RESEARCH ARTICLE

Transgenerational effects of pyriproxyfen in a field strain of *Musca domestica* L. (Diptera: Muscidae)

Naeem Iqbal [1]*, **Nauman Sadiq**[1,2]*, **Muhammad Nadir Naqqash**[1]*, **Muhammad Usman**[3], **Hafiz Azhar Ali Khan**[4], **Allah Ditta Abid** [3], **Muhammad Sohail Shahzad**[3]

1 Institute of Plant Protection, MNS University of Agriculture, Multan, Pakistan, 2 Department of Plant Protection, Ministry of National Food Security & Research, Pakistan, 3 Department of Agronomy, MNS University of Agriculture, Multan, Pakistan, 4 Institute of Zoology, University of the Punjab, Lahore, Pakistan

* naeem.iqbal@mnsuam.edu.pk, naeemiqbal18@yahoo.com (NI); nadir.naqqash@mnsuam.edu.pk (MNN); naumansadiq13@gmail.com (NS)

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Abstract

Musca domestica L. (Muscidae: Diptera) is a human and livestock pest especially in tropical and sub-tropical areas. Different insecticides have been used to control this pest that pose serious harmful effects on humans and the environment. The current study was planned to investigate the effects of two concentrations (LC_{25} and LC_{50}) of pyriproxyfen on biological and population parameters of a field strain of *M. domestica*. The exposed parents (F_0) and their progeny (F_1) were studied to examine the transgenerational effects. The results indicated that preadult duration was higher in control (13.68 days) compared to LC_{50} treated individuals (12.44 days). The male and female longevity was relatively lower in the LC_{25} treated population i.e. 24.62 and 26.62 days, respectively. The adult pre-oviposition period (APOP) and total pre-oviposition period (TPOP) values were higher in the LC_{25} treated individuals than those of control. Moreover, oviposition days and fecundity were reduced in the treated individuals as compared to the control treatment. A gradual decrease in the net reproductive rate (R_0) was observed (8.46–14.07 per day) while the value of R_0 was significantly higher in control. The results suggested that pyriproxyfen can be effectively utilized and incorporated in the management programs of *M. domestica*.

Introduction

The common housefly, *Musca domestica* L. (Muscidae: Diptera) is a major pest of humans and animals in the tropical and sub-tropical areas of the world. It serves as a mechanical vector for numerous pathogenic bacteria and other microorganisms [1, 2]. Some of the important diseases spread by house fly include salmonellosis, shigellosis, and cholera [3]. They are also involved in rickettsial and viral syndromes including helminthic (e.g. roundworms, hookworms, pinworms, and tapeworms) and protozoan pathogens (e.g. amoebic dysentery) [4]. All these pathogenic microorganisms are spread when house flies defecate and/or regurgitate on the food items [5].

House flies are mainly controlled by the use of highly toxic and residual insecticides. However, the injudicious use of insecticides to control house fly populations has led to the development of resistance to most of the insecticides. *M. domestica* was the first insect in which resistance was reported against organochlorine (DDT) [6]. Later, it developed resistance to organophosphates (45.4–62.5 folds to dichlorvos), carbamates (>290-fold to propoxur) and many other insecticides from different groups [7–9]. In addition, the unwise use of pesticides caused serious harmful effects on the environment, humans and other non-target organisms. Consequently, the use of non-conventional insecticides like insect growth regulators (IGRs) has received considerable attention in recent years, especially in the indoor environment [10, 11]. It has been well documented that IGRs are comparatively safer to the environment and mammals and break down easily in nature. These features make them viable alternatives to traditional insecticides for house fly control [12].

Pyriproxyfen is an IGR which acts on the endocrine system of insects by mimicking the naturally occurring juvenile hormone. It hinders molting and adult formation in insects, and can be an important component of Integrated Pest Management program [13]. It has been found very effective against a wide variety of insects belonging to Lepidoptera, Hemiptera and Diptera orders [14–16]. Pyriproxyfen lowers female mosquito fecundity by interfering with egg maturation or transferring eggs to the larval environment [17]. A new method, enhanced sterile insect technique (SIT), involves dusting radiation-sterilized males with pyriproxyfen before release. In semi-field conditions, the deployment of pyriproxyfen-dusted cloths within test huts led to a 96% decrease in indoor pests (mosquitoes) compared to control [18]. Though, the effects of pyriproxyfen are studied on larval stages of *M. domestica* [19, 20], but little is known about the sublethal effect of pyriproxyfen on adults. The studies involving lethal and sublethal effects of pyriproxyfen on the demographics and biological parameters of *M. domestica* after exposure to adults are lacking. The sublethal exposure induces variation in the P450s [21, 22] which in turn increases pesticide metabolism and may result in pest outbreak [23]. Generally speaking, insects receiving lethal and sublethal exposure to insecticides usually result in their mortality, altered physiology and biological traits or change in their feeding behavior [20]. Lethal and sublethal exposures may also result in pest's population suppression by lengthening developmental time, reducing survival and fecundity. Therefore, the changes induced by lethal and sublethal doses should be taken into account for pest management [24]. The current study was aimed to evaluate transgenerational effects of pyriproxyfen in a field strain of *M. domestica*. The transgenerational analysis will be helpful to explore hormetic effect (low dose stimulation) of pyriproxyfen which may lead to increase biotic potential in exposed insect pest populations. Age-stage, two sex life table was utilized to compare the exposed and unexposed progeny of *M. domestica*.

Materials and methods

Insecticides and *Musca domestica* strain

A commercial formulation of pyriproxyfen 10.8 EC (Syngenta Pakistan) was purchased from a local pesticide market (Multan, Pakistan) to use in bioassays.

Adults of *M. domestica* were collected from an agricultural field (30.163458˚N, 71.441871˚E) in Multan, Punjab, Pakistan. These adults were shifted to mesh cages (30 × 30 × 60 cm) and placed in the laboratory at 25 ± 2˚C temperature, 60±5% relative humidity and 16:8 light-to-dark ratio. They were fed on 20% sugar solution on a cotton plug. Plastic cups (5 cm high, top Ø 8.3 cm, bottom Ø 7.2 cm) containing larval diet were placed in the cages for egg laying. The larval diet consisted of rice husk, wheat bran, sucrose, milk powder and yeast in ratio of 40: 10: 3: 1: 1 along with small quantity of water [20].

Lethal concentration estimation

The acute toxicity of pyriproxyfen to *M. domestica* was tested using a food-incorporated bioassay method developed by Subaharan et al. [25] with little modification. Serial dilutions (ranging from 1 μg/ml to 10 μg/ml) of pyriproxyfen were prepared in 20% sugar solution [20]. Each concentration was replicated thrice and 10 adult *M. domestica* were released in each replication. The bioassay was conducted in mesh cages at 25 ± 2˚C temperature, 60±5% relative humidity and 16:8 light-to-dark ratio. A cotton plug was dipped in a specific concentration and placed inside the mesh cage as adult food. The adult mortality was recorded after 72 h of the treatment.

Transgenerational studies

The transgenerational studies were conducted using the methodology of Ghramh et al. [26]. Briefly, LC_{25} and LC_{50} concentrations of pyriproxyfen were prepared in 20% sugar solution and 50 adults (4–5 days old) were exposed to each concentration. The similar numbers of adults were also kept as control. After 72 hours of exposure, a larval medium or egg laying material (as described above) was provided to the treated adults (F0) or the control group in plastic cups (5 cm high, top Ø 8.3 cm, bottom Ø 7.2 cm). The numbers of eggs laid in each cup were counted on daily basis. For transgenerational studies, 50 eggs from each group were separated very carefully and placed in new cups containing larval diet. The egg hatching in each group was observed on daily basis. After hatching, the larvae were reared in the same groups in small plastic boxes (4×15×10 cm) until adult eclosion at 25± 2˚C under a 16:8 h light–dark photoperiod and 60±5% relative humidity. After adult emergence (F1), pre-oviposition period, oviposition period and fecundity were determined following the methodology of Ghramh et al. [26].

Statistical analyses

The probit analysis was used to estimate lethal concentrations (LC_{25}, LC_{50}), their fiducial limits (FL), the slope and other related parameters using POLO-PLUS $^{®}$ software program (LeOra Software 2003, Inc., Berkeley, CA). To develop a life table, the data were evaluated by using the theory of age-stage, two sex life table. The population parameters, included in this study were the age-stage specific survival rates (s_{xj}, where x is age and j is stage), net reproductive rate (R_0), the mean generation time (T), finite rate of increase ($\lambda_{\circ}$) age-specific survival rate (l_x), and intrinsic rate of increase (r). Additionally, based on the population parameters, other parameters viz. age-specific fecundity (m_x), age-stage specific life expectancy (e_{xj}), and age-specific maternity ($l_x m_x$) were also calculated [27–31].

For assessment of reproductive/biotic potential in a better way, total pre-oviposition period (TPOP), adult pre-oviposition period (APOP), reproductive days (R_d) (i.e., the number of days that adult produced offspring), reproductive value (v_{xj}), were computed using the computer program TWOSEX-MSChart [29]. The variances and standard errors of the population parameters were estimated using the bootstrap procedure with 100,000 random resampling and difference of population parameters between control and insecticide treatment groups was compared by using the paired bootstrap test based on the confidence intervals of differences implemented in TWOSEX-MSChart [29]. All graphics were created using SigmaPlot 12.0 (Systat Software Inc., San Jose, CA, USA).

The population parameters were estimated by following equations [27–31]:

The value of $l_x m_x$ was calculated by using the equation [29]:

$$\sum_{x=0}^{\infty} e^{-r(x+1)} l_x m_x = 1$$

Age-stage specific survival can be calculated using this statistical formula [27]:

$$lx = \sum_{j=1}^{k} S_{xj}$$

R_o was calculated by using the equation [29, 30]:

$$R_0 = \sum_{x=0}^{\infty} l_x m_x$$

The equation to determine the T value was [29]:

$$T = (\text{In } R_o)/r$$

The value of r was estimated by:

$$\sum_{x=0}^{\infty} e^{-r(x+1)} l_x m_x = 1$$

Finite rate of increase was calculated by:

$$\lambda_o = e^r$$

The value e_{xj} was determined by [30]:

$$e_{xj} = \sum_{i=x}^{\infty} \sum_{y=j}^{k} s'_{iy}$$

V_{xj} value was determined with equation [31]:

$$v_{xj} = \frac{e^{-r(x+1)}}{S_{xj}} \sum_{i=x}^{\infty} e^{-r(x+1)} \sum_{y=j}^{k} S'_{iy} f_{iy}$$

Results

Lethal concentration estimation

The field strain of *M. domestica* was exposed to concentrations of pyriproxyfen, and LC_{50} and LC_{25} values were calculated based on the data collected after 72 h of exposure to various concentrations. LC_{10} and LC_{25} values for the pyriproxyfen were 0.83 and 1.94 µg/ml, respectively. Whereas, the LC_{50} value of pyriproxyfen against *M. domestica* was 4.99 µg/ml (Table 1).

Basic parameters

The effects of concentrations of pyriproxyfen on the field strain of *M. domestica* are presented in Table 2. The pre-adult duration was much higher in the control treatment (13.68) while it was lower in the LC_{25} (12.50) and LC_{50} treated (12.44) individuals. The female longevity in the control treatment was higher those of LC_{25} and LC_{50} treated individuals. Similarly, male

Table 1. Toxicity analysis of pyriproxyfen against *Musca domestica* after 72 h of exposure.

Sr. No.	Lethal Concentration	Doses (µg/ml)	Fiducial limit	χ^2	DF	Slope
1	LC_{10}	0.83	0.27–1.55	2.15	4	1.72±0.28
2	LC_{25}	1.94	0.90–3.06	2.15	4	1.72±0.28
3	LC_{50}	4.99	3.21–6.85	2.15	4	1.72±0.28

χ^2 = Chi Square, DF = Degree of Freedom

longevity was significantly higher (27.89) in the control group and significantly lower (24.62) in both the LC_{25} and LC_{50} groups.

The adult pre-oviposition period (APOP) values were significantly higher (5.50 days) in the population treated with LC_{25}, while the value of APOP in the control group was significantly lower (1.87 days). The total pre-oviposition period (TPOP) of the progeny of population treated with LC_{25} was significantly higher (19.75) compared to control (18.26). The oviposition days were significantly higher in the control population (7.93). While, the group that was treated with LC_{25} and LC_{50} had a significantly lower number of oviposition days. The fecundity was significantly higher in the control group, whereas the fecundity was significantly lower in individuals that had been treated with LC_{50} (Table 2).

Population parameters

Transgenerational effect of pyriproxyfen on the population parameters is shown in Table 3. The intrinsic rate of increase (*r*) in the control was significantly higher (0.12/day) while it was lower in LC_{50} treated individuals (0.09/day). The net reproductive rate (R_0) was significantly higher in the control group (17.3 offspring/day) and significantly lower in the LC_{50} treated individuals (8.46 offspring/day). The values of mean generation time in control and treated individuals were statistically at par. The LC_{25} treated group had comparatively higher mean generation time (*T* = 22.84 days) than the control (*T* = 21.23 days) and LC_{50} treated groups (*T* = 21.45 days). Lambda (⋏) values indicated that finite rate of increase (per day) was also not significantly affected (Table 3).

Age-stage specific maternity ($l_x m_x$)

The results of age-stage specific maternity ($l_x m_x$) indicated that the fecundity (f_x) was at its highest point (23/day) in 20 days old female and then decreasing trend was observed from day

Table 2. Transgenerational effect of pyriproxyfen on different life table parameters of *Musca domestica* (F1).

Parameters	Control	LC_{25}	LC_{50}
Pre-adult duration	13.68± 0.07a	12.50± 0.07b	12.44± 0.07b
Female longevity	28.80± 0.78a	26.41±0.75a	27.56± 0.91a
Male longevity	27.89±0.54a	24.62± 0.53b	24.62± 0.53b
APOP	1.87±0.09b	5.50±0.22a	2.56±0.29b
TPOP	18.26± 0.26b	19.75 ±0.30a	18.56±0.41b
Oviposition days	7.93±0.37a	5.25±0.39b	5.00±0.00b
Fecundity	61.25 ± 1.24a	57.67± 1.79a	47.00±1.36b

TPOP: Total pre-oviposition period of female counted from birth, APOP: Adult pre-oviposition period of female counted from adult eclosion from pupae.

Means in the same row followed by the same letter are not significantly different (P > 0.05) using bootstrap test.

Table 3. Transgenerational effect of pyriproxyfen on population parameters of *Musca domestica*.

Parameters	Control	LC_{25}	LC_{50}
r (per day)	0.12±0.01a	0.11±0.01a	0.09±0.01b
R_o (per day)	17.30± 3.83a	14.07± 3.84a	8.46±2.54b
T (days)	21.23±0.43a	22.84±0.38a	21.45± 0.44a
λ_o (per day)	1.13±0.01a	1.12±0.01a	1.10±0.01a

r = The intrinsic rate of increase (per day); λ = Limiting rate of growth (per day)

R_0 = The net reproductive rate (offspring/individual); T = The mean generation time (days).

Means in the same row followed by the same letter are not significantly different (P > 0.05) using bootstrap test.

20 to day 24. The age-stage specific survival rate (l_x) also decreased over time and it was around 30 days in LC_{25} treated population and 32 days in control. Age-specific fertility (m_x) was 4.32 days in the LC_{50}-treated individuals and 2.60 days in the control treatment. Age-specific maternity ($l_x m_x$) was maximum (2.08 offspring/day) in the control group while significantly less in LC_{25} treated individuals (1.26 offspring/day) (Fig 1).

Age-stage specific survival rate (S_{xj})

Age-stage survival rate (S_{xj}) was higher for larvae in the control group (13.25 days) while it was lower in the LC_{25} treated group (11.99 days). The pupal survival was also higher in control compared to treated individuals. The male age stage-specific survival was higher in the LC_{25} treated individuals than those of LC_{50} treated individuals. For females, S_{xj} value was higher in LC_{50} treated individuals (32 days) while it was lower in the LC_{25} treated individuals (31 days) (Fig 2).

Age-stage-specific life expectancy (e_{xj})

The age-stage-specific life expectancy (e_{xj}) calculates an individual's post-age-x life expectancy based on the age-stage, two-sex life table. The life expectancy (e_{xj}) was the highest on day 3 and then gradually declined in all groups. The values of e_{xj} were higher for control individuals (33.03 days) followed by LC_{25} (31.94 days) and LC_{50} (30.91 days) treated individuals (Fig 3).

Age-specific maternity (V_{xj})

It is the age-specific maternity or reproductive value (v_{xj}) that contributes to future population growth for individuals of stage j and age x. The control treatment showed the lowest peak of v_{xj} on day 18 which was decreased to 0 by day 30. The highest peak was observed in the LC_{25} treated population on day 19 compared to other treatments (Fig 4).

Discussion

Pyriproxyfen is one of the most commonly used Insect Growth Regulator (IGR) against a broad range of insect pests. The current study aimed to explore the sub-lethal effects of pyriproxyfen on the biological and population parameters of *M. domestica*. The results indicated that pyriproxyfen significantly reduced preadult duration, male and female survival rate, fecundity and net reproductive rate in treated populations. Current study revealed higher preadult duration in the control population compared to LC_{25} and LC_{50} treated populations. Similar results were obtained by Iftikhar et al. [14] and Mahmoudvand et al. [32] who reported decline in preadult duration in *Plutella xylostella* and *Hippodamia convergens*. Richardson & Lagos [33] reported reduced developmental time in 1st and 4th nymphal instar of *Aphis glycines*
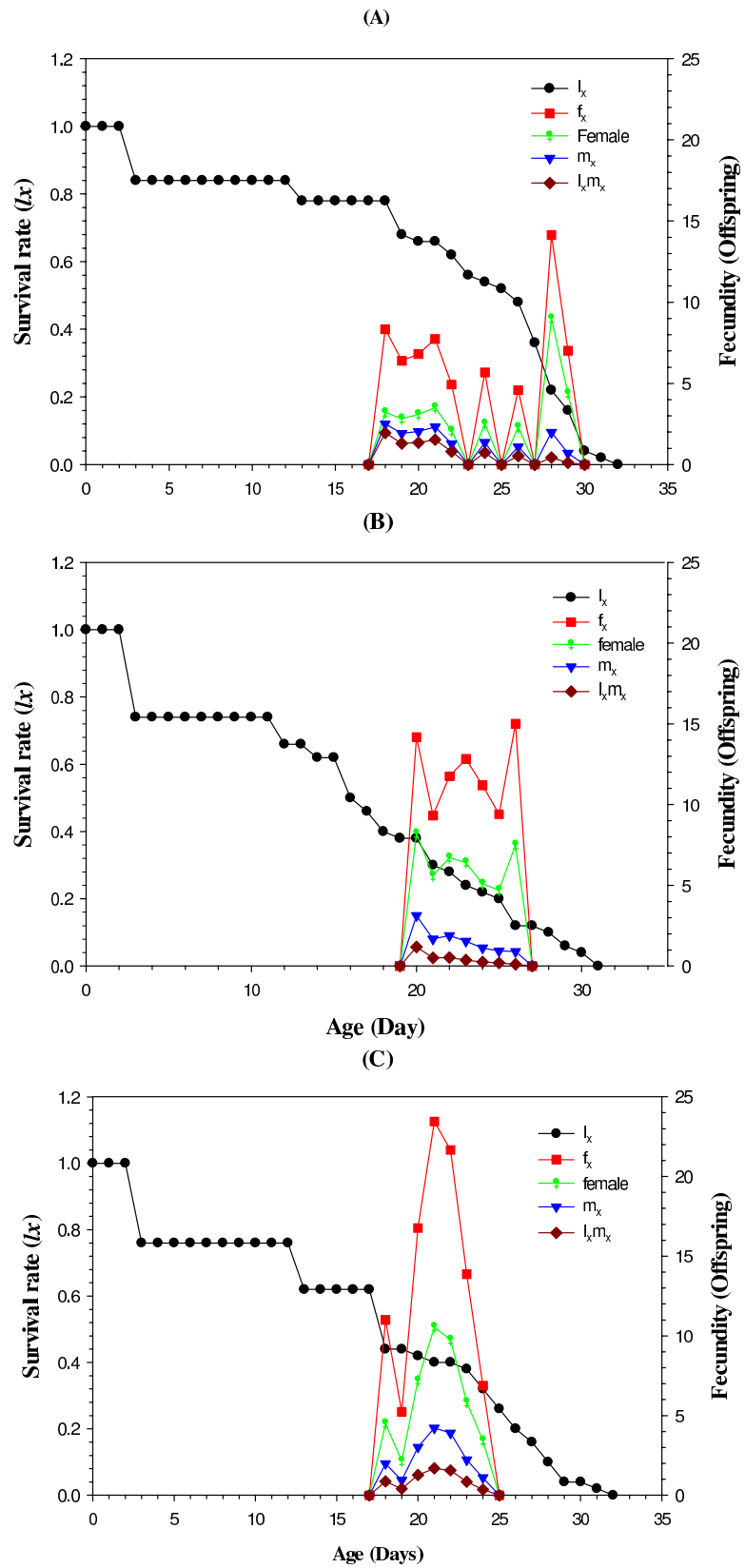
(A)

(B)

(C)

Fig 1. Survival rate (l_x), maternity rate (m_x) and their product ($l_x m_x$) in the progeny of pyriproxyfen treated adults; A) Control B) LC$_{25}$ C) LC$_{50}$.
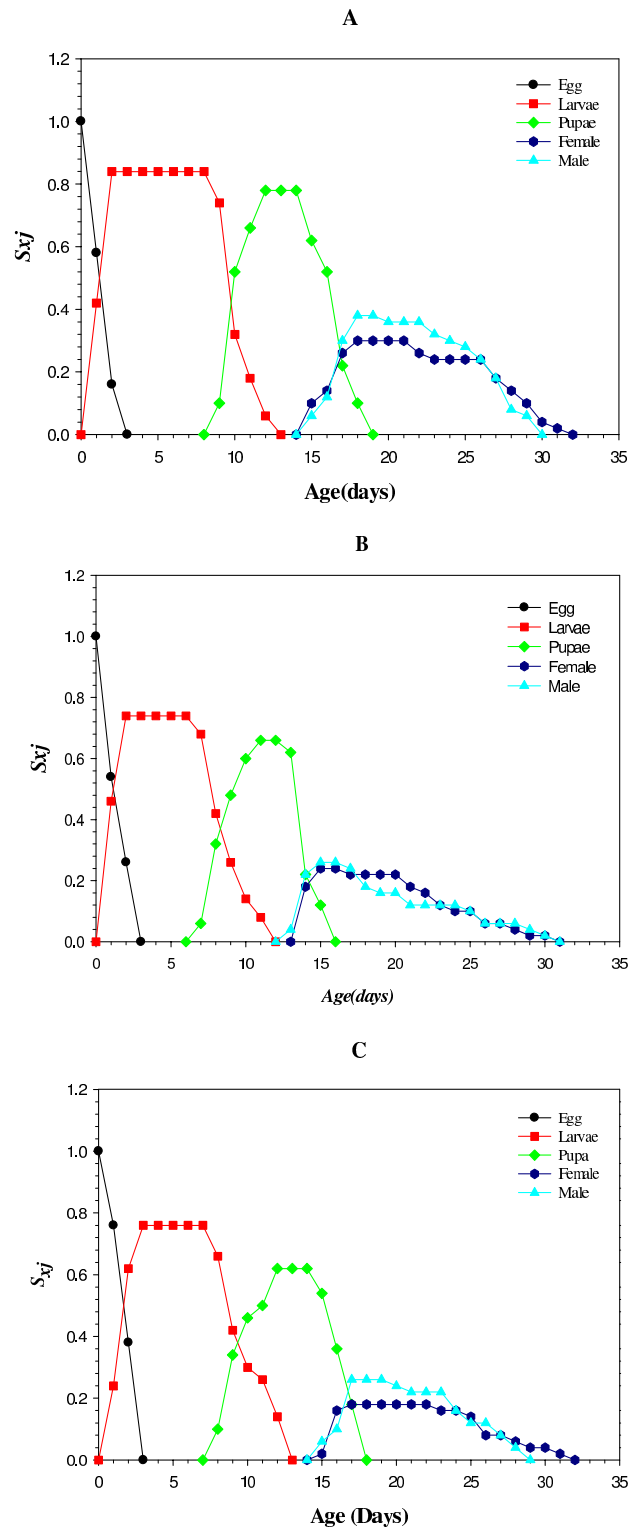
Fig 2. Age stage-specific survival of *Musca domestica* in treated and control groups A) Control B) LC$_{25}$ and C) LC$_{50}$.
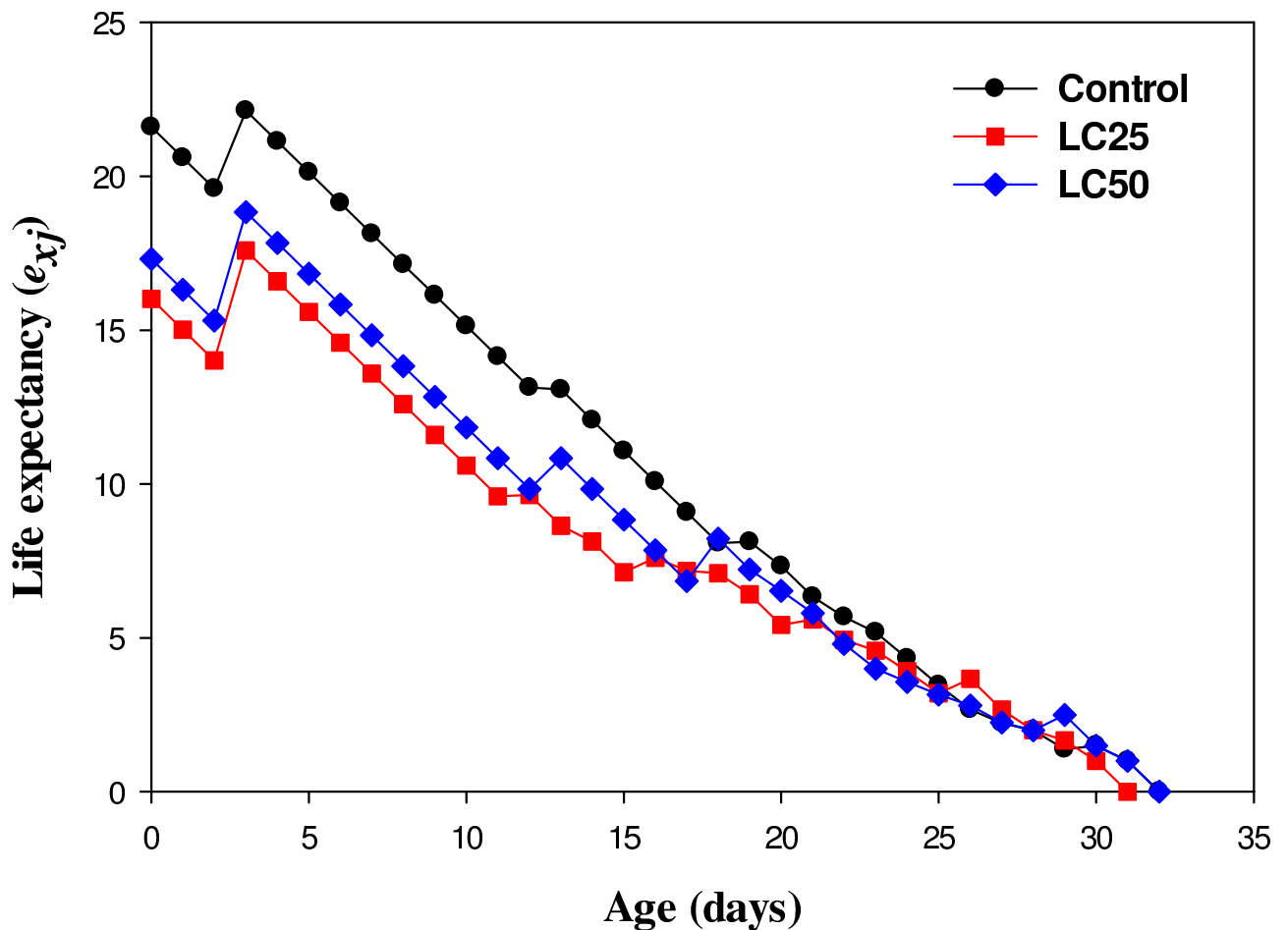
Fig 3. Life expectancy *(e_xj)* of *Musca domestica* in control, LC_{25}, and LC_{50}.

as a result of pyriproxyfen exposure. The results of the present study such as toxicity values, response of biological traits and life table parameters after exposure to pyriproxyfen varied from the previous study on *M. domestica* [20]. The most probable reason for these variations could be due to differences in geographical origins of strains tested, their susceptibility to insecticides, bioassay conditions and chemicals used in the present and previous studies.

Male and female longevity was low in the treated population while it was higher in control population. A similar trend was observed by Steigenga et al. [34] in *Bicycles anynana* and by Rahmani and Bandani [35] in *H. variegata* as a result of sublethal concentrations of pyriproxy-fen and thiamethoxam, respectively. Khan [20] also reported decreased longevity of females of an insecticide susceptible strain of *M. domestica* when larvae were subjected to sublethal doses of pyriproxyfen. Two other studies reported reduction in female longevity in *P. xylostella* and *H. convergens* [14, 15].

The sublethal doses of insecticide induce changes in APOP and TPOP values [15]. APOP and TPOP values were considerably greater in the treated population while these values were significantly lower in the control. These findings are in accordance with the findings of Lee et al. [36] and Alizadeh et al. [15]. Alizadeh et al. [15] and Huang et al. [37] also reported increased APOP and TPOP in *P. xylostella* as a result of sublethal exposure of pyriproxyfen. Oviposition days were reduced in treated population and the same reduction was observed in
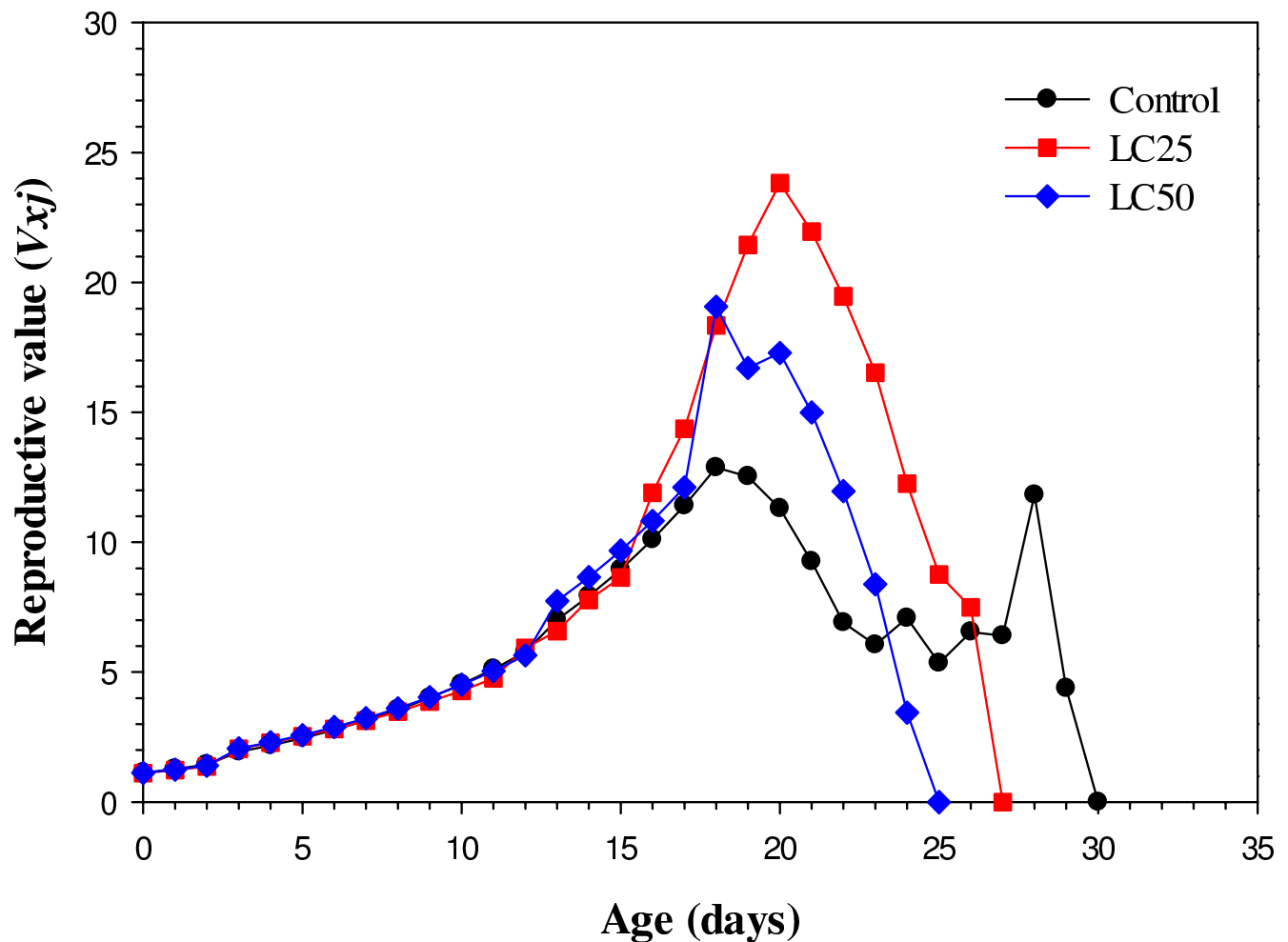
Fig 4. Reproductive value (*Vxj*) of *Musca domestica* in control, LC$_{25}$, and LC$_{50}$.

Spodoptera litura [38]. Fecundity was also decreased in treated populations compared to control, and these variations are confirmed by Alizadeh et al. [15]. Mahmoudvand et al. [32] also reported that sublethal doses of pyriproxyfen reduce the fecundity of *P. xylostella*. Khan [20] reported that sublethal exposure of pyriproxyfen to the larval stage of *M. domestica* significantly reduces fecundity. These studies showed that pyriproxyfen negatively affected the biological parameters. In addition to this, population parameters are also affected by the sublethal exposure of pyriproxyfen [39].

Net reproductive rate is an important parameter that helps in population determination. The net reproductive rate (R_0) was reduced in the treated population. These results are comparable to the previous findings of Mahmoudvand et al. [32] and Shah et al. [39] who reported that the net reproductive rate was reduced in *M. domestica* due to sublethal exposure to pyriproxyfen. Reduced net reproductive rate was observed as a result of sublethal exposure of pyriproxyfen to *Anopheles gambiae* [40]. The result of the present study reveals that mean generation time (T) was increased in treated population and its value was lower in control. The current findings are consistent with the findings of many previous studies conducted to evaluate the sublethal effects of pyriproxyfen on mean generation time in different insects

[15, 41]. Khan [20] reported that there was no significant increase in mean generation time when *M. domestica* larvae were treated with the sublethal doses although a slight increase in value was observed in the treated individuals in the present study. The finite rate of increase (ʎ) was decreased in the treated population and these findings were verified by previous studies [32]. The decrease in value of ʎ was also studied in *Oxycarenus hyalinipennis* by Naeem et al. [42]. Iftikhar et al. [14] also reported a similar decline in ʎ while studying the effect of sublethal doses of pyriproxyfen on *H. convergens*. These sublethal effects of pyriproxyfen on adults of *M. domestica* could be due to its negative effects on hormone production which affect growth and reproduction [43]. Results of current experiment suggest that pyriproxyfen can be effectively used to control *M. domestica* populations in different habitat due to lack of hormesis effects [20].

Conclusion

The present studies showed that the basic parameters (TPOP, APOP, oviposition days, fecundity, male and female longevity) and population parameters (r, R_0, $\lambda_\circ$, T) were negatively affected when adults of *M. domestica* were exposed to concentrations of pyriproxyfen. The mean generation time (T) was higher in the treated population compared to the control population. The current results showed that *M. domestica* can be controlled by pyriproxyfen. Based on this data, field trials should be conducted to further ensure the potential of pyriproxyfen against *M. domestica*.

Supporting information

S1 Data.
(ZIP)

Acknowledgments

Author Contributions

Conceptualization: Naeem Iqbal, Muhammad Nadir Naqqash.

Data curation: Nauman Sadiq, Muhammad Usman.

Formal analysis: Naeem Iqbal, Nauman Sadiq, Muhammad Nadir Naqqash.

Methodology: Allah Ditta Abid.

Resources: Naeem Iqbal.

Software: Hafiz Azhar Ali Khan, Muhammad Sohail Shahzad.

Supervision: Naeem Iqbal, Muhammad Nadir Naqqash.

Writing – original draft: Naeem Iqbal, Nauman Sadiq, Muhammad Nadir Naqqash, Muhammad Usman, Allah Ditta Abid, Muhammad Sohail Shahzad.

Writing – review & editing: Naeem Iqbal, Nauman Sadiq, Muhammad Nadir Naqqash, Hafiz Azhar Ali Khan, Allah Ditta Abid, Muhammad Sohail Shahzad.

References

1. Gioia G., Freeman J., Sipka A., Santisteban C., Wieland M., Gallardo V.A., et al., 2022. Pathogens associated with houseflies from different areas within a New York State dairy. JDS Communications 3, 285–290. https://doi.org/10.3168/jdsc.2021-0200 PMID: 36338025

2. Rauf I., Khuhro N.H., Memon R.M., Khan I.A., 2022. Parasitism preference of Chalcid hymenopteran *Dirhinus giffardii* (Silvestri) confirms higher parasitism against housefly (*Musca domestica*) (Diptera: Muscidae) pupae. Plos One 17, e0262034.

3. Hiremath B., 2022. Review on Study of Insecticidal Activity of *Cymbopogon citratus*, and Aloe vera against *Musca domestica*. Energy Sustain. Soc. 2, 1–9.

4. Malik A., Singh N., Satya S., 2007. House fly (*Musca domestica*): a review of control strategies for a challenging pest. J. Environ. Sci. Health–B. 42, 453–469.

5. Olagunju E.A., 2022. Housefly: Common zoonotic diseases transmitted and control. J. Zoonotic Dis. 6, 1–10.

6. Metcalf RL (1989) Insect resistance to insecticides. Pestic Sci 26:333–358.

7. Acevedo GR, Zapater M, Toloza AC (2009) Insecticide resistance of house fly, *Musca domestica* (L.) from Argentina. Parasitol Res 105:489–493.

8. Liu N, Yue X (2000) Insecticide resistance and cross-resistance in the house fly (Diptera: Muscidae). J Econ Entomol 93:1269–1275. https://doi.org/10.1603/0022-0493-93.4.1269 PMID: 10985042

9. Shi J, Lan Z, Zhang XG (2011) Characterisation of spinosad 1 resistance in the housefly Musca domestica (Diptera: Muscidae). Pest Manag Sci 67:335–340.

10. Nighat B., Sharma B., Pandey R.S., 2013. *Calotropis procera* and *Annona squamosa*: potential alternatives to chemical pesticides. Br. J. Appl. Sci. Technol. 3, 254–267.

11. Al-Ghamdi K.M., Saleh S.M., Mahyoub J.A., Asid A.N., Al-Najada A.R., Mamdouh I.N., et al. 2014. Potential studies of nonconventional chemicals against the housefly larvae *Musca domestica* L. Life Sci. Journal 11, 1046–1049.

12. Devi V. and Awasthi P., 2022. Juvenile hormone mimics with phenyl ether and amide functionality to be insect growth regulators (IGRs): synthesis, characterization, computational and biological study. *Journal of Biomolecular Structure and Dynamics*, 40(23), pp.13246–13264. https://doi.org/10.1080/07391102.2021.1985614 PMID: 34622740

13. Francesena N., Schneider M.I., 2018. Selectivity assessment of two biorational insecticides, azadirachtin and pyriproxyfen, in comparison to a neonicotinoid, acetamiprid, on pupae and adults of a Neotropical strain *Eretmocerus mundus* Mercet. Chemosphere 206, 349–358.

14. Iftikhar A., Hafeez F., Hafeez M., Farooq M., Aziz M.A., Sohaib M., et al. 2020. Sublethal effects of a juvenile hormone analog, Pyriproxyfen on demographic parameters of non-target predator, *Hippodamia convergens* Guerin-Meneville (Coleoptera: Coccinellidae). Ecotoxicology 29, 1017–1028.

15. Alizadeh M., Karimzadeh J., Rassoulian G.R., Farazmand H., Hoseini-Naveh V., Pourian H.R., 2012. Sublethal effects of pyriproxyfen, a juvenile hormone analogue, on *Plutella xylostella* (Lepidoptera: Plutellidae): life table study. Arch. Phytopathol. Plant Prot. 45, 1741–1763.

16. Oouchi H., 2005. Insecticidal properties of a juvenoid, pyriproxyfen, on all life stages of the diamond-back moth, *Plutella xylostella* (Lepidoptera: Yponomeutidae). Appl. Entomol. Zool. 40, 145–149.

17. Itoh T., Kawada H., Abe A., Eshita Y., Rongsriyam Y., Igarashi A., 1994. Utilization of bloodfed females of *Aedes aegypti* as a vehicle for the transfer of the insect growth regulator pyriproxyfen to larval habitats. Journal of the American Mosquito Control Association-Mosquito News, 10(3), pp.344–347.

18. Bouyer J. & Lefrançois T., 2014. Boosting the sterile insect technique to control mosquitoes. Trends in parasitology, 30(6), pp.271–273. https://doi.org/10.1016/j.pt.2014.04.002 PMID: 24746400

19. Sulaiman S., Syed M.S., Jeffery J., 2008. Effect of Triflumuron and Pyriproxyfen on *Musca domestica* L. Larval Stages in the Laboratory. Iran J. Arthropod Borne Dis. 2, 1–6.

20. Khan H.A.A., 2021. Pyriproxyfen induces lethal and sublethal effects on biological traits and demographic growth parameters in *Musca domestica*. Ecotoxicology 30, 610–621.

21. Wu M.C., Chang Y.W., Lu K.H., Yang E.C., 2017. Gene expression changes in honey bees induced by sublethal imidacloprid exposure during the larval stage. Insect Biochem. Mol. Biol. 88, 12–20. https://doi.org/10.1016/j.ibmb.2017.06.016 PMID: 28732753

22. Valbon W.R., Hatano E., Oliveira N.R., Ataíde Á.D., Correa M.J.M., Gomes S.F., et al., 2021. Detrimental effects of pyriproxyfen on the detoxification and abilities of *Belostoma anurum* to prey upon *Aedes aegypti* larvae. Environ. Pollut. 284, 117130.

23. Bantz A., Camon J., Froger J.A., Goven D., Raymond V., 2018. Exposure to sublethal doses of insecticide and their effects on insects at cellular and physiological levels. Curr. Opin. Insect. Sci. 30, 73–78. https://doi.org/10.1016/j.cois.2018.09.008 PMID: 30553488

24. Pekár S., 2012. Spiders (Araneae) in the pesticide world: an ecotoxicological review. Pest Manag. Sci. 68, 1438–1446. https://doi.org/10.1002/ps.3397 PMID: 22945871

25. Subaharan K., Senthoorraja R., Manjunath S., Thimmegowda G.G., Pragadheesh V.S., et al., 2021. Toxicity, behavioural and biochemical effect of *Piper betle* L. essential oil and its constituents against housefly, *Musca domestica* L. Pestic. Biochem. Physiol. 174, 104804.

26. Ghramh H.A., Sadiq N., Naqqash M.N. et al. Transgenerational effects of lambda-cyhalothrin on *Musca domestica* L. (Diptera: Muscidae). *Sci Rep* 12, 19228 (2022). https://doi.org/10.1038/s41598-022-23492-3 PMID: 36357409

27. Chi H., & Liu H. (1985). Two new methods for the study of insect population ecology. Bulletin of the Institute of Zoology, Academia Sinica, 24, 225–240.

28. Chi H. (1988). Life-table analysis incorporating both sexes and variable development rates among individuals. Environmental Entomology, 17(1), 26–34. https://doi.org/10.1093/ee/17.1.26

29. Chi H. (2020). TWOSEX-MSChart: a computer program for the age-stage, two-sex life table analysis. National Chung Hsing University in Taiwan. R.O.C. (http://140.120.197.173/Ecology/Download/TWOSEX-MSChart-B100000.rar).

30. Chi H., and Su H. Y. 2006. Age-stage, two-sex life tables of *Aphidius gifuensis* (Ashmead) (Hymenoptera: Braconidae) and its host *Myzus persicae* (Sulzer) (Homoptera: Aphididae) with mathematical proof of the relationship between female fecundity and the net reproductive rate. Environ. Entomol. 35: 10–21.

31. Tuan S. J., Lee C. C., and Chi H. 2014. Population and damage projection of *Spodoptera litura* (F.) on peanuts (*Arachis hypogaea* L.) under different conditions using the age-stage, two-sex life table. Pest Manag. Sci. 70: 805–813.

32. Mahmoudvand M., Moharramipour S., Iranshahi M., 2015. Effects of pyriproxyfen on life table indices of *Plutella xylostella* in multigenerations. Psyche 2015, 1–7.

33. Richardson M.L. and Lagos D.M., 2007. Effects of a juvenile hormone analogue, pyriproxyfen, on the apterous form of soybean aphid (*Aphis glycines*). *Journal of Applied Entomology*, 131(5), pp.297–302.

34. Steigenga M.J., Hoffmann K.H. and Fischer K., 2006. Effects of the juvenile hormone mimic pyriproxyfen on female reproduction and longevity in the butterfly *Bicyclus anynana*. *Entomological science*, 9 (3), pp.269–279.

35. Rahmani S., Bandani A.R., 2013. Sublethal concentrations of thiamethoxam adversely affect life table parameters of the aphid predator, *Hippodamia variegata* (Goeze) (Coleoptera: Coccinellidae). Crop Prot. 54, 168–175.

36. Lee Y.S., Lee S.Y., Park E.C., Kim J.H., Kim G.H., 2002. Comparative toxicities of pyriproxyfen and thiamethoxam against the sweetpotato whitefly, *Bemisia tabaci* (Homoptera: Aleyrodidae). J. Asia-Pac. Entomol. 5(1), pp.117–122.

37. Huang Z., Wang Y., Zhang Y., 2015. Lethal and sublethal effects of cantharidin on development and reproduction of *Plutella xylostella* (Lepidoptera: Plutellidae). J. Econ. Entomol. 108, 1054–1064.

38. Xu Q., Tang B., Zou Q., Zheng H., Liu X., Wang S., 2015. Effects of pyriproxyfen on female reproduction in the common cutworm, *Spodoptera litura* (F.) (Lepidoptera: Noctuidae). PLoS One 10, e0138171.

39. Shah R.M., Shad S.A., Abbas N., 2015. Mechanism, stability and fitness cost of resistance to pyriproxyfen in the house fly, *Musca domestica* L.(Diptera: Muscidae). Pestic. Biochem. Physiol. 119, 67–73.

40. Ngufor C., N'Guessan R., Fagbohoun J., Odjo A., Malone D., Akogbeto M., et al. 2014. Olyset Duo® (a pyriproxyfen and permethrin mixture net): an experimental hut trial against pyrethroid resistant *Anopheles gambiae* and *Culex quinquefasciatus* in Southern Benin. PloS One 9, e93603.

41. Moadeli T., Hejazi M.J., Golmohammadi G., 2014. Lethal Effects of Pyriproxyfen, Spinosad, and Indoxacarb and Sublethal Effects of Pyriproxyfen on the 1st Instars Larvae of Beet Armyworm, *Spodoptera exigua* Hübner (Lepidoptera: Noctuidae) in the Laboratory. J. Agric. Sci. Technol. 16, 1217–1227.

42. Naeem A., Hafeez F., Iftikhar A., Waaiz M., Güncan A., Ullah F., et al. 2021. Laboratory induced selection of pyriproxyfen resistance in *Oxycarenus hyalinipennis* Costa (Hemiptera: Lygaeidae): Cross-resistance potential, realized heritability, and fitness costs determination using age-stage, two-sex life table. Chemosphere 269, 129367.

43. Devillers J. and Devillers H., 2020. Lethal and sublethal effects of pyriproxyfen on apis and non-apis bees. *Toxics*, 8(4), p.104. https://doi.org/10.3390/toxics8040104 PMID: 33212791