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Sublethal Effects of Insecticides on Mating Performance of Melon Fly, *Zeugodacus cucurbitae* (Coquillett) (Diptera: Tephritidae)

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Abstract

The melon fruit fly, *Zeugodacus cucurbitae*, is a major pest of cucurbitaceous fruits. Insecticides are commonly used to control this pest. However, environmental factors can reduce lethal doses to sublethal levels. Additionally, adult flies might only receive sublethal exposure due to limited contact during spraying. This study aimed to determine the sublethal effects of fipronil 5 SC, spinosad 45 SC and malathion 50 EC insecticides on mating and remating performance in *Z. cucurbitae*. Adults were treated with LC_{20} of insecticides through topical application and the experiment included four combinations: (1) treated female and treated male (F+M+); (2) treated female and untreated male (F+M-); (3) untreated female and treated male (F-M+); (4) untreated female and untreated male (F-M-). Fipronil treated flies exhibited significantly lower mating percentage (75%-females, 60%-males). However, mating percent in spinosad and malathion was on par with control group. Contrary to this, a significant decrease in remating percentage was noticed in all the insecticide treatments. These findings underscore the adverse effects of sublethal insecticide exposure on reproductive performance in melon fruit fly, which may alter population dynamics and the efficacy of pest management strategies.

Keywords : Melon fly, Insecticides, Sublethal effects, Mating, Remating

The melon fruit fly, *Zeugodacus cucurbitae* (Coquillett), is recognized as one of the most destructive pests affecting vegetables and fruits in temperate, tropical and subtropical regions, including areas across Asia, Africa, the Americas and Australia (Fletcher, 1987; Dhillon *et al.,* 2005 and Sapkota *et al.,* 2010). This pest has an extensive host range, infesting over 81 different plant species, but shows a particular preference for cucurbit crops such as bitter gourd (*Momordica charantia* L.), muskmelon (*Cucumis melo* L.), snap melon (*C. melo* L. var. *momordica*), snake gourd (*Trichosanthes anguina* L.), cucumber (*Cucumis sativus* L.) and ridge gourd (*Luffa acutangula* L.) (Dhillon *et al.,* 2005; Siderhurst & Jang, 2010 and Shivaramu *et al.,* 2022). Female flies oviposit their eggs deep within the fruit pulp, where the developing larvae feed on the fruit from the inside, leading to significant reductions in both market value and crop yield. In addition, this pest facilitates the entry of pathogens, which can exacerbate crop losses and increase the risk of secondary infections. The extent of damage caused by the melon fruit fly can vary widely, ranging from 30 to 100 per cent, depending on seasonal conditions and the vulnerability of the crops to infestation (Dhillon *et al.,* 2005; Amin *et al.,* 2011; Subedi *et al.,* 2021; Devaiah *et al.,* 2022 and Pradhan *et al.,* 2023). Chemical insecticides remain the primary method of controlling tephritid fruit flies globally. But, there are high chances that, flies are exposed to sublethal doses in the field condition. Because, adult flies don't feed on fruits and come in contact with fruits only during oviposition. Additionally abiotic factors such as sunlight, rainfall and temperature may reduce lethal dose of insecticides to sublethal dose over period of time (Stark *et al*., 1995). Sublethal exposure may not result in immediate mortality, but it can adversely affect the pest's behaviour, reproductive capacity and resilience to natural enemies, thereby altering population dynamics and overall pest management efficacy (Desneux *et al.,* 2007). In melon fly, copulation starts at dusk and terminates at dawn, with a mating duration of more than 10 hours (Tsubaki and Sokei, 1988). Female and male fruit flies mate multiple times, as repeated mating can increase the likelihood of successful fertilization and improve overall reproductive output (Teruya and Isobe, 1982; Kuba and Ito, 1993). Sublethal dose of insecticides may alter intricate mating dynamics, such as courtship rituals, mate choice and rematings. Therefore, this study aims to explore the sublethal effects of insecticides on the mating and remating performance of *Z. cucurbitae*, with a focus on how these effects may alter reproductive biology and impact pest management strategies. Understanding the implications of sublethal insecticide exposure on mating dynamics, is crucial for designing integrated pest management approaches that consider the ecological and evolutionary consequences of chemical use.

Material and Methods

Insect culture

Fruit fly-infested fruits were collected from bitter gourd and ridge gourd fields in GKVK campus (13° 4' 49.6" N and 77° 33' 58.8" E). The infested fruits were brought to the laboratory and placed in plastic container (29 cm diameter × 12 cm height) with a 2-3inch layer of sand to facilitate pupation. Pupae were collected from the sand using a sieve. These collected pupae were later transferred to a wooden insect cage (30 x 30 x 30 cm). Upon adult emergence, the flies were provided with a food supplement consisting of yeast hydrolysate enzymatic (a protein source from MP Biomedicals™), sugar and water. After 10-15 days, pumpkins were placed inside the cage, as an oviposition substrate. After pupation, the pupae were harvested and the F_1 adults were subsequently used in the experiments.

Insecticides

Several studies have highlighted the efficacy of spinosad, fipronil and malathion in reducing fruit infestation from *Z. cucurbitae*, thereby providing higher yields (Srinivas *et al*., 2018; Sharma & Gupta, 2019; Abhishek *et al*., 2021 and Nehra *et al.,* 2021). In this context, commonly used insecticides *viz.,* fipronil 5 SC, spinosad 45 SC and malathion 50 EC were used to study the sublethal effects on mating performance of fruit flies.

Determination of sublethal concentrations

Bioassays were conducted by topical application method. Preliminary assays were carried out in the laboratory to find the appropriate dose, giving mortality in the range of 10-90 per cent. Later, five concentrations of each insecticide were finalized and used in the experiment. 5 days old, male and female flies were treated topically with selected insecticides on the body of insect through Multipette® M4 - Multi-Dispenser Pipette (Eppendorf™). Prior to application, flies were immobilised by 3 min exposure to 4 °C and 5 µl of insecticide solution was applied on the thorax of each fly. This method was adapted from the approach reported previously (Busvine, 1980 and Hsu *et al.,* 2012) and is the preferred method of measuring toxicity in arthropods as it is efficient and accurate. After exposure, the treated adults were transferred to new plastic containers along with food supplement and mortality was recorded till 168 hours. Later, the mortality was corrected using Abbott's formula and corrected mortality was subjected to probit analysis and LC_{20} was estimated. The flies were then, treated with sublethal dose (LC_{20}) of insecticides

(Table 1) to determine the impact on mating performance.

TABLE 1
Insecticides used in the experiment

Insecticide	LC_{20} (ppm)
Fipronil 5 SC	7.62
Spinosad 45 SC	1.06
Malathion 50 EC	33.78

Treatment Groups

Here, the flies were treated with LC_{20} of the insecticides on thorax using topical application method as described above. Later, male and female flies of the same treatment were mixed in single container (mating cum oviposition chamber) for mating in different combinations as follows:

1. Treated female and treated male (F+ M+)
2. Treated female with untreated male (F+ M-)
3. Untreated female with treated male (F- M+)
4. Untreated female with untreated male (F- M-)

In each treatment, 20 flies for each sex were used and each pair of fly was considered as one replication. The untreated group (F- M-) was considered as control. The same procedure was followed for all the insecticides and observations were recorded accordingly.

Impact of Sublethal Dose of Insecticides on Mating and Remating per cent

All the containers were regularly checked and monitored every night to confirm the mating status of flies. The mating pairs in the mating chambers were collected with vials, without disturbing mating activity and were transferred to separate boxes. The mated flies remained in pairs throughout the night until sunrise of the next day. Later, mated male and female flies were marked on the thorax with the help of paint (Fig. 1). Then, the marked flies (mated) were released back into the mating chamber and were observed daily for additional mating events. If a marked fly (previously mated) has paired with an unmarked fly (virgin), the latter was marked with a different colour and monitored for subsequent matings. Each pair of flies were marked with a distinct paint colour to avoid misidentification. Every time a marked fly mated; it was recorded. Further, remating percentage (per cent of flies remated) was calculated based on the number of times the marked fly mated. The same procedure was followed in all the treatment groups and observation were recorded separately.

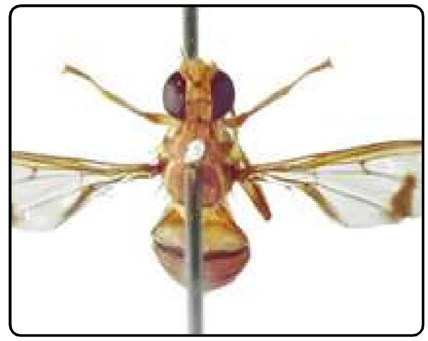

Fig. 1 : Mated fly marked on thorax with paint

Statistical Analysis

The data was analysed and statistical significance among treatments were estimated at 5 per cent probability level using one way analysis of variance (ANOVA) followed by Tukey's as post hoc test. The data was analysed using IBM SPSS statistics ver. 27 (IBM, Armonk, NY, USA).

RESULTS AND DISCUSSION

Effect of Sublethal Dose of Insecticides on Mating (%)

Mating (%) varied significantly between treatment groups. In F+ M+ treatment group, per cent of female and male flies mated was significantly lower (75%-females, 60%-males) (F-46.13, P<0.01) in fipronil treated flies compared to control flies (100%-females, 100%-males). Whereas, in spinosad treated flies, 90 per cent of females and 60 per cent of males mated. However, no significant difference was observed in malathion (80%-females, 90%-males) (F- 46.13, P-0.14) treated flies compared to control flies (100%-females, 100%-males) (Fig. 2).

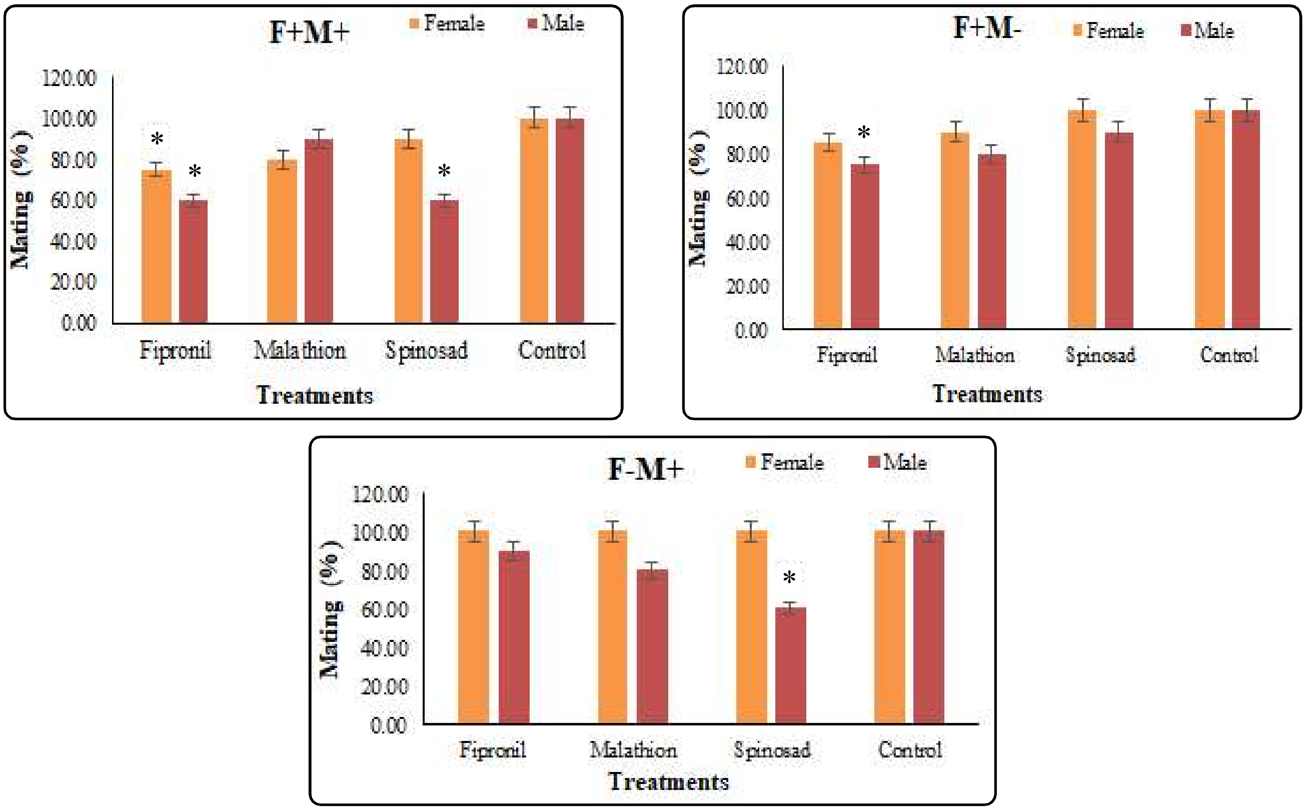

Fig. 2 : Mating (%) recorded in fipronil, spinosad and malathion treated flies (LC_{20}) in different treatment groups

In F+M- treatment group, mating (%) was significantly lower in fipronil treated males flies (75%) (F- 36.38, P<0.02), but no effect was observed on females, where 85 per cent of females mated. Whereas, mating (%) observed in, malathion (90%-females and 80%-males) and spinosad (100%-females and 90%-males) treated flies were on par with control group (100%-females and 100%-males) (Fig. 2).

In F- M+ treatment group, significantly lower mating (%) was observed in spinosad treated males (60%) (F-61.2, P<0.001), but no effect was observed on females, where 100 per cent of females mated. Whereas, no significant difference (F-61.2, P-0.9) was observed in fipronil (100%-females and 90%-males) and malathion treated flies (100%-females and 80%-males) compared to control (100%-females and 100%-males) (Fig. 2).

Overall, only fipronil treated male and female flies and spinosad treated male flies recorded lower mating (%) in F+M+ group compared to control flies.

Effect of Sublethal Dose of Insecticides on Remating (%)

Remating (%) varied significantly among treatment groups. In F+ M+ treatment group, significantly lower remating per cent was observed in fipronil (20%-females, 25%-males) and malathion (20%-females, 25%-males) (F-94.4, P<0.0001) treated flies compared to control flies (90%-females, 80%-males). Whereas, in spinosad treated flies, 30 per cent of females and 50 per cent of males mated (F-94.4, P<0.0001), which was significantly lower than control (Fig. 3).

In F+ M- treatment group, significantly lower remating (%) was observed in malathion treated flies (30%-females and 30%-males) (F- 53.9, P<0.001) followed by spinosad (60%-females and 60%-males) and fipronil (45%-females and 45%-males) treated flies compared to control group (90%-females and 80%-males) (Fig. 3).

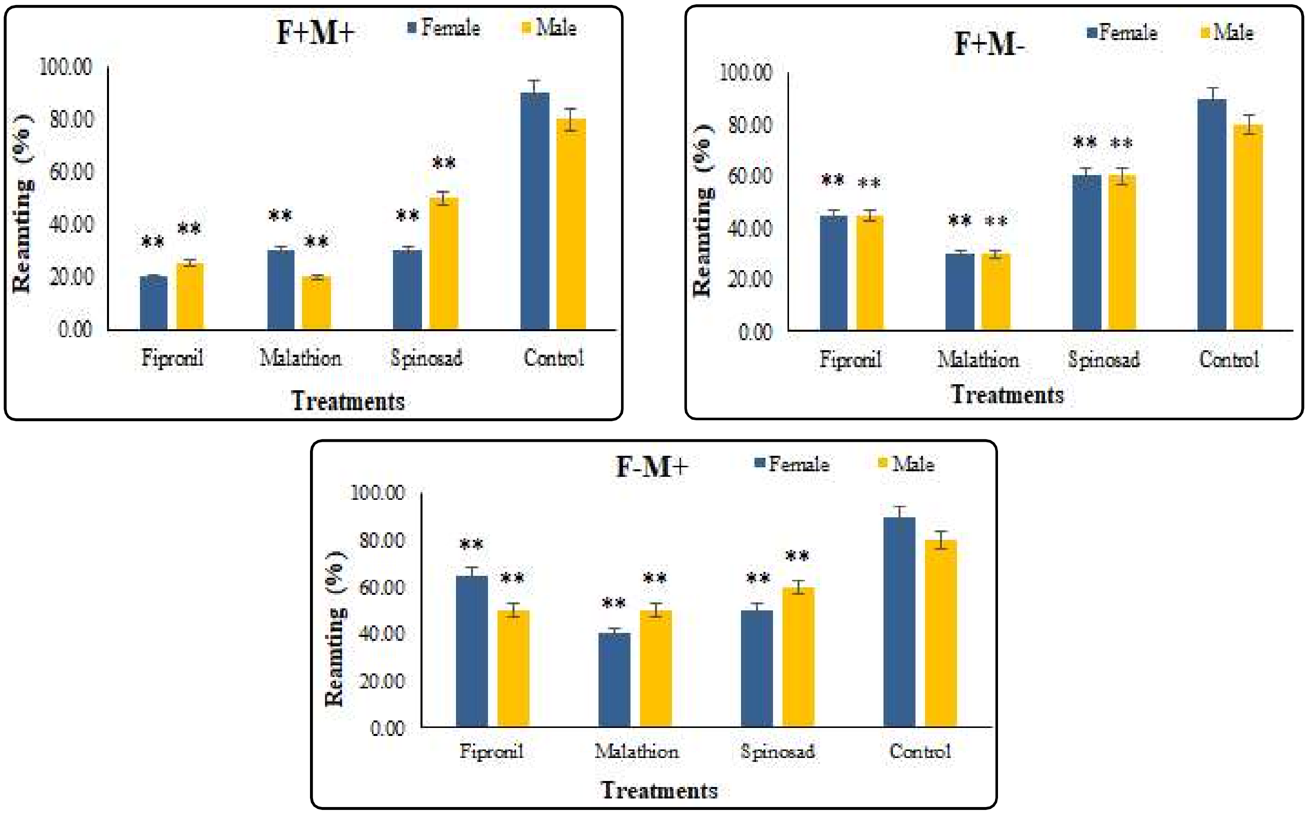

Fig. 3 : Remating (%) recorded in fipronil, spinosad and malathion treated flies (LC_{20}) in different treatment groups

In F-M+ treatment group, significantly lower remating (%) was observed in malathion treated flies (40%-females and 50%-males) (F- 36.38, P<0.001) followed by spinosad (50%-females and 60%-males) and fipronil (65%-females and 50%-males) treated flies compared to control group (90%-females and 80%-males) (Fig. 3).

Overall, remating (%) was significantly lower in flies of all the insecticidal treatment, compared to control flies.

Spinosad, fipronil and malathion insecticides are widely used for management of fruit flies. There are high chances that, flies are exposed to sublethal doses in the field condition. Because, adult flies don't feed on fruits and come in contact with fruits only during oviposition. Additionally abiotic factors such as sunlight, rainfall and temperature may reduce lethal dose of insecticides to sublethal dose over period of time (Stark *et al.*, 1995). Sublethal dose/ concentrations of insecticides may change the chemical communication system and therefore, decrease chances of reproduction in insects (De Franca *et al.,* 2017). Sublethal doses of insecticides on insects can vary by sex and species (Tejeda *et al.,* 2014 and Margus *et al.,* 2019). Thus, in the present study, sublethal effects of insecticides on both the sexes was evaluated. Sublethal doses of tested insecticides had varied effect on the mating and remating in melon fly, *Z. cucurbitae*. Fipronil treated flies recorded lower mating (%) compared to malathion and spinosad treated flies. Fipronil, a phenyl pyrazole, is a potent inhibitor of the gamma-aminobutyric acid (GABA)-gated chloride channel (Cole *et al.,* 1993). It is known to induce oxidative stress, reducing fecundity, fertility and longevity in *Spodoptera litura* (Jameel *et al.,* 2019). Fipronil interacts and binds with DNA which might be responsible for higher DNA damage and cell death (Jameel *et al.,* 2019). The oxidative stress or the DNA damage could be the one of the reasons for reduced mating observed in treated flies. In contrast, remating (%) was significantly reduced in all the treatment groups. Spinosad acts as nicotinic acetylcholine receptor (nAChR) allosteric modulators. Spinosad causes direct or indirect changes in the complex physiological mechanisms that regulate reproductive behaviour by modulation of certain genes (Gomulski *et al.,* 2012). Whereas, malathion is an organophosphate, which acts as acetyl cholinesterase inhibitor. Malathion may reduce neurophysiological responses by acting on the CNS at particular synapses between neurons. The blocking or disruption of neural signals by insecticides will thus, affect courtship behaviour (Zhang, 1987). Even though, insecticides failed to reduce mating (%), rematings were significantly inhibited in flies. Female and male fruit flies mate multiple times to enhance their reproductive fitness (Teruya & Isobe, 1982 and Kuba & Ito, 1993) and remating is crucial for maintaining genetic diversity and population resilience. Inhibiting remating in such flies would greatly limit the reproductive potential, thus inhibiting pest build up. Several studies have reported sublethal effects of various insecticides on *Z. cucurbitae* such as reduced fecundity, fertility and longevity (Rana *et al*., 2015; Nian *et al.,* 2022 and Li *et al.,* 2023).

The results suggest that, insecticides such as fipronil, malathion and spinosad significantly reduced remating in melon flies. These findings contribute to a broader understanding of how chemical control methods influence insect populations and highlight the necessity of considering sublethal effects when designing pest management programs. Further studies are necessary, to elucidate the specific physiological and molecular mechanisms underlying the observed behavioural changes.

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