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SUSCEPTIBILITY OF DIAMONDBACK MOTH, *PLUTELLA XYLOSTELLA* (LEPIDOPTERA: PLUTELLIDAE) TO SOME EUPHORBIACEAE PLANT EXTRACTS UNDER LABORATORY CONDITIONS

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ABSTRACT

Diamondback moth, *Plutella xylostella* is a serious insect pest of brassica crops. Its larvae skeletonize the leaves of host plants. Considering safer to environment, five plant extracts of *Ricinus cummunis*, *Euphorbia heliscopia*, *E. hirta*, *E. milii* and *E. pulcherrima* with a standard insecticide (chlorpyrifos) were tested. Second instar larvae of field collected *P. xylostella* were used for toxicity tests under laboratory conditions and changes in their biological parameters. Chlorpyrifos showed maximum mortality with LC_{50} of 1.13 ppm whereas *R. cummunis* was most effective with LC_{50} of 28.8 ppm followed by *E. heliscopia*, *E. milii*, *E. hirta and E. pulcherrima* of 32.9, 43.7, 44.6 and 40.5 ppm, respectively after 72 hrs exposure. Potential of Euphorbiaceae plant extracts needs further field testing for effective management tool against this important insect pest as safe and alternate bio-insecticide.
Keywords: Diamondback moth, toxicity, Euphorbiaceae, chlorpyrifos

INTRODUCTION

Diamondback moth, *Plutella xylostella* (Lepidoptera: Plutellidae) is a serious worldwide chewing insect pest of many cultivated and non-cultivated Brassicaceae plants comprising mainly cabbages, cauliflower, broccoli and ornamentals (Capinera, 2001; Reddy et al., 2004). It is also found to infest non-cruciferous crops like *Amaranthus viridis* (Vishakantaiah and Viseweshwargowda, 1975). However, cabbages and cauliflowers are preferred because of their thick and succulent leaves giving important olfactory and gustatory stimuli for better selection and proper colonization (Dubey and Chand, 1977). Severe infestation is caused on crucifers reported from at least 128 countries of the world with losses of about 16 million dollars (Mohan and Gujar, 2003).

Damage on cabbage and cauliflowers by *P. xylostella* is the major problem faced by growers. The degree of infestation changes according to the type of plant, location, level of natural enemies and management techniques utilized (Talekar and Shelton, 1993). Their short generation time and high fecundity rate enable them to become significant insect pest of brassica crops damaging almost all plant growth stages from seedling to head formation (Gujar, 1999). Larvae damage the plant by feeding on leaves and construction of tunnels in fruit head causing defoliation along with contamination and malformation of heads in cabbages, broccoli, cauliflower etc (Talekar and Shelton, 1993). Larvae skeletonize the leaves firstly feeding on leaves and then peep inside the curd (Sexena et al., 1989). It may reduce the production from 31-100% (Abraham and Padmanabhan, 1968).

Management of this important insect pest mainly relies on use of insecticides due to which it has developed resistance against almost every insecticide (Kumar and Gujar, 2005; Bhatti et al., 2013; Nasir et al., 2013). The use of synthetic insecticides is also causing environmental hazards, food contamination and mortality of naturally occurring biological control agents. Rapidly evolved resistance in DBM is due to its short generation time, large number of offspring and genetic changes in any given population (Freeman et al., 2006). This situation, stresses to find out the alternatives to manage this pest.

Significant reduction in *P. xylostella* population has been observed with application of certain plant extracts including *Alpinia galangal*, *Amomum cardamom*, *Cypeus rotundus* and *Gomphrena globosa* on cultivated cabbage reduced their damage to much extent (Dadang and Ohsawa, 2001). Antifeedant effect of neem and *Lantana camara* extracts was prominent on their larvae with reduced damage of treated cabbage leaves (Liang et al., 2003; Dong et al. 2005). Bing *et al*. (2008) tested the antifeedent activity of momordicine I and Π against their

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larvae and found momordicine II more active. For Euphorbiaceae plant extracts, extracts of *Euphorbia antiquorum* and *E. pulcherrima* were more active than others (Karnataka, 2009). Chloroform extract of *Acalypha fruticosa* showed antifeedant and larvicidal activities for their third instar larvae (Lingathurai *et al.*, 2010).

Studies regarding information on deleterious effects of plant extracts as bio-insecticide can contribute in pest management. Sublethal effects responsible for physiological or behavioral changes in individuals are also helpful in their effective control. Reduction in life span, development rate, fertility, fecundity, changes in sex ratio, feeding deterrence etc are important parameters necessary to provide wide picture of their role and contribution in pest management (Wei et al., 2010). Considering the importance of insecticidal properties of some Euphorbiaceae plants, susceptibility of 2nd instar larvae of *P. xylostella* was performed against their plant extracts under laboratory conditions.

MATERIALS AND METHODS

Insect culture: *Plutella xylostella* larvae (about 200) were collected randomly from cauliflower farmer fields of Taxila for further laboratory rearing at 25±2ºC, 50-60±5% RH and 14 hours light phase. Leaves of cauliflower were provided as natural diet. Pupae developed from these larvae were collected on daily basis and kept in a separate Petri dish. Adult emerged were reared in a rearing cage (12"×9"×6") covered with muslin cloth and provided with 10% honey solution. Two to three fresh leaves of cabbage were provided in cage for oviposition on daily basis. Eggs laid on cabbage leaves were collected and placed in 1kg plastic jars labeled with age information.

Preparation of plant extracts: Plant extracts of *Euphorbia helioscopia, E. milii, E. hirta, E. pulcherrima,* and *Ricinus communis* were used to test their lethal effects against *P. xylostella* larvae. Chlorpyrifos was used as standard to compare the toxicity of botanical extracts. Leaf samples of each plant were kept for 2-3 weeks for drying and then ground to make powder which was stored in glass flasks at 25°C. Samples of dehydrated plants were placed into 250ml flasks with 100ml water and 100 gram powder for 100% stock solution. Flasks were tightened with aluminum foil and kept in shaker for 24h in dark at 24°C. Plant extract was purified first through two-layered cheese cloth followed by vacuum purification. The terminus was further used for bioassays.

Bioassays: Toxicity of plant extracts was assessed against second instar larvae of *P. xylostella* by using no choice leaf dip method. 5cm diameter plastic Petri dishes were used. Stock solution in 50 ml distilled water was prepared and then diluted in half serial concentrations in 6-8 levels. Moist filter papers were placed at the base of Petri dishes. Fresh non-treated leaves of cauliflower were dipped in stock solution for 5-10 seconds (s) and then removed with the help of forceps and air-dried. Treated leaves were then placed in petri plates lined with moist filter paper. Five second instar larvae were released in each Petri dish. Similar process was performed with other concentrations including control. Petri dish was covered with lid to avoid escape of *P. xylostella* larvae. Mortality of *P. xylostella* was recorded after 48, 72 and 168 hr of treatment

Statistical analysis: The data observed were subjected to the statistical analysis using POLO-PC (LeOra, 1987) and SPSS for their respective LC_{50}, fiducial limits and slope ± SE based on mortality data. Based on LC_{50} value, comparative rate for change with standard LC_{50} value of chlorpyrifos was used to find out the relative toxicity effect of plant extracts at the respective time of observations.

RESULTS AND DISCUSSION

LC_{50} values comparison of the plant extracts and chlorpyrifos used as standard showed the highest toxicity of chlorpyrifos (3.78 ppm) against second instar larvae of *P. xylostella* at 48 hr observation. *R. cummunis* plant extract was better with LC_{50} of 32.8 ppm followed by *E. pulcherrima*, *E. hirta*, *E. milii* and *E. helioscopia* with LC_{50}s of 34.5, 35.2, 40.5 and 40.6, respectively after 48 hours. When compared with chlorpyrifos, the comparative ratios (CR) of *R. cummunis*, *E. pulcherrima*, *E. hirta*, *E. milii* and *E. helioscopia* were 8.67, 9.12, 9.31, 10.7 and 10.7 times less, respectively (Table 1). Comparison of LC_{50} values of five plant extracts and chlorpyrifos used as standard showed the highest toxicity of chlorpyrifos with 1.13 ppm value against

second instar larvae of *P. xylostella* at 72 hr observation. *R. cummunis* was the most effective plant extract with LC_{50} of 28.84 followed by *E. helioscopia*, *E. milii*, *E. hirta* and *E. pulcherrima* with LC_{50S} of 32.9, 43.7, 44.6 and 40.5, respectively after 72 hours (Table 1). When compared to the CR of chlorpyrifos, toxicity of *R. cummunis*, *E. helioscopia*, *E. hirta*, *E. milii* and *E. pulcherrima* were 28.8, 32.9, 44.6, 39.9 and 40.5 times less , respectively (Table 1).

After 168 hours of exposure, chlorpyrifos showed the highest toxicity (0.60 ppm LC_{50}) against second instar larvae of *P. xylostella*. *R. cummunis* was the most effective plant extract with almost insignificant LC_{50} than that of chlorpyrifos. *E. pulcherrima*, *E. helioscopia*, *E. hirta* and *E. milii* followed the toxicity of *R. cummunis*. Comparative ratio with chlorpyrifos showed *R. cummunis*, *E. pulcherrima*, *E. helioscopia*, *E. hirta* and *E. milii* with respective decrease trend in their toxicity (Table 1). According to to our results, Euphorbiaceae plant extracts of *R. cummunis*, *E. pulcherrima*, *E. helioscopia*, *E. hirta* and *E. milii* showed variable toxic response with respect to exposure time. The toxicity increased with time duration and took more time to exert toxic effect against target pest when compared with chlorpyrifos. Previously *E. pulcherrima* was found more active than other Euphorbiaceae plant extracts against *P. xylostella* (Karnataka, 2009).

Nature has provided plants with certain allelochemicals which make them to defend themselves against pest problems. Such plant chemicals affecting biology and behavior of certain insect pests have insecticidal, larvicidal and ovicidal properties including Euphorbiaceae plant family as observed against other lepidopteran insect pests (Karnataka, 2009). Present study was based on use of these plant extracts in water, however, organic solvents like hexane, chloroform and ethyl acetate had shown prominently higher toxicity of *Hygrophila auriculata*, *Citrus sinensis*, *Azadiractin indica*, *Zingiber officinale* and *Vitex negundo* (Baskar *et al.*, 2011). Significant reduction in *P. xylostella* population has been observed with *Alpinia galangal*, *Amomum cardamom*, *Cypeus rotundus* and *Gomphrena globosa* on cabbages with reduced their damage (Dadang and Ohsawa, 2001). They have also shown antifeedant and growth inhibitory effects on different insect pests (Sahayaraj, 1998; Lingathurai *et al.*, 2010). Methanol based extracts of *Synedrella nodiflora* proved toxic against *S. litura* (Rathi and Gopalakrishnan, 2005).

Different aspects like antifeedant, ovicidal and larvicidal effects to different biological parameters of different insect pests has shown promising results in past (Liang et al., 2003; Dong et al., 2005; Bing et al., 2008). However, there exist merely 1% of plant based pesticides worldwide focusing isolates and essential elements of active plant extracts (Isman and Grieneisen, 2014). Studies including deleterious effects of plant extracts responsible for physiological or behavioral changes in insect pests can be helpful in pest management (Wei et al., 2010). Considering the importance of insecticidal properties *R. cummunis*, *E. pulcherrima*, *E. helioscopia* and others, further efficacy trials to test different biological and behavioral responses of the pest may be performed. It will be helpful to conduct further field trials and selection for possible utilization of these botanicals for pest control.

Table 1: Susceptibility of second instar larvae of *Plutella xylostella* against some plant extracts of Euphorbiaceae under laboratory conditions

Plant extracts	After 48 hours			After 72 hours			After 168 hours		
	LC_{50} (FL at 95%)	CR	Slope±SE	LC_{50} (FL at 95%)	CR	Slope±SE	LC_{50} (FL at 95%)	CR	Slope±SE
Ricinus cummunis	32.8 (11.9-602)	8.67	0.75±0.19	28.8 (9.87-953)	25.4	0.63±0.18	0.71 (0.09-1.51)	1.18	0.49±0.16
Euphorbia helioscopia	40.6 (17.1-1162)	10.7	1.53±0.48	32.9 (14.3-280)	29.1	1.10±0.26	4.91 (2.34-33.4)	8.18	0.51±0.16
Euphorbia hirta	35.2 (16.0-1212)	9.31	1.80±0.60	44.6 (17.1-706)	39.4	1.09±0.28	4.90 (2.34-33.0)	8.16	0.51±0.16
Euphorbia milii	40.5 (16.5-547)	10.7	1.19±0.31	39.9 (16.5-702)	35.3	1.02±0.26	6.6 (2.87-134)	11.0	0.47±0.16
Euphorbia pulcherrima	34.5 (15.7-428)	9.12	1.50±0.42	40.5 (16.5-547)	35.8	1.19±0.31	4.91 (2.18-61.5)	8.18	0.46±0.16
Chlorpyrifos (standard)	3.78 (1.81-6.48)	1.00	0.70±0.16	1.13 (0.16-2.38)	1.00	0.61±0.16	0.60 (0.07-1.37)	1.00	0.71±0.18

hr = hour
LC_{50} = lethal concentration at 50% level
FL = Fiducial limits
CR = Comparative ratio with respective standard LC_{50}
SE = Significant error

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