TOXICITY OF SELECTED INSECTICIDES TO *PIERIS BRASSICAE* L. (LEPIDOPTERA: PIERIDAE)

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ABSTRACT: The toxicity of carbaryl, pirimicarb (pirimor) and commercial formulation of neem using leaf dip and larval-dip techniques against second and third instars of *Pieris brassicae* L. larvae was determined. Larval mortalities rates were significantly higher with carbaryl and pirimor compared to the neem in the larval-dip bioassays 24-h after treatment. In the leaf-dip bioassays, the highest concentrations of carbaryl and pirimor caused 80% to 100% mortalities of larvae. Neem exhibited a significant lethal and antifeedant effects on second and third instars larvae, although the effect was slow and varied among the different larval instars. Quick cessation of food consumption of larvae on treated leaves was observed. Consequently, there was a negligible damage on the insecticide treated leaves. Based on the data collected in the current study it could be speculated that carbaryl and pirimor may have unduly residue on treated plants, therefore; neem extract is merit to be considered as a suitable control agent against *P. brassicae* larvae.

KEY WORDS**:** Bioassay, Cabbage, Large white butterfly, Leaf-dip technique**.**

Cabbage (*Brassica oleracea* L. var. *capitata*) is an important vegetable crop grown in many countries in the worldwide (Mazlan & Mumford, 2004). Several pests attack cabbage and the most serious damage is caused mainly by the larvae of several species such as: small white butterfly (*Pieris rapae* L.), large white butterfly (*Pieris brassicae* L.), cabbage moth (*Mamestra brassicae* L.), and diamondback moth (*Plutella xylostella* L.). All larval stages of large butterflies feed on foliage (Jankowska, 2006). Among them cabbage butterfly, *Pieris brassicae* L., is a cosmopolitan, polyvoltine insect (Spieth, 2002) and found wherever cruciferous vegetables are grown (Younas et al., 2004). Sometimes massive outbreaks of *P. brassicae* may occur and injury on cabbage cultures may be extensive (Metaspalu et al., 2009). The control of *P. brassicae* on vegetables is usually accomplished with the use of conventional chemical insecticides (Zafar et al., 2002). The damage notably affects the value of this crop because its consumption and sale happen when it is still fresh (Cartea et al., 2009). Synthetic insecticides have been in use for more than 50 years and have resulted in fast, economical and effective pest control (Gossa, 2007). Insecticides application is the dominant method for controlling *Pieris brassicae* in cruciferous crops because of a low market tolerance for pest damage and the lack of reliable alternative pest control options (Lundgren & Heimpel, 2003). Because larvae feed on the marketable portion of the crop, synthetic insecticides likely will remain an essential tool for successful cabbage production. However, their use should be minimized to prevent or at least delay development of resistance (Hill & Foster, 2000). The sole use of biological control may not always be sufficient to manage insect pest populations and supplementary insecticide treatments may be needed. Undoubtedly, compatibility between natural enemies and insecticides is a primary concern in programs of integrated pest management (Jalali & Leeuwen, 2009). Pirimor has good activity on aphids and also offer bee safety (Palumbo & Tickes,

2003). Pirimor is a selective systemic insecticide with contact, stomach and respiratory action, has been found to be useful in the implementation of IPM against aphids (Cabral et al., 2007).

Neem extracts, a very complex tetranortriterpenoids, has been effectively used against >400 species of insects, including many key crop pests, and has proved to be one of the most promising plant ingredients for integrated pest management at the present time. It is being used for the control of forest and crop pests, as an alternative to chemical insecticides. Several reports have described the antifeedant, repellent and growth modifying neem properties on the insects (Mikami & Ventura, 2008). Effects on Lepidopteran larvae have been documented, and this insect order seems to be particularly sensitive (Calvo & Molina, 2003). Neem extracts are usually safe for beneficial organisms, such as bees, predators and parasitoids, mammals, for the environment and with minimal residual effects (Pavela, 2009).

Concerns about the further development of resistance and undue residue have made the search for less hazardous chemical imperative. Our interest for this study stemmed from reports in which they reported neem extract with good characteristics in pest control programs. With retrospect, the objectives of this study were to compare the effects of insecticides in question on *Pieris brassicae* larvae. Also determine the effects of commercial neem extract antifeedant activity to the larvae under laboratory conditions.

MATERIALS AND METHODS

For laboratory experiments, eggs of *P. brassicae* (second generation) were collected from cabbage fields, at Urmia (a town in West Azarbadijan province) in July and August 2009. Resultant larvae were reared on fresh leaves of cabbage plants in the laboratory under short-day conditions (L12:D12) at 20–22 ^{o}C. Pirimor and carbaryl water suspension in five concentrations (250, 500, 1000, 1500 and 2000 ppm) and neem water emulsions in five concentrations (1, 2, 4, 8, and 16 %) and untreated control groups were tested. Freshly cut leaves of cabbage were individually dipped in a prepared insecticide solution for 10 seconds and after air-drying for 30 minutes, each leaf was placed in a separate 14.5 cm diameter Petri dish. Control leaves were treated similarly with tap water. Filter paper was placed inside a plastic petri dish and treated leaf tissue was placed on top of the filter paper. Fifteen randomly selected second and third instar larva were released in each petri dish and allowed to feed on fresh leaves. Each test was replicated four times. During the experiment, temperature varied from 22 to 24°C, day/night light period was 12/12 h and relative air humidity was 80— 85%. For carbaryl and pirimor, larval mortality was determined 24-h after treatment and for neem the mortality rate was scored 48, 72, 96 and 120 h post treatment.

One-way analysis variance (ANOVA) was employed to compare the means. LC_{50} values were estimated by likelihood program of probit analysis using SPSS 16 software.

RESULTS

Statistical analysis of the data indicated that there were significant differences among the means of treatments ($P < 0.01$). Larvae exposed to carbaryl and pirimor in the leaf- dip bioassay technique had significantly higher mortality rates when compared to the neem extract treatment.

The duration of the bioassay was an important factor affecting larval response

to tested insecticides. The responses of second and third instars larvae did differ in bioassays with pirimor and carbaryl at 24-h post treatment. Third instar larvae were significantly less susceptible to the insecticides compared to the second instar cohorts. LC_{50} values of carbaryl and pirimor on second instar were lower than LC_{50} values for third instar (Table 1 and 2).

Neem extract exhibited a significant lethal effect on larvae, although it was slow and varied among the larval instars. Forty-eight h. post treatment mortality increased and a direct positive relationship between mortality rates and concentrations was detected. The result of treated food consumption for second and third instars larvae showed that within first 24-h post treatment, no mortality has been recorded. Whereas, after 48-h, at the highest concentration of neem, dead larvae were observed. During this period of time, the larval color became brown and also food consumption cessation was detected. At 96-h post-treatment the toxicity increased corresponding to the applied concentration of neem (Table 3).

Larval weight was a significant factor in bioassays which affecting the response of *P. brassicae* larvae. When second instar larvae were introduced on treated leaf-disks, they stopped feeding, became moribund and experienced dying process. Therefore, the larvae did not cause noticeable damage. When third instar larvae were introduced on treated leaf-disks, they normally initiated food consumption for a certain period of time, but after a while, a permanent food consumption cessation was occurred. Therefore, some degree of damage occurred on the treated leaves.

DISCUSSION

Among the cabbage pests, *P. brassicae* is one the most destructive pests worldwide. The larvae feed on the foliage of plants and can completely defoliate and destroy the plant. Several biological control agents have been recognized, however in the outbreak of the pest, application of insecticide is the only tool to suppress the pest population.

Larval age and time length of exposure were significant factors affecting the response of *P. brassicae* to insecticides. Larval mortalities rates were significantly higher with chemical insecticides when compared with neem extract (botanical insecticide). Hill et al. (2000) showed that carbaryl was highly toxic to *P. xylostella* larvae. In the present study in leaf-dip bioassays, larval mortality on leaves treated with pirimor or carbaryl was significantly higher than those treated with neem. It has been reported that an application of a tank mix of crude *Plxy*GV inoculum and Pirimor (a selective pesticide, specific to aphids) caused a reduction in efficacy on *P. xylostella* larvae of the *Plxy*GV. In this case it is speculated that Pirimor could be acting as a feeding deterrent to *P. xylostella* (Ogutu et al., 2002). Botanical products are useful and desirable tools in most pest management programs because they can be effective and often complement the actions of natural enemies (Jogar et al., 2009). Generally, neem extract is effective against *P. brassicae* larvae with significant lethal and antifeedant effects accompanied by significant reduction in food consumption. Lethal and antifeedant effects of neem extracts or neem-based insecticides on *P. xylostella* larvae have been well documented (Perera et al., 2000). These results are well in agreement with the findings of the current study. Likewise, Zabel et al. (2002) reported high antifeedant effect of neem on *Lymantria dispar* and *Leptinotarsa decemlineata*. According to Liang et al. (2003) results, Agroneem, Ecozin and Neemix had lethal effects on the diamondback moth larvae, and neem oil reduced larval survival of

Helicoverpa armigera (Ma et al., 2000). Our data showed that neem was toxic to larvae and all larvae died priori to pupation. A direct relationship between concentration and mortality rate was detected. In conclusion, the older larvae (third instar) could inflect some degrees of damage on the treated leaves. Therefore, in order to prevent foliage damage, insecticides such as neem should be applied as early as possible against second instar larvae.

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Table 1. Mortality (Mean ± SE) and LC_{50} values of second instar larvae of *P. brassicae* exposed to two different insecticides.

Concentration (ppm)	Mean ± SE	LC_{50}	χ^2	Sig	Intercept ± SE	CV
Pirimori						
250	1.75 ± 0.47					
500	4.5 ± 0.64					
1000	9.2 ± 0.47	703	8.5	0.00	0.476 ± 0.09	12.34%
1500	12 ± 0.7					
2000	15 ± 0.0					
Carbaryl						
250	1. 5±0.64					
500	4±0.41					
1000	10.25±0.47	693	2.58	0.00	0.537±0.095	10.66%
1500	13±0.41					
2000	14.5±0.28					
Controls	-					

Table 2. Mortality (Mean ± SE) and LC_{50} values of third instar of *P. brassicae* larvae exposed to two different insecticides.

Concentration (ppm)	Mean ± SE	LC_{50}	χ^2	Sig	Intercept ± SE	CV
Pirimori						
250	1.75±0.47					
500	4.5±0.64					
1000	9.25±0.47	762	8.11	0.00	0.414±0.093	12.34%
1500	12±0.7					
2000	15±0.0					
Carbaryl						
250	1±0.41					
500	3.25±0.47					
1000	8.75±0.47	801	8.11	0.00	0.414±0.093	11.78%
1500	12.25±0.47					
2000	14.25±0.47					
Controls	-					

Table 3. Mortality (Mean ± SE) of second and third instars of *P. brassicae* larvae exposed to neem extract.

Concentration (%)	48 h		72 h		96 h		120 h
	L2	L3	L2	L3	L2	L3	L3
1	0.33±0.33	0.42±0.2	0.66±0.33	1.42±0.2	1.33±0.33	2±0	2.57±0.29
2	2.33±0.33	1.42±0.2	3.33±0.33	2.85±0.34	4±0.57	3.42±0.36	4.28±0.28
4	5 ± 0.57	2±0.21	8±0.57	5±0.37	10.33±0.33	7.1±0.4	7.71±0.28
8	8.33±0.33	3.28±0.35	12.66±0.33	6.42±0.2	15.66±0.33	8.7±0.42	10.28±0.35
16	12±0.57	5.28±0.47	16.66±0.33	9.57±0.68	19.66±0.33	11.85±0.67	13.42±0.36
Controls	-	-	-	-	-	-	-