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Synergistic Effect of Three Medicinal Plant Extracts and Their Nutritional Indices on the *Tribolium castaneum* Herbst and *Ephestia kuehniella* Zeller

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ABSTRACT

Chemical control of stored pests causes environmental pollution, contamination of stored products and endangering human health. Botanical compounds are one of the suitable alternatives for this aim. In this study effects of eucalyptus, rosemary and mentha extracts alone and in mixed of them on *T. castaneum* and *E. kuehniella* were evaluated. Due to our research, the most effect of rosemary extract on the *T. castaneum* adult, and eucalyptus on the 3rd and 4th instars larvae of *E. kuehniella* observed. The most significant synergetic on the *T. castaneum* adult was the combination of ME, MR and ER. Also, the most synergetic effect of extracts on the 3rd and 4th instar larvae of *E. kuehniella* were related to ME, and MR and ME respectively. The lowest amount of *T. castaneum* adult Feeding deterrence percentage indices (FDI) and Approximately Digestibility (AD) were observed in rosemary extract and the lowest amount of Efficiency of conversion of ingested food (ECI), Relative consumption rate (RCR) and The Relative growth rate (RGR) was related to eucalyptus extract. The lowest amount of *E. kuehniella* larvae FDI was observed in rosemary extract and the lowest amount of ECI, RGR and AD was related to eucalyptus extract. Therefore, these botanical extracts can be used to control these two important stored pests.

Keywords: Botanical extract, Combination, Feed, Mortality.

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INTRODUCTION

The Red flour beetle, *Tribolium castaneum* Herbst from the Tenebrionidae family is one of the most common and destructive pests of stored products all over the world, which are usually found abundantly in stored legumes and grains (Patil, 2020). Adult insects and larvae of this pest feed on flour, broken and stored seeds in factories and silos, and cause considerable duge (Arthur et al, 2019). Also, due to the rapid increase in population, stored products are contaminated with larval shells and feces, and its quality is greatly reduced (Kumar, 2023). It is estimated that about 10-30% post-harvest damage is caused by insects, microorganisms and other factors on stored products every year (Gustavsson, Cederberg, Sonesson, Van Otterdijk, & Meybeck, 2011).

The Mediterranean flour moth, *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) is another important storage pest, which is highly polyphagous and has a global distribution (Javanshir, Karimzadeh, Hejazi, & Shayestehmehr, 2022).

Larvae of this pest prefer stored products such as beans, grains, dried fruits and even dried mushrooms (Razmjou, Afshari, & Abedi, 2022). While feeding, larvae cause the loss of food due to the secretion of silk threads (Shahriari, Sahebzadeh, & Zibaee, 2017). Today, to control stored pests, fumigant chemical pesticides and sometimes radioactive rays are used, which have irreparable risks for humans and the environment (Ahmadi & Zolfagharieh, 2015). Also, the resistance of stored pests to methyl bromide and phosphine fumigants has been reported in many countries (Singh, Nebapure, Taria, Sagar, & Subramanian, 2023). Therefore, a lot of research is currently being done in order to use new compounds of plant origin that have less environmental risks. Many plants and their secondary metabolites have physiological and behavioral effects such as repelling and deterring insects (Ṣengül Demirak & Canpolat, 2022). Therefore, the use of plant compounds is considered one of the best ways to control stored pests (Papachristos & Stamopulos, 2002).

Plant extracts and essential oils have metabolites that affect the nutritional, biological and immune properties of insects. Also, these compounds are a mixture of monoterpenoids (phenol, ketone, hydrocarbon acid, etc.) that usually affect the growth, reproduction, and survival of insects and can be used against agricultural pests (GaiGaire, Scharf, & Gondhalekar, 2020). These compounds are lipophilic and somewhat volatile. For this reason, it quickly enters the body of insects and by affecting the nervous system, interferes in their physiological and behavioral processes and causes changes in biological processes such as reduced nutrition, delayed molting, death of larvae and pupae, and sterility of adults (Jankowska, Rogalska, Wyszkowska, & Stankiewicz, 2017).

Research has also been conducted on the killing and repellent effects of plant extracts on storage pests, including the red flour beetle (Campolo et al, 2014). Researchers have reported that in many countries, in order to prevent pest attacks, stored products are mixed with plant extracts and powders, thus preventing their damage (Bezabih, Satheesh, Workneh Fanta, Wale, & Atlabachew, 2022). Eucalyptus, with the scientific name *Eucalyptus globulus* Labil from the Mirtaceae family, is one of the most important species in the world, which is a native of southeastern Australia, which was imported to Iran more than a hundred years ago (Abdossi, Yaghooti Moghaddam, & Hadipanah, 2015). Eucalyptus extract has insecticidal properties due to the presence of several monoterpenes (cineole, linalool and citronellol) (Khedhri et al, 2023). The compound extracted from the leaves and seeds of eucalyptus has shown insecticidal and repellent effects on *Trogoderma granarium* Everts (Mostafa, Hossain, Hossain, Biswas, & Hague, 2012). Rosemary (*Rosmarinus officinalis* L.) is one of the medicinal, herbaceous and aromatic plants of the Lamiaceae family. This plant belongs to the mint family and is distributed almost all over the world and is also cultivated in Iran. Rosemary has antioxidant activity and this characteristic is related to carnosol and carnosic acid compounds. Also, the activity of lipoxygenase inhibiting enzyme and the inhibitory effect of rosemary extract on arachidonic acid metabolism have also been reported (Salehi Sardoei, Falahaimani, & Gholamshahi, 2020). Mentha with the scientific name *Mentha piperita* L. is an herbaceous and perennial plant belonging to the Lamiaceae family. Although this plant is scattered all over the world, its spread is more in temperate and humid regions such as the Mediterranean (Sadat & Ladan Moghadam, 2019). Mentha leaves contain 0.5-4% volatile oil, which consists of 50-78% free menthol and 5-20% menthol combined with other compounds. This species produces terpenes and a variety of other compounds that are mainly stored in the epidermal glands of leaves, stems and reproductive organs (Karimi & Mohsenzadeh, 2013). The lethal, repellent, anti-nutritional and anti-spawning effects of peppermint extracts have been reported (Wubie, Bezabeh, & Kefelegn Kebede, 2014).

Food quality is calculated with nutritional indices parameters. Favorable foods rich in carbohydrates and proteins are preferred and affect growth, immunity, survival and reproduction indices of *E. kuehniella* larvae (Ghasemi, Jalali Sendi, & Zibaee, 2018). Due to the limitation of the use of chemical pesticides in the control of stored pests, in this research the insecticidal activity and synergistic activities of eucalyptus, rosemary and mentha extracts against *T. castaneum* and *E. kuehniella* were evaluated. Also, one of the important impact of pesticides on pests is the effect on nutritional indicators. In this research, the effect of plant extracts on nutritional indicators, effective factors such as relative growth rate, relative consumption rate efficacy of conversion of ingested food and feeding deterrent indices were calculated.

MATERIAL AND METHODS

Collection of studied plants

Eucalyptus, rosemary and mentha leaves that were identified by botanists of Urmia University's botany department were collected from Urmia region in spring. Then, they were dried in the laboratory of Urmia University in the shade and at a temperature of 24˚C, then they were powdered using an electric mill and stored in a refrigerator at a temperature of 4˚C.

Extraction method

Soxhlet was used for extraction. Each time, 50 gr powder of the desired part of the plants was poured inside the tubed filter paper and placed inside the Soxhlet finger. Then, about 280 ml of 95% ethanol was poured into the balloon. After turning on the device, the balloon was gradually heated from the bottom and reached the boiling point. A little before reaching the boiling point was opened the cold water. The extraction of all plants continued for at least 3 hours. After obtaining the extracts, the solvent was evaporated using a rotary device. The extracts were poured into glass containers and kept in the refrigerator at a temperature of 4˚C.

***T. castaneum* rearing**

The primary population of adult insects was prepared from Jihad Agriculture Insectarium of Urmia and grown on food containing 10 parts of white flour and one part of yeast at a temperature of 27±1˚C, relative humidity of 60±5% a light to dark period of 14:10 hours.

***E. kuehniella* rearing**

The initial colony of Mediterranean flour moth was obtained from Jihad Agriculture Insectarium of Urmia. The rearing of insect larvae was conducted by meal flour and 2% yeast. Also, sugar solution was used to adult feed. The flour containing insect eggs were placed in plastic containers kept in the growth chamber with a temperature of 25±1˚C and a relative humidity of 60±5% and a light to dark period of 14:10 hours. When eggs hatched, flour was available to feed the larvae. After one generation, the eggs obtained from adults were used in the experiments of this research.

Contact toxicity bioassay of extracts

In order to obtain the minimum and maximum concentrations (20-80% mortality) of the extracts used in the experiment, first a series of preliminary experiments were conducted on the *T. castaneum* adult and the *E. kuehniella* 3^{rd} and 4^{th} instar larvae. Based on the preliminary experiments, five concentrations for each extract were calculated logarithmically. And along with control (distilled water) in a volume of 0.5ml of each concentration of the mixture in water were used in experiment (Robertson, Russell, Preisler, & Savin, 2007). Twenty insects were transferred into each petri dish and were kept at a temperature of 25±2°C and a relative humidity of 65±5%. To feed insects, one gr of flour, without mixing with the extract, was placed on filter paper in the Petri dish. The number of dead in each treatment was recorded 6, 12, 18 and 24 hours after treatment. This experiment was repeated 3 times. To evaluate toxicity, toxicity indices and relative toxicity were used from equations 1 and 2 (Sun. 1950). And data means were compared using Tukey's test.

Equation (1): Relative toxicity= $\left(\frac{LC50\ \text{The least effective poison}}{LC50\ \text{of another compound}}\right)$

Equation (2): Toxicity indices= $\left(\frac{LC50\ \text{of the most potent poison}}{LC50\ \text{of another compound}}\right)*100$

Synergistic activity extract mixtures

Synergistic interactions between extracts to evaluate potential synergies between the extracts (eucalyptus, rosemary and mentha), mixtures were prepared maintaining the same concentration of single extract, following 1:1 ratio of the oils. Mixtures were applied to *T. castaneum* adults, *E. kuehniella* 3rd and 4th instar larvae and their LC_{50} values were estimated after 24 hours. Relationships of mixtures and comparison of expected and observed LC_{50} values it was determined using Wadley's statistical model. Where E refers to expected LC_{50} and A is the proportion of oil A in the mixture. LC_{50}(a) is the LC_{50} of oil A and b is the proportion of oil B in the mixture, as well as LC_{50}(b) is the LC_{50} of oil B and the rest according to Wadley, theoretical LC_{50} values were calculated from:

Equation 3: $$E=\frac{a+b+c+\cdots+n}{\frac{a}{LC50(a)}+\frac{b}{LC50(b)}+\frac{c}{LC50(c)}+\cdots\frac{cn}{LC50(cn)}}$$

Where E, a, b, c… and n are as described above. The interaction between the observed and theoretical LC_{50} values (Equation 4) was compared:

Equation 4: $$R=\frac{Expected\ LC50}{Observed\ LC50}$$

Where R represents synergistic interaction: the relationship between the constituents of the mixture is defined as either synergistic (when $R > 1.5$), additive ($1.5 \geq R > 0.5$), or antagonistic ($R \leq 0.5$), based on this model.

Effect of extracts on the nutritional indices

In order to investigate the effect of different concentrations of extracts on nutritional indicators, according to the method of Huang, Lam, & Ho (1998), the diet of each insect was prepared. Before starting the experiment, the number of 10 *E. kuehniella* 3rd and 4th larvae and *T. castaneum* adult were weighed separately and added to the petri dish containing treated food as a repetition. Insects to be treated were starved for 24 hours. Insects and treated food residue were reweighed after 72 hours. This test was done in three repetitions. The feeding inhibition indices was done separately in the same conditions. The nutrition indices used by Farrer, Barbour, & Kennedy (1989) method. Obtained data modified by Huang et al., 2018 is as follows:

The Relative growth rate (RGR) = (A - B) / (B × day)

Relative consumption rate (RCR) = D / (B × day)

Efficiency of conversion of ingested food (ECI) (%) = (RGR / RCR) × 100

Feeding deterrence percentage indices (FDI) (%) = (C - T) / C × 100

Approximately Digestibility (AD) = $[(W_i - W_f) / Wi] \times 100$

Where W_i is dry weight of food eaten per larva after t time (mg), W_f is dry weight of produced waste (mg).

A: Weight of live insect after three days (mg per person insect)

B: Initial weight of insect (mg per person insect)

C: Weight of food consumed in the control condition (mg per insect)

D: The amount of food eaten after three days (mg of food eaten per person insect)

T: Weight of food consumed in the treatment condition (mg per insect)

Data means were compared using Tukey's test.

RESULTS

Contact Toxicity

The lethal effects of eucalyptus, rosemary and mentha extracts on *T. castaneum* adult using contact bioassay are shown in the table 1. The results showed that all extracts contact toxicity against *T. castaneum* adult in a time- and dose-dependent manner, and a concentration of 5% resulted in the highest mortality. The highest mortality rate of 96.66% was observed for rosemary at 24 hours of exposure.

Table 1. Mortality of *Tribolium castaneum* adult after contact treatment with three extract oils.

Treatments	**Concentration(μL/mL)**	**Mortality ± SD^b (%) at Different Time (h) of Treatment**			
		6	12	18	24
Mentha	1	3.3±1.2^e	6.6±1.8^e	10.00±1.3^d	10.00±1.7^d
	2	13.33±0.6^d	13.33±0.7^d	13.33±1.2^d	13.33±1.2^d
	3	20.00±0.9^c	20.00±0.9^c	26.66±1.6^c	43.33±2.3^c
	4	30.00±1.3^b	36.66±2.4^b	40.00±3.0^b	70.00±1.9^b
	5	43.33±3.3^a	50.00±2.9^a	66.66±3.6^a	80.00±4.0^a
Control		0.00±0.0^e	0.00±0.0^f	0.00±0.0^e	0.00±0.0^e
Rosemary	1	3.33±1.2^d	6.66±2.4^e	6.66±2.4^e	13.33±2.2^e
	2	3.33±1.2^d	16.66±2.2^d	20.00±1.9^d	23.33±1.6^d
	3	16.66±1.2^c	33.33±1.2^c	43.33±1.3^c	46.66±2.8^c
	4	43.33±1.3^b	53.33±3.3^b	53.33±3.3^b	73.33±4.3^b
	5	53.33±2.3^a	76.66±3.6^a	86.66±4.6^a	96.66±4.6^a
Control		0.00±0.0^d	0.00±0.0^f	0.00±0.0^f	0.00±0.0^f
Eucalyptus	1	3.33±1.2^e	6.66±0.5^e	6.66±2.4^e	6.66±2.4^e
	2	13.33±1.2^d	20.00±2.1^d	20.00±1.9^d	23.33±1.6^d
	3	20.00±1.9^c	33.33±1.1^c	36.66±1.4^c	43.33±1.3^c
	4	30.00±1.0^b	56.66±2.6^b	56.66±2.6^b	56.66±2.6^b
	5	56.66±2.6^a	63.33±2.3^a	63.66±2.3^a	63.66±2.3^a
Control		0.00±0.0^e	0.00±0.0^f	0.00±0.0^f	0.00±0.0^f

*Means with common letters are in the same statistical group at 5% probability level (Tukey test).

Also, the lethal effects of extracts on the 3rd and 4th instar larvae of *E. kuehniella* results showed that contact toxicity is time, larval instar and dose-dependent and a concentration of 5% resulted in the highest mortality. The highest mortality rates of 3rd and 4th instar larvae were observed in eucalyptus with 63.33% and 53.33% after 24 hours. (Table 2, 3).

Table 2. Mortality of 3rd instar larvae of *Ephestia kuehniella* after contact treatment with three extract oils.

Treatments	Concentration (µL/mL)	Mortality ± SD^{b} (%) at Different Time (h) of Treatment			
		6	12	18	24
Mentha	1	6.66±1.4^{c}	6.66±1.4^{d}	13.33±1.2^{d}	13.33±1.2^{d}
	2	6.66±0.4^{c}	23.33±1.6^{c}	23.33±1.6^{c}	26.66±2.6^{c}
	3	20.00±1.9^{b}	26.66±1.6^{bc}	26.66±1.6^{bc}	30.00±2.0^{bc}
	4	26.66±1.6^{ab}	30.00±2.0^{ab}	30.00±2.0^{b}	33.33±2.2^{b}
	5	33.33±1.2^{a}	36.66±1.4^{a}	53.33±2.3^{a}	56.66±2.6^{a}
Control		0.00±0.0^{d}	0.00±0.0^{e}	0.00±0.0^{e}	0.00±0.0^{e}
Rosemary	1	3.33±1.0^{d}	6.66±2.1^{d}	10.00±1.0^{d}	13.33±1.2^{d}
	2	6.66±0.4^{cd}	23.33±1.6^{c}	30.00±1.0^{c}	30.00±1.0^{c}
	3	13.33±1.2^{c}	33.33±1.9^{b}	36.66±1.4^{bc}	36.66±1.4^{bc}
	4	23.33±2.6^{b}	36.66±1.4^{b}	40.00±2.0^{ab}	46.66±2.8^{ab}
	5	33.33±1.2^{a}	43.33±1.3^{a}	46.66±2.8^{a}	50.00±2.0^{a}
Control		0.00±0.0^{d}	0.00±0.0^{e}	0.00±0.0^{e}	0.00±0.0^{e}
Eucalyptus	1	0.00±0.0^{d}	6.66±0.4^{e}	13.33±0.2^{d}	20.00±0.9^{d}
	2	6.66±0.4^{c}	13.33±1.2^{d}	26.66±1.6^{c}	30.00±2.0^{cd}
	3	20.00±1.9^{b}	26.66±2.6^{c}	26.66±2.6^{c}	36.66±2.4^{c}
	4	30.00±2.0^{ab}	33.33±1.2^{b}	36.66±2.4^{b}	46.66±2.8^{b}
	5	36.66±2.4^{a}	43.33±2.3^{a}	46.66±2.8^{a}	63.33±2.3^{a}
Control		0.00±0.0^{d}	0.00±0.0^{f}	0.00±0.0^{e}	0.00±0.0^{e}

*Means with common letters are in the same statistical group at 5% probability level (Tukey test).

Table 3. Mortality of 4th instar larvae of *Ephestia kuehniella* after contact treatment with three extract oils.

Treatments	Concentration (µL/mL)	Mortality ± SD^{b} (%) at Different Time (h) of Treatment			
		6	12	18	24
Mentha	1	0.00±0.0^{d}	3.33±0.2^{d}	6.66±0.4^{d}	6.66±0.4^{d}
	2	10.00±1.0^{c}	16.66±1.2^{c}	23.33±1.6^{c}	24.33±1.9^{c}
	3	20.00±1.9^{b}	23.33±1.6^{bc}	23.33±1.6^{c}	25.66±1.0^{bc}
	4	26.66±2.6^{ab}	30.33±1.9^{b}	30.00±1.0^{b}	33.33±1.2^{b}
	5	33.33±2.2^{a}	43.33±2.3^{a}	46.66±2.8^{a}	46.66±2.8^{a}
Control		0.00±0.0^{d}	0.00±0.0^{d}	0.00±0.0^{e}	0.00±0.0^{e}
Rosemary	1	0.00±0.0^{d}	3.33±0.2^{e}	10.00±1.0^{d}	13.33±2.2^{d}
	2	3.33±0.2^{d}	13.33±1.2^{d}	20.00±1.9^{c}	23.33±2.6^{c}
	3	10.00±0.8^{c}	16.66±1.2^{cd}	26.66±1.6^{bc}	30.00±2.0^{b}
	4	16.66±1.2^{b}	26.66±2.6^{b}	36.66±2.4^{ab}	40.00±2.0^{ab}
	5	26.66±1.6^{a}	36.66±2.4^{a}	40.00±2.0^{a}	43.33±2.3^{a}
Control		0.00±0.0^{d}	0.00±0.0^{e}	0.00±0.0^{e}	0.00±0.0^{e}
Eucalyptus	1	0.00±0.0^{e}	13.33±1.2^{c}	23.33±1.6^{c}	26.66±2.6^{d}
	2	6.66±0.4^{d}	16.66±1.2^{bc}	23.33±1.6^{c}	30.00±1.5^{cd}
	3	13.33±1.2^{c}	20.00±1.9^{b}	26.66±1.6^{bc}	36.66±2.4^{c}
	4	20.00±1.9^{b}	30.00±2.0^{ab}	36.66±2.4^{ab}	46.66±3.8^{b}
	5	30.00±2.0^{a}	33.33±2.2^{a}	40.00±2.8^{a}	53.33±3.3^{a}
Control		0.00±0.0^{e}	0.00±0.0^{d}	0.00±0.0^{d}	0.00±0.0^{e}

*Means with common letters are in the same statistical group at 5% probability level (Tukey test).

Synergistic activity extract mixtures on the *T. castaneum* adults

Insecticidal effect of tree extracts (eucalyptus, rosemary, mentha) were evaluated (Table 4). The most significant synergy based on Wadley's determination was the combination of ME, MR and ER, and the synergy ratio was R > 1.5. Also no one of the extract mixtures showed antagonistic interaction R < 0.5 or additive 1.5 ≥ R > 0.5 based on Wadley's calculation.

Table 4. Synergistic interaction of three binary combinations of three extract oils against *Tribolium castaneum* after 24 h contact application.

Treatments	LC_{50}	Intercept+5	Slope± SE	df [b]	X^2	Expected LC_{50} [e]		
						Wadley[f]	R[h]	S[i]
M	8.41 (6.02-18.02)	3.14	2.01±0.44	3	1.94	-	-	-
R	9.05 (6.41-20.44)	2.88	2.22±0.49	3	3.60	-	-	-
E	11.80 (7.54-48.39)	2.39	2.43±0.67	3	3.34	-	-	-
ME	9.20 (6.52-21.50)	2.71	2.38±0.55	3	1.61	687.97	3.1	Syn
ER	10.43 (7.04-30.89)	2.53	2.43±0.61	3	1.88	848.05	2.2	Syn
MR	7.39 (5.54-13.59)	3.17	2.11±0.43	3	2.04	407.12	2.3	Syn

[a] Lethal concentration (LC50); [b] df = Degree of freedom; [c] X2 = Chi square; [e] Expected LC50 based on each calculation model; [f] Wadley's calculation of expected LC50; [h] R = Determination of interaction of the mixture based on Wadley's determination method: when R > 1.5, synergistic (Syn) interaction; when 1.5 _ R > 0.5, additive (Add) interaction; when R _ 0.5, antagonistic (Anta) interaction; [i] S = Synergy ratio from Wadley's calculation.

Synergistic activity extract mixtures on the 3rd and 4th instar larvae of *E. kuehneilla*

Insecticidal effect of tree extracts (eucalyptus, rosemary, mentha) on the 3rd instar larvae of *E. kuehniella* were evaluated (Table 5). The most significant synergy based on Wadley's determination was the combination of ME the synergy ratio was R > 1.5. And ER and MR of the extract mixtures showed additive interaction 1.5 ≥ R > 0.5 based on Wadley's calculation.

Table 5. Synergistic interaction of three binary and one binary combinations of three extract oils against 3rd instar larvae of *Ephestia kuehniella* after 24 h contact application.

Treatments	LC_{50} [a]	Intercept+5	Slope± SE	df [b]	X^{2c}	Expected LC_{50} [e]		
						Wadley[f]	R[h]	S[i]
M	4.46 (4.15-9.51)	3.84	1.57±0.34	3	4.30	-	-	-
R	4.15 (3.24-6.38)	4.11	4.45±0.32	3	0.81	-	-	-
E	2.86 (2.22-3.81)	4.37	1.38±0.31	3	2.75	-	-	-
ME	1.66 (1.17-2.08)	4.64	1.63±0.31	3	1.63	3.48	2.10	Syn
ER	5.03 (3.91-8.18)	3.87	1.61±0.38	3	0.06	3.38	0.67	Add
MR	4.03 (3.16-6.13)	4.13	1.44±0.32	3	0.95	4.30	1.06	Add

[a]Lethal concentration (LC50); [b]df = Degree of freedom; [c]X2 = Chi square; [e]Expected LC50 based on each calculation model; [f]Wadley's calculation of expected LC50; [h]R = Determination of interaction of the mixture based on Wadley's determination method: when R > 1.5, synergistic (Syn) interaction; when 1.5 _ R > 0.5, additive (Add) interaction; when R _ 0.5, antagonistic (Anta) interaction; [i] S = Synergy ratio from Wadley's calculation.

Insecticidal effect of tree extracts (eucalyptus, rosemary, mentha) on the 4th instar larvae of *E. kuehniella* were evaluated (Table 6). The most significant was the combination of ME and MR. And ER of the extract mixtures showed additive interaction.

Table 6. Synergistic interaction of three binary and one binary combinations of three extract oils against 4th instar larvae of *Ephestia kuehniella* after 24 h contact application.

Treatments	LC_{50}[a]	Intercept+5	Slope± SE	df [b]	X[2c]	Expected LC_{50}[e]		
						Wadley[f]	R[h]	S[i]
M	5.08 (4.06-7.61)	3.68	1.87±0.35	3	0.86	-	-	-
R	4.79 (3.67-8.10)	4.02	1.44±0.32	3	2.55	-	-	-
E	3.47 (2.46-6.67)	4.47	0.97±0.30	3	1.56	-	-	-
ME	2.22 (1.68-2.78)	4.48	1.51±0.31	3	2.01	4.12	1.85	Syn
ER	5.69 (4.45-9.10)	3.58	1.88±0.37	3	4.39	4.02	0.71	Add
MR	4.31 (3.41-6.46)	4.01	1.57±0.33	3	0.22	4.93	1.14	Syn

[a] Lethal concentration (LC50); [b] df = Degree of freedom; [c] X2 = Chi square; [e] Expected LC50 based on each calculation model; [f] Wadley's calculation of expected LC50; [h] R = Determination of interaction of the mixture based on Wadley's determination method: when R > 1.5, synergistic (Syn) interaction; when 1.5 _ R > 0.5, additive (Add) interaction; when R _ 0.5, antagonistic (Anta) interaction; [i] S = Synergy ratio from Wadley's calculation.

Investigating the effect of eucalyptus, rosemary and mentha extracts on the nutritional indeces of *T. castaneum* and *E. kuehniella*

The effect of extracts on nutritional indicators of *T. castaneum* adult were showed (Table 7). The lowest amount of FDI was observed in rosemary extract and the lowest amount of ECI, RCR and RGR was related to eucalyptus extract. The lowest amount of AD was observed in rosemary and control treatments.

Table 7. Feeding indices of *Tribolium costaneum* adult with extract oil.

Extracts	FDI	ECI	RCR	RGR	AD
	%			mg/day^{-1}	
Mentha	21.14±1.23[b]	35.07±2.06[bc]	0.17±0.01[ab]	0.06±0.00[b]	54.34±2.02[b]
Rosemary	14.73±0.92[c]	44.67±2.24[ab]	0.20±0.02[a]	0.09±0.01[a]	49.23±1.34[c]
Eucalyptus	38.34±2.04[a]	27.11±1.13[c]	0.11±0.00[b]	0.03±0.00[b]	62.24±2.34[a]
Control	-	47.74±1.67[a]	0.23±0.01[a]	0.11±0.00[a]	47.64±4.74[c]

Columns with same letters were not significantly different at p<0.01

The effect of extracts on nutritional indicators on the 3rd and 4th instar larvae of *E. kuehniella* were showed (Table 8, 9). The lowest amount of FDI was observed in rosemary extract and the lowest amount of ECI, RGR and AD was related to eucalyptus extract. Also, no significant difference was observed between different treatments in the RCR index.

Table 8. Feeding indices of 3rd instar larvae of *Ephestia kuehniella* with extract oil.

Extracts	FDI	ECI	RCR	RGR	AD
	%			mg day^{-1}	
Mentha	30.03±2.87[b]	37.69±2.86[c]	0.29±0.03[a]	0.11±0.01[a]	87.64±2.86[ab]
Rosemary	12.24±0.86[c]	45.31±3.04[b]	0.33±0.01[a]	0.15±0.01[a]	91.12±3.11[a]
Eucalyptus	46.26±2.14[a]	23.52±1.21[d]	0.21±0.01[a]	0.05±0.01[b]	70.26±2.72[b]
Control	-	53.04±3.04[a]	0.34±0.01[a]	0.18±0.01[a]	94.16±3.14[a]

Columns with same letters were not significantly different at p<0.01

Table 9. Feeding indices of 4th instar larvae of *Ephestia kuehniella* with extract oil.

Extracts	FDI	ECI	RCR	RGR	AD
	%			mg mg day^{-1}	
Mentha	28.73±1.53^{b}	21.32±1.01^{c}	0.20±0.01^{a}	0.43±0.03^{b}	87.14±3.51^{ab}
Rosemary	11.24±0.92^{c}	30.62±2.16^{b}	0.24±0.01^{a}	0.74±0.04^{a}	90.23±3.42^{a}
Eucalyptus	42.34±2.03^{a}	15.46±0.84^{d}	0.19±0.01^{a}	0.03±0.01^{c}	72.34±2.13^{b}
Control	-	39.08±3.41^{a}	0.29±0.03^{a}	0.11±0.02^{c}	92.82±4.53^{a}

Columns with same letters were not significantly different at p<0.01

CONCLUSION AND DISCUSSION

The results of our contact experiments showed that the most effect of rosemary extract was on adult *T. castaneum*. And on eucalyptus, it was observed on 3rd and 4th instar larvae of *E. kuehniella*. In the study of the effect of several plant extracts on *Phthorimaea operculella* (Zeller), results showed that eucalyptus and angelica extracts can be used in the integrated control of this pest (Nouri Ganbalani, Teymouri Bilesavar, Rafiee-Dastjerdi, Mardani-Talaee, & Mansouri, 2020). In the present study, eucalyptus had the best effect on *E. kuehniella* larvae. Eucalyptus essential oil can control *T. castaneum* adult by contact and fumigation method (Bagheri, Mohammadi Sharif, Hadizadeh, & Amiri-Besheli, 2011). In our contact experiment, eucalyptus had the least effect among the three plant extracts on *T. castaneum* adult. In the investigating of toxicity effect of cumin and rosemary essential oils and cumin extract on *E. kuehniella*, the results showed that the use of these extracts and plant essential oils can be suitable choice for stored pest control (Heydari Moghadam, Azimizadeh, & Mohammadi, 2018). In this study, rosemary extract also had effect on this pest. The fumigation, repellent and contact effects of eucalyptus essential oil on *T. castaneum*, due to the harmlessness of this compound for the environment and proper efficiency, this botanical composition is a suitable alternative for controlling stored pests (Bagheri et al, 2011). Similar results were obtained in the contact experiments of this study. The insecticidal effect of *Artemisia annual* L. and *Sambucus ebulus* L. extracts on *T. castaneum*, showed that all the used concentrations of both plants have high mortality, so they can be a good substitute for artificial insecticides (Jalali sendi, Haghighian, & Ali Akbar, 2003). In this study, all plant extracts studied had a lethal effect on both pests. Effect of thyme and rosemary extracts on *Bemisia tabaci* (Genn.) showed that both extracts have repellent and mortality on the adults in different concentrations (Sertkaya, Kaya, & Soylu, 2010). In this study, rosemary extract had a lethal effect on *T. castaneum* adult and on 3rd and 4th instar larvae of *E. kuehniella*. Investigations show that the lethality of aqueous extracts of the leaves of three plants, tobacco, mentha and rosemary, on the *Callosobruchus machulatus* Fabricius adult has a good control effect on pests (Asgharnejhad, & Belvasi, 2018). Similar results were obtained in this experiment. Mentha extract on *Aphis fabae* Scop caused the death of this pest, and the higher concentration of the extract, had beneficial effect (Biniaś, Gospodarek, & Rusin, 2017). In this study, eucalyptus extract had a lethal effect on both studied pests. Amazu et al. investigated the insecticidal effect of mentha on *Callosobruchus maculatus* (Fabricius). The results indicated that mentha leaves have lethal effect on the adult of this pest (Amazu et al, 2021). Similar results were obtained in this experiment. The results of Saeidi & Moharramipour research on the effect of *Artemisia khorassanica* Podl. *Rosmarinus officinalis* L. and *Mentha longifolia* L. essential oils on *T. confusum* based on LC_{50} values showed that adult insects of this pest have similar sensitivity to *A. khorassanica* and *R. officinalis* oils (Saeidi & Moharramipour, 2013). In the present study, based on LC_{50} values obtained from experiments, *T. castaneum* adult and of *E. kuehniella* larvae had different sensitivities to the plant extracts studied. In the insecticidal effects of rosemary extracts on the *Rhyzopertha dominica* (F.) and *Sitophilus oryzae* (L.) it was seen that rosemary extracts can be used in controlling the pests of stored grains (Yaman & Şimşek, 2019). Similar results were obtained in this experiment.

Essential oils represent a green alternative in agricultural fields due to reported insecticidal properties. The combination of plant extracts showed enhanced activity, with synergy rates. The nutritional indices in insects depends on the type of their food. Undesirable compounds affect the indicators of growth, immunity, survival and reproduction (Adamo, Davies, Easy, Kovalko, & Turnbull, 2016). In our research, studied plant extracts, decreased the relative growth rate, relative consumption rate and conversion efficiency of the food eaten by insects. Several researches have been conducted on the effect of plant extracts on the mortality and nutritional parameters of stored pests. Antifeedant activity of cinnamon (*Cinnamomum aromaticum* Nees.) on adult and larvae of *T. castaneum* has been reported (Huang et al, 1998). In research, the feeding inhibition effect of the extracts of two medicinal plants *Alhagi maurorum* Medik and *Berberis thunbergii* DC. on *T. castaneum* adult was investigated. The results showed that increasing the concentration of the extract of two plants was significantly effective on the indicators nutrition of pest and *A. maurorum* extract has significantly reduced the relative growth rate, relative consumption rate and conversion efficiency of food eaten by *T. castaneum* more than *B. thunbergii* extract (Taghizadeh & Mohammadkhani, 2016). In our study, the lowest amount of *T. castaneum* adult Feeding deterrence percentage indices (FDI) and Approximately Digestibility (AD) were observed in rosemary extract and the lowest amount of Efficiency of conversion of ingested food (ECI), Relative consumption rate (RCR) and The Relative growth rate (RGR) was related to eucalyptus extract. The lowest amount of *E. kuehniella* larvae FDI was observed in rosemary extract and the lowest amount of ECI, RGR and AD was related to eucalyptus extract. Findings Taghizadeh (2018) on the effect of ethanol extract of three medicinal plants *Achillea millefolium* L., *Euphorbia helioscopia* L. and *Fumaria parviflora* (Lamark) on the nutritional indicators of *T. castaneum* showed that *E. helioscopia* extract has better performance than other extracts (Taghizadeh, 2018). In our research, the studied plant extracts had different effects on the nutritional indices of the two pests.

Study the synergistic effect of *Lantana camara* L. and *Tithonia diversifolia* (Hemsl) extracts on the *T. castaneum*, the results showed that the combination of both extracts significantly increases the mortality rate. Therefore, it can be concluded that these extracts have a synergistic effect on *T. castaneum* (Henagamagea, 2023). In the present research, the results of plant extracts on the *T. castaneum* adult showed that the binary combination of all extracts has synergistic effects. And in the 3rd instar larvae, combined ME and in 4th instar larvae of *E. kuehniella* ME and MR had synergistic effects. In the investigation of the contact and fumigation toxicity of *Elsholtzia ciliata* (Thunb.) essential oil on the *T. castaneum* larvae the results showed that carone and limonene in a ratio of 1:7 have synergistic fumigant activity on the *T. castaneum* larvae (Liang et al., 2020). Similar results were obtained in this experiment.

According to the results of our research, eucalyptus, rosemary and menthe extracts alone and in mixed of them have lethal effects on *T. castaneum* and *E. kuehniella*. And they have a negative effect on their nutritional indicators, so they can be used to control these two important stored pests.

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