scientific reports

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Effects of Indoxacarb on life history traits and population performance of the house fly, *Musca domestica*

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The house fly, *Musca domestica* L. is an important pest of livestock and humans, especially in subtropical and tropical regions worldwide. Efficacy of indoxacarb has been shown against a wide range of insects, including *M. domestica*. This study evaluated the impact of three concentrations of indoxacarb (LC_{10}, LC_{30} and LC_{50}) on the population and biological parameters of a *M. domestica* strain. Results showed that pre-adult development was prolonged in the treated population with maximum duration observed in LC_{50} treated population (13.88 days). Longevity of male and female *M. domestica* was the lowest in the LC_{50} treated population (23.06 and 25.14 days, respectively). The pre-oviposition period (APOP) was maximum in the LC_{50} treated population (3.71 days) while the value of total pre-oviposition period (TPOP) was the highest in the LC_{30} treated population (21.12 days). The adults exposed to the LC_{50} value of indoxacarb had reduced fecundity (42.00), net reproductive rate (R_o = 5.88), mean generation time (T = 21.66) and finite rate of increase (λ = 1.09). Similarly, age-specific maternity, age stage specific survival rate, age-stage reproductive values and age-stage life expectancy were higher in LC_{10} and LC_{30} treatments, while their values were lower in population that was treated with the LC_{50} value of indoxacarb. The findings suggested that sub-lethal concentrations (LC_{10}, LC_{30}) of indoxacarb had a hormetic effects, while the higher concentration (LC_{50}) had a non-hormetic effect on the biology of *M. domestica*.

Keywords House fly control, Indoxacarb, Lethal concentration, Sub-lethal concentrations, Two-sex life table

The house fly, *Musca domestica* L. is a serious pest impacting both agricultural environments and residential areas worldwide[1]. It is a notorious vector of various pathogens, contributing to the spread of numerous food-borne infections[2,3]. Behavior of *M. domestica*, including the retention of pathogens in its digestive tract and the potential for contamination of surfaces through regurgitation, defecation, and feeding, facilitates the transmission of these pathogens[4]. This insect plays a crucial role in the dissemination of diseases such as cholera and salmonellosis, as well as other severe food borne illnesses[5]. Beyond bacterial infections, *M. domestica* is also responsible in the transmission of protozoan diseases like amoebic dysentery, as well as rickettsia, and viral disorders[6]. Houses, food points and animal farms demand instant control of *M. domestica*.

Insecticides are utilized in public places as sprays, baits and fogs[7]. The residual concentrations and insecticide persistence, due to long-term of sublethal exposure, have been led to environmental concerns[8,9]. Furthermore, the ubiquitous presence of pesticides often drives the development of resistance or tolerance in pest populations[10–12]. Exposure to sublethal doses may also induce hormesis which in turns stimulate particular biological processes such as increased rate of reproduction[13]. Additionally, the population reduction of natural enemies, behavioral changes in arthropods and reduction in competition can lead to the resurgence and secondary outbreaks of pests, all these problems may link hormesis effect[14].

Indoxacarb is known as pro-insecticide which activate metabolically within the target insect. It acts as a sodium-channel blocker in the target insect[15]. Efficacy of indoxacarb has been shown against a wide range of insects, including *M. domestica*[16]. However, there are limited studies showing the effects of sublethal and lethal exposure of indoxacarb on the biology of insect pests. For the control of *M. domestica*, an effective chemical with prolong product life is required. Relying solely on bioassays is inadequate for ensuring product stability and managing *M. domestica* effectively. To overcome this limitation, transgenerational studies are regarded as a more reliable approach for selecting insecticides[17]. These analyses offer a detailed understanding of how insect

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behavior changes throughout all developmental stages: egg, larva, pupa, and adult. By comparing these stages, we can devise more effective pest control strategies. This research is focused on assessing lethal and sublethal effects of indoxacarb by performing a transgenerational analysis on the progeny of *M. domestica*. The results will inform whether indoxacarb is a viable option for controlling *M. domestica* or not.

Materials and methods

Collection and rearing of *Musca domestica*

Adult *M. domestica* used for this experiment were captured by using aerial net near agriculture field of Multan (30.1864° N, 71.4886° E), Punjab, Pakistan. They were shifted to the laboratory of Entomology for rearing in wooden-mesh cages (30 × 30 × 60 cm) and population homogenization. The population of *M. domestica* was reared for five generations before starting experiments. Adults were fed on powdered milk and sugar mixture (1:1).The photoperiod was adjusted to 14-light hour: 10-dark hour, while temperature and humidity were maintained at 25 ± 2 °C and 60 ± 5%, respectively. For oviposition, transparent plastic cups (100 ml capacity) were partially-filled with a larval diet. The main constituents of larval diet were wheat bran, rice husk, sugar and yeast in the proportions of 60:20:15:5, respectively. The larval diet was placed in the cages for two days. After that the cups containing eggs were shifted to new cages for further rearing and experimentation.

Insecticide

A commercial formulation of indoxacarb (Steward® EC30WG, FMC) was obtained from a local market and used in bioassays.

Lethal and sublethal concentrations estimation

The toxicity of indoxacarb to *M. domestica* was assessed using a food-incorporated bioassay as described by Khan[18]. The bioassays were conducted in small cages measuring 30 × 30 × 60 cm, with mesh panels installed on the sides to ensure adequate ventilation. Five concentrations of indoxacarb viz., 1.5, 3.0, 6.0, 12.0 and 24.0 ppm along with a control (0.0 ppm) were prepared in 20% sugar-water solution. Cotton plugs soaked in specific concentration was dipped and placed inside the cage and 10 adult female *M. domestica* (2–3-day old) were released in the cage using a siphon tube. There were a total of three cages for each concentration. The laboratory conditions were same as for rearing experiment. The mortality was recorded after 72 h of exposure to determine the LC_{10}, LC_{30} and LC_{50} values.

Transgenerational studies

Based on the bioassay experiment, three concentrations (LC_{10}, LC_{30}, and LC_{50}) were selected for further experiment (see result section). The transgenerational studies were conducted by following the methodology of Sadiq et al.[11] with a few modifications. Briefly, 50 adults (25 male and 25 female) of *M. domestica* (2–3-day old) were exposed to each concentration and control inside cages (30 × 30 × 60 cm). For this purpose, a cotton plug was soaked in a specific concentration and placed in the cage for 24 h. Inside each cage, adults were fed on powdered milk and sugar mixture (1:1). In control, flies were fed on powdered milk and sugar mixture (1:1), and water through soaked cotton. After 24 h, the larval diet was placed in the cage for egg laying. To observe the transgenerational effects, 50 eggs were separated from the larval diet of each treatment, labeled and placed on fresh larval diet. The data of egg hatching, larval and pupal durations were recorded daily. Newly emerged adults from each treatment were kept in pairs in meshed cages to record total preoviposition period (TPOP), fecundity, oviposition period and longevity of adults. Population and demographic features were tracked and compared from hatching to adulthood until the mortality of individuals. The entire experiments were conducted under the laboratory conditions: 25 ± 2 °C, 60 ± 5% relative humidity, and a 14-light hour: 10-dark hour photoperiod. The collected data were used to construct an age-stage, two-sex life table. To calculate the intrinsic rate of growth (r) in the context of age-stage two-sex life table theory, the Euler-Lotka equation was used as described previously[11]. This equation relates the intrinsic rate of increase to the life table parameters and is derived from the basic principles of population dynamics.

Statistical analyses

The mortality data, if needed, were corrected by the Abbott's formula[19]. The lethal and sublethal concentrations were calculated by probit analysis using software (IBM SPSS Statistics for Windows, Version 26.0, IBM Corp, Armonk, NY, USA). Life history parameters were analyzed statistically following the method of Chi and Su[20]. For this purpose, age-stage, two sex life table was used. The reproductive parameters were computed computer program TWOSEX-MSChart[21]. Variance, standard error, and mean were estimated using 100,000 bootstrap procedure. All graphics were created using SigmaPlot 12.0 (Systat Software Inc., San Jose, CA, USA). The population parameters were estimated by following equations:

The age-specific maternity ($l_x m_x$) value was calculated with equation[22]:

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

Age-stage specific survival (l_x) was calculated by formula[23]:

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

To equation utilized to calculate the R_o is given below[23]:

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

T value was determined by the following equation[23]:

$$\boldsymbol{T} = (\boldsymbol{InRo}) / \boldsymbol{r}$$

Equation to determine the value r[22]:

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

Finite rate of increase (λ) was calculated by:

$$\lambda\, o = \ e^{r}$$

e_{xj} value was calculated by[23]:

$$e_{xj} = \sum_{i=x}^{\infty} \sum_{y=j}^{K} S_{iy}^{t}$$

The equation for determination of V_{xj}[23]:

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

Results

Lethal and sublethal concentration estimation

The lethal concentrations of indoxacarb were determined through concentration-mortality bioassays. For indoxacarb, LC_{10}, LC_{20} and LC_{50} values were 1.57 µg/ml, 3.08 µg/ml and 4.09 µg/ml, respectively. Whereas the value of Chi-square was calculated as 2.34 (Table 1). Mortality of *M. domestica* in control group was less than 4%.

Effect of indoxacarb on life history traits of *Musca domestica*

Table 2 indicated the life table parameters, such as adult pre-oviposition period (APOP), total pre-oviposition period (TPOP), oviposition, preadult duration, female longevity, male longevity, and fecundity. All the parameters of control group were compared with experimental groups, exposed to different concentrations of indoxacarb (LC_{50}, LC_{30}, and LC_{10}). The pre-adult duration was statistically at par in each concentration with a small increase observed in LC_{50} treated population (13.88 days). Maximum fecundity was observed in the LC_{30} treated population (60.96) as compared to other populations. Significantly lower female longevity was observed in the LC_{50} treated group (25.14 days) than that observed in the LC_{10} treated population (27.36 days). The male longevity was higher in control (28.17 days) and the lowest in the LC_{50} treated population (23.06 days). The APOP duration was similar in control, LC_{10}, and LC_{30} (3.00 days), and its value was slightly higher in the LC_{50} treated group (3.71 days). The TPOP was also the highest in LC_{30} treated population (21.12 days), while LC_{50} treated population exhibited the shortest TPOP (19.14 days). The number of oviposition days were significantly higher in the control group (6.11 days), whereas the LC_{50} treated group showed a substantially lower value (4.00 days) (Table 2).

Effect of indoxacarb on population parameters of *Musca domestica*

Population parameters were evaluated against different concentrations of indoxacarb. The population parameters includes the mean generation time *(T)*, finite rate of increase *(λ)*, intrinsic rate of increase *(r)*, and net reproductive

Insecticide	LC_{10} (95% FL) (µg mL^{-1})	LC_{30} (95% FL) (µg mL^{-1})	LC_{50} (95% FL) (µg mL^{-1})	χ^2	DF	*P*
Indoxacarb	1.57 (0.83–2.28)	3.08 (2.07–4.06)	4.09 (3.67–6.42)	2.341	4	0.673

Table 1. Lethal concentration Estimation of Indoxacarb against *Musca domestica* adults after 48 h. *LC* lethal concentration, *DF* degree of freedom, χ^2 Chi square.

Parameters	Control	LC_{10}	LC_{30}	LC_{50}
Pre-adult duration (days)	12.02 ± 0.05a	12.26 ± 0.15a	13.38 ± 0.07a	13.88 ± 0.06a
Female longevity (days)	26.56 ± 0.66b	27.36 ± 1.65a	27.13 ± 1.11a	25.14 ± 1.15b
Male longevity (days)	28.17 ± 0.78a	27.00 ± 1.91b	27.33 ± 1.48b	23.06 ± 0.05c
APOP (days)	3.00 ± 0.00a	3.00 ± 0.00a	3.00 ± 0.00a	3.71 ± 0.19a
TPOP (days)	19.83 ± 0.22a	20.00 ± 0.00a	21.12 ± 0.12a	19.14 ± 0.51a
Oviposition days	6.11 ± 0.30a	6.04 ± 0.58a	4.63 ± 0.26a	4.00 ± 0.00a
Fecundity (per female)	44.05 ± 2.47c	50.38 ± 4.54b	60.96 ± 3.10a	42.00 ± 2.86c

Table 2. Effect of Indoxacarb on different life history traits of *Musca domestica*. *TPOP* total pre-oviposition period of female counted from birth, *TPOP* total pre-oviposition period of female counted from birth. Means in the same row followed by the same letter are not significantly different ($P > 0.05$) using bootstrap test.

Parameters	Control	LC_{10}	LC_{30}	LC_{50}
r (per day)	0.11 ± 0.01c	0.11 ± 0.01c	1.51 ± 0.52a	1.11 ± 0.42b
R_o (per day)	14.06 ± 5.12a	13.10 ± 2.46a	9.80 ± 3.01b	5.88 ± 3.03b
T	23.84 ± 0.10a	23.73 ± 0.27a	23.98 ± 0.20a	21.66 ± 1.52b
λ (per day)	1.12 ± 0.09a	1.11 ± 0.01a	1.10 ± 0.01a	1.09 ± 0.01a

Table 3. Effect of Indoxacarb on different population parameters of *Musca domestica*. r = The intrinsic rate of increase (per day); $\circ\lambda$ = The finite rate of increase (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.

rate (R_0), as shown in Table 3. The value of r was significantly lower in control and LC_{10} treated groups (0.11/day) compared to the LC_{30} treated population (1.51/day). Significantly higher value of R_o was observed in the control group (14.06/day) while it lowered in the LC_{50} treated group (5.88/day). The value of T and λ was comparatively less in the LC_{50} treated populations (Table 3).

Effect of indoxacarb on the age-specific maternity ($l_x m_x$)

The important parameter for determining the population parameter is $l_x m_x$. The age is $l_x\ m_x$, which is the combination of l_x (age-specific survival rate) and m_x age-specific fecundity. The four components (A, B, C, and D) of Fig. 1 show the difference in $l_x m_x$ values of treated and control group populations of *M. domestica*. The peak for age-stage specific fecundity (m_x) was significantly higher at 3.30 on day 21 in the LC_{30}-treated population and the process ended on the day 25. At the same time, the maximum peak value of m_x was 22.40 in the LC_{30}-treated population on day 22. Moreover, the maximum peak value of $l_x m_x$ was 13.30 on the day 27 in the population, which was treated with LC_{10} of indoxacarb. Contrarily the $l_x m_x$ was observed at its lowest peak observed in control (6.03 at day 21) and LC_{50} treated population (6.08 at day 21). A minor difference was observed in the LC_{50}-treated population and the control group. Similarly, the lowest f_x value (10.98 on day 17 and 25) was observed in the LC_{10}-treated population. The peak value of m_x was 2.22 on day 21 which is the lowest value among all treatments.

Effect of indoxacarb on the age-stage specific survival rate (S_{xj})

The S_{xj} shows the age-stage specific survival rates of *M. domestica* in four different treatments. The comparative observation of Fig. 2(A), 2(B), 2(C), and 2(D) shows that there is no significant difference in the egg hatching duration. However, the highest larval survival (13 days) was recorded in the control group while it significantly reduced to 11 days in the LC_{50} treated population. The female survival is crucial for population dynamics and in the current study the maximum survival of female (30.94) was recorded in population which was subjected to LC_{10}. The female survival in the control group was almost same to those of LC_{10} treated population. The female survival was the lowest in population treated with LC_{50} of indoxacarb. The male survival was higher in control and population treated with LC_{10} of indoxacarb. While minimum male survival 27 days was observed in the LC_{50} treated population.

Effect of indoxacarb on the age-stage reproductive values (V_{xj})

Figure 3 shows the value of age-stage reproductive (V_{xj}). The increase in V_{xj} value (24.54 on day 21) was recorded in the LC_{30} treated population which gradually reduced to zero on day 25. Opposite to this the minimum peak value of *Vxj* was observed in the control group (17.67 on day 18) and this value was reduced to zero on day 27. The age stage reproductive days were higher in control and LC_{10} treated populations and the value in both cases decreased to zero on the 27th day. The increased reproductive rate in a short period may lead to pest outbreaks.

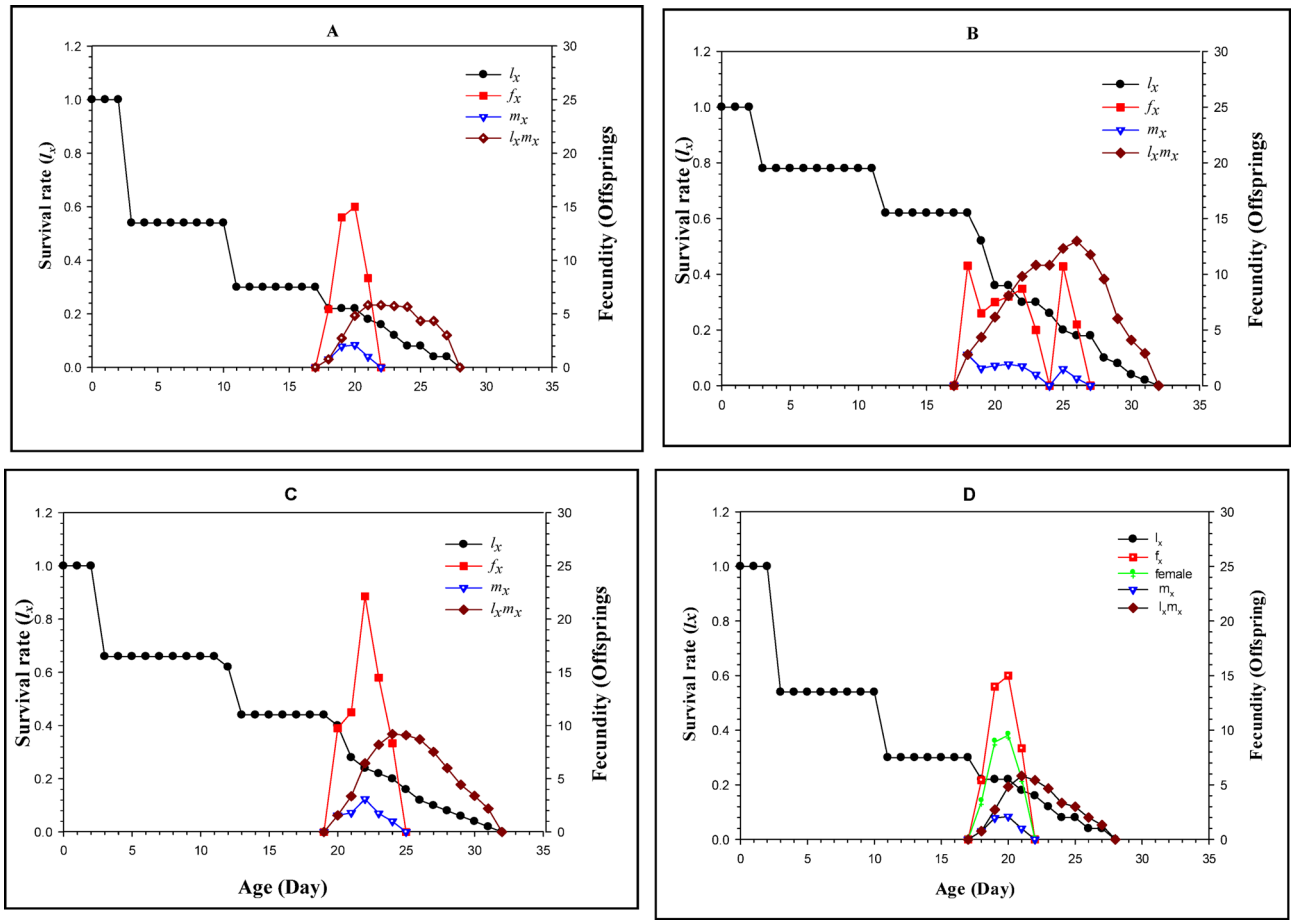

Fig. 1. Age-specific survival rate (l_x), age-specific fecundity of total population (m_x), and age-specific maternity ($l_x m_x$) in control (**A**), LC_{10} (**B**), LC_{30} (C) and LC_{50} (**D**) treated populations of *Musca domestica*.

Effect of indoxacarb on the age-stage life expectancy (e_{xj})

The e_{xj} metric represents the anticipated time duration for *M. domestica* individuals at age x and stage *j* (Fig. 4). The peak of *exj* curve was higher in control (20.19 on day 3) and the value of the life expectancy curve reduced to zero on day 32. The lowest peak value (14.40 on day 3) of e_{xj} curve was recorded in the population subject to LC_{50} of the indoxacarb. This curve was reduced to zero earlier than other curves present in the graph.

Discussion

Indoxacarb is a neurotoxic insecticide that belongs to the oxadiazine-class, which control insect pests by hampering sodium channels, causing paralysis, affecting the transmission of neural signals, and ultimately causes mortality of target insect pests[16]. Its efficacy has established it as a widely used insecticide in both agricultural and residential pest control, specifically for managing insect populations that have evolved resistance to other insecticides[24–26]. Indoxacarb pose minimal risk to non-target species, such as birds, mammals, and aquatic organisms, due to its selective mode of action[27]. However, current studies have suggested potential sublethal effects on exposed organisms under certain exposure scenarios, particularly with repeated applications in ecosystems where bioaccumulation may occur. Findings of the present study underscore the need for more research to completely understand the sublethal implications of indoxacarb on *M. domestica*, especially the long-term effects on population and life table parameters. Indoxacarb is highly toxic to dipterans because it damages the nervous system by blocking the sodium channels[28].

The pre-adult duration of *M. domestica* is significantly affected due to exposure of sublethal doses of insecticides[11,29]. In current study, the preadult duration was not significantly higher in control compared with indoxacarb treatments. These findings can be justified by comparing the current study with the study of Iqbal, et al.[30]. The fecundity was increased when population was exposed to sublethal doses and similar findings were reported previously by Rodrigues et al.[31] and Zhang et al.[32]. Male longevity was decreased in the treatments compared to the control, these findings aligns with the results of previous research[11,29]. Moreover, the values for adult pre-oviposition period (APOP) and total pre-oviposition period (TPOP) were at par in all the treatments including control. In contrast, Liu et al.[33] and Iqbal et al.[30] reported that sublethal exposure of insecticides to flies extend their APOP and TPOP duration. This delayed oviposition effectively reduces population pressure,

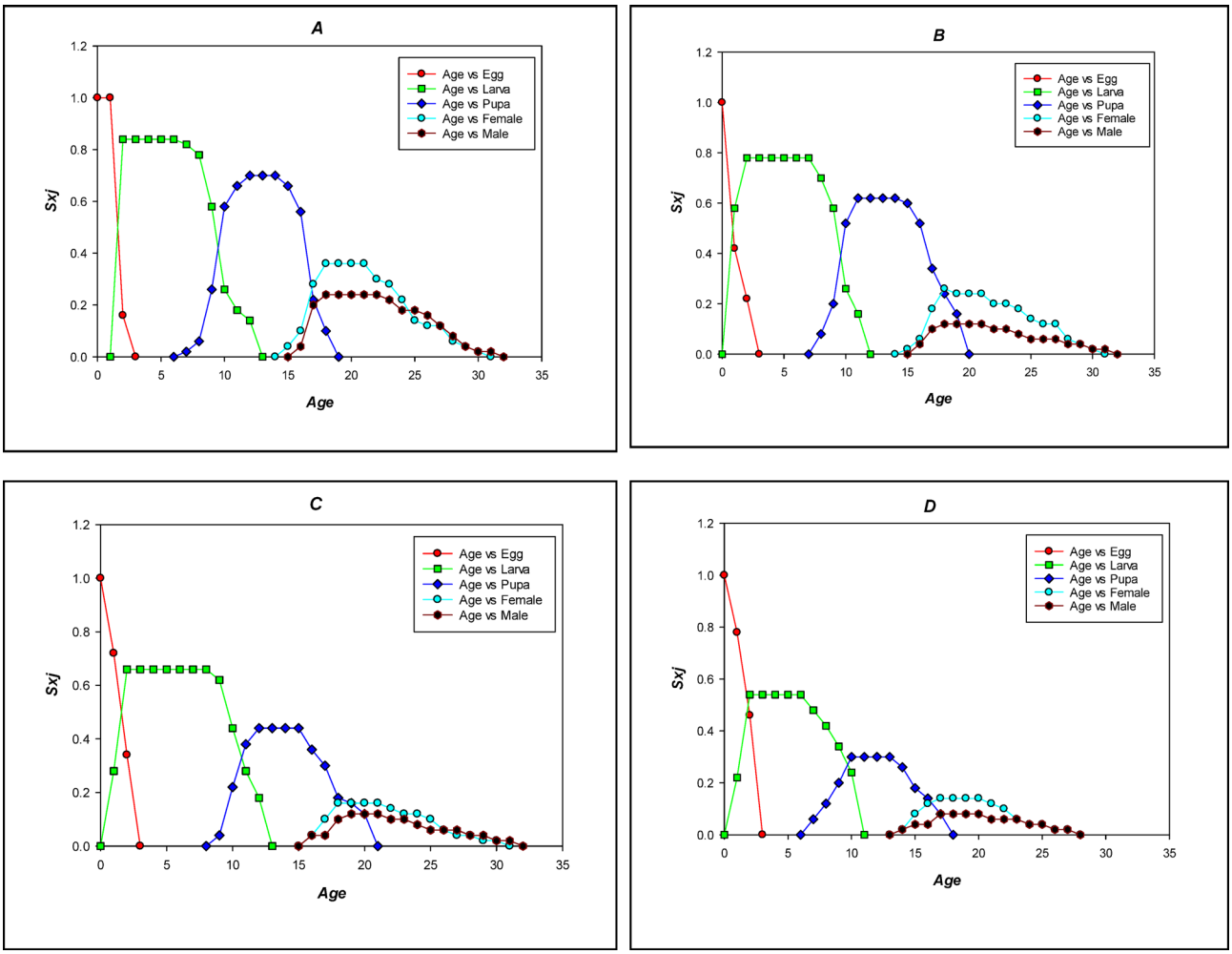

Fig. 2. Age stage specific survival rate (S_{xj}) in control (**A**), LC_{10} (**B**), LC_{30} (**C**) and LC_{50} (**D**) treated populations of *Musca domestica*.

supporting the notion that increased APOP and TPOP are indicators of indoxacarb's transgenerational effects on *M. domestica*.

Moreover, the value of *T (*Mean generation time) and *λ (*finite rate of increase) were comparatively lower in LC_{50} treated populations. These findings are consistent with Khan[18] and suggest that variations in *T* and *λ* are crucial in assessing the potential of indoxacarb. These variations are also helpful to decide whether indoxacarb should be included in integrated pest management (IPM) of *M. domestica* or not. The age-stage maternity ($l_x m_x$) was increased in population exposed to sublethal doses of indoxacarb and findings relates with those of Iqbal et al.[30]. The value of S_{xj} was reduced after sublethal exposure in males and females, results can be justified on the basis previous findings of Sadiq et al.[11], Khan[18], Khan[29] and Farooq & Freed[34]. The reproductive potential of *M. domestica* was boosted when the population was exposed to sublethal concentrations (LC_{10} and LC_{30}). Wu et al.[35] reported the similar findings when *Spodoptera frugiperda* was exposed to sublethal doses. Age-stage life expectancy (e_{xj}) was reduced due to sublethal exposure and current findings are comparable with the results of Shoukat et al.[36]. The variations in population and life table parameters could be utilized to evaluate indoxacarb.

Overall, exposure of LC_{10} and LC_{30} of indoxacarb revealed hormetic effects while LC_{50} had a non-hormetic effect on the performance of most of the life history traits of *M. domestica*. Better performance of insect pests after exposure to low lethal or sublethal concentrations of insecticides; whereas, weak performance of life history traits after exposure to lethal concentrations collectively termed as 'the hormetic concentration-response model"[37,38]. In the present study, stimulatory effects of LC_{10} and LC_{30} and inhibitory effects of LC_{50} of indoxacarb on the biology of *M. domestica* is in broad agreement with some previous studies. For example, exposure of *M. domestica* to tebuconazol at the LD_5 level showed hormetic effects as compared with LD_{10} and LD_{20} levels that showed non-hormetic effects on the performance of life history traits[38]. Gowda et al.[39] reported that insect pests usually develop an adaptive mechanism after exposure to low lethal or sublethal concentrations of insecticides. The presence of insecticide-induced hormesis in insect pests has important implications for the management of insect pests under field conditions. For instance, populations of insect pests suddenly resurge in the presence of insecticide induce hormesis, which ultimately increase the cost of controlling insect pest along with the increased rate of pesticide applications[37,38].

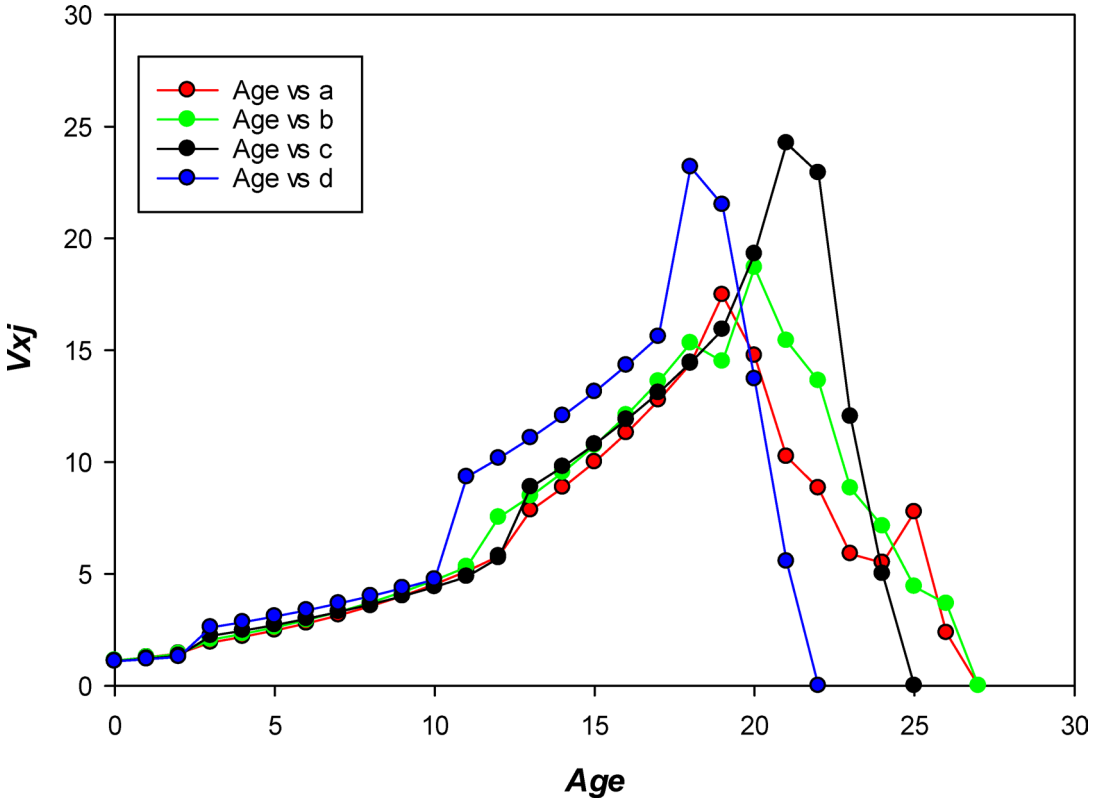

Fig. 3. Reproductive value (V_{xj}) in control (**A**), LC_{10} (**B**), LC_{30} (**C**) and LC_{50} (**D**) treated populations of *Musca domestica*.

Conclusion

Based on the results it is concluded that indoxacarb altered the population and growth parameters of *M. domestica*. The findings of the present study revealed that exposure of LC_{10} and LC_{30} of indoxacarb revealed hormetic effects while LC_{50} had a non-hormetic effect on the performance of most of the life history traits of *M. domestica*. The results suggested that indoxacarb at the LC_{50} level can be included in the management program of *M. domestica*. However, future studies should also be conducted to see the effects of indoxacarb on a wide range of non-target organisms before its inclusion in the management programs.

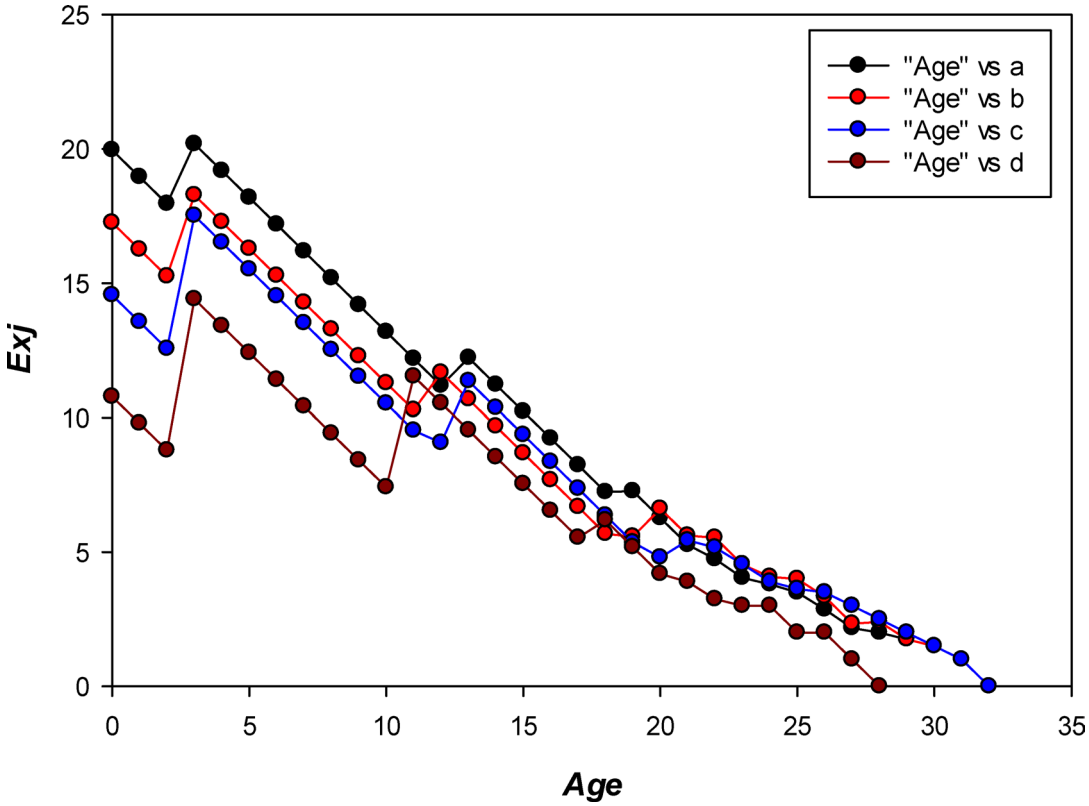

Fig. 4. Life expectancy (e_{xj}) in control (**A**), LC_{10} (**B**), LC_{30} (**C**) and LC_{50} (**D**) treated populations of *Musca domestica*.

Data availability

Datasets used and/or analysed during the current investigation are available from the corresponding author upon reasonable request.

Received: 1 January 2025; Accepted: 9 June 2025
Published online: 03 July 2025

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Acknowledgements

The authors received no specific funding for this research.

Author contributions

NS and NI conceived and designed the study. NS conducted performed the experiment and recorded the data. NS and NI performed statistical analyses. NI, HAAK, ADA, MSS and MNN helped in interpretation of the data. NS, NI and HAAK prepared the first and revised drafts of the manuscript. NI, HAAK, ADA and MSS approved the final version of the manuscript.

Declarations

Competing interests

The authors declare no competing interests.

Ethical statement

All methods related to plants were conducted in accordance with relevant institutional and national guidelines in the method section.

Additional information

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