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Comparative Toxicity of Neem and Seven Insecticides on Hazelnut Weevil (*Curculio nucum* Col.: Curculionidae) with Laboratory Bioassays

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Hazelnut weevil (*Curculio nucum* L. Col., Curculionidae) is common pest in hazelnut (*Corylus avellanae*) and insecticides are the primary tool used to manage infestation of it. The insecticides are applied for its control for many years; therefore, to evaluate their effectiveness and establishing baseline insecticide mortality for future resistance monitoring programs are needed. In this study, efficacy of 7 insecticides including benfurocarb, carbaryl, carbosulfan, furathiocarb, lambda-cyhalothrin, methiocarb and 1 botanical pesticide with azadirachtin were evaluated in laboratory bioassays and lethal concentrations were determined against hazelnut weevil. There was no acute toxicity of azadirachtin on hazelnut weevil in 72 h. The highest toxicity based on LC_{50} values was observed in lambda-cyhalothrin (3.06 mg AI/L) and followed by carbosulfan (12.4 mg AI/L). The LC_{50}'s (mg AI/L) for furathiocarb, methiocarb and benfurocarb in residual filter paper method were 36, 44.5 and 90.2, respectively. The least efficacy were determined in carbaryl, LC_{50}'s were 224.3 and 277.5 mg AI/L for two carbaryl preparations. The lowest slopes of probit lines were determined for carbaryl whereas it was steepest for carbosulfan. LC_{99}'s were close or upper than recommended field doses in carbaryl while they were lower for other compounds.

Key Words: Hazelnut weevil, *Curculio nucum*, insecticides, azadirachtin, toxicity, hazelnut.

INTRODUCTION

Hazelnut is one of the main agricultural export products of Turkey, drives hundred millions dollars of income each year. About 400,000 families live on hazelnut and about 70 % of the worldwide hazelnut production is supplied by Turkey. Almost 584000 hectare land is used for hazelnut production in Turkey[1]. Hazelnut weevil (HW) (*Curculio nucum* L. Col.: Curculionidae) is the most serious pest of hazelnut in Turkey as well as other hazelnut growing countries[2-10]. Hazelnut weevil cause

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substantial damage on hazelnut and most of the hazelnut farmers have to take control measurements against this pest every year. Various conventional chemicals are currently used to control this insect. It is responsible for huge amount of insecticide used in main hazelnut growing area, Black sea region of Turkey. There is no effective natural enemies and no other alternative management tactics except chemical control against this pests yet[10,12]. Hazelnut weevil complete one generation per year. It spends its egg and larval stages in nuts and pupate in soil. Only vulnerable biological stage for spray interventions are seen as adult. Adult stage last almost 3 months and feed on female flowers and premature nuts until lay eggs in developed nuts. The farmers generally spray once or twice after mid-may against adult stage[6].

Sometimes some producers complain about that they can't get satisfactory results of applying insecticide against the *Curculio nucum* and claim that they are in doubt about some preparations. It is known that some of these preparations are used against this insect in this region for about 40 years[13].

In this laboratory study, we evaluated acute toxicity of 7 licensed insecticides which have been used on farmlands for a long time and 1 azadirachtin preparation on hazelnut weevil adults. Their lethal doses were determined and compared each other. The purpose of these studies was to examine the comparative toxicity of selected conventional and experimental insecticides to hazelnut weevil adults in laboratory bioassays.

EXPERIMENTAL

Test insects: In toxicity tests, adult individuals of hazelnut weevil were used. The adults were collected from hazelnut orchards in Ordu (Ünye) province which mostly have high insect populations and usually orchards are sprayed every year. Hazelnut bushes were shaken on sheet at the dimension of 3.5 × 3 m and adults were collected in perforated plastic bags that have fresh hazelnut leaves inside. They were transferred in laboratory and experiments carried out in less than 24 h after collecting. Preliminary tests were performed on adults collected on 21 April 2000 at first and the doses producing 0-100 % mortality were determined for each insecticides. After than almost 1 week later (1 May 2000) again adults were collected by using same method mentioned above from same locations. The adults that are used in each insecticide test were randomly selected from gathared adult population.

Insecticides in tests: A hazelnut weevil population was tested with a botanical insecticides Azadirachtin (Neem Azal T/S 1%, 10.000 ppm a.i, Trifolio-M company,Germany) as well as 7 recommended insecticides including carbaryl (Sevin 85 WP and Sevin XLR Plus, Bayer), carbosulfan

(Marshal 25 EC) ,benfurocarb (Oncol 200 EC), furathiocarb (Deltanet 400 EC), lambda-cyhalothrin (Karate 5 EC) and methiocarb (Mesurol WP 50). Insecticides used in the tests were selected from those currently registered commercial formulations for hazelnut weevil and have been used for a long time in orchards. Preparations were obtained from local offices of authorized companies. Also, Neem Azal T/S 1 % including Azadirachtin (botanical insecticide) were supplied from Verim Ülkü Ins. Ltd. Std which is representative of Trifolio-M company in Turkey (Table-1). The concentrations used for insecticides are given Table-1.

TABLE-1
INSECTICIDES USED IN THE EXPERIMENTS

Compound	Trade name	Recommended application rate g/da-mL/da	Concentrations used in bioassay (mg AI/L)
Azadirachtin 1 %	Neem Azal T/S	1-2 L	50, 100, 250, 500, 750, 1000, 2000
Benfurocarb 200 gr/lt	Oncol EC 200	150 mL	20, 50, 60, 70, 80, 90, 100, 125
Carbaryl 85 %	Sevin 85 WP	150 g	85, 170, 212.5, 425, 637.5, 850
Carbaryl 480 gr/lt	Sevin XLR Plus	200 mL	120, 240, 360, 480, 600, 720, 960
Carbosulfan 250 gr/lt	Marshal 25 EC	125 mL	5, 10, 15, 20, 25
Furathiocarb 400 gr/lt	Deltanet 400 EC	100 mL	10, 20, 30, 40, 50, 60
L-cyhalothrin 50 gr/lt	Karate 5 EC	50 mL	1, 2, 3, 4, 5, 10
Methiocarb 50 %	Mesurol WP 50	100 g	12.5, 25, 37.5, 50, 125, 250

Bioassay with insecticides: Topical application of pesticides to adult stage of hazelnut weevil was considered impractical, so LC_{50} values were determined from treated filter paper test[14] closely approximates the commercially recommended operational application rate of each insecticide tested in toxicity tests. Tests were carried out in 9 cm diameter petri dishes lined bottom with filter paper of 8.9 cm in diameter. Doses were prepared as mg AI/L. Stock solutions of each insecticide were developed by dissolving preparation samples in de-ionised water. Dilutions were made from each stock solution to yield the desired insecticide concentrations as mg AI/L. Solutions were stirred on hot plate for 2 min before use. No food source was provided for insect during toxicity experiments. In preparing and applying chemical dosages digitally regulated micropipettes have been used. Spouts of pipettes have been changed after each dosage application. Deionized water were used in all dilutions.

For each insecticide tested, serial dilutions of formulated material were transferred onto the filter paper in petri dish by digital pipete, agitated to distribute evenly and allowed to dry for *ca.* 1 h. Petri dish was treated with 1 mL of freshly shaken insecticide suspension of sufficient volume to wet to filter paper with no run-off. Adults were placed into a series of petri dishes that contained 5-8 different concentrations of formulated insecticide, along with controls treated with only de-ionised water to determine LC's for a given insecticide. Doses were diluted and applied from lower to higher doses during experiments. De-ionised water were used in all dilutions.

Each petri dishes contained 5 adults and replicated 4-5 times for each doses. Sex was not separated. The petri dishes were sealed with corresponding lids and bioassay were conducted under constant light at 22 ± 1°C and 60-70 % R.H. A minimum 20 adults per dose were tested for each insecticide and mortality was assessed at 24 h after treatment. There was no mortality in the first 24 h in the azadirachtin application and hence this chemical has a low acute toxicity, observations were extended up to 72 h. For each chemical compound at least 120 adults using were planned due to the reasons[15] and this number changed between 140 to 195 from chemical to chemical.

Adults were considered as dead if no movement was observed after leaving dead adults under 500 Watt light bulp for 1 min because of their tanatozis behaviour. Control mortality never exceeded 10 %.

Analysis of statistics: Data were analyzed and LC_{50}'s and LC_{99}'s generated with POLO-PC using probit analysis (LeOra Software,1987). Other statistical data such as confidence intervals of doses, relative potency and parallelism tests were also calculated by same programme. Only one lowest dose caused 100 % mortality were taken care and others were discarded[15]. LC_{50}'s were considered significantly different from one another if the 95 % confidence limits did not overlap.

RESULTS AND DISCUSSION

Because of hazelnut weevil is only serious pest of hazelnut and hazelnut growing is limited in a few countries, there is a very limited studies on this pest and effectiveness of chemical compounds on it. Therefore, concentration-response data using filter paper method as standart procedure provided important baseline information under laboratory conditions and could be a useful tool in evaluating in differences of insecticide susceptibility in further studies.

In the 7 of the 8 preparation used in the experiment, it was observed that mortality rate of hazelnut weevil adults increased dependent on doses (Figs. 1 and 2).

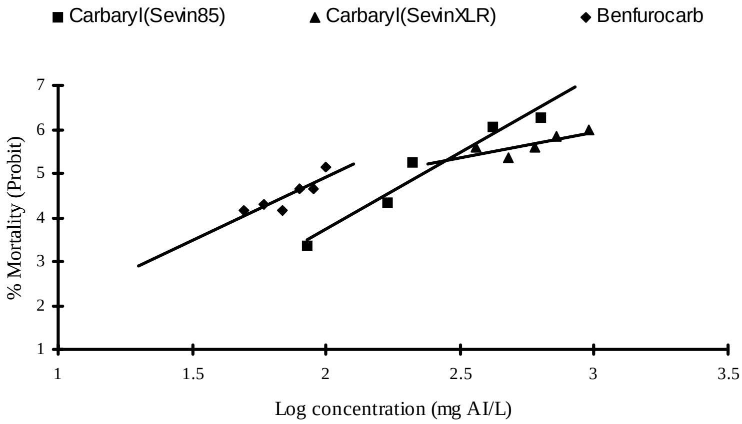

Fig. 1. Dose response relationship of hazelnut weevil adults to carbaryl and benfurocarb in bioassay, 24-hr after exposure

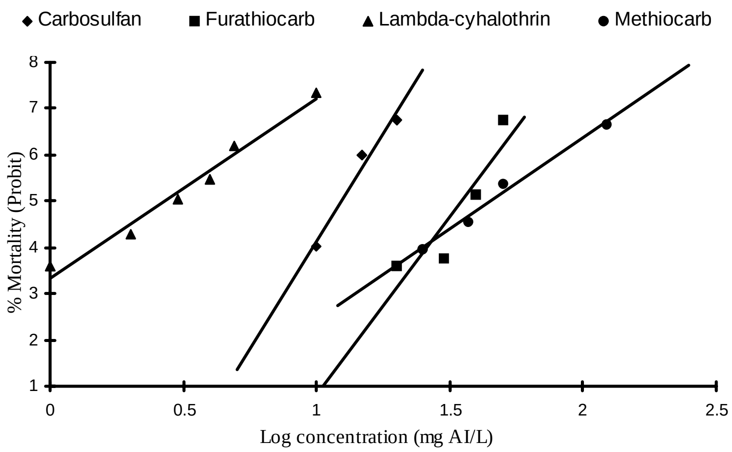

Fig. 2. Dose response relationship of hazelnut weevil adults to carbosulfan,furathiocarb, L-cyhalothrin and methiocarb in bioassay, 24 h after exposure

Toxicity of azadirachtin: As a result of the experiment, the study of ascertaining lethal concentrations were succeeded at all chemicals except Azadirachtin. In the 72 h observations on Azadirachtin, no mortality was observed at doses between 100-2000 mg AI/L and so lethal concentration could not be reached. This result showed that azadirachtin doesn't have acute contact toxicity to hazelnut weevil at these doses. In some of the other preliminary studies in which azadirachtin was used for hazelnut weevil, low acute efficiency was observed[16]. It is well known based on many researches that azadiractin has low acute toxicity for most pests but effective in a chronic way[16,17]. It is clear that there is a need to study on chronic effects of azadirachtin on hazelnut weevil in order to assess of this botanical pesticide that has potential use in organic and environmentally sound hazelnut production. According to our later research carried in semi-field cage experiments over the efficiency of azadirachtin on hazelnut weevil, it was observed that azadirachtion caused 75-86 % mortality at different application doses at the end of 10 d.

Toxicity of carbamates and pyrethroid: Six carbamates and one pyrethroid registered compounds were evaluated in toxicity tests. These compounds are effective against hazelnut weevil adults under laboratory conditions and that some appeared more effective than others. Toxicity of seven insecticides to hazelnut weevil adult stage is presented in Table-2. Hazelnut weevil adults were most sensitive to λ-cyhalothrin and least sensitive to carbaryl. The LC_{50} values ranged from 3.06 mg AI/L for λ-cyhalothrin, to 277.5 mg AI/L for carbaryl (Sevin XLR Plus). According to lethal concentaration values, λ-cyhalothrin was most toxic to adults with the value of 3.06 mg AI/L. Carbosulfan was the second compound in effectiveness with 12.4 mg AI/L LC_{50} value. They were followed by furathicarb and methiocarb with 36 mg AI/L and 44.5 mg AI/L of LC_{50}'s, respectively. Benfurocarb and two carbaryl formulations (Sevin 85 WP and Sevin XLR Plus) had the lowest toxicity with LC_{50} values of 90.2 mg AI/L, 224.5 and 277.5 mgAI/L, respectively (Table-2). The responses of hazelnut weevil adults to λ-cyhalothrin and carbosulfan were significantly different from each other's and all other compounds ($p > 0.05$). There was no difference in LC_{50} values of furathiocarb and methicarb and they were seen equally effective ($p < 0.05$). Benfurocarb differed in potency from all compounds except carbaryl (Sevin XLR Plus). Two carbaryl formulations were also seen as equally effective against hazelnut weevil adults.

The slopes of the probit regression lined in our test carbaryl (Sevin XLR Plus) had the least slope while carbosulfan had the steepest. When insecticides were evaluated all together parallelism test was rejected but when the compounds were compared to each other one by one, some of them had statistically no difference in their slopes. There was no significant difference in slopes between two carbaryl formulations indicating that the tested population exhibited similar responses to two carbaryl preparations. Similarly, no significant difference was determined between the slopes of benfurocarb, methiocarb and λ-cyhalothrin. Carbosulfan and furathiocarb had similar steep slopes and also no difference was found (Figs. 1 and 2). It is well known that carbaryl is common compound that being used for a long time in region. Therefore least slopes in carbaryl may be caused by its common usage for years. All other carbamate compounds differed from carbaryl in slopes while three of them were in same group. When slopes are not parallel, it can demonstrate that detoxification enzymes are different both in quality and quantity. As the dose-response function slope is negatively correlated with variability in susceptibility of a population to a compound, the relatively low slope in reponse to carbaryl compounds indicates a low degree of homogenecity of toxicological response, whereas the higher slopes in response to carbosulfan, furathiocarb, benfurocarb, λ-cyhalothrin and methiocarb indicates a comparatively narrow range of susceptibilities within the tested population. On the other hand, parallel slopes can show that organisms have enzymes that are similar in quality but different in quantity[15].

So, it is possible to interpret that hazelnut weevil uses similar detoxification enzyme against the chemicals in the same group with regard to slopes and uses different detoxification enzyme against the chemicals in different groups.

TABLE-2
RESPONSE OF HAZELNUT WEEVIL ADULTS TO INSECTICIDES AT 24 HOURS AFTER EXPOSURE IN RESIDUAL FILTER PAPER TEST

Compounds	Tested samples No.	Slope (S.E)	LC_{50} (95% confidence limits, mg AI/L)	χ^2
Benfurocarb	195	5.15(1.02)b	90.2 (77.2-123.5)d	10.2
Carbaryl (85 WP)	140	3.64(0.54)c	224.3 (182.5-270.1)e	3.55
Carbaryl(XLR Plus)	195	2.87(0.51)c	277.5 (114.4-389)de	9.29
Carbosulfan	150	9.94(1.72)a	12.4 (11.2-13.5)b	0.95
Furathiocarb	175	8.62(1.23)a	36 (29.3-42.9)*c	13.1
λ-Cyhalothrin	175	4.89(1.08)b	3.06 (3.53-6.75)a	0.81
Methiocarb	135	4.21(0.76)b	44.5 (36.9-54.7)c	0.91

*The difference between the lethal conc. and slopes having the same letter is not important ($p < 0.05$).

Comparative toxicity: When the chemicals were compared to each other with considering their relative potency, depending on pyrethroid, λ-cyhalothrin LC_{50} value which was the most effective, carbamate formulations were found 4-90.9- fold less effective against hazelnut weevil, based on LC_{50} values (Table-3). Among the carbamate formulations, carbosulfon was the most effective compound with 4-fold least effectiveness. Similarly hazelnut weevil adults were 11.8-, 14.5- and 29.4-fold less sensitive to furathiocarb, methiocarb and benfurocarb, respectively. Two carbaryl compounds were least toxic (71.4 and 90.9-fold) to hazelnut weevil adults than λ-cyhalothrin. As the parallelism test was rejected, simple mathematical proportion made between LC_{50} doses instead of efficiency coefficients figured out by POLO-PC[15].

Recommended application rate and laboratory efficiency: When the recommended application rate of preparations and LC_{99} values calculated from laboratory bioassays are compared to each other (recommended application rate/LC_{99} value), the highest ratio (14.7-fold) was obtained from carbosulfan and furathiocarb has followed it with 5.97-fold. Of the two chemicals with carbaryl active ingredient, Sevin XLR Plus was the only preparation appeared having higher laboratory LC_{99} dose than filed recommended rate with.0.54-fold. Methiocarb and λ-cyhalothrin had values of 3.15 and 2.74-fold, respectively. Another carbaryl formulation Sevin 85 WP and benfurocarb showed very close LC_{99} values to recommended application rate, with 1.31 and 1.17-fold (Table-4).

TABLE-3
RELATIVE POTENCIES AND COMPARATIVE EFFICIENCY OF SEVEN INSECTIDES AGAINST HW ADULTS

Compound	Trade name	Efficacy* (LC_{50}) Rate	
		Relative potencies	Comparative toxicity (1/fold)
λ-Cyhalothrin	Karate 5 EC	1.0	-
Benfurocarb	Oncol EC 200	0.034	29.4
Carbaryl	Sevin 85 WP	0.014	71.4
Carbaryl	SevinXLR Plus	0.011	90.9
Carbosulfan	Marshal 25 EC	0.247	4.0
Furtahiocarb	Deltanet 400 EC	0.085	11.8
Methiocarb	Mesurol WP 50	0.069	14.5

*As the parallelism is rejected according to POLO-PC, calculation has been made through mathematical proportion based on LC_{50}'s.

TABLE-4
COMPARISON OF RECOMMENDED APPLICATION RATES OF BIOASSAYED INSECTICIDES WITH LABORATORY EFFICIENCY RESULTS AGAINST HW

Compound	Trade name	Recommended application rate (mg AI/L water)	LC_{99} (%95 confidence limits) (mg AI/L)	Recommended dose (LC_{99})
Benfurocarb	Oncol EC 200	300.0	255(160.1-1947.7)	1.17
Carbaryl	Sevin 85 WP	1275.0	975.5(686.2-1799.8)	1.31
Carbaryl	Sevin XLR Plus	960.0	1789(975.6-19523.0)	0.54
Carbosulfan	Marshal 25 EC	312.5	21.2(18.4-27.8)	14.7
Furathiocarb	Deltanet 400 EC	400.0	67(52.5-141.8)	5.97
λ-Cyhalothrin	Karate 5 EC	25.0	9.1(6.75-18.9)	2.74
Methiocarb	Mesurol WP 50	500.0	158.5(107.7-343.3)	3.15

When the 95 % CL's of LC_{99} values were considered, CL's of two carbaryl formulations and benfurocarb were extended farther than field recommendation rate. Large confidence limits mean that heterogeneity of bioassayed population against these compounds are high. Although laboratory bioassay can give us a rough result about compounds' field performance, still we can say that these chemicals should be followed closely by further studies for resistance occurance. Perhaps for this reason, some growers complain about chemical control in poor effectiveness, especially when wide spread carbaryl usage for a long time[13], *ca.* 40 years in hazelnut orchards is considered.

There are a few results from field and laboratory studies regarding efficiency of insecticides used against hazelnut weevil. While some of them were conducted in field conditions, some others were about lethal dose determination of carbaryl and methiocarb with the method of topical application[13,18-21] used carbaryl against hazelnut weevil adults with topical application method and resulted in 2.34-fold resistance in population from Ordu province and 4.34-fold resistance in population from Trabzon when those compared with population of Kastamonu province Methiocarb is more effective to hazelnut weevil adults than carbaryl and 3.7-fold resistance was detected in the experiment 3 years later[13].

It is not possible to compare the studies made in the past with our research results in point of resistance as their application methods differ. But, the results[13,21] have raised doubts about carbaryl that used widely against hazelnut weevil since 1963. Similarly, the results presented here caused same kind of suspect about carbaryl. Also all other conventional insecticides evaluated in this study, except λ-cyhalothrin are being used against HW for many years in hazelnut growing area; therefore, we hope that evaluation their effeciacy and establishing base-line insecticide mortality will be useful for future resistance monitoring programs.

Conclusion

As result of this research, it was found that Azadirachtin did not have acute toxicity against hazelnut weevil adults in 72 h. Since azadirachtin has chronic effects on many insects depend on previous researches, it will be good idea to study on chronic effects of this chemical on hazelnut weevil in future. The results presented here show that λ-cyhalothrin has highest efficiency against hazelnut weevil adults. It is followed by carbosulfan, furathiocarb, methiocarb and benfurocarb in effectiveness. These data indicate that these recommended insecticides are effective in controlling hazelnut weevil adults if adults come in contact with these insecticides. Carbaryl compounds that being used for a long time and common pesticide against hazelnut weevil in hazelnut orchards were least toxic. This result was not surprise because carbaryl have already been used against hazelnut weevil for *ca.* 40 years in hazelnut growing area. Therefore, it is important to continually evaluate the field and laboratory performance of this insecticide for future insect control recommendations in hazelnut. More field and laboratory toxicity experiments on adults from different locations are needed to be done with this chemical in order to reveal resistance possibility. When recommended dosage and LC_{99} dose obtained are compared, a similar doubt raises for benfurocarb. This chemical also seems to deserve attention in future evalutions.

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