



FITNESS COST ASSOCIATED WITH DIAMIDE-RESISTANT POPULATION OF BRINJAL SHOOT AND FRUIT BORER *LEUCINODES ORBONALIS* GUENEE

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ABSTRACT

Brinjal shoot and fruit borer, *Leucinodes orbonalis* is a serious pest of brinjal and has evolved resistance to commonly used diamide insecticides such as flubendiamide and chlorantraniliprole. Lifetable parameters of the field populations of *L. orbonalis*, collected from brinjal fields of Kullarayanpalayam (Palakkad) (PKD) and Anchal (Kollam) (KLM), Kerala, reported resistance to diamides was studied and compared with that of laboratory-reared susceptible population of *L. orbonalis* (Lo-S) procured from ICAR- NBAIR, Bengaluru to determine the fitness cost associated with diamide resistance. The results revealed that the PKD population was found to have a significantly longer larval period with 12.7 ± 0.82 days as compared to the Lo-S population (10.6 ± 2.71 days), while the KLM population showed a larval period of 11.1 ± 0.87 days. The pupal duration was significantly longer in both KLM and PKD populations with 10.3 ± 0.48 and 11.8 ± 0.63 days respectively; whereas, the Lo-S population showed only 9.6 ± 0.69 days. The longevity of female adults of PKD population was (3.80 ± 0.63 days), while KLM and Lo-S populations showed 4.20 ± 0.63 and 4.40 ± 0.51 days respectively. The fecundity of KLM (178.9 ± 6.08 eggs/ female) and PKD (171.40 ± 5.64 eggs/ female) populations were significantly lower when compared with the Lo-S population (194.1 ± 4.38 eggs/ female). Only 89.11% eggs hatched in the PKD population while 89.34 ± 2.73 and $91.87 \pm 4.01\%$ of eggs hatched in KLM and Lo-S populations, respectively. However, the fitness parameters like the incubation period, pupation rate, adult emergence rate, female ratio, and male longevity were not significantly different. The PKD and KLM populations showed both negative and positive biological trade-offs exhibiting a relative fitness of 0.74 and 0.82 respectively compared to the Lo-S population (1).

Key words: *Solanum melongena*, *Leucinodes orbonalis*, lifetable parameters, diamide resistance, fitness, pupa, larva, adult, longevity, incubation, sex ratio, eggs, fecundity

Leucinodes orbonalis Guenee native to south and southeast Asia, with India considered as the centre of origin (Karthika et al., 2019), is a major pest of *Solanum melongena* (eggplant) in Asia. Calendar-based prophylactic spraying of insecticides belonging to different groups is practiced by the farmers to manage *L. orbonalis*. Diamide insecticides are extensively used among farmers due to their great efficacy against lepidopteran pests and low mammalian toxicity (Larson et al., 2014). However, over-reliance on diamide insecticides has resulted in the development of resistance in many lepidopteran pests including *L. orbonalis* in India (Kariyanna et al., 2020; Thomas et al., 2024). Fitness cost studies play a crucial role in the insecticide resistance management (IRM) program. According to Kilot and Ghanim (2012), the development of insecticide resistance is often correlated with the existence of fitness costs. To adapt and survive in a toxic environment, the pests develop resistance by target site mutation or metabolic detoxification which requires a higher resource allocation and energy consumption.

Insects own a variety of behavioural, physiological, and genetic strategies to deal with hazardous pesticides. These strategies can occasionally result in resistance by constituting overexpression of detoxifying enzymes or causing mutations in the target locations. Such activities might hinder the insect's capacity to reproduce, its ability to disperse, and have several other negative repercussions on its fitness. Hence, when an insect develops resistance to an insecticide, it often comes at a cost. This cost is known as a "fitness cost" and can include increased energy expenditure or other disadvantages that make the resistant insect less fit than its non-resistant counterparts in the same population. Resistance management strategies benefit from the existence of fitness costs related to resistance. A pest population exhibiting fitness costs linked to pesticide resistance shows a decrease in the frequency of resistant individuals in the absence of selection pressure. In contrast, the frequency of resistant individuals tends to grow in the absence of a fitness cost linked to insecticide, favouring the evolution of resistance (Kilot

and Ghanim, 2012). Hence, the present study aimed to investigate the fitness cost associated with diamide-resistant population of *L. orbonalis* which has not been studied earlier.

MATERIALS AND METHODS

Leucinodes orbonalis population maintained in the laboratory of the Indian Council of Agricultural Research - National Bureau of Agricultural Insect Resources (ICAR- NBAIR), Bengaluru since 2012 without any exposure to insecticides showing an LC_{50} value of 0.50 ppm to flubendiamide and of 0.119 ppm to chlorantraniliprole was considered as the susceptible population (Lo-S). The diamide-resistant populations of *L. orbonalis* PKD (Palakkad) and KLM (Kollam) used in the study were collected from brinjal fields of Kullarayanpalayam (Palakkad) ($10^{\circ}45'28.2''$ N; $76^{\circ}49'43.7''$ E) and Anchal (Kollam) ($8^{\circ}57'47.6''$ N; $76^{\circ}52'14.6''$ E), Kerala, which exhibited an LC_{50} value of 110.29 ppm and 23.99 ppm for flubendiamide and 29.19 ppm and 2.17 ppm for chlorantraniliprole. A resistance ratio of 218.84-fold and 47.59-fold for flubendiamide and 244.75-fold and 18.22-fold for chlorantraniliprole was expressed by PKD and KLM populations, respectively. The two populations were maintained under laboratory conditions at $27\pm 2^{\circ}C$, 60-70% RH, and a photoperiod of 14:10h (L:D). The larvae were reared on a natural diet (potato) without any exposure to insecticides and adults were fed with 10% honey.

For the fitness cost study, first-generation larvae of the field populations were screened for susceptibility to flubendiamide, and larvae that survived LC_{90} were further reared for further studies. The populations were maintained in the laboratory on healthy potatoes without any further exposure to insecticides to study the fitness cost. Ten neonate larvae of the F_2 generation of PKD, KLM, and Lo-S population were transferred into a plastic container (5 cm in height x 3.5 cm in diameter) with potato pieces (ten neonate larvae as a replicate, a total of ten replicates) labelled with the date of hatching. The larvae were maintained on the potato until they reached the sixth instar and came out for pupation. The larval period and the number of larvae undergone pupation were observed. Emerged pupae were transferred into a container (7 cm height x 21 cm dia) labelled with the date of pupation and maintained until the adult emergence. The pupal period, the number of adults emerged, and the sex ratio were observed. Ten pairs of freshly emerged male and female adults were transferred into ten cylindrical containers (15cm

height x 10 cm dia) lined with black paper and were fed with 10% honey solution. The mouth of the cylindrical containers were covered with a black net of fine mesh for egg-laying purposes which was replaced daily. The adult life span, oviposition period, and fecundity were recorded. The black net cloth with eggs were transferred into plastic containers (7 cm height x 21 cm dia) labelled with the date of egg laying. The incubation period of the eggs was observed and the number of newly emerged larvae (hatchability) was observed.

The lifetable parameters like larval developmental duration (L), pupation rate (Pr), pupal duration (P), emergence rate (Er), female ratio (Fr) and adult longevity (A), fecundity (F), egg period (E), and hatchability (Ha) were analyzed by analysis of variance (ANOVA) in GRAPES 1.0.0 in CRD (Gopinath, 2021) and means were separated by Tukey's test. The population trend index (R_0), and relative fitness (R_f) were calculated as follows: $Nt = N_0 \times Pr \times Fr \times Er \times Fd \times Ha$; $R_0 = Nt/N_0$; $R_f = R_0T/R_0S$, where, N_0 and Nt are the number of individuals in the initial population and the next generation, respectively. R_0T and R_0S are the increased trend index of the field-collected populations and LoS population, respectively (Sun et al., 2023).

RESULTS AND DISCUSSION

Lifetables of the Lo-S, KLM, and PKD populations were established to compare the fitness parameters (Table 1). The results showed that the PKD population had a significantly longer duration of larval period with 12.7 ± 0.82 days when compared with the Lo-S population showing only 10.6 ± 2.71 days. While with a developmental period of 11.10 ± 0.87 days, the KLM population showed statistical similarity with the Lo-S population. The pupal period of both KLM and PKD populations were 10.3 ± 0.48 and 11.8 ± 0.63 days respectively which differed significantly from the pupal period of the Lo-S population (9.6 ± 0.69 days). The female adult longevity was the lowest for the PKD population with 3.80 ± 0.63 days which was significantly different from the Lo-S population showing 4.40 ± 0.51 days, followed by KLM with 4.20 ± 0.63 days of female longevity. The fecundity of the Lo-S population was 194.1 ± 4.38 eggs/ female, while the PKD and KLM populations showed fecundity rates of 171.40 ± 5.64 and 178.9 ± 6.08 eggs/ female which was significantly lower compared to the Lo-S population. The hatchability also differed significantly among the populations with the lowest rate of $89.10\pm 3.87\%$ for the PKD population and the highest of $91.87\pm 4.01\%$ for the Lo-S population.

Table 1. Lifetable parameters of Lo-S, PKD and KLM population of *Leucinodes orbonalis*

Life parameters	Population		
	Lo-S	PKD	KLM
Larval period (Days)	10.60± 2.71 ^b	12.70± 0.82 ^a	11.10± 0.87 ^b
Pupation rate (%)	90.00± 0.00 ^a	89.00± 5.67 ^a	90.00± 0.00 ^a
Pupal period (Days)	9.60± 0.69 ^c	11.80± 0.63 ^a	10.30± 0.48 ^b
Adult emergence rate (%)	92.22± 5.37 ^a	87.72± 5.92 ^a	88.88± 5.23 ^a
Male longevity (Days)	3.80± 0.42 ^a	3.40± 0.84 ^a	3.60± 0.51 ^a
Female longevity (Days)	4.40± 0.51 ^a	3.80± 0.63 ^b	4.20± 0.63 ^{ab}
Female emergence rate (%)	56.67± 5.51 ^a	52.69± 3.51 ^a	53.94± 4.52 ^a
Fecundity (No. of eggs/female)	194.10± 4.38 ^a	171.40± 5.64 ^c	178.90± 6.08 ^b
Incubation period (Days)	4.40± 0.84 ^a	4.40± 0.69 ^a	4.40± 0.69 ^a
Hatchability (%)	91.87± 4.01 ^a	89.11± 3.87 ^b	89.34± 2.73 ^{ab}
Relative fitness	1.00	0.74	0.82

However, no significant difference was observed among the PKD, KLM, and Lo-S populations in the pupation rate (89.00± 5.67, 90.00± 0.00, and 90.0± 0.00%), adult emergence rate (87.72± 5.92, 88.88± 5.23, and 92.22± 5.37%), male longevity (3.40± 0.84, 3.6± 0.51, and 3.80± 0.42 days), female emergence rate (52.69± 3.51, 53.94± 4.52, and 56.67± 5.51%), and incubation period (4.4± 0.69, 4.4± 0.69, and 4.4± 0.84), respectively. Compared with the Lo-S population the relative fitness of the PKD and KLM populations were estimated to be 0.74 and 0.82, respectively which suggested the association of fitness costs with diamide resistance. These results indicated a survival disadvantage with the resistant populations compared to the Lo-S population and the results are in conformity with the observation of Oliveira Padovez et al. (2022), who reported a relative fitness of 0.64 for chlorantraniliprole-resistant strain of *Spodoptera frugiperda*. The variation in relative fitness may stem from the difference in diamide resistance among field populations. The considerable fitness cost suggests that the development of diamide resistance can be delayed under effective insecticide resistance management (IRM) programs. According to Liu et al. (2015), cyantraniliprole selected laboratory strains of *Plutella xylostella* showed longer larval developmental time, decreased rate of pupation and adult emergence, and a lower fertility with a lower relative fitness. Shah et al. (2017) also reported that insecticide-resistant insects show a shorter life span, delayed maturation period, and lower survival rates than insects not exposed to insecticides. Sun et al. (2023) stated that the reduced fitness in *Chilo suppressalis* displaying a fitness cost of 0.53 can lead to the reversion of tetraniliprole resistance in the absence of the insecticide. Multiple generations of treatment with diamide insecticides have been reported

to significantly modify pests' biological parameters and result in prolonged larval duration, reduced pupation and adult emergence rate, and decreased fecundity and hatchability (Sun et al., 2022; Shah and Shad, 2020; Ribeiro et al., 2014). The present study emphasizes that the diamide-resistant population of *L. orbonalis* showed a reduced relative fitness with lower survival duration of female adults, lower fecundity, and reduced hatchability. Also, to manage insecticide resistance, a study on the fitness cost of insect pests exposed to insecticides is necessary which plays a crucial function in IRM programs.

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AUTHOR CONTRIBUTION STATEMENT

Conceived and designed the analysis (AT, SMS, and BP); Performed work (AT); contributed reagents (BP); wrote the manuscript (AT); Corrected the paper (SMS, BP, and MC). All authors read and approved the manuscript.

CONFLICT OF INTEREST

No conflict of interest.

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