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Effects of Various Plant Extracts on the Development of the Potato Beetle under Laboratory and Field Conditions: A Combined Study

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ABSTRACT

In this study, we examined the effects of ethanolic extracts obtained from the various parts of *Liquidambar orientalis*, *Buxus sempervirens*, *Alnus glutinosa*, *Artemisia absinthium*, *Aesculus hippocastanum* and *Rhus coriaria* on the egg-laying behavior of the potato beetle, *Leptinotarsa decemlineata*, under laboratory conditions at 25±2°C and 60±5 % relative humidity, and tested the antifeedant and toxic effects of the two extracts leading to the smallest number of egg-laying, *Liquidambar orientalis* and *Buxus sempervirens*, with a field study. The field study showed that the average number of 6.2 larvae and 7.2 adults before treatment of the *Liquidambar orientalis* extract decrease to 1.2 larvae and 3.2 adults after treatment. With the use of the *Buxus sempervirens* extract, the 10.2 larvae and 8.4 adults that existed before the treatment were reduced to 2.6 larvae and 2.8 adults after treatment. As for the eggs laid in the plants, the control plants had 13 while *Liqudambar orientalis* decreased this number to 3 and *Buxus sempervirens* to 2. These results suggest that these two plant extracts can be used in the field as a potential alternative to chemical pesticides.

Key words: *Buxus sempervirens*, botanical pesticides, *Liquidambar orientalis*, plant extracts, potato beetle, pest management.

INTRODUCTION

Biotic and abiotic factors prevent the present agricultural areas from being used effectively. One of these biotic factors is that, pests are known to decrease the quality and productivity in forests and agricultural areas (Fenemore, 1984; Ecevit, 1988; Thacker, 2002; Isman, 2006; Matthews, 2006; Zehnder *et al.*, 2007; Bianchi *et al.*, 2013). Synthetic insecticides have been used for the last 50 years to control these pests. There are five main classes of synthetic insecticides; namely organochlorides, organophosphates and carbamates, pyrethroids, neonicotinoids and ryanoids (Ware, 1982; Dorow, 1993; Palmquist *et al.*, 2011). The most commonly used one of these are the organochlorines. Developed countries have banned their use along with various other pesticides such as the synthetic DDT, therefore leading to a search for alternative methods in management of the pests (Franzen, 1993; Thacker, 2002; Scott *et al.*, 2003; Isman, 2006; Matthews, 2006; Zehnder *et al.*, 2007). Management of pests in Turkey is undertaken largely by using chemicals, the use of which not only leads to chemical resistance in pests, but also cause the death of useful insects such as bees, or animals such as birds and fish. These chemicals may even reach humans via the food chain and eventually lead to a number of permanent or fatal diseases (Fenemore, 1984; Ecevit, 1988; FAO, 1992; Matthews, 2006; Handal *et al*., 2016).

An alternative method of minimizing the harms of pests may be the use of poisonous and healthy plant extracts which can be found in nature (Alberto *et al.*, 2010). These extracts contain anti-feedant substances and substances which regulate or inhibit beetle growth and development. They also contain substances inhibiting the effects of juvenile and the moulting hormones in beetles and/or substances imitating their functions (Whittaker and Feeny, 1971; Berenbaum, 1985).

There are many plants which may be used as pesticides. Plants were used for the first time for pesticide activities in the mid-17th century (Smith and Secoy, 1975). These plants are seen today as a potential source of natural pesticides due to the various substances they contain. Plant pesticides are effective only on the pests themselves and they have the advantage of not harming non-targeted organisms or humans. Besides, they are easily degradable and environment-friendly (Berenbaum, 1985). As opposed to traditional pesticides which managed pests only with an active substance, plant pesticides contain extracts effective on both the behaviors and the physiologies of pests (Saxena, 1987).

The main purpose of the agricultural studies is to increase the yield of product per hectare. The primary damage of Colorado potato beetle, *Leptinotarsa decemlineata* (Say, 1824) (Coleoptera: Chrysomalidae), is leaf feeding by larvae and adults, although young fruits can also be eaten when the hosts are eggplant or tomato. Leaf-feeding has the greatest effect on potato growth, if occurring within two weeks of peak flowering, whereas during the last few weeks before harvest or very early in the growth of the crop it has little effects on yield in Turkey. Up to now, chemical substances of the pyrethroid group, such as deltamethrin (Deltamethrin) have been utilized to control this pest (Ertürk and Uslu, 2007; Turkish Ministry of Agriculture and Rural Affairs, 2008).

This study identified the effects of various plant extracts on this pest in a laboratory, and then examined the two most effective ones by spraying them on potatoes in a field to test the behavior of the potato beetle, its feeding, egg-laying, larvae and adult density.

MATERIAL AND METHODS

Selection and maintenance of the pest

L. decemlineata larvae and adults were carefully collected between May and June 2002 in the areas used for growing cultures around Ordu province and its towns in Turkey. Without damaging the larvae and adults using a tool Spraying bottle, they were brought to the laboratory in plastic boxes allowed airflow. Throughout the study, *L. decemlineata* larvae and adults fed on fresh potato leaves. The larvae and adults of the potato beetle destroyed the plant by affecting its leaves and young branches as well as by laying eggs. Therefore, these two features were selected for the evaluation of results (Ertürk and Uslu, 2007). Agria varieties were used as the potato variety in this study.

Selection of the plants

In this study, we used extracts obtained from various parts of *Buxus sempervirens*, *Aesculus hipopcostonum*, *Rhus coriaria*, *Alnus glutinusa* and *Artemisia absinthium*. These plants collected from Black Sea Region of Turkey in 2003 in field. *Liquidambar orientalis* was bought from a herbalist. The identification of these plants was done according to Davis (1966-1988). The literature survey of plants used in this study has chosed. The literature survey of plants used in this study was conducted.

Preparation of plant extracts

Fresh leaves, flowers and seeds were washed under running distilled water. The fresh stems and leaves of plants were dried at 45°C for 5-6 hours. The leaves from *Lq. orientalis* were cut into pieces and grinded into powdery form using pestle and mortar and shade dried. The extract of the leaves, flowers, and seeds were prepared according to the methods described (Holopainen *et al.*, 1988; Yildirim *et al.*, 2000; Mohan *et al.*, 2009). The powders were stored in air tight plastic tube. Water and ethanolic extracts were prepared of sterile distilled water and ethanol mixing 250 gr of dry powder of plants and 1250 ml solvent, at room temperature in a round bottom flask with occasional shaking. The extracts were kept at 4°C for 5 days. Both water and ethanolic extracts were then filtered through a muslin cloth for coarse residue and they finally filtered through were filtered through 100 μm membrane filter, and then the solutions were dried with an evaporator. The crude extracts were stored at -20°C until they were used. Before their use, they were dissolved in 25% ethanol.

Bioassays

1. Laboratory study

The varieties potatoes of agria, the seed potatoes, were used in this study. According to the Provincial Directorate of Ordu, it is the most suitable variety for this region. They were planted in plastic pots (approximately 35 cm wide and 45 cm deep). The soil used was chosen carefully and mixed with natural animal fertilizers. Then an equal number and weight of potatoes were planted in the pots which were kept under controlled laboratory conditions until they leaf out. All pots were exposed to normal weather conditions. All pots were kept at 25±2°C and 60±5 % relative humidity. The potted plants were covered with veils to avoid contamination from the outside extra insects.

When the potato plants in the pots were grown enough, approximately four to five weeks after the potato plant height of 50 cm. 50 ml of each previously prepared plant extract (see, preparation of plant extracts) was transferred into plastic glass and applied to the plants homogeneously. Two pots were formed for each plant extract. Following treatment, three pairs of *L. decemlineata* adults (six in total) were placed in each pot. In order to prevent beetle crossings between pots, each pot was tied by tulle cloth allowing airflow. When they arrive, plant height 50 cm, 2 male and 4 female was left on the plants healthy potato beetle and the beetles laid their eggs. After 7 days, plant extracts were applied for a second time in a similar to the first one. These pots were then controlled weekly and prepared experiment reports. All operations were performed as triple repetition under the same conditions all the same parent plant.

2. Field study

Field study was carried out in a quarter-acre potato growing area near the town named Kabadüz in Ordu province. The Kabadüz (40°59'01"N, 37°53'02"E) is located in the south of Ordu, at an altitude of about 600 meters with an annual average temperature of 4.3°C and average relative humidity of 74.7%. Soil before planting was organized as agricultural. The plants were watered individually with drip irrigation and fertilized according to the farmer planting. Potatoes were planted in groups of 25 and the experiment was made to coincide with the chemical spraying time.

The field in farmland was divided into three plots separated by a barrier of 3-mm thick clear polyethylene film. The potatoes were planted in three blocks with 25 potato roots in each block. Each plot was further divided into three sections of 3 rows each, and each section served as a replicate for sampling. The potatoes were planted in the plot in mid-March 2006 in rows of 70 cm spacing, each with 25 plants spaced 40 cm apart. Experiment was designed with three replications and for each plant extract a total of 75 potato roots were used. These potatoes were grown until the experiment. Care was taken to ensure equal amount of plant extract application to foliage of potato plants. After determination of leaves on the ground and in the period following the first insect to be seen on a potato leaf, spray was applied in the field. Once every 7 days to monitor the population dynamics of *L. decemlineata*, larvae, adults and eggs were observed. A total of 4 samplings were conducted from 15 April 2006 to 15 May 2006. The control block and the blocks which were to receive the plant extract applications were labeled as Part 1, 2 and 3.

The two extracts found to be most effective in the laboratory study (*Lq. orientalis* and *B. sempervirens*) were chosen to be used in the field study (Table 1). The plant extract to be tested in each block was prepared in the laboratory and transferred to the field in dark-colored bottles. Following the identification of the existence of *L. decemlineata* adults, larvae and eggs on the potato plants in the marked blocks, 250 g per liter plant extracts were applied homogeneously to each potato plant with a back pulverizator (about 10-15 liters capacity, a suitcase model). The control group was sprayed with the solvent (water and ethyl alcohol) in which plant extracts were dissolved. The solvent was diluted so as not to damage the plant. After a week, the experiment was repeated in the same way. Each block was controlled weekly and experiment reports were prepared. Samples of *L. decemlineata*, larvae, adults and eggs were randomly collected from each section in a paper towel to prevent moisture, placed in plastic bags, and returned to the laboratory. The effectiveness of two extracts under field study was calculated with the Handerson-Tilton formula (Lamparski and Wawrzyniak, 2005).

Statistical analyses

The statistical analyses of the data were performed by using the SPSS for Windows (version 12.0) package program. In order to determine the effects of the two plant extracts before and after treatment on the potato beetle's larvae and adults, the significance of difference between groups was tested by one way variance analysis (one way ANOVA). When there was a difference between groups, the means was tested by post HOC tests (Tukey HSD). In the evaluation of data, if p-value is less than or equal 0.05, it was accepted as significant.

Table 1. Parts used of plants and effect of its extracts on egg-laying behavior of potato beetle, *Leptinotarsa decemlineata*, in laboratory conditions.

Plant used	Family name	Local name	Ecozone in Turkey	Part(s) used	Number of laying egg	Situation of eggs
Liquidambar orientalis	Altingiaceae	Turkish sweetgum (*Turkish*, Sığla ağacı)	Muğla-Fethiye	Rasin	45	3 egg pocket clear yellowish
Buxus sempervirens	Boxaceaea	Boxwood tree (*Turkish*, Şimşir)	North Anatolia	Seed and leaf	47	2 egg pocket darker yellowish
Alnus glutinosa	Betulaceae	Common alder (*Turkish*, Adi kızılağaç)	Northen Anatolia	Leaf	59	-
Artemisia absinthium	Asteraceae	Wormwood (*Turkish*, Pelin otu)	The whole Anatolia	Flower and leaf	65	-
Aerulus hippocastanum	Sapindaceae	Horsechesnut (*Turkish*, At kestanesi)	The whole Anatolia	Fruit and leaf	93	-
Rhus coriaria	Anacardiaceae	Sumac (*Turkish*, Sumak)	West and South Anatolia	Leaf	104	-
Control (Water only)	-	-	-	-	9	13 egg pocket clear yellowish
Control (Ethyl alcohol)	-	-	-	-	0	-

RESULTS

Table 1 presents findings about the effects of the eggs of *L. decemlineata* adults on the potato plants grown in laboratory pots. The table shows that, among the six different plant extracts used, the most successful extract against the egg-laying behavior of *L. decemlineata* was *Lq. orientalis*, and the least successful *R. coriara* (Table 1).

The *Lq. orientalis* extract, which was not found to be toxic in beetles, acted as an effective anti-feedant thus preventing beetles from feeding and laying many eggs (Table 1). The beetles placed in the pot with this extract were not able to consume the majority of the plant's leaves until harvesting, and not able to lay more eggs (Table 1). Those placed in the pot with *B. sempervirens* did not consume the majority of the plant's leaves until harvesting and did not lay many eggs (Table 1). The *A. hippocastanum* extract was not able to prevent beetle eggs from hatching. This plant extract did not have an adequate anti-feedant effect on any life stages of the beetle. Thus, these beetles were well-fed and laid many eggs (Table 1). This extract was observed not to be very effective in the biological control against the potato beetle. The *Ar. absinthium* and *Al. glutinosa* extracts had similar effects and led to the laying of a similar number of eggs (Table 1). These extracts did not fully prevent adult beetles from destroying the leaves of the potato plant. Therefore, they were decided not to be useful in biological control. The *R. coriaria* extract was similarly found to be not very effective against the potato beetle (Table 1). Due to the excessive damage caused by the adult beetles in the control groups, the plants in these pots were left without leaves, which stopped beetles from laying their eggs.

Lq. orientalis and *B. sempervirens*, the two plant extracts leading to the fewest number of *L. decemlineata* eggs in the laboratory, were chosen for field study. Table 2 and Figure 1 show the results of the field study. Before the treatment of the *Lq. orientalis* extract, the average number of larvae and adults was 6.2 and 7.2, respectively (Table 2), but after the treatment these numbers decreased to 1.2 and 3.2, respectively, (Table 1 and Figure 1). Also, a small number of eggs were identified in the 3 seedbeds. When compared to the eggs in the control block, the ones in this group were of a darker yellowish color and only 3 dead adult beetles were found in the whole area (Table 2). The appearance of potato seedbeds was respectable (Figure 2). In the potato blocks before the treatment of *B. sempervirens*, an average number of 10.2 larvae and 8.4 adults were seen. These values decreased to an average of 2.6 larvae and 2.8 adults after the treatment of the extract. A small number of eggs were identified in the two seedbeds. The appearance of the eggs resembled those in the block with *Lq. orientalis* (Table 1). In the count in this block, no adult beetles were seen. The potato seedbeds looked healthy (Figure 2).

In the potato seedbeds sprayed with 25% ethanol (control group), the potato leaves were almost fully eaten, the number of larvae was dense, many eggs were laid, and the number of adult beetles too much that of the other two blocks. The potato seedbeds were damaged (Figure 2). While the numbers of larvae and adults were 10 and 9.6 respectively before the treatment of ethanol, the numbers were 10 and 7.4 after the treatment. Before and after the treatment, the difference in the numbers of both larvae and adults was statistically insignificant, ($p < 0.05$).

Table 2. Effects on potato beetle, *Leptinotarsa decemlineata* of extracts obtained from *Buxus sempervirens*, and *Liquidambar orientalis*, in field.

Plant	Before treatment		After treatment	
	Number of Larvae (mean*±SEM)**	Number of Adults (mean*±SEM)**	Number of Larvae (mean*±SEM)**	Number of Adults (mean*±SEM)**
Control	10.0±1.67 a	9.6±1.03 a	10.0±1.38 a	7.4±0.68 a
Liquidambar orientalis	6.2±1.02 b	7.2±0.58 b	1.2±0.49 b	3.2±0.37 b
Buxus sempervirens	10.2±1,56 a	8.4±0.51 a	2.6±0.75 b	2.8±0.58 b

*Each represents three repeats

**The differences between the values in the same columm fallowed by the same letter are not statistically significant, p>0,05.

Extraction efficiency

Extract from *Lq. orientalis* was more effective than extract from *B. sempervirens*. On the contrary, the effects of the extract from *B. sempervirens* at adults of the pest were more than extract from *Lq. orientalis*. Efficiency of the extract from *Lq. orientalis* at larvae of the pest was 80.65 %, and was 42.34 % at adults of the pest. On the contrary, efficiency of the extract from *B. sempervirens* at larvae of the pest was 74.51 %, and was 56.76 % at adults of the pest.

In the potato seedbeds sprayed with 25% ethanol (control group), the potato leaves were almost fully eaten, while in the potato seedbeds sprayed with extract from *B. sempervirens* and *Lq. orientalis* the potato leaves were not fully eaten by potato beetle.

Fig. 1. General aspect of potato blocks after treatment in field: *B. sempervirens* (A), *Liquidambar orientalis* (B) Control (only ethanol solvent) (C).

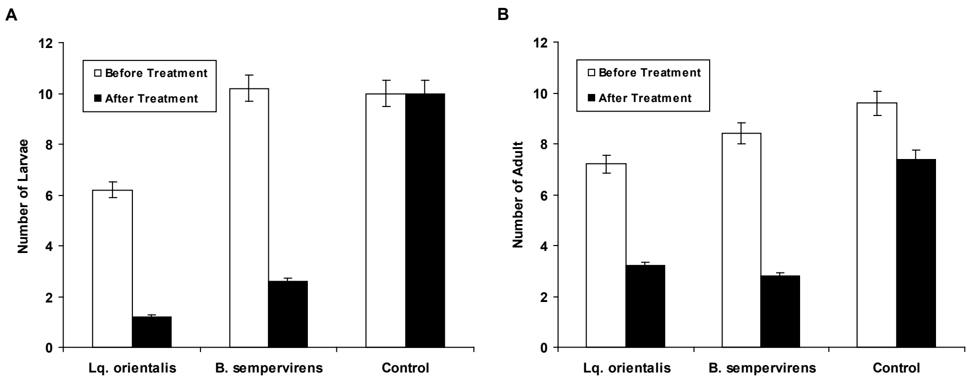

Fig. 2. Comparison results of before treatment and after treatment of extracts, number of larvae (A) and number of adult beetle (B).

DISCUSSION

Natural substances found in plants are an efficient alternative for the chemicals used in the controls of phytophagous insects. These substances have been shown to suppress the growth potential of the pest as well as its feeding (Whittaker and Feeny, 1971; Berenbaum, 1985). Plant extracts generally realize these effects with the active substances they contain. The effects of these components on pests depend on many variables such as the solvent used or environmental factors such as the temperature, climate or humidity (Ertürk, 2002). As an added difficulty, determining the most active substance in these extracts is not always easy. The isolated extract may not always have the desired effect (Van Beek and De Groot, 1986). The majority of these substances are thought to be anti-feedants. The chemicals in various plants which inhibit pest feeding are known as natural anti-feedants. Until today, triterpens, lactons, alkaloids, cucurbitacins, quinins and phenols have been used as anti-feedants in pest control (De Groot and Van Beek, 1987; Wieczorek, 1996). Nowadays, some plant pesticides are commonly used as food, spices or medical treatment in developed countries. For instance, in North America and European countries, plant pesticides such as *Chrysanthemum cinerariifolium* (Trevir.) Vis. pyrethrum, *Derris* spp. and *Lonchocarpus* spp. rotenone, *Nicotiana tabacum* L. nicotine, *Ryania speciosa* Vahl ryania and *Azadirachta indica* neem are used instead of chemical pesticides and are sold as agricultural products (Nawrot *et al.*, 1986; Norris, 1986).

The larvae and adults of the potato beetle damage the leaves of the plant with the eggs they lay on the leaves and young branches (Turkish Ministry of Agriculture and Rural Affairs, 2008). On GC–MS analysis of the Styrax liquidus from *Lq. orientalis*, overall, 31 compounds representing 99.8% of the total oil were identified where 1-8 Cineole, α-pinene, piperitone were identified as the major components by Gurbuz *et al.*, (2013). Lee *et al.*, (2004) reported that *Eucalyptus nicholii* and *Metrosideros fulgens* showed them to be rich in 1,8-cineole (82.19%, 77.50%, respectively) with lower amounts of other mono- and sesquiterpenoids. The oriental sweetgum from *Lq. orientalis* containing 1,8-cineole, α-pinene, piperitone concentrations showed the highly in toxicity in the presence of the larvae and adults of potato beetle. It is well known that variation in the composition of essential oils depends on genetics, type and age of leaf source, environment and oil analysis. Boland *et al.*, (1991) also found that *E. nicholii* contained 1,8-cineole (84%), isovalic aldehyde and α-pinene, *E. codonocarpa* contained ρ-cymene (30%) and piperitone (39%), and *E. blakelyi* contained 1,8-cineole (55%), and limonene (11%). Under our methods of extraction and analysis essential oils from similar sources are similar in composition. Eucalyptus oil (*E. globulus* Labill.) and eucalyptol (1,8-cineole) are accepted as food flavourings in a number of countries (Ash and Ash, 1995). The extracts mentioned above suppress the appetite of the pests with their anti-feedant substances and strong smell. Not feeding enough, they therefore do not complete their development. The extracts also avoid the eggs from hatching, make them turn a darker color, inhibit the feeding of the larvae and adults, and reduce nutrient consumption by probably destroying the metabolism of the larvae. All of these prevent the potato beetle from eating the leaves of the plant. If the plant extract is effective against the pest, it is natural that the beetle will lay fewer eggs on the plants which receive a treatment of this extract.

In our study, the pest was seen to lay the smallest number of eggs in the potato plants which received *Lq. orientalis* and *B. sempervirens* extracts, which protected the plants against the damage made by this pest (Table 1). Ertürk and Uslu (2007) have also identified these two extracts as the best anti-feedant for the potato beetle in a study conducted with 20 plant extracts in a laboratory. They were therefore used in the field study (Table 2). Ertürk and Uslu (2007) reported that these two extracts decreased the nutrient consumption of the potato beetle larvae and adults, and reduced their body weight under laboratory conditions. They also showed that the toxicity level of these two extracts were high. However, they concluded that these extracts acted as antifeedants and repellents, more than their toxicity (Saxena, 1987; Rembold, 1994; Ertürk and Uslu, 2007). As is widely known, such components in plant drugs inhibit the effects of juvenile and the moulting hormones in insects and imitate their functions (Bowers *et al.*, 1976). As the extracts studied here also contained similar substances, the number of the eggs and larvae in the experimental group was observed to be much smaller than the control group (Table 2 and Figure 2). The extracts had a toxic effect on eggs and thus decreased the number of larvae. Under field conditions, these two extracts also inhibited female pests' oviposition (Table 1). Probably due to the aromatic components in two extracts, this may help the plant protect itself against massive damage from pests (Table 2). Mukherjee (2003) reported that the influence of seven plant allelochemicals on growth rate, nutritional physiology and mid-gut esterase activity was studied in fifth instar larvae of *Spodoptera litura*. Relative growth rates were highest in larvae fed on the control diet followed by diet treated with β-sitosterol (0.618± 0.012 mg d−1 mg−1) and cineole (0.618 ± 0.054 mg d−1 mg−1) (Abramson *et al.*, 1973). Furthermore the activity of the leaves of *B. sempervirens* extracs is likely due to the high content of sitosterol, stigmasterol, cycloartenol, lupeol, germanicol and β-amyrin in the freestate. This is the first report of significant insecticidal activity by these extracts and compounds against the larvae and adults of potato beetle.

Turkey that is known to have a very rich flora may use this potential natural resource in agriculture as well as in medical treatments, landscape architecture, constructions or as food and spices (Davis, 1966-1988). We are of the opinion that more studies of this nature will help a better understanding of the active substances in plant extracts and thus benefit agricultural areas. With similar studies, we may identify alternative substances to chemicals which contaminate our natural resources and threaten our future. In addition to reducing environmental pollution, the use of these cheaper natural pesticides would have an economic benefit as well.

According to our field results, *Lq. orientalis* and *B. sempervirens* may be used as plant pesticides, due to the substances in their extracts, with the aim of inhibiting the population and feeding of *L. decemlineata* larvae (Table 2, Figure 2). Therefore, we offer to use together under field condition these two extracts according to pest's development stages.

CONCLUSION

Plants could be an alternative source to prevent damage to crops caused by the potato beetle because they constitute a potential source of bioactive chemicals and are generally free from harmful effects. Using of these botanical derivatives in potato beetle control instead of synthetic insecticides could reduce the cost and environmental pollution. Further studies on identification of active compounds, toxicity and field trials are needed to recommend the active fraction of these plant extracts for development of eco-friendly chemicals for control of insect vectors. These two plants extracts from our study results, because of the secondary compounds they included, it showed toxic and lethal effects on pests. Especially sweet gum tree has provided away from the potato plant which was damaged because of the smell of spring. This plant should be done utilized after a purified active substance is isolated, essence of which we believe would be more effective in pest control. For use in the field of plants used in different areas will increase the interest in these plants. The use of sweet gum tree more carefully, which is considered an endemic plant and it would be good to expand.

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