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Sublethal Dose Impact of λ Cyhalothrin on Life Table Parameters of Ladybird Beetle, *Coccinella septempunctata* (Coleoptera: Curculionidae) Reveals Tolerance in Field Population

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Muhammad R, BA and Misbah R conducted the experiment, Muhammad R analyzed the data. Muhammad R, BA, Muhammad A, MT, SA and Muddassir A wrote the article. AMS critically reviewed and revised the manuscript.

Key words
***Coccinella septempunctata*, λ cyhalothrin, Generalist predator, Life table parameters, Sublethal dose**

ABSTRACT

Insecticides are a quick tool to suppress insect pests and an important element of integrated pest management (IPM). λ cyhalothrin, a pyrethroid, is commonly used to manage economic insect pests in agricultural crops since long. An assay was designed to evaluate the efficacy of λ cyhalothrin on biological parameters of generalist predator, *Coccinella septempunctata*. Results indicated that λ cyhalothrin has no significant impact on developmental life stages of larvae of ladybird beetle but it significantly reduced the span of total adult longevity (30.8 to 26.91 d), female adult longevity (31.5 to 27.79 d), and male adult longevity (29.4 to 25.56 d) as compared to control. Among population parameters, λ cyhalothin had a non-significant impact on adult preoviposition (APOP), total preoviposition period (TPOP), oviposition period, intrinsic rate of increase, finite rate of increase, and mean generation time (T). However, it had significantly reduced the fecundity of females from 294.00 to 262.43 eggs/female and net reproductive rate (R_0) (174 to 91.85 d). Our results showed that *C. septempunctata* had adopted to manage λ cyhalothrin and it could be used in experiments involving *C. septempunctata* as a natural biocontrol agent.

INTRODUCTION

Ladybird beetles are the member of class insecta and belongs to the family Coccinellidae (Atta *et al*., 2019). The lady bird beetle (Coleoptera: Coccinellidae) is common to a wide range of natural and agricultural habitats with worldwide distribution. Coccinellids are Holometabolous insects possess four stages in their life cycle i.e., egg, larva, pupa, and adult. There are three molting and four larval instars (Pervez, 2004). All motile stages are predator but the larval and adult stages are very good predators of aphids and other small insects (Hangay and Zborowski, 2010). Its 3rd and 4th instars larvae are more voracious as compared to 1st and 2nd instar (Atta *et al*., 2019). Predatory potentials of females are more than males and laboratory reared adults are also more voracious than the field collected. Wheat (*Triticum aestivum* L.) is the main cereal staple crop of Pakistan and affected by aphids (Anonymous, 2018; Atta *et al*., 2019). Therefore, aphids attained the status of regular pest in Pakistan and regular monitoring of wheat crop is very important during the crop season (Abdulkhairova, 1979; Atta *et al*., 2019). Biological control is the action of parasitoids, predators and pathogens in maintaining density at a lower average than would otherwise occur. According to Sathe and Bhosale (2001), predators are the organism which directly attack, kill, and eat one of the other species (prey of host). Biological control agents comprises an important elements of many integrated pest management (IPM) program but many synthetic pesticides affect them negatively (Mordue and Blackwell, 1993). Natural enemies/ biological control agents are the most important factors to regulate the pest population for keeping the insect pests below economic threshold level (Atta *et al*., 2019). Ladybird beetles are very common for controlling many insects and different studies have been done on them (Singh and Bras, 2004; Ullah *et al*., 2012; Farooq *et al*., 2018; Atta *et al*., 2019). λ

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cyhalothrin is commonly used insecticides for insect pest management on wheat, rice, and other crops (Atta *et al.*, 2019).

Arthropods resistance to insecticides is of great concern in integrated pest management programs for applied entomologist. More than 600 species of insects and mites have been reported to develop resistance to one or more chemicals (Whalon *et al.*, 2012). The development of resistance in insect pests lower the agronomic value of the insecticides (Jiang *et al.*, 2011; Whalon *et al.*, 2015). On the other hand, fewer insects from natural enemies have shown resistance to insecticides (Croft, 1990; Whalon *et al.*, 2012). The repeated exposure to same to pesticides can evolve resistance in natural enemies like agricultural insect pests (Croft and Morse, 1979; Pathan *et al.*, 2008; Pree *et al.*, 1989; Rodrigues *et al.*, 2013a). The development of resistance to certain pesticide could be attributed to intrinsic factors such as genetic makeup, behavioural patterns, and metabolic physiology, in addition to extrinsic factors such as pesticide properties and exposure frequency and coverage (Forgash, 1984; Georghiou and Taylor, 1977a; Rosenheim and Tabashnik, 1990; WHO, 1957). It is therefore of interest that certain predator insects appear less susceptible as compared to other insect/ groups (Tillman and Mulrooney, 2000; Williams *et al.*, 2003), their prey (Gesraha, 2007), or even key pest species (Spíndola *et al.*, 2013).

To use beneficial arthropods as bio-control agents or preserve their local natural populations in integrated pest management, their susceptibility to the pesticides used must be taken into account. In order to save released or local beneficial fauna and to augment and exploit their performance, several well-known strategies needed to be exploited. Looking for preparations harmless to biological agents among the existing pesticides; developing novel selective active ingredients finding (collecting/selecting) tolerant or resistant strains of natural enemies. The success of biological control may be enhanced by preventing the careless use of pesticides by having direct and indirect toxic effects on natural enemies. These adverse effects on the bio-agents can be minimized by considering and implementing some tactics which may play important role in expanding the function of biological control. The goal of IPM is to select such chemicals that are having compatibility. So, this study had been carried out to evaluate the sublethal impact of λ cyhalothrin on biological parameters of ladybird beetle.

MATERIALS AND METHODS

Insects

Coccinella septempunctata adults were collected from experimental area Rice Research Institute Kala Shah Kaku, Pakistan during the season 2018. Adults collected were reared in laboratory and *Aphis gossypii* were supplied as diet on daily basis collected from field. One generation (F_0) was reared in the laboratory. Forty-five eggs were used in this study for each treatment. Grubs were supplied with sufficient number of *A. gossypii* as food. 2nd stage in stars were introduced into treated petri dishes to record the impact of insecticide life table parameters.

Insecticide

λ cyhlothrin (Karate 5EC), a commercial product of Syngenta, Pakistan, was used for sublethal studies against *C. septempunctata* larvae for biological parameters studies. Stock solution of 0.1% ml/L was prepared in distilled water. Then further two dilutions were made viz., 0.05 and 0.025 ml/L. Central doses 0.05 ml/L was selected for sublethal studies. For control only distilled water was used.

Bioassay

15 ml of selected dose were poured in the perti dishes and then it was shaked for 10-15seconds so that dose may be distributed to entire surface of the flask. The remaining liquid was wasted and petri dish was kept in front of fan to let it dry. Distilled water was used for control treatment. 45 eggs were used in this study. Due to higher mortality of 1st instar, 2nd instar larvae were put inside petri dish. Data for life stage and mortality was collected after 12 h (0900 and 2100 h). Adults of the same treatment were sexed to record the fecundity and life span for each treatment till death of each individual.

Statistical analysis

The basic life table parameters such as age-stage survival rate (Sxj), reproductive value (Vxj), age-stage specific life expectancy rate (Exj), intrinsic rate of increase (r), reproductive rate (R0), Finite rate of increase (λ), and mean generation time (T), were analyzed using the computer program TWOSEX-MS Chart (Chi and Liu, 1985; Chi, 2016a, b). The standard errors were calculated using the bootstrap technique included in the program with 100,000 random sampling (Efron and Tibshirani, 1993). Adult longevity, adult pre-oviposition period (APOP), total pre-oviposition period (TPOP), fecundity and population parameters (r, λ, R0, and T) were compared using the paired bootstrap test based on the confidence interval of the differences. Survival rate and reproductive value curves were plotted using MS Word software-2013.

RESULTS AND DISCUSSION

Age-stage two sex life tables

The development duration for each stage of *C.*

septumpunctata are presented in Table I. The development period was the shortest in λ cyhalothrin (33.52d) followed by control (33.6 d). Moreover, the females' life periods were longer than males in both treatments. Adult male longevity decreased in λ cyhalothrin treatment (25.56 d) as compared to control (29.40). In the same way, female longevity and total adult longevity was significantly lower (27.79 d) and (26.91 d) for insecticide treated grubs as compared to control (31.5 d) and (30.80 d), respectively. Age-stage, two sex life tables parameters describes the probability of a new born to survive to specific age (x) and stage (j) (Figs. 1-3). The age-stage curve (S_{xj}) describes a higher survival rate on control as compared to treatment. The l_x, f_{xj}, and m_x curves indicate that *C. septempunctata* had higher survival on control as compared to lamnda cyhalothrin. While the highest fecundity was recorded in control treatment as compared to sublethal dose of λ cyhalothrin.

Table I. Effect of λ cyhlothrin on duration of development of *C. septumpunctata* (days) reared on *A. gossypi*.

Development stage	N	Control	N	λ cyhalothrin
Egg	45	5.00 ± 0.00 a	45	5.00 ± 0.00 a
L1	44	4.00 ± 0.00 a	43	4.00 ± 0.00 a
L2	40	5.10 ± 0.12 a	40	5.08 ± 0.12 a
L3	34	5.56 ± 0.20 a	33	5.61 ± 0.17 a
L4	30	6.53 ± 0.15 a	28	6.64 ± 0.15 a
Pupa	30	7.07 ± 0.15 a	23	7.17 ± 0.16 a
Preadult	30	33.6 ± 2.30a	23	33.52 ± 0.32 a
Adult longevity		30.80 ± 0.57 a		26.91 ± 0.63 b
Female	20	31.50 ± 0.60 a	14	27.79 ± 0.79 b
Male	10	29.40 ± 1.13 a	9	25.56 ± 0.88 b

SEs were estimated by bootstrapping (100,000 replications). N, number of individuals completing a specific stage.

Age stage specific life expectancy curves (E_{xj}) were plotted in Figure 2. The newly hatched eggs of *C. septempunctata* were expected to survive for a longer period in control as compared to treated larvae. Both, males and females are expected to live a longer life when treated with distilled water as compared to λ cyhalothrin.

Age-stage-specific reproduction rate (V_{xj}) for different treatments plotted in Figure 3. Adult females contributed more to the population as they are the most productive stages of a population. Moreover, the successful adult emergence recorded more females as compared to males in all treatments. The highest age-stage reproductive value was recorded in control (1.1427) treatment, followed by λ cyhalothrin (1.1295). The fecundity of an individual is affected by the conditions in which it is raised. The f_{xj} curve explains the highest fecundity in λ cyhalothrin treated individuals was 19.8 eggs/day at 40th day while in control peak value 17.8 eggs was recorded on 42th day (Fig. 4).

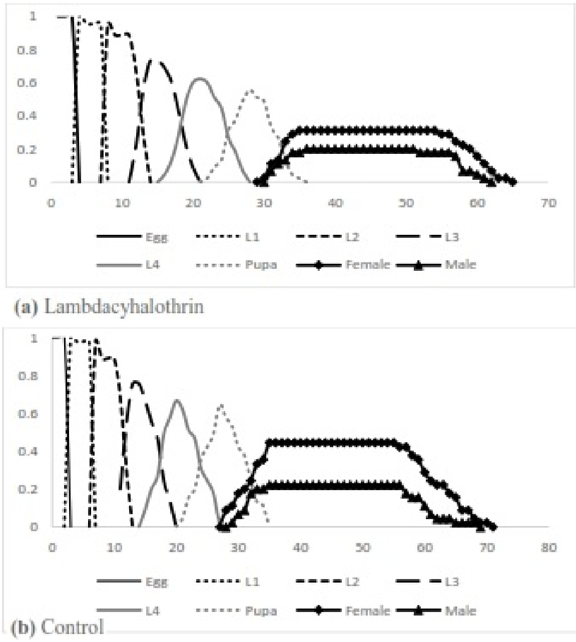

Fig. 1. Effect of λ cylothrin on age stage specific survival rate (*Sxj*) of *Coccinella septumpunctata*.

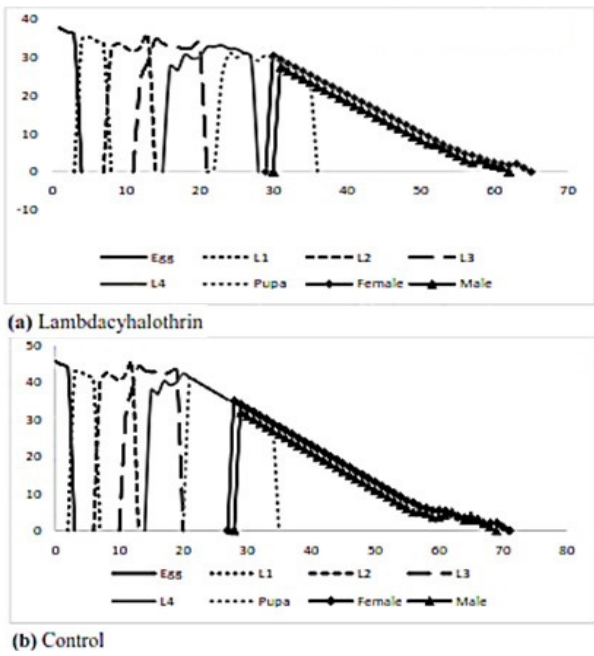

Fig. 2. Effect of λ cylothrin on age stage specific life expectance (*exj*) of *Coccinella septumpunctata*.

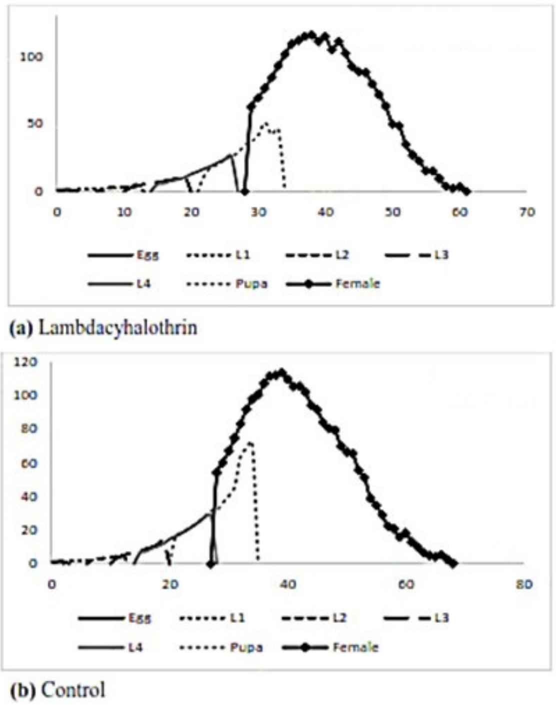

Fig. 3. Effect of λ cyhlothrin on age stage specific reproductive rate (*Vxj*) of *Coccinella septumpunctata*.

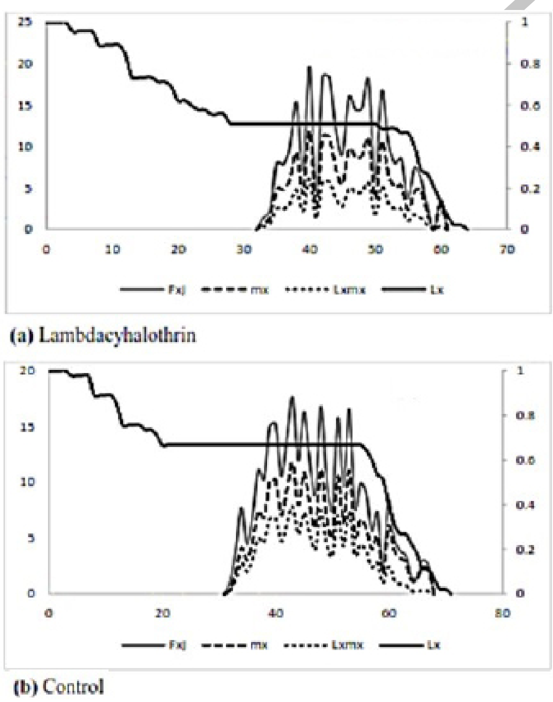

Fig. 4. Effect of λ cyhlothrin on age stage specific survival rate (*lx*), age stage specific fecundity (*fxj*), age specific fecundity (*mx*), and age specific maternity (*lxmx*) of *Coccinella septumpunctata*.

Population parameters

Population were recorded for control and sublethal dose of λ cyhalothin insecticide. SEs were estimated with bootstrap technique with 100,000 replicatons. Net Reproductive rate (R_0) was highest for control (147.00) followed by λ cyhalothin (91.85). Intrinsic rate of increase (r) was maximum for control (0.1339) followed by λ cyhalothin (0.1218). Mean generation time (T) was maximum for control (37.41) followed by λ cyhalothin (37.13) but statistically insignificant. Finite rate of increase (λ) values were highest to lowest for control (1.1427) as compared to λ cyhalothin (1.1295) (Table II).

Table II. Effect of λ cyhlothrin on fecundity and life table parameters (Mean ± SE) of *C. septumpunctata* reared on *A. gossypi.*

Parameters	Control	λ cyhalothrin
APOP	4.60 ± 0.11 a	4.14 ± 0.23 a
TPOP	29.20 ± 0.533 a	28.57 ± 0.40 a
Oviposition period	14.20 ± 0.2634 a	13.36 ± 0.41 a
Fecundity (eggs/female)	294.00 ± 5.835 a	262.43 ± 4.98 b
R_0 (offspring individual^{-1})	147 ± 23.4808 a	91.85 ± 43.72 b
T (d)	45.41 ± 0.652 a	45.13 ± 0.365 a
r (d^{-1})	0.13387±0.0052a	0.1218±0.0066 a
λ (d^{-1})	1.14269±0.0059a	1.1295±0.0074a

APOP, adult pre-oviposition period; TPOP, total pre-oviposition period; R0, net reproductive rate; T, mean generation time; r, intrinsic rate of increase; λ, finite rate of increase. SEs were estimated by bootstrapping (100,000 replications).

DISCUSSION

IPM includes the integration of insecticides with other natural controlling agents such as predators, parasitoids, and parasites (Bilal *et al*., 2019), but it has been seldom achieved due to incompatibility of parts of IPM especially insecticides and biocontrol agents (Tabashnik and Johnson, 1999). Insecticides are found equally toxic to natural enemies (Bilal *et al*., 2019) and less toxicity to natural enemies other than pest is viewed as a rare exception (Croft, 1990). With the advancement in insect pests' management techniques, insecticides are still primary tool for insect pest management. The impact of insecticides on beneficial fauna helping in pest management without an additional cost to IPM is an important matter (Atta *et al*., 2019) (Fig. 1).

λ cyhalothrin is a pyrethroid used on a wide scale for insect pest controlling programs. The results indicated that sublethal dose of λ cyhalothrin had a non-significant on developmental stages of ladybird beetle, however, preadult period was shorter than control. These results suggest development of tolerance in *C. septempunctata* against λ cyhalothrin, a pyrethroid. The development of resistance in coccinellid species field populations against λ cyhalothrin and pyrethroids was reported earlier by researchers in other countries (Torris *et al*., 2015; Rodrigues *et al*., 2013a, b). Bozsik (2006) reported that λ cyhalothrin is moderately harmful to *C. septempunctata* adults that is fairly similar to our results. The less survival of *C. septempunctata* as compared to the control might be attributed to the outbreak of aphids after pyrethoids application in field (Deguine *et al*., 2000; Godfrey *et al*., 2000) (Fig. 2).

λ cyhalothrin is among type-II pyrethroids which are more toxic due to presence of cyano group in molecule as compared to type-I pyrethroids such as permthrin and bifenthrin (Sattelle and Yamamoto, 1988; Khambay and Jewess, 2010; Torres *et al*., 2015). Wilis and Jepson (1994) reported the same for deltamethrin pyrethroid that *C. septempunctata* moved to lower parts of the shelter when exposed to deltamethrin. It may be due to that it had adopted to manage with the situation when exposed to λ cyhalothrin. Our results indicated that λ cyhalothrin had long term impact on *C. septempunctata* such as adult longevity of the male and female adult. This may be attributed to variable biological, operational, and genetic influences (Georghiou and Taylor, 1977a, b) (Fig. 3).

The sublethal application may have a suppressive or vice versa impact on fecundity of an insect. It means that it may reduce or augment its fecundity (Ali *et al*., 2017). The reduced fecundity of the female may be attributed to this factor. However, the population parameters such as generation time (T), intrinsic rate of increase (r), finite rate of increase (λ), and net reproductive rate were remained non-significant for control and λ cyhalothrin which may be attributed to development of resistance in field population of *C. septempunctata* against λ cyhalothrin. The development of resistance to an insecticide is not a permanent character in case of insects. An insect resistant to a certain insecticide may become susceptible again if raised in an insecticide free environment over generations (Ya-jun *et al*., 2014). The number of generations have not been reported for an insect for loss of resistance against an insecticide. This may be due to specific biology of an insect. The loss of resistance could be evaluated in the laboratory by producing a resistant biotype for a specific chemical. However, this evaluation may require an additional cost which depends on the biology of biocontrol agent under study (Fig. 4).

CONCLUSION

Our results revealed that *C. septempunctata* may have developed to manage λ cyhalothrin, and the insecticide may have not a significant damaging impact upon field population of ladybird beetle, *C. septempunctata*. It can be used in IPM programs involving *C. septempunctata* as a biological control agent.

Statement of conflict of interest

The authors have declared no conflict of interest.

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