Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2016; 4(4): 1122-1125

Received: 23-05-2016
Accepted: 24-06-2016

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Comparative study on the efficacy of *Bacillus thuringiensis* var. *tenebrionis* and a neem based insecticide on adults and larvae of *Xanthogaleruca luteola* (Mull) (Col: Chyrsomelidae) in laboratory Conditions

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Abstract
The elm leaf beetle, *Xanthogaleruca luteola (*Müll). (Col: Chrysomelidae) is one of the most important pests on elm trees in Iran. Due to environmental issues of synthetic insecticides, biorational agents have been advised in control management programs of elm leaf beetle in urban green spaces. The susceptibility of adults and 3rd instar larvae of this pest was evaluated to Bithiran® (*Bacillus thuringiensis* var. *tenebrionis*) and NeemAzal®-T/S (Neem based insecticide) using elm leaves dipped in aqueous insecticidal solutions. The mortality was recorded 24 h after treatment. The LC_{50} values for the third instar larvae and adults were respectively estimated to be 106.83 and 57.6 ppm for BT and 357.17 and 107.61 ppm for Neem. Bithiran® was more effective against *X. luteola* larvae and adults when compared to NeemAzal®-T/S. Further research on these biorational agents is recommended in field conditions before making any decision on their incorporation in control management programs.

Keywords: Elm leaf beetle, Bithiran®, NeemAzal®–T/S

1. Introduction

Among a series of urban pests, the elm leaf beetle *Xanthogaleruca luteola* (Müll) (Coleoptera: Chrysomelidae), as a defoliating insect, causes important damage on elm (*Ulmus* spp.) in farms and urban areas [1, 2] This pest was first recorded to be found in Iran in 1945 and it has become one of the most significant urban trees' pests [3]. Both adults and larvae feed on the emergent leaves of the elm. The larvae skeletonize the leaves, destroying the tree's ability to achieve photosynthesis, adults beetle damage the foliage evidenced by a shot hole appearance in the leaves, and this damage continues throughout the growing period [4]. Repeated heavy infestation does not kill the tree outright, rather it usually weakens it, rendering it vulnerable to attack by insects and diseases. However, the beetle does transmit Dutch elm disease [2, 5]. Pesticides of biological origin have been intensively investigated for the past 30 years. An effort has been made to find an alternative to conventional insecticides. The alternative should be able to reduce health and environmental impacts [6]. There has been a worldwide interest in the development of alternative strategies, including the re-examination of using plant derivatives against agriculturally important insect pests. Plant derived materials are more readily biodegradable. Some have low toxicity to humans as well as natural enemies, and are more selective in action [7]. The application of such insecticides in urban areas holds special risks since most of them are not very selective, a realization that has led to the search for safe and environmentally friendly alternatives. Growing interest has been devoted to the development of control strategies with low environmental impact, such as botanical pesticides derived from naturally occurring plant compounds [8] and microbial agents, which are generally highly specific against target pests, thus facilitating the survival of beneficial insects in treated crops [9]. Among the most promising biological control agents, the entomopathogens such as viruses, bacteria and fungi provide a huge potential to control plant pests and diseases. The pesticide activity of these naturally occurring micro-organisms is mostly mediated by mechanisms highly specific to the target species, and typically based on modes of action, which are unique and usually not relevant to humans. Discovery of *B. thuringiensis* var.

tenebrionis was announced by Krieg *et al* [10] the "San Diego" strain was later identified by Herrnstadt *et al* [11], and Bithiran strain was announced in 2001 in Iran. These studies were conducted to determine the performance characteristics of the new *B. thuringiensis* strains for use in protecting elms against elm leaf beetle. Activity of the "tenebrionis", "San Diego" and "Bithiran" strains is apparently similar and is limited to larvae and adults of some beetles including the yellow mealworm, elm leaf beetle, and Colorado potato beetle. NeemAzal®-T/S contains NeemAzal®, the purified active ingredient of the seed kernels of the tropical Neem tree *Azadirachta indica* A. Juss. The active substance permeates into the leaves and is distributed partially systemic in the plant; the pest insects take it up orally upon feeding (sucking or biting). NeemAzal®-T/S has a special mode of action. It stops the insect´s feeding and plant damaging activity. However, a "knock down" effect should not be expected. Within a few hours after application of NeemAzal®-T/S, pest insects become inactive and after a few days, the population does not develop any longer and collapses. The present study examined the toxicity of the Bithiran® and NeemAzal®-T/S against the 3rd instar larval and adult stages of *X. luteola*.

2. Materials and Methods

2.1 Insect culture

The eggs, neonates and pupae of *X. luteola* were collected from elm trees in the campus of Ferdowsi University of Mashhad, Iran, where no pesticides were used. Eggs and neonates collection were used to obtain the newly emerged 3rd instar larvae and pupae for obtaining the adults. Fresh leaves were daily provided for feeding. The insect colonies were maintained in the laboratory at 25 ± 2 °C; 75± 5% RH and 16:8 L: D photoperiods. The adults and larvae were used in the corresponding tests 48 h after occlusion. Each individual was used only once to avoid pseudo replication.

2.2 Bioassay methods

The insecticides tested for oral toxicity bioassays were conducted using five different concentrations of the biorationals, *Bacillus thuringiensis* var. *tenebrionis* (Bithiran®, Mehr Asia Biotechnology Company) and Azadirachtin (NeemAzal®-T/S, Trifoio_M Company), thereafter abbreviated as BT and Neem, respectively. The concentration ranges of 50–200 and 20–150 ppm for BT and of 100-1000 and 50-200 ppm for Neem were used against larvae and adults, respectively. Each experiment was repeated 5 times with groups of 10 insects. Two equal-sized leaves of elm tree were dipped into the desired concentration for 30 seconds, dried in air for 30 minutes and placed at the bottom of each Petri-dish (90 mm diameter). Control leaves were dipped in distilled water and dried as above. Ten adults or larvae were transferred onto leaves of each Perti-dish, considered as experimental unit. Mortality was recorded 24h after treatment.

2.3 Statistical analysis

Mortality data for each developmental stage were analyzed with the probit model using the Maximum Likelihood Program [12, 13]. The results include estimation of the LC_{50} (and other LCs, if required) and the 95% confidence limits, slope and intercept of probit mortality regression, and the relevant statistical tests (such as "t" ratio, 'g' factor and heterogeneity). For comparison of the probit mortality lines, the program also provides the likelihood ratio tests of equality and parallelism [14]. Estimated median lethal concentration to kill 50% of insects was expressed as the LC_{50} (ppm). The estimates of values of parameters needed for computing confidence limits were provided by individual probit analysis from the POLO-PC output.

3. Results

The result showed that the mortality of larvae and adults of *X. luteola* increased as the concentrations of the biorationals increased (Figure 1). BT caused 81.67% mortality on larvae at the concentration of 200 ppm, while mortality of 85% on adults achieved at the concentration of 150 ppm. Neem caused 85% mortality on larvae at the concentration of 1000 ppm and 88.3% mortality on adults at the concentration of 200 ppm (Figure 1).

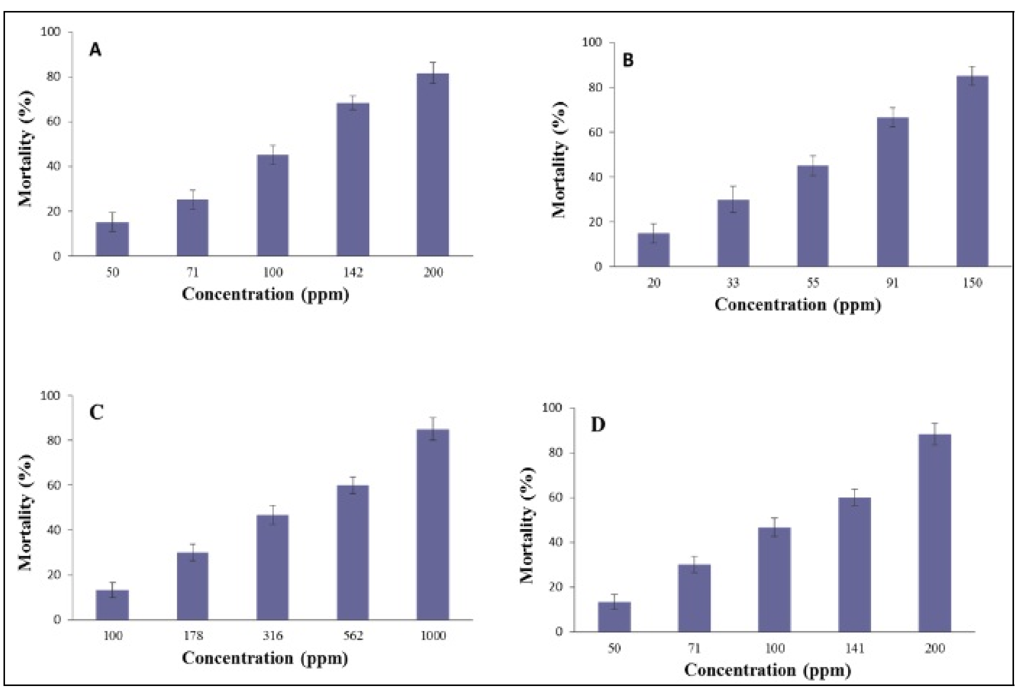

Fig 1: Mean percent mortality (± SE) of the 3rd Instar larvae (left) and adults (right) of *X. luteola* (Col: Chrysomelidae) after 24h treatment by various concentrations of BT (A-B) and Neem (C-D)

A more appropriate comparison among toxicities of the insecticides could be obtained using probit analyses of data. The results showed that both insecticides had a lethal effect on adult and 3rd instar larvae, but the adults were more susceptible than larvae (Table 1). The slope values of probit regressions were in the range of 1.94–3.37. The heterogeneity factors less than 1 indicated that there was no sign of systematic deviations in the chi-square (χ^2) values. For both insecticides, the regression tests (*t* ratio) were greater than 1.96 and the potency estimation tests (*g* factor) were less than 0.5 at all probability levels (Table 1).

The dose mortality responses of *X. luteola* larvae and adults were compared in terms of differences in slopes and intercept of probit regressions, and LC_{50}s values. The slopes of probit mortality regressions differed significantly among four biorational-life stage treatments, as revealed by rejecting the likelihood ratio test of parallelism (χ^2=14.05, *df*=3, *P*=0.003), as did the intercepts, as revealed by the likelihood ratio test of equality (χ^2=88.48, df=5, *P*<0.001). The slopes of probit mortality regressions for the BT against Larvae (3.37) was significantly greater than that on adults (2.32), as revealed by rejection of the likelihood ratio test of parallelism (χ^2=4.76, df=1, P=0.029). While similar comparisons for Neem showed that the slope of probit mortality regression on larvae (1.94) was significantly lower than those of adults (3.23), as revealed by rejection of the likelihood ratio test of parallelism (χ^2=7.89, df=1, *P*=0.005)

Table 1: Probit analysis of toxicity of insecticides to the 3rd instar larvae and adults of *X. luteola*

Life stage	Insecticide	Slope ± SE	Intercept ±SE	"t" ratio	Heterogeneity	g(0.95) factor	Lethal concentration (95% CL) (ppm)	
							LC_{50}	LC_{90}
3rd Instar larvae	BT	3.371±0.398	-6.84±0.80	8.47	0.17	0.05	106.83	256.36
Adults		2.322±0.275	-4.09±0.49	8.45	0.15	0.05	57.60	205.30
3rd Instar larvae	Neem	1.947±0.239	-4.97±0.61	8.16	0.36	0.058	357.17	1625.73
Adults		3.233±0.396	-6.57±0.80	8.16	0.36	0.058	107.61	268.07

BT and Neem applied as Bithiran® and NeemAzal®-T/S, respectively. SE: Standard error CL: Confidence limit

Based on probit analysis, the highest LC_{50} value (357.17 ppm) was obtained on larvae by Neem and the lowest value (57.60 ppm) on adults by BT (Table 1). Toxicity comparison using the LCs ratios and 95% confidence limits indicated that the toxicity of BT was significantly higher than that of neem, based on either the LC_{50} or LC_{90} values (Table 2).

Table 2: BT/Neem LC_{50}s ratios and their respective 95% confidence limits to compare insecticidal activity against larvae and adults of *X. luteola* ¥

Life stage	Ratio	95% CL of ratio€
3rdInstar larvae	0.30	2.69–4.15 *
Adult	0.54	1.54–2.27 *

BT and Neem applied as Bithiran® and NeemAzal®-T/S, respectively.
¥ Bioassay were conducted by leaf dipping method
€ Lower and upper 95% CL calculated as described by Robertson & Preisler (1992)
* Significant difference at *P*<0.05.

4. Discussion

Our results showed that adults of *X. luteola* were more susceptible to the biorationals than larvae. In a similar study, Shekari *et al*. also reported that adults were more sensitive than larvae to methanolic extract of *Artemisia annua* L. (Asteraceae) [3]. The findings from this study revealed that *B. thuringiensis* was more effective on both life stages of *X. luteola* compared to Neem under laboratory conditions. The toxicity of BT has been attributed primarily to the presence of delta-endotoxin [15]. Whitney *et al*., [16] have investigated the efficacy of *B. thuringiensis* (formulation of San Diego) in greenhouse and field conditions on the elm leaf beetle, *X. luteola*. According to their results in the greenhouse, BT caused 96.4% mortality on larvae and 97.6% on adults after 72 hour at the concentration of 2 lb/100gal (2500 ppm). In present study, BT (formulation of Bithiran) caused 81.67% mortality on larvae at the concentration of 200 ppm, and 85% mortality on adults at the concentration of 150 ppm after 24 hour. The observed differences between these studies might be due to variation in the type of formulation and/or bioassay conditions. The toxicity of *B. thuringiensis* (Biobit) and neem extract (*Azadirachta indica*) were evaluated against *Plutella xylostella* L. (Lepidoptera: Plutellidae) larvae under laboratory conditions using leaf dip method. For Biobit (*Bacillus thuringiensis* 1% WP) 5 mg, 7 mg and 10 mg were weighed and mixed with 10 ml of distilled water. For neem oil (*Azadirachta indica* 3% EC; Meliaceae), three different doses were prepared: 0.3 ml (low), 0.6 ml (medium) and 1 ml (high) per 100 ml of distilled water [17]. Their results showed that at high dose, Biobit caused 100% mortality after 5 days of exposure. At medium dose, the percentage of larval mortality was constant from 5 to 7 days and was 96%. At low dose, the percentage of larval mortality was the highest recorded at 7 days and was 92%. At high dose, neem oil showed a percentage of larval mortality up to 92% after 7 days of exposure. At medium dose, the percentage of larval mortality reached 88% after 8 days of exposure. At low dose, the maximum larval mortality rate was 40% between 6 and 8 days of exposure. The insecticidal activity of plant extracts has been shown to vary with dose and duration of exposure [18].

In a study on the insecticidal and antifeedant activity of different plant parts of *Melia azedarach* on *Xanthogaleruca luteola*, Defagó *et al*. [19] demonstrated strong antifeedant activity and fully inhibiting feeding at many of the concentrations tested. Several other studies have demonstrated the impact of neem on mortality of *X. luteola* [20, 21].

Hiromi *et al.* [22] stated that the low toxicity of Neem compared to *B. thuringiensis* could be attributed to its antifeedant and repellant effect on insects. This antifeedant and repellant effect of neem extracts has been reported to be accompanied by a significant reduction in food consumption by the herbivorous insect, which might be the cause of its low toxicity in this study.

The use of these insecticidal products could provide an alternative approach to conventional insecticides. Further experiments are needed to clarify the nature of the compounds involved in their insecticidal activity to optimize the effective doses. For practical purposes, the application of these biorationals should be evaluated in the field conditions as part of an integrated pest management (IPM) package.

5. Conclusion

With the increasing demand for alternatives to broad-spectrum chemical control agents for insect pests, particularly in urban environments, research into possible integrated pest management options is increasing. The use of natural pathogens such as bacteria and botanical pesticides derived from naturally occurring plant compounds for insect control is becoming more attractive. *Bacillus thuringiensis* var. *tenebrionis* was shown to be effective against the elm leaf beetle and is likely to play an important role in managing this insect's populations. Combining these control strategies into an integrated management program, in conjunction with a campaign to eliminate attractive overwintering sites, could be very important in relieving elm leaf beetle pressure on urban elms and reducing the mortality rate of susceptible trees.

6. Acknowledgments

This research is a part of the MSc studies of the first author. We would like to acknowledge the financial support provided by the Research council of Faculty of Agriculture, Ferdowsi University of Mashhad, Iran.

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