreva in Belokobylskij & Lelej, 2017), Georgia (Japoshvili, 2022), Sweden, United Kingdom (Kieffer, 1916 as *Hemilexis perplexa*).

Host records: Recorded by Nixon (1980) as a parasitoid of the ceratopogonid *Forcipomyi bipunctata* (Linnaeus) (= *F. picea* Winnertz).

Genus *Monelata* Förster, 1856

Monelata Förster, 1856: 123. Type species: *Diapria parvula* Nees, 1834, designated by Ashmead (1893).

***Monelata aegyptiaca* Priesner, 1953**

Monelata aegyptiaca Priesner, 1953: 451, 453, ♀.

Distribution in the Middle East: Egypt (Priesner, 1953).

Extralimital distribution: Known only from Egypt.

Host records: Unknown.

Genus *Plagiopria* Huggert & Masner, 1983

Plagiopria Huggert & Masner, 1983: 67, 72. Type species: *Plagiopria passerai* Huggert & Masner, 1983, by original designation.

***Plagiopria besucheti* Huggert & Masner, 1983**

Plagiopria besuchetti Huggert & Masner, 1983: 74, 75, ♀.

Distribution in the Middle East: Turkey (Huggert & Masner, 1983).

Extralimital distribution: Known only from Turkey.

Host records: Unknown.

***Plagiopria huberi* Huggert & Masner, 1983**

Plagiopria huberi Huggert & Masner, 1983: 74, 76, ♀.

Distribution in Iran: Tehran (Huggert & Masner, 1983).

Distribution in the Middle East: Iran (Huggert & Masner, 1983).

Extralimital distribution: Known only from Iran.

Host records: Unknown.

Genus *Psilus* Panzer, 1801

Psilus Panzer, 1801: Heft 83, plate 11. Type species: *Psilus cornutus* Panzer, 1801, by monotypy.

***Psilus carinatus* (Kieffer, 1911)**

Galesus (*Galesus*) *carinatus* Kieffer, 1911a: 846, ♂.

Distribution in the Middle East: Syria (Kieffer, 1911a, 1916 both as *Galesus carinatus*).

Extralimital distribution: Known only from Syria.

Host records: Unknown.

Genus *Spilomicrus* Westwood, 1832

Spilomicrus Westwood, 1832: 129. Type species: *Spilomicrus stigmaticalis* Westwood, 1832, by monotypy.

Spilomicrus formosus **Jansson, 1942**

Spilomicrus formosus Jansson, 1942: 215, ♂, ♀.

Distribution in Iran: Ardabil (Samin et al., 2018).

Distribution in the Middle East: Iran (Samin et al., 2018).

Extralimital distribution: Europe (Northern, Western, Eastern), Canada, Japan, Russia, USA (Chemyreva, 2018).

Host records: Unknown.

Genus *Trichopria* Ashmead, 1893

Trichopria Ashmead, 1893: 407, 431. Type species: *Trichopria pentaplasma* Ashmead, 1893, by original designation.

Trichopria aegyptiaca **Priesner, 1940**

Trichopria aegyptiaca Priesner, 1940: 71, 72, ♂, ♀.

Distribution in the Middle East: Egypt (Priesner, 1940).

Extralimital distribution: Known only from Egypt.

Host records: Unknown.

Trichopria aegyptorum **(Priesner, 1953)**

Phaenopria aegyptorum Priesner, 1953: 444, 447, ♀.

Distribution in the Middle East: Egypt (Priesner, 1953 as *Phaenopria aegyptorum).*

Extralimital distribution: Known only from Egypt.

Host records: Unknown.

Comments: Collected from detritus of an irrigation canal (Priesner, 1953).

Trichopria alexandrina **(Priesner, 1940)**

Phaenopria alexandrina Priesner, 1940: 72, ♀.

Distribution in the Middle East: Egypt (Priesner, 1940, 1953, as *Phaenopria alexandrina).*

Extralimital distribution: Known only from Egypt.

Host records: Unknown.

Trichopria atomaria **(Priesner, 1953)**

Phaenopria atomaria Priesner, 1953: 446, 446, ♀.

Distribution in the Middle East: Egypt (Priesner, 1953 as *Phaenopria atomaria).*

Extralimital distribution: Known only from Egypt.

Host records: Unknown.

***Trichopria bifoveata* Ashmead, 1895**

Trichopria bifoveata Ashmead, 1895: 898, ♀.

Distribution in the Middle East: Turkey (Petrov & Beyarslan, 1996).

Extralimital distribution: England, Germany, Sweden (Nixon, 1980).

Host records: Unknown.

***Trichopria cheopis* Priesner, 1940**

Trichopria cheopis Priesner, 1940: 71, 74, ♀.

Distribution in the Middle East: Egypt (Priesner, 1940).

Extralimital distribution: Known only from Egypt.

Host records: Unknown.

Comments: This species was collected from detritus of the Nile inundation (Priesner, 1940).

***Trichopria crassifemur* Nixon, 1980**

Trichopria crassifemur Nixon, 1980: 38, ♂.

Distribution in the Middle East: Turkey (Petrov & Beyarslan, 1996).

Extralimital distribution: England, Sweden, Switzerland (Nixon, 1980).

Host records: Unknown.

***Trichopria delicatula* Priesner, 1940**

Trichopria delicatula Priesner, 1940: 71, 76, ♀.

Distribution in the Middle East: Egypt (Priesner, 1940).

Extralimital distribution: Known only from Egypt.

Host records: Unknown.

Comments: Collected from detritus material (Priesner, 1940).

***Trichopria hannai* Priesner, 1940**

Trichopria hannai Priesner, 1940: 71, 77, ♀.

Distribution in the Middle East: Egypt (Priesner, 1940).

Extralimital distribution: Known only from Egypt.

Host records: Recorded by Priesner (1940) bred from pupae of *Drosophila* sp. (Diptera: Drosophilidae).

***Trichopria helouanensis* Priesner, 1940**

Trichopria helouanensis Priesner, 1940: 72, 81, ♀.

Distribution in the Middle East: Egypt (Priesner, 1940).

Extralimital distribution: Known only from Egypt.

Host records: Unknown.

***Trichopria inermis* Kieffer, 1909**

Trichopria inermis Kieffer, 1909: 386, sex not stated.

Distribution in Iran: Khuzestan (Rabee et al., 1993; Modarres Awal, 2012).

Distribution in the Middle East: Iran (Rabee et al., 1993; Modarres Awal, 2012), Turkey (Petrov & Beyarslan, 1996 as *T. intermis*).

Extralimital distribution: France, Germany, Sweden, Switzerland, United Kingdom (Nixon, 1980).

Host records: Recorded as a parasitoid of the calliphorid *Lucilia sericata* (Meigen); the muscid *Mesembriana meridiana* (Linnaeus), and the sarcophagid *Brachicoma devia* (Fallén) (Nixon, 1980). In Iran, recorded as a hyperparasitoid of the tachinid *Linnaemya neavi* Curran (Rabee, Siahpoush, Nazemi, & Mozaffari, 1993; Modarres Awal, 2012).

***Trichopria longicornis* (Thomson, 1858)**

Diapria longicornis Thomson, 1858: 362, ♂.

Distribution in Iran: Kermanshah (Samin et al., 2018).

Distribution in the Middle East: Iran (Samin et al., 2018), Turkey (Petrov & Beyarslan, 1996).

Extralimital distribution: Sweden (Kieffer, 1916 as *Ashmeadopria longicornis*), United Kingdom (Nixon, 1980; Notton, 2004).

Host records: Recorded by Nixon (1980) as being taken flying over nest of ant *Formica rufa* Linnaeus.

***Trichopria major* (Priesner, 1953)**

Phaenopria major Priesner, 1953: 441, 448, ♂, ♀.

Distribution in the Middle East: Egypt (Priesner, 1953 as *Phaenopria major*).

Extralimital distribution: Europe, Russia (Chemyreva in Belokobylskij & Lelej, 2017).

Host records: Unknown.

***Trichopria masrensis* Priesner, 1940**

Trichopria masrensis Priesner, 1940: 72, 79, ♀.

Distribution in the Middle East: Egypt (Priesner, 1940).

Extralimital distribution: Known only from Egypt.

Host records: Unknown.

Comments: Specimens of this species were collected in the detritus of an irrigation canal at Maadi (Priesner, 1940).

***Trichopria minor* (Priesner, 1953)**

Phaenopria minor Priesner, 1953: 443, 448, ♂, ♀.

Distribution in the Middle East: Egypt (Priesner, 1953 as *Phaenopria minor*).

Extralimital distribution: Known only from Egypt.

Host records: Unknown.

Trichopria myrmecobia **(Kieffer, 1911)**

Diapria (*Tropidopria*) *Myrmecobia* Kieffer, 1911b: 962, 978, ♂, ♀.

Distribution in Iran: West Azarbaijan (Samin et al. 2018).

Distribution in the Middle East: Iran (Samin et al., 2018), Turkey (Petrov & Beyarslan, 1996).

Extralimital distribution: Bulgaria (Petrov & Beyraslan, 1996), United Kingdom (Kieffer, 1916 as *Ashmeadopria myrmecobia*).

Host records: Recorded by Kieffer (with some doubt) (1916 as *A. myrmecobia*) in association with the *Formica* sp. (Hymenoptera: Formicidae).

Trichopria revelata **(Priesner, 1953)**

Phaenopria revelata Priesner, 1953: 445, 447, ♀.

Distribution in the Middle East: Egypt (Priesner, 1953 as *Phaenopria revelata*).

Extralimital distribution: Known only from Egypt.

Host records: Unknown.

Trichopria simulatrix **(Priesner, 1953)**

Phaenopria simulatrix Priesner, 1953: 443, 448, ♂, ♀.

Distribution in the Middle East: Egypt (Priesner, 1953 as *Phaenopria simulatrix*).

Extralimital distribution: Known only from Egypt.

Host records: Unknown.

Comments: Specimens of this species were swept from Poaceae (= Graminaceae) and taken from detritus of an irrigation canal (Priesner, 1953).

Family Ismaridae Thomson, 1858

Genus *Ismarus* Haliday, 1835

Ismarus Haliday, 1835: 467. Type species: *Cinetus Dorsiger* Haliday, 1831, by monotypy.

Ismarus dorsiger **(Haliday, 1831)**

Cinetus Dorsiger Haliday, in Curtis, 1831: 380, ♂.

Distribution in Iran: Guilan (Izadizadeh et al., 2021b).

Distribution in the Middle East: Iran (Izadizadeh et al., 2021b).

Extralimital distribution: Andorra, Bulgaria, China, Czech Republic, Denmark, Finland, France, Germany, Ireland, Italy, Montenegro, Netherlands, Norway, Russia, South Korea, Spain, Sweden, Switzerland, United Kingdom (Kim et al., 2018a), Georgia (Japoshvili, 2022).

Host records: Recorded as being a hyperparasitoid of the dryinid *Aphelopus serratus* Richards (Chemyreva in Belokobylskij & Lelej, 2017).

Ismarus halidayi **Förster, 1850**

Ismarus halidayi Förster, 1850: 285, ♀.

Distribution in Iran: Mazandaran (Izadizadeh et al., 2021b).

Distribution in the Middle East: Iran (Izadizadeh et al., 2021b).

Extralimital distribution: Azerbaijan, Bulgaria, Canada, China, Czech Republic, Denmark, Finland, Georgia, Germany, Hungary, Ireland, Japan, Mongolia, Netherlands, North Africa, Norway, Russia (European, Far East, Siberia), Scotland, South Korea, Sweden, United Kingdom, USA (Kim et al., 2018a; Chemyreva in Belokobylskij & Lelej, 2017).

Host records: Recorded as a hyperparasitoid of the dryinids *Anteon jurineanum* Latreille (Chambers, 1955; Olmi, 2000; Chemyreva in Belokobylskij & Lelej, 2017), and *Anteon infectum* (Haliday) (Chambers, 1981).

Ismarus rugulosus **Förster, 1850**

Ismarus rugulosus Förster, 1850: 284, ♀.

Distribution in Iran: Golestan (Izadizadeh et al., 2021b), Kermanshah, Northern Khorasan, Southern Khorasan (Rahmani et al., 2019).

Distribution in the Middle East: Iran (Rahmani et al., 2019; Izadizadeh et al., 2021b).

Extralimital distribution: Austria, Bulgaria, Canada, Czech Republic, Denmark, Finland, France, Germany, Ireland, Italy, Kazakhstan, Kyrgyzstan, Netherlands, Norway, Russia, Slovakia, Sweden, Ukraine, United Kingdom, USA (Kim et al., 2018a), Korea (Chemyreva in Belokobylskij & Lelej, 2017).

Host records: Recorded as a hyperparasitoid of the dryinids *Anteon pubicorne* (Dalman) (Waloff, 1975; Perkins, 1976), and *Lonchodryinus ruficornis* (Dalman) (Waloff, 1975; Olmi, 2000). It was also reared from female of cicadellid *Streptanus sordidus (*Zetterstedt) (Chemyreva in Belokobylskij & Lelej, 2017).

DISCUSSION

Taxonomic and faunistic knowledge of Diaprioidea in the Middle East is very poor due to the paucity of regional studies. The wasp fauna of most of the Middle Eastern countries is largely ignored despite of the rich and diverse flora in most of them. For example, for the fauna of Iran as the richest in the 18 Middle East countries, only two papers on Ismaridae (Rahmani et al., 2019; Izadizadeh et al., 2021b), and seven papers on Diapriidae (Rabee et al., 1993; Amini et al., 2014; Samin et al., 2018; Izadizadeh et al., 2020; Izadizadeh et al., 2021a, 2023a, b) have been published so far.

In the present checklist, totally 48 species of the superfamily Diaprioidea in 18 genera and two families (Diapriidae and Ismaridae), have been reported from four of the Middle Eastern countries (Egypt, Iran, Syria, and Turkey). An undetermined *Trichopria* sp. (of the *Trichopria keralensis* species group) was also first recorded for Saudi Arabia and Yemen by Kim et al. (2016). *Psilomma dubia* Kieffer, 1908 is newly recorded for the Middle East. In total, 28 species of Diaprioidea in 15 genera and two families (Diapriidae: 25 species; Ismaridae: three species) have been reported from Iran so far. This is followed by Egypt with 15 species in three genera, Turkey with seven species in three genera, and Syria with two species in two genera (all in the family Diapriidae). Fourteen diapriid species are so far only known from Egypt (endemic or subendemic to Egypt): *Basalys steueri* (Kieffer), *Monelata aegyptiaca* Priesner, *Trichopria aegyptiaca* Priesner, *T. aegyptorum* (Priesner), *T. alexandrina* (Priesner), *T. atomaria* (Priesner), *T. cheopis* Priesner, *T. deliculata* Priesner, *T. hannai* Priesner, *T. helouanensis* Priesner, *T. masrensis* Priesner, *T. minor* Priesner, *T. revelata* (Priesner), and *T. simulatrix* (Priesner). On the other hand, only a single species, *Psilus carinatus* (Kieffer) is endemic to the Syrian fauna, and one species (*Plagiopria huberi* Huggert & Masner) is known to be endemic or subendemic to the Iranian fauna. Among the 18 genera of the Middle East Diaprioidea, the genus *Trichopria* (Diapriinae) with 18 recorded species is the most diverse, followed by *Belyta* (Belytinae) with seven species.

Among the countries adjacent to Iran, Russia with 153 species of Diapriidae in 29 genera, and nine species of Ismaridae in the genus *Ismarus* (Belokobylskij & Lelej, 2017) is more diverse than the other countries. Additionally, among the Middle East and adjacent countries to Iran, Russia shares the greatest number of species with Iran (14 species), followed by Turkey (four species), Azerbaijan (three species), Kazakhstan and Turkmenistan (each with two species), and Armenia (one species). None sharing between Iran and Egypt with 28 and 15 species, respectively proves a great difference between the fauna of these two countries which are located in two various geographically regions (Ethiopian and Palaearctic).

Among the 31 provinces of Iran, Diaprioidea have been recorded from 14 provinces (Alborz, Ardabil, Chaharmahal & Bakhtiari, Golestan, Guilan, Kermanshah, Khuzestan, Lorestan, Mazandaran, Northern Khorasan, Qazvin, Southern Khorasan, Tehran, and West Azarbaijan), in which Guilan with 14 species was found to be the richest, followed by Mazandaran and Golestan with 13 and 11 species, respectively. These results are biased towards the more sampled provinces, and without any faunistic survey in the most regions of Iran. Also, parasitoid-host relationships were recoreded for only two Iranian diapriid species: *Coptera silvestrii* as a parasitoid of *Carpomya vesuviana* Costa (Diptera: Tephritidae) (Amini et al., 2014), and *Trichopria inermis* as a hyperparasitoid of *Linnaemya neavi* Curran (Diptera: Tachinidae) (Rabee et al., 1993; Modarres Awal, 2012).

From the data provided, many more species are expected to occur in the Middle East countries. Therefore, further collections and studies are needed to clarify the distribution of this group of wasps in the other Middle Eastern countries.

ACKNOWLEDGEMENTS

The authors would like to express gratitude to C.-J. Kim and J.-W. Lee (South Korea) for editing the first draft of the manuscript, L. Masner (Canada) and V. Chemyreva (Russia) for providing some papers. We thank the anonymous reviewers for insightful comments and suggestions. This research was supported by Islamic Azad University (Yadegar-e Imam Khomeini (RAH) Shahre Rey Branch), and Cairo University (Egypt).

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For faunistic research follow this order, Distribution:.., Material examined:…, Host plant:….etc.

Example:
Sphex oxianus Gussakovskij, 1928
Distribution: Central and South West Asia, Afghanistan, Iran, Israel, Turkey (Bohart and Menke, 1976; Menke and Pulawski, 2000; Kazenas, 2001), Turkey: Artvin (De Beaumont, 1967).
Material examined: Ankara, Altındağ, Çubuk Dam Lake, 900 m, 29.06.1998, 1 ♂; Kalecik, 600 m, 24. 07. 2001, 2 ♀♀, Kalecik, 800 m, 25. 07. 2001, 3 ♀♀
Host plant: *Echinophora* sp.
Please use ♀, ♂ symbols. Please write upper genus categories with capital letters.

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