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Toxicity and sub lethal effects of diamide insecticide (Cyantraniliprole 10.26 OD) on the biology of Diamondback moth, *Plutella xylostella* L. (Lepidoptera: Plutellidae)

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ABSTRACT : Toxicity of Cyantraniliprole to the diamondback moth *Plutella xylostella* L. was assessed using leaf dip bioassay method. The results of the probit regression analysis of mortality data revealed that the LC_{25}, LC_{50} and LC_{75} for 72 h, was 0.00012 ppm, 0.00708 ppm and 0.40624 ppm, respectively. The mean slope value of the probit regression analysis was 0.3835. The effects of sub lethal concentrations of Cyantraniliprole 10.26 OD applied at LC_{25}, LC_{50} and LC_{75} on the biological characteristics of the diamondback moth, *P. xylostella* were investigated, which shown the extended development time of all life stages, reduced larval weight, pupation rate, pupal weight, adult emergence, oviposition period, fecundity and fertility and higher number of malformed adults with the increased concentration of the insecticide. The net reproductive rate, intrinsic rate of increase and finite rate of increase of *P. xylostella* were significantly reduced when treated with increasing doses of Cyantraniliprole compared to control. The results suggest that sub lethal concentrations of Cyantraniliprole reduce population growth of *P. xylostella* by affecting its survival, development and reproduction.

Keywords: Cyantraniliprole, *Plutella xylostella,* Sub lethal effects, Biological parameters

INTRODUCTION

The diamondback moth *Plutella xylostella* (L.) has a great economic importance in Brassicaceae crops in many parts of the world. Agricultural intensification and increased production of cruciferous vegetable crops over the past decades have raised the pest status further, the estimated annual total costs associated with damage and management of DBM worldwide was 4-5 billion USD (Zalucki *et al.* 2012) and in India, it is about 168 million USD (Sandur, 2004). Due to intensive application of insecticides and unique biological properties such as genetic plasticity, high fecundity and short life cycle, this pest has developed resistance to over 93 compounds and become the more resistant pest (APRD, 2015).

The anthranilic diamides belong to group 28 (ryanodine receptor modulators) of the IRAC mode of action classification. The two distinct classes of diamide insecticides *viz.,* phthalic acid diamide (flubendiamide) and anthranilic diamides (chlorantraniliprole and cyantraniliprole) are newer class of insecticides which selectively target insect ryanodine receptors (RyR), a distinct class of homo-tetrameric calcium release channels and play a pivotal role in calcium homeostasis in numerous cell types resulting in feeding cessation, muscle paralysis, and ultimately death.

Cyantraniliprole is an o-amino-benzamide insecticide in which a cyano group replaced the 4-halo substituent of the former anthranilic diamide chlorantraniliprole. Cyantraniliprole, a third member of diamide group, possesses a cross spectrum activity against caterpillars, foliage feeding beetles, fruit flies, leaf miners and also sucking pests like aphids, whiteflies and thrips etc. The trans-laminar activity of Cyantraniliprole resulted in improved efficacy against sucking pests and low toxicity to fish, birds and mammals. In addition to directly killing insects, insecticides may have sub lethal effects. The repeated application of diamide group of insecticides to manage *P. xylostella* presently may result in the increase of resistance level in the near future. Preserving the efficacy of diamide insecticides as pest management tool is one of the most important challenges currently faced in agriculture.

Sub lethal effects are defined as physiological and/or behavioral effects on individuals that survived from exposure to a pesticide at low or sub lethal concentration. Sub lethal exposure to various insecticides could result in increased fecundity, fertility, and longevity in some insect taxa. However, reduced fecundity and longevity, and altered behavior were usually observed in most insect pests upon sub lethal exposure to insecticides. Because sub lethal effects could increase or decrease insect population growth, assessing such effects is important. The current research therefore determined the lethal and sub lethal effects of cyantraniliprole on *P. xylostella*.

MATERIALS AND METHODS

Insects

The field populations of *P. xylostella* were collected from Iruttupallam (11130^0N; 76158^0E) near Coimbatore and were mass cultured. Insects were collected in the form of larvae, maintained in the laboratory on cabbage or cauliflower leaves. Mustard seedlings were provided to facilitate oviposition. Adult moths were maintained in oviposition cages with 10% honey solution fortified with multivitamins as food.

Bioassay method and determination of sub lethal concentrations

Leaf disc bioassay as described by Tabashnik *et al.* (1991) was used. The cauliflower leaves were washed with Triton X-100 and air dried. Leaf discs of 6 – 8 cm were cut and dipped in six different concentrations of Cyantraniliprole 10.26 OD *viz.,* 0.0001, 0.0003, 0.001, 0.05, 0.1 and 0.3 ppm for 5 – 10 seconds and air dried thoroughly for an hour. The leaves were placed in bioassay container and 10 third instar larvae were released to feed on each treated leaf disc in three replicates with appropriate distilled water control. The mortality was counted every 24 hours, consecutively for 3 days.

LC_{50} values for different bioassays were estimated using probit analysis. All the experiments were carried out in a room with a photoperiod of 12:12 (L: D) and experiments with control mortality more than 20% were discarded and repeated. The mortality of larvae was observed up to 72 hours for LC_{25}, LC_{50} and LC_{75} and until adult emergence for growth and development studies. According to the bioassay results, application of LC_{25}, LC_{50} and LC_{75}values for 72 h were selected as sub lethal doses for the subsequent experiments.

The corrected percent mortality in case of single concentration toxin test was calculated using Abbott's formula (Abbott, 1925). Probit regression analysis and ANOVA were carried out by Finney (1971) method.

Sub lethal doses of cyantraniliprole 10.26 OD to *P. xylostella*

The development and reproductive behaviours of *P. xylostella* were studied by continuous exposure to sub lethal doses of Cyantraniliprole 10.26 OD. Hundred neonates were obtained from the stock culture were starved for 3 h prior to exposure to treated leaf discs. Cauliflower leaves were dipped in Cyantraniliprole solution at LC_{25}, LC_{50} and LC_{75} equivalent concentrations and the water control.

The survivors after 72 hours were fed with treated cauliflower leaves and continued until pupation. Pupae were weighed and placed in adult emergence cage. Pupae were examined daily for eclosion and the numbers of normal and deformed adults were recorded. Pupae were considered dead if adults had not emerged after 7 days. Five pair of male and female moths that were introduced into a single plastic container and provided with 10 % sugar solution. Cauliflower leaves were used as egg laying substrate and leaves were replaced daily until the cessation of egg laying.

Data on developmental parameters *viz.,* egg, larval and pupal periods, adult longevity, adult emergence, larval, and pupal weights as well as the reproductive behaviour *viz.,* fecundity, fertility and oviposition period of *P. xylostella* were recorded. Life-history parameters such as the intrinsic rate of increase (rm), finite rate of increase, net reproductive rate (R0) and the generation time (T) of the offspring were calculated from the data on survival rates and fecundity were obtained. The analysis of the raw data was facilitated by TWOSEX-MSChart (Chi, 2005).

RESULTS AND DISCUSSION

Toxicity of cyantraniliprole 10.26 OD to *P. xylostella*

The corrected mortalities of *P. xylostella,* when the late second instars were treated with six concentrations of Cyantraniliprole for 72 hours were shown in Fig. 1. The mortality obtained for 0.0001, 0.0003, 0.001, 0.05, 0.1, 0.3 ppm and 0.00 after 72 hours were 20.00, 30.00, 40.00, 53.33, 66.67, 80.00 and 6.67 % respectively. A perusal of the data depicted in Table 1 indicated that the LC_{25}, LC_{50} and LC_{75} values were 0.00012, 0.00708 and 0.40624 ppm respectively. The mean slope value of the probit regression analysis was 0.3835 as shown in Fig. 2. The results of the present study are in agreement with the findings of Liu *et al.,* (2015) where the LC_{50} values of the unselected populations of diamondback moth to Cyantraniliprole ranged from 0.069 mg l^{-1} to 0.15mg l^{-1}. Similarly, Dong *et al.,* (2016) Wang *et al.,* (2014) and Wang *et al.,* (2017) reported that the LC_{50} of Cyantraniliprole against *H. armigera* (0.175 mg l^{-1}); third-instar larvae of *Spodoptera exigua, Ostrinia nubilalis,* and *Agrotis ipsilon* (0.11, 0.05, and 0.13 mg/L, respectively) and *Bemisia tabaci* (0.00205 mg/ml), respectively.

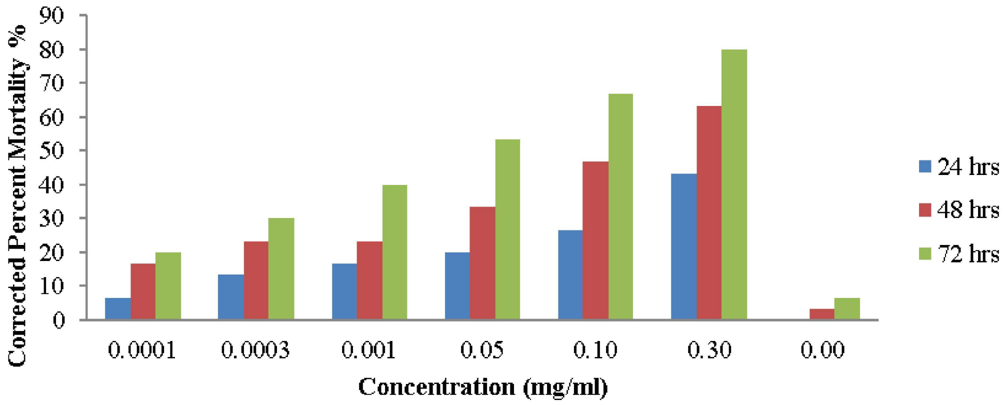

Fig 1. Toxicity of cyantraniliprole 10.26 OD against *P. xylostella*

Table 1: Probit regression analysis of dose response mortality data to Cyantraniliprole against *P. xylostella*

Sub lethal dose (ppm) (72 hrs)	95% fiducial limits		Slope	χ2*	Degree of Freedom (df)
	Lower	Upper			
LC_{25} (0.00012)	0.00001	0.00157	0.3835	1.8824	6
LC_{50} (0.00708)	0.00194	0.02578			
LC_{75} (0.40624)	0.05369	3.07378			

* In each case χ2 value from the goodness-of-fit test was less than the tabular value, (p = 0.05), indicating that the data fit the probit model.

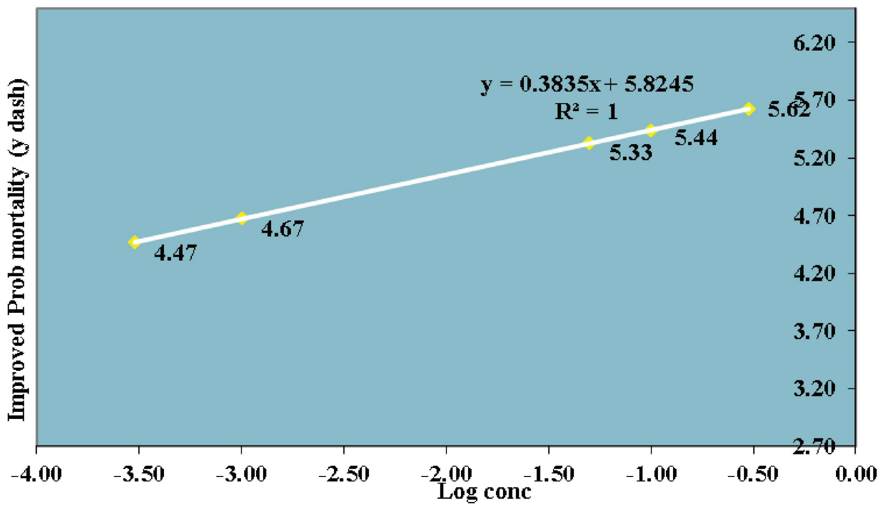

Fig. 2. Probit regression analysis of mortality data to Cyantraniliprole for *P. xylostella*

Sub lethal effects of Cyantraniliprole on the developmental and reproductive parameters of *P. xylostella*

The sub lethal concentrations of Cyantraniliprole had a considerable effect on the developmental as well as the reproductive parameters compared to untreated populations which are depicted in Table 2 and 3. The increased concentrations of Cyantraniliprole extended the developmental period of egg (3.4, 3.7, 3.9 and 3.4 days for LC_{25}, LC_{50}, LC_{75} and control, respectively), larva (6.03, 6.56, 6.63 and 6.03 days for LC_{25}, LC_{50}, LC_{75} and control, respectively), pupa (4.56, 4.58, 4.8 and 4.41 days for LC_{25}, LC_{50}, LC_{75} and control, respectively), adult male (7.52, 8.17, 9.25 and 7.2 days for LC25, LC50, LC_{75} and control, respectively) and adult female (8.52, 9.45, 9.57 and 8.17 days for LC_{25}, LC_{50}, LC_{75} and control, respectively). The results of the above study are in agreement with Kannan *et al.*, (2017), they have reported that the Bt endotoxin confirmed the prolonged duration of egg, larva and adult insects and reduced the pupal weights and pupation in *P. xylostella*.

Table 2. Sub lethal effects of Cyantraniliprole on the developmental parameters of *P. xylostella*

Sub lethal dose (mg/ml)	Egg period (days)*	Larval period (days)*	Pupal period (days)*	Larval weight (mg/larva)*	Pupation (%)*	Pupal weight (mg/pupa)*	Oviposition Period (days)*
LC_{25}	3.4c	6.03b	4.56b	0.42a	76.67b	4.9a	6.4b
LC_{50}	3.7b	6.56a	4.58b	0.38c	63.33c	4.3c	6.11c
LC_{75}	3.9a	6.63a	4.8a	0.38c	53.33d	4.1d	7.00a
Control	3.4c	6.04b	4.41c	0.41b	83.33a	4.7b	7.00a
SED	0.0646	0.0747	0.0612	0.0031	0.8947	0.0677	0.0519
CD (0.05)	0.1407	0.1627	0.1333	0.0067	1.9495	0.1474	0.1131

* Mean of 30 observations; Means followed by different letters indicate significant differences ($P<0.05$, LSD)

There was a significant reduction in the larval weight (0.42, 0.38, 0.38 and 0.41 mg per larva for LC_{25}, LC_{50}, LC_{75} and control, respectively) and pupal weight (4.9, 4.3, 4.1 and 4.7 mg per pupa LC_{25}, LC_{50}, LC_{75} and control, respectively). The results of the present study were in concordance with the findings of Yin *et al.* (2008) and Kannan *et al.*, (2017) concluded that the sub lethal concentrations of Spinosad and Bt prolonged the development duration and reduced the pupal weight, respectively.

The sub lethal doses of Cyantraniliprole also suppressed the pupation rates, adult emergence and oviposition but there was a significant increase in the malformed adults (12.9, 22.45 and 45.45 per cent for LC_{25}, LC_{50} and LC_{75} respectively) compared to control (4.17%). The fecundity of the females treated with LC_{50} and LC_{75} were 59.00 and 43.00 respectively, which were significantly lower than that of control (72.00). In *Spodoptera exigua* (Hübner), sub lethal effects of cyantraniliprole markedly shortened adult longevity, reduced pupation rate, and survival rate after exposure to LC_{10} and LC_{25} concentrations (Yu *et al.,* 2015). Similar results were reported by Yin *et al.* (2008) where the fecundities of females when treated with Spinosad the LC_{25} and LC_{50} doses for 48 h were significantly lower than those given the control treatment, at 32.6% and 49.2%, respectively.

Similarly, the fertility (%) was also reduced in the treated insects (83.29, 77.25, 75.9 per cent for LC_{25}, LC_{50}, LC_{75}, respectively) compared to control (85.6%). A study conducted by Liu *et al.* (2015) in comparison with the laboratory selected and untreated populations of *P. xylostella*, revealed the Cyantraniliprole selected populations showed significantly lower fertility and hatchability. The sub lethal effects of Bt endotoxin Cry 1 Ac to *H. armigera* (Kannan and Uthamasamy, 2006) also showed reduction in healthy adult emergence, fertility and increased malformed adults which were in agreement with the current findings.

Table 3. Sub lethal effects of Cyantraniliprole on the reproductive parameters of *P. xylostella*

Sub lethal dose (mg/ml)	Adult emergence (%)*	Healthy adult emergence (%)*	Malformed adult emergence * (%)	Adult longevity		Fecundity (No. of eggs laid) **	Fertility ** (%)
				Male	Female		
LC_{25}	82.61b	85.71b	14.29c	7.52c	8.52b	69b	83.29b
LC_{50}	78.95c	73.33c	26.67b	8.17b	9.45a	59c	77.25c
LC_{75}	68.75d	54.55d	45.45a	9.25a	9.57a	43d	75.9c
Control	88.00a	95.45a	4.54d	7.2d	8.17c	72a	85.6a
SED	0.7650	0.6531	0.1235	0.1315	0.1320	0.8101	0.7313
CD (0.05)	1.6667	1.4230	0.2691	0.2866	0.2877	1.7651	1.5935

*- Mean of 30 observations; Means followed by different letters indicate significant differences (P<0.05, LSD), **No. of observation = 5 pairs

The effects sub lethal doses of Cyantraniliprole on the life table parameters were studied (Table 4). Significant reduction was resulted in the intrinsic rate of increase (r_m) when the concentration of insecticide increases. The finite rate of increase for LC_{25}, LC_{50}, LC_{75} and control were 1.229, 1.193, 1.145 and 1.239 also resulted in significant reduction. The above reduction indicated that the sub lethal effects of Cyantraniliprole may influence the population dynamics of the pest. Lashkari *et al.* (2007) reported that intrinsic rates of increase of cabbage aphids were lower in populations treated with sub lethal concentrations of imidacloprid and pymetrozine than in controls.

Table 4. Sub lethal effects of Cyantraniliprole on the life table parameters of *P. xylostella*

Sub lethal dose (mg/ml)	Life table parameters			
	Intrinsic rate of increase (r_m)	Finite rate of increase	Net reproductive rate (R_0)	Generation time (T)
LC_{25}	0.206	1.229	34.5	17.18
LC_{50}	0.176	1.193	26.55	18.6
LC_{75}	0.135	1.145	15.05	20.07
Control	0.214	1.239	36.00	16.72
SED	0.0025	0.0154	0.1068	0.1836
CD (0.05)	0.0054	0.0337	0.2327	0.3999

The net reproductive rate also tended to reduce in the sub lethal concentrations LC_{25}, LC_{50}, LC_{75} 34.5> 26.55> 15.05 and control (36.00) (Table 4). On the other hand, Kerns and Stewart (2000) reported that bifenthrin increased the net reproductive rate of aphids treated with sub lethal doses (resurgence). This may be due to difference in insect species as well as chemical group. In the present study, the larval, pupal and adult development were extended with the sub lethal doses indicating the increased generation time (T) of the treated populations compared to control. Similar reports were reported by Yin *et al.* (2008) that the mean generation times of *P. xylostella* were prolonged when treated with sub lethal concentrations of Cyantraniliprole.

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