INVESTIGATIONS ON THE EFFECTS OF *XANTHIUM STRUMARIUM* L. EXTRACTS ON COLORADO POTATO BEETLE, *LEPTINOTARSA DECEMLINEATA* (SAY, 1824) (COLEOPTERA: CHRYSOMELIDAE)

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ABSTRACT: The effects of *Xanthium strumarium* L. extracts obtained methanolic were investigated on (*Leptinotarsa decemlineata* Say), Colorado potato beetle larvae and adults were treated in the laboratory conditions. As a result of the investigation, in all methods other than leaf disk method during larva instar, depending on the rising rate of concentration of these extracts prolonged larvae and pupae instar, causing a high death rate of larvae and pupae during instar, malformed larvae and pupae occured, decreased number of adults from pupae, and led health females to lay fewer eggs. In disk method antifeed effect was observed in larvae and adults. In dipping methods in adults, it was determined that females laid much fewer eggs than the control individuals and that less larvae occured.

KEY WORDS: *Leptinotarsa decemlineata* Say, *Xanthium strumarium* L., repellent antifeed, growth effect, extract.

CPB (*Leptinotarsa decemlineata* Say), is one of the insect pests of potato in Turkiye which has caused an economic impact. In recently years broad spectrum insecticides are used to control CPB as in the past in Turkiye. Usage of broad spectrum insecticides has led to widespread resistance in Turkiye. Last some years, various insecticides are often used to reduce resistance that occured in pests.

The appliction of plant extracts to be used to control insects can be a possible alternative. In this plant *Xanthium strumarium* L. belonging to Compositae family and having various effects on insects. It is determined that the root, body and water of the oil extract which are transparent form *X. strumarium* led to the deaths of *Tylenchulus semipenetrans* larvae (Malik et al. 1988).

In another study the effects of the extract from *X. strumarium* leaves against *Meloidogyne javanica* which is found in the plants roots showed hindered development and negative effects on the eggs opening on *M. javanica* (Nandal and Bhatti 1986).

Studies done in Turkiye using the liquid extract from *X. strumarium* fruits had a repellent affect on Colorado potato bettle adults and larvae (Çetinsoy et al. 1998).

In the present study, results of the study on efficacy of *X. strumaruim* fruit extract against *L. decemelineata* on potato leaves in laboratory conditions. The study was carried out in 1998 between 2002 years in Plant Protection Central Research Institute .

MATERIALS AND METHODS

Extracts

Breuer and Devkota (1990) method was used in preparation of *X. strumarium* methanolic fruit extract. Ripe and sundried drupes of *X. strumarium*, collected in Ankara in September 1998. The material was stored in the go down to dry. The fruits were ground in methanol in a Waring Blender then after drying completely they were compacted and ground to powder. After this methanol was added in the mixture was mixed for 72 hours. This mixture was extracted in 5-6 hours using the Soxhlet machine. Methanol was removed from the extract obtained in a rotary evaporator (50 –60 dergees).

Insect

CPB was bred on potato leaves for several months in the Plant Protection Central Research Institute laboratory. Wardojo (1969) arrangement method was used to culture the stock.

Effect on larva stage of the extract was tested on the third-instar larvae (30-35 weight) with leaf dipping, larva dipping, topical application and leaf disc methods at four concentration levels ranging 1 to 7%.

Leaf-dip feeding

Potato leaves were treated by dipping into extract concentrations (1%, 3%, 5%, 7%) then left to dry for 30-minutes. The threated leaves and larvae were placed into plastic containers that were lined with moister filter paper. After 24 h, the larvae were given threated leaves again. After 48 h, the larvae were provided with fresh, untreated leaves to feed (Prijiono and Hassan 1993).

Larvae-dipping

Larvae were 1-3 s directly dipped into extracts with (1%, 3%, 5%, 7%) prepared concentrations. Control larvae were dipped in distilled water (Oroumchi and Lorra 1993).

Topical application

20% concentration prepared extract was microapplied to the dorsal thorax of the larvae in 0.5µl/larva, 1µl/larva, 2µl/larva (Kaethner 1992). Later the 10 larvae were placed in each labeled and covered containers with potato leaves in them. Each experiment was repeated 6 times. For the maturing larvae to become pupae, they were shifted into containers with the same dimensions containing 1/3 sterile soil, sand and manure mixture. The experiments were checked daily until adult emergence. Extracts were prepared using distilled water and 0.01% Tritonx 100 was added. Only distilled water was used in the control group. The results for all the three groups are given below.

- The period of larva stage [period from beginning of the experiment larvae (3. stage) to until they became pupae]
- The rate death of larva stage[number of life larvae from beginning of the experiment to number of live at the end of larvae stage]
- The period of pupa stage[period from pupa to adult stage]

- The rate death of pupa stage [number of pupae at the beginning and number of adults at the end gave death rate]

Leaf disc method

In the experiment, 5%, 10%, 20% concentrations of *X. Strumarium* methanol fruit extract were prepared using distilled water and 0.01% spread adhesive TritonX100 was added. TritonX100 and distilled water was used in the control. Potato leaves were cut in 2.2 cm diameter discs and dipped in into the prepared concentrations for 3-5 s and then dried for 30 minutes. 2 dried leaf discs were put into each 9cm petri dishes with moister filter paper. The experiment was repeated 10 times, and continued for 72 h in which larvae death rate and leaf discs consumed were monitored daily.

In adult experiments

Each petri dish 2 10-15 day adults were placed. 30 pieces 2.2 cm leaf discs that were dipped into distilled water were placed into petri dishes as a control. Digital planimeter was used to measure area consumed on the leaves, and remaining leaf area was subtrcted from total leaf area (Zehnder and Warthen 1988).

Fecundite

Potato leaves were dipped for 3-5 s in prepared concentrations (%1, 2.5, 5, 10) then dried for 30 minutes and placed into shifon covered plastic containers with 5 female adults, after 24 hours of feeding on treated potato leaves, untreated potato leaves were given to feed. Then females were placed into bigger shifon covered plastic containers with 5 male adults, the experiment was repeated 6 times. A two month period followed where daily monitoring was done and the total number of eggs were recorded. The eggs were then placed into petri dishes inlaid with filter paper and number of hatched eggs were monitored and recorded daily (Schmutterer 1986).

RESULTS

Leaf dipping

Studying the table 1. shows us that concentrations alter larvae stage periods, smallest average period being 4.24±0.161 days in the control group and the longest average being 9.65±0.359 days in the 3% concentration group. Statistical data shows $P<0.05$ importance between the control and all the other groups.

Smallest larvae death rate was 1.67±0.068% in the control group while greatest larvae death rate was 55.00±0.418% in the 5% concentration group. Statistically the control group was found different to all groups $P<0.05$ but no importance between the other concentration groups.

The stage of pupa was completed shortest in the control group in 9.43±0.178 days while the longest completion period was in the 3% group which took 10.80±0.304 days, statistics show that 3% group was $p<0.05$ significantly different to all the groups, while the other groups were similar to the control group. Pupa death rate was lowest in the control group 3.34±0.086% and in the $p<0.05$ statistics it was found significant that *X. strumarium* extract had no effect on pupa death rates.

Larvae dipping

The period of larva stage was found to be dependant on concentrations. Table 1. shows that The period of larva stage was shortest 4.10±0.153 days in the control group, and longest 7.78±0.671 days in the 5% groups. Statistical analysis showed that the 1%, 5% and 7% groups were non different P<0.05 the 3% group showed significant differences..

The rate death of larva stage was least at 3.33±0.211% in the control group while in the 5% and 7% groups the average rate was 36.67±0.172% in deaths.

L decemlineata dipped into different concentrations of *X. strumarium* methanolic fruit extract took a short period of 9.00±0.155 days to change from larvae to pupae in the control group while in the higher concentration groups this period was extended, the longest pupa stage period of 11.23±0.737% was found in the 5% group. Statistical finds suggest that there was no significant differnce between the 3%, 5% and 7% groups (p<0.05).

Pupa death rate was least at 1.670±0.368 % in the control group. In this period death rates were similar in the 1% and 3% groups and again in the 5%and 7% groups concentrations were found insignificant in pupa death rates (p>0.05).

Topical application

Larva stage periods were smallest at 3.81±0.165 days in the control group while the longest period was 6.52±0.414 days in the 2µl/individual group.(4.62). Statistics performed showed singificant difference in all groups (p<0.05).

Larva stage death rates were least at 3.33±0.211 in the control and ascending in the 0.5µl/individual, 1µl/individual, 2µl/individual dozes. Statistics showed significant differences in all groups.

L. decemlineata larvae used in the topical application using *X. strumarium* methanoilc fruit extract turned to pupae and the pupa stage period took longest in the 2µl/larva (10.75±0.479 days), and shortest in the 0.5µl/larva (9.48±0.173 days). In the control study the pupa stage period was 9.86±0.141 days. Statistics showed a significant differnce (p<0.05) in the control and the 2µl/larva groups but non significant in the other groups. As for pupa stage death rate statistcs showed significant differences in concentrations, the smallest average being 3.44±0.245% and greatest being 18.00±0.376% in the 1µl/larva group (p<0.05).

Antifeeding effect

Maximum consumed area after 72 hours was 3.80±0.0000cm² in the control and the least area in that time was 0.16±0.117cm² in the 15%concentration group. Statistics show p<0.0 significance in consumed leaf area in all concentrations and thus non different in the concentrations.

According to this maximum consumed leaf area is in the control at 5.70±0.123cm² and the least 0.08±0.0.053cm² in the 20% concentration group (Table). Statistics show significant difference (p<0.05) between the control group and all concentration groups.

Fecundite

Eggs layed by the potato beetle were found to be significant statistically ($p<0.05$) for all concentrations, in accordance with this an average of 1565.16±141.00 eggs were layed n the control, least number of eggs 561.30±46.50 in the 1% group while the data collected from the other 2.5%, 5% and 10% groups were almost similar. Most eggs hatched in the control group 1475.33±57.15 while the least number (average 419.80±37.90) of eggs hatched in the 1% group.

Conclusions and Opinions

X. strumarium methanol fruit extract prolonged the larvae stage period compared to the control. In the control larva period was 4.24 days while in the 3% extract group it averaged 9.65 days. In the 5% and 7% extract groups the period were 7.13 days and 6.98 days respectively. In the larva dipping experiment the larva period was longer than the control. Control was 4.10 days while in the 7% extract group it was an average of 7.80 days. In the topical application method like the previous methods used the larva stage periods was longer compared to the control group, control average was 3.81 days while in the 2µl/individual concentration group it was 6.52 days.

There were no references found on other studies done using *X. strumarium* methanol extract against Colorado potato beetle or other pests. In the leaf dipping method larva stage death rates were lesser 1.67%, 7% and 53.33% in all the concentration levels compared to the control group. Larvae death rates were higher in all groups other than control (control 3.34%, 7% concentration 36.67%).

Topical application method also showed high larvae death rates compared to control group, in the 2µl / individual concentration had 56.68% rate. In leaf dipping with *X. strumarium* extract in it the pupa stage was longer than the control, it took 9.43 days in the control while in the 3% group it took 10.80 days, in the other concentrations pupa stage completed close to the value of the control group.

Larva dipping method the pupa stage was again longer than the control group, control completion took 9.00 days while in the 5% concentration group it took 11.23 days. In the topical method like the other two methods pupa stage was less in the control stage (9.86 days) and took 10.75 days in the 2µl/individual concentration group. No study hs been found with regard to this extract. *X strumarium* methanol fruit extract showed pupa death rates in the with extract. In the control its was 3.34% while in the 3% group it was 8.34%. in the larva dipping method the death rate was found to be 10%. Pupa death rate in the topical application was 3.44% in the control group while in the highest concentration levels it was noted to be 16.68%.

Leaf area with *X strumarium* consumed with time wasn't found to be significant but there was a slight effect of concentration.

Leaf with *X strumarium* extract in all concentrations showed decrease in consumed area and thus could be said that it has a strong antifeeding effect. Çetinsoy et al., (1998) shows that extract obtained from the *X strumarium* fruit in the 1/16 concentration hindered feeding in *L decemlineata* adults and larvae, and this effect was proportion to time.

Eggs left in 1%, 2.5%, 5% and 10% concentrations of *X. strumarium* methanol fruit extract yielded a decreased number of hatched eggs proportional to the concentration. In this laboratory study it was seen that *X. strumarium* fruit extract had different effects on different stages of *L. decemlineata*. Moreover before the effects of floral extracts on *L decemlineata* can be fully accepted more study has to be done in farm conditions and from there data and results collected compared with those obtained in the laboratory.

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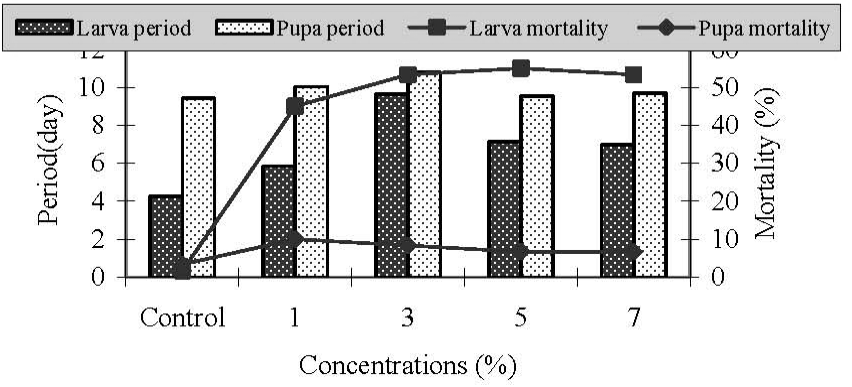

Fig. 1. Larva of *Leptinotarsa decemlineata* Say fed on treated plants with different concentration of *Xanthium strumarim* L. fruit methanolic extract was determined larva and pupa stage period and mortality .

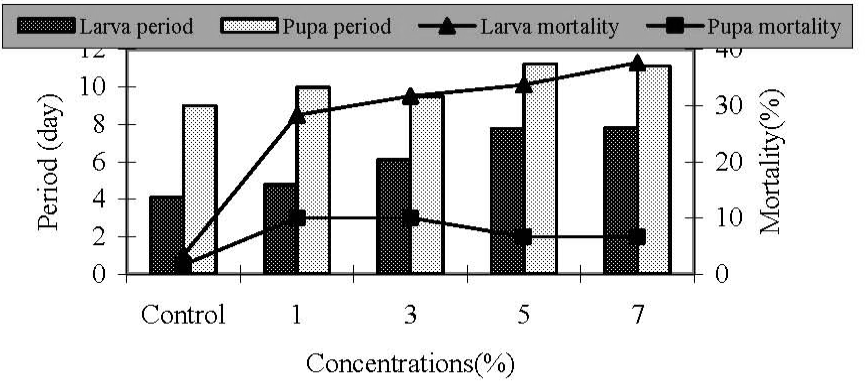

Fig. 2. Larva of *Leptinotarsa decemlineata* Say dipped with different concentration of *Xanthium strumarim* L. fruit methanolic extract was determined larva and pupa stage period and mortality .

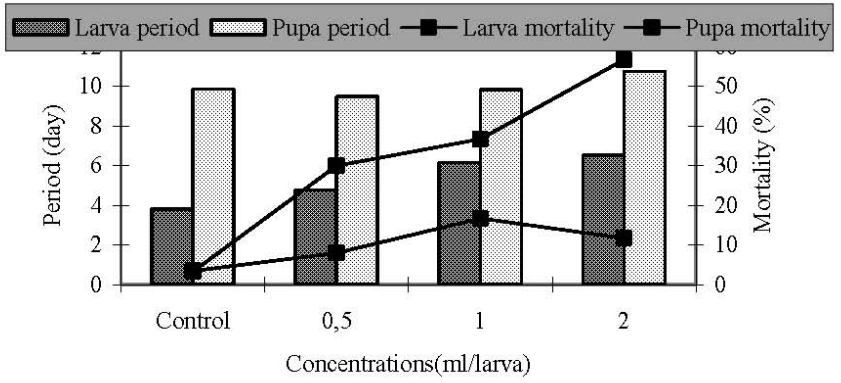

Fig. 3. Larva of *Leptinotarsa decemlineata* Say topical application with different concentration of of *Xanthium strumarim* L. fruit methanolic extract was determined larva and pupa stage period and mortality .

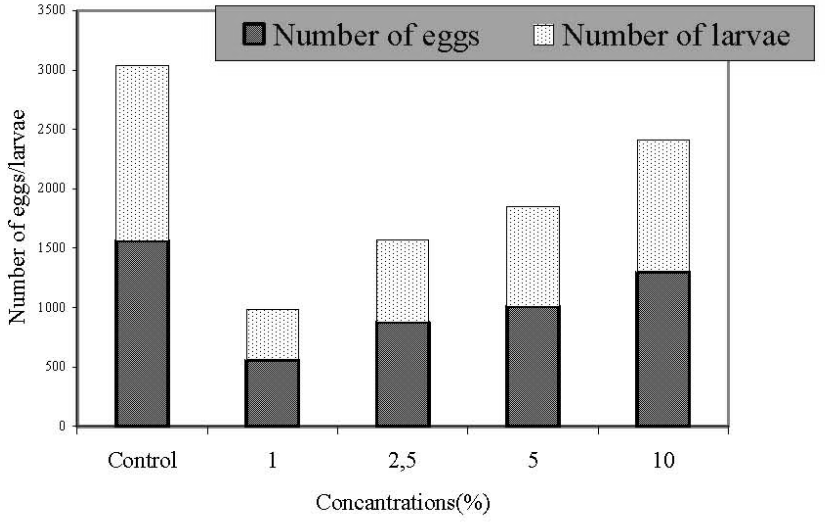

Fig. 4. Total number of eggs laid by groups of 30 fameles of *Leptinotarsa decemlineata* Say per treatment during 2 months and of emerged larvae.

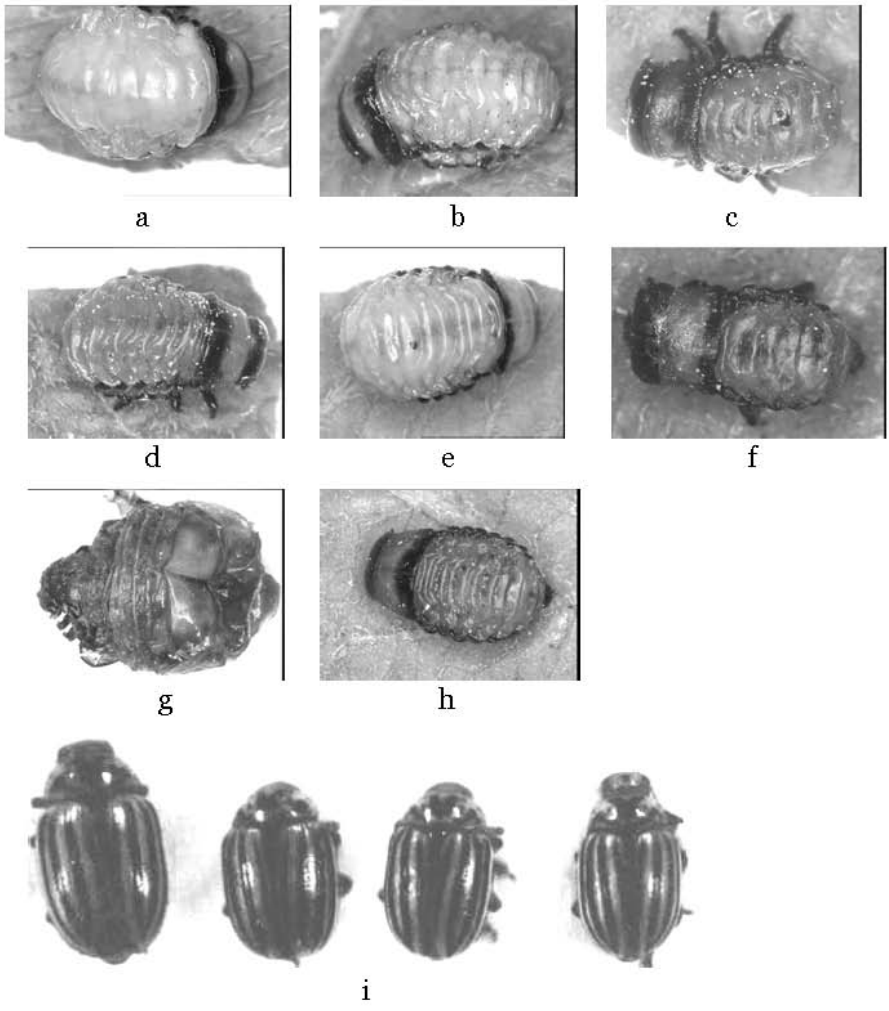

Fig. 5. Abnormal larvae of *Leptinotarsa decemlineata* Say showing, developed from larvae fed on treated with different concentration of *Xanthium strumarium* L. fruit methanolic extract, 1% C 20 days (a) and 3% C 17 days(b), 7% black spots appear on dorsal part of larva (c) and 27 days (d) later, abnormal larvae of *L. decemlineata* showing, developed from larvae dipped into different concentration of *X. strumarium* fruit methanolic extract, black spots on dorsal of larva 1% C 20 days (e), darkening areas on larva 5% C 23 days (f), 7% C (g) and 3% C 32 days (h) late, abnormal adults of *L. decemlineata* developed from larva topical application with *X. strumarium* fruit metanolich extract of 2µl/ larva (a) and normal adult from control (b).

Table 1. *Leptinotarsa decemlineata* Say Larva stage period and pupa stage period and the death of rate determined after leaf dipping, larvae dipping methods using *Xanthium strumarium* L. methanolic fruit extract of larvae during larva stage.

Extract name	Conc. (%)	Leaf-dip feeding				Larvae dipping			
		p.l.s (day)	r.d.l.s(%)	p.p.s(day)	R.d.p.s(%)	p.l.s (day)	r.d.l.s (%)	p.p.s (day)	r.d.l.s (%)
X. strumarium methanol fruit extract	control	4.24±0.16d (3-7)	1.67±0.068 b (0-10)	9.43±0.178b (7-12)	3.34± 0.086 (0-10)	4.10± 0.153c (3-7)	3.33± 0.211b (0-10)	9.00± 0.155b (7-11)	1.67± 0.368 (0-10)
	1	5.85± 0.276c (5-9)	45.00± 0.224a (40-50)	10.04± 0.528b (7-11)	10.00± 0.316 (0-20)	4.77±0.229c (3-8)	28.33±0.169a (10-40)	9.96± 0.264b (8-14)	10.00± 0.316 (0-20)
	3	9.65± 0.359a (8-13)	53.34± 0.667a (40-70)	10.80± 0.304a (9-13)	8.34± 0.307 (0-20)	6.14± 0.170b (4-8)	31.67±0.323a (0-50)	9.50± 0.354b (6-16)	10.00± 0.285 (0-20)
	5	7.13± 0.604bc (5-15)	55.00± 0.418a (40-70)	9.55± 0.283b (8-11)	6.67± 0.211 (0-10)	7.78± 0.674a (4-25)	36.67±0.250a (20-60)	11.23± 0.737a (7-19)	6.67± 0.211 (0-20)
	7	6.98± 0.573b (4-18)	53.34± 0.667a (30-70)	9.71± 0.220b (8-12)	6.67± 0.211 (0-10)	7.81± 0.347a (2-11)	36.67±0.172a (30-50)	11.11± 0.386a (8-16)	6.67± 0.333 (0-20)

*Means within columns followed by the same letter are not significantly different (P>0.05,Duncans's multiple range test).
P.l.s: The period larva stage
R.d.l.s: The rate death larva stage
P.p.s: The period pupa stage
R.d.p.s: The rate death larva