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TOXICITY AND CHEMOSTERILITY IMPACT OF INSECT GROWTH REGULATORS BAITED DIET ON ADULT PEACH FRUIT FLY, *Bactrocera zonata* (SAUNDERS) (DIPTERA: TEPHRITIDAE)

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Application of synthetic-insecticides against tephritid fruit flies on horticultural-crops increases cost of production and results in biomagnification of their toxic residues in man through food chain. This necessitates to investigate some biorationals like Insect Growth Regulators (IGRs) as their ecofriendly alternate.A laboratory bioassay was conducted to determine toxicity and chemosterility impact of five IGRs *viz.,* Methoxyfenozide, Lufenuron, Buprofezin, Pyriproxyfen and Fenoxycarb on *Bactrocera zonata* through IGR treated adult diet. The results of sterility impacts of five IGRs indicated that Methoxyfenozide, Fenoxycarb, Lufenuron, Pyriproxyfen and Buprofezin demonstrated approximately 37.5, 34.8, 30.9, 25.1 and 22.4% reduction in fecundity; 65.9, 67.3, 67.8, 72.2, and 72.9% less hatchability; 29.4, 25.8, 22.2, 17.6 and 16.1% less sperm concentration liberated from testes; 31.2, 28.4, 25.9, 19.2 and 17.5% less sperm concentration liberated from spermatheca; and 36.2, 32.2, 27.8, 20.8 and 19.6% less egg concentration liberated from ovaries of *B. zonata* over control treatment, respectively. Similarly, lethal concentration (LC) values for fifty percent reduction in fecundity, testes sperm concentration, spermathecal sperm concentration and ovarian egg concentration in *B. zonata* were 0.44, 0.43, 0.68, 1.31 and 0.85% for Methoxyfenozide; 0.50, 0.51, 1.19, 1.75 and 1.06% for Fenoxycarb; 0.50, 0.62, 1.25, 2.52 and 1.54% for Lufenuron; 0.97, 0.83, 2.26, 4.11 and 2.52% for Pyriproxyfen; and 1.05, 0.91, 2.38, 4.33 and 2.68% for Buprofezin, respectively. The mortality results indicate that Methoxyfenozide, Fenoxycarb, Lufenuron, Pyriproxyfen, and Buprofezin explained 37.5 (0.44% LC_{50}), 34.7 (0.50% LC_{50}), 30.9 (0.50% LC_{50}), 25.1 (0.97% LC_{50}) and 22.5% (1.05% LC_{50}) mortality in adult *B. zonata*, respectively. Overall, Methoxyfenozide proved more toxic and had higher chemosterility impacts on *B. zonata* followed by Fenoxycarb, Lufenuron, Pyriproxyfen, and Buprofezin. On the basis of these findings, it is concluded that Methoxyfenozide has maximum toxic and chemosterility impacts on both male and female *B. zonata*, so can be a better eco-friendly biorational for fruit fly management in Pakistan.

Keywords: Chemosterilization, IGRs, laboratory bioassay, mortality, peach fruit fly, sperm and egg concentration.

INTRODUCTION

Peach fruit fly, *Bactrocera zonata* (Saunders) (Diptera: Tephritidae), is a serious polyphagous pest of fruits and vegetables (El-Minshawy *et al.,* 2018). It globally attacks over 50 cultivated and wild plants, mainly those with fleshy fruits including guavas, mangoes, peach, apricots, figs, and citrus (Hossain *et al.,* 2017; El-Minshawy *et al.,* 2018). This fruit fly species is indigenous to Asia and is widely distributed in Southeastern countries like India, Sri Lanka, Bangladesh, Thailand, Mauritius (El-Minshawy *et al.,* 2018) and Egypt (Hossain *et al.,* 2017; El-Minshawy *et al.,* 2018). *Bactrocera zonata* has attained the status of economic and quarantine pest globally. It accounts for 10 to 20% losses in the north-western Himalayan region and up to 89.50% in Pakistan (Hossain *et al.,* 2017). It has been reported that *B. zonata* causes 3-100% fruit losses in different regions, seasons and fruits or vegetables (Ahmad and Begum, 2017). Its pest status is more or less equal to *B. dorsalis* and *B. cucurbitae* in fruits and vegetables in India, and in *Mangifera indica* L. (mango), *Averrhoa carambola* L.(carambola) and *Psidium guajava* L. (guava) in Bangladesh (Hossain *et al.,* 2017). Various published reports reveal that *B. zonata* is the most dominant, devastating and abundantly found fruit fly species in different ecological regions of Pakistan where it infests a variety of fruits and vegetables (Ahmad and Begum, 2017). Fruits flies also cause direct damage of 40-80% to the export of leading crops (Dias *et al.,* 2018) and their detection inside fruits limits exports of fruits in international markets due to imposition of sanitary and phytosanitary, and quarantine restrictions by the importing countries (Lanzavecchia *et al.,* 2014; Dias *et al.,* 2018). Huge amount (millions of dollars) is spent annually on fruit-fly control, management of pre-harvest and post-harvest losses and stringent pre-export treatments of horticultural produce in fruit-fly hot-spot regions/countries of the world (Dhami *et al.,* 2016; Dias *et al.,* 2018).

The management of tephritid fruit flies, including *B. zonata*, is becoming difficult in many countries due to the behavioral, feeding and biological adaptability of various life stages of tephritid fruit flies and elimination of effective broad-spectrum fruit-fly-specific insecticides from markets (Böckmann *et al.*, 2014; Dias *et al.*, 2018). A wide range of research on various aspects of fruit fly monitoring and management strategies has been reported in the literature. The available literature on tephritid fruit flies management demonstrates that maximum research has been published on management of these fruit flies with biological-control (29%), chemical-control (20%), behavioral-control (18%), bioinsecticides (17%), natural product insecticides (13%), mechanical-control (7%) and genetic control (6%) tactics. However, only 14% research was conducted on the monitoring of fruit fly with different monitoring techniques (Dias *et al.*, 2018). In developing countries like Pakistan, the management of tephritid fruit flies totally depends upon the cover spray of synthetic insecticides (Williams *et al.*, 2003; Yee, 2007; De Bon *et al.*, 2014) because of their quick knockdown impacts (Oerke, 2006; Nicholson, 2007). Mostly, organophosphorus insecticides (malathion) and spinosad are used for effective control of tephritid fruit flies (Williams *et al.*, 2003; Urbaneja *et al.*, 2009), but they have high biomagnification properties. This property makes these insecticides very hazardous to human health and persistent environmental pollutants. The cover spray of such insecticides not only causes ecological backlashes in fruit flies against insecticides but also induces lethality to non-target beneficial arthropods and phytotoxic effects on plants (Williams *et al.*, 2003; Yee *et al.*, 2007; Urbaneja *et al.*, 2009; Mostafalou and Abdollahi, 2013; Li *et al.*, 2018). Insecticides application also increases the cost of production and leaves toxic residues in fruits and vegetables causing biomagnification of residues in man (Klungness *et al.*, 2005; Gogi *et al.*, 2010). There is need to investigate some ecofriendly and target-specific biorationals like IGRs as an alternate to such persistent synthetic insecticides.

Chemosterilization of tephritid fruit flies with insect growth regulator (IGR) has been successful in both the laboratory and field levels (Alam *et al.*, 2001). Successful results of various IGRs as chemosterilants have been reported against tephritid fruit flies in both in-vivo and in-vitro conditions because they effectively induce infertility in these fruit flies (Jemaa and Boushih, 2010; Chang *et al.*, 2012). It has been concluded by some researchers that IGRs affect the reproductive system, reproduction, growth, and metamorphosis of many pests (Riddiford and Truman, 1978; Magoc *et al.*, 2005). Navarro-Llopis *et al.* (2010) reported more significant decline in the population and infestation of *C. capitata* in persimmon orchard treated with chemosterilant-traps (24 traps/ha) than malathion aerial-treatment. The area-wide management of tephritid fruit flies includes Sterile Insect Technique (SIT) which is not economical and practically convenient in developing countries like Pakistan. It is being replaced with new lethal systems and other methods like chemosterilization (Navarro-Llopis *et al.*, 2004). Chemosterilants, for example, lufenuron (Alemany *et al.*, 2008; Bachrouch *et al.*, 2008), apholate (Wendell and Ruth, 1964), Hexamethylphosphoramide, hexamethylmelamine (Chang *et al.*, 1964), tepa, hempa, tretamine and ethanesulfonates (Chance *et al.*, 1969) have been studied as spray against tephritid fruit flies but for their safe, practical, and effective application, bait stations have been proposed (Mangan and Moreno, 2007; Robert *et al.*, 2009). It is, therefore, imparative to assess different IGRs formulations marketed in Pakistan for their toxicity and chemosterility impacts on *B. zonata*; so that effective IGRs can be screened out and recommended for bait-station application against *B. zonata* and other tephritid fruit flies.

Keeping in view the importance of IGRs as chemosterilants against tephritid fruit flies, present research was conducted to evaluate the chemosterility impacts of five Insect Growth Regulators (IGRs) *viz.*, Methoxyfenozide, Lufenuron, Buprofezin, Pyriproxyfen and Fenoxycarb on *B. zonata* through IGR treated adult diet under controlled conditions.

MATERIALS AND METHODS

***Mass rearing of Bactrocera zonata*:** The experiment was conducted in Integrated Pest Management (IPM) Laboratory, Department of Entomology, University of Agriculture, Faisalabad (31.4303° N, 73.0672° E), Punjab, Pakistan. Guava fruits infested with *B. zonata* were collected from different orchards in Faisalabad. The infested fruits were brought into IPM laboratory and kept in card boxes half-filled with sieved and sterilized sand. The sand was oven-sterilized at 160 °C for one hour. Pupae were sieved out from sand by using a fine-mesh sieve after a week. The pupae were kept in the dome-shaped rearing cages till the adult emergence. The cages were provided with the spongy strips soaked with the adult diet containing protein-hydrolysate, molasses, guava-juice, yeast-powder and water in 1:1:1:1:6 ratio. These strips were suspended after soaking in adult diet solution. The fresh, properly cleaned, and washed, guava fruits were brought in laboratory, surface-sterilized by 70% alcohol and suspended inside the rearing cage for egg collection. After three days, fruits were shifted from rearing cage to card boxes having sterilized sand for attaining the next progeny. This procedure was used to mass culture *B. zonata* upto 5th generation in IPM laboratory maintained at 26±2 °C, 65±5% rh and 12 L:12 D photoperiod. Adult flies of 5th generation were used for experimentation.

***Preparation of pesticides dilutions*:** Five formulations of IGRs were used in this study (Table 1). The glass beakers were cleaned with bleach and distilled water, air-dried and then were used in this experiment. A stock solution (D-1) of the highest concentration (1.28%) was prepared for each IGR.

Table 1. List of Insect Growth Regulator (IGRs) used for studying their chemosterilant effects on the male and female adult of *Bactrocera zonata* through laboratory bioassay.

IGRs	Active ingredients	Mode of action	Company	Concentrations
Runner®	Methoxyfenozide	Ecdysteroid agonist	Dow AgroSciences	0.02, 0.04, 0.08, 0.16, 0.32, 0.64, 1.28%
Match®	Lufenuron	Chitin synthesis inhibitor	Syngenta, Pakistan Ltd.	0.02, 0.04, 0.08, 0.16, 0.32, 0.64, 1.28%
Applaud®	Buprofezin	Chitin synthesis inhibitor	Dow AgroSciences	0.02, 0.04, 0.08, 0.16, 0.32, 0.64, 1.28%
Admiral®	Pyriproxyfen	Juvenile hormone antagonists	Sumitomo Chemical Co., Ltd.	0.02, 0.04, 0.08, 0.16, 0.32, 0.64, 1.28%
Insegar®	Fenoxycarb	Juvenile hormone antagonists	Syngenta, Pakistan Ltd.	0.02, 0.04, 0.08, 0.16, 0.32, 0.64, 1.28%

The quantity of formulated solution of each IGR needed to prepare the required stock-solution was calculated using equation: $C_1V_1 = C_2V_2$. Then, next lower dilution (D-2) (0.64%) was prepared from D-1 in another measuring cylinder by taking half of the stock solution and diluting it with distilled water to attain the original volume. Sequential dilutions were prepared for each of the IGR until last dilution was achieved (Atta *et al.*, 2015). Seven concentrations of each IGR (0.02, 0.04, 0.08, 0.16, 0.32, 0.64 and 1.28%) were prepared and used for studying their chemosterilant effects on *B. zonata* through laboratory bioassay at 26±2 °C, 65±5% rh and 12 L:12 D photoperiod.

***Bioassay*:** A total of thirty-two experimental units (glass jars with diet soaked spongy strips) were prepared for each IGR and both sexes of *B. zonata*. The experiment consisted of seven concentrations of IGRs and one control treatment which were replicated four-times in completely randomized design. Volume of each glass jar was 4224 cm^3 or mL (8 cm radius and 21 cm height). The adult diet (protein-hydrolysate, molasses, guava-juice, yeast-powder and water in 1:1:1:1:6 ratio) was admixed with IGR concentration. Spongy strips were soaked in IGR admixed adult diet and then suspended in the plastic jars. A counted number of newly emerged starved adult fruit flies (10 males+10 females) of *B. zonata* were released in each experimental unit and kept there for force-feeding on the adult diet for 24 hours. After feeding for 24 hours on treated diet, the IGR-admixed-diet soaked strips were replaced with normal-diet soaked strips for the rest of the experimental period. The adult flies were observed after three days and considered dead if these didn't show any movement in their legs and/or antenna after gentle touch with soft camel-hair brush. The adult flies found dead after 3 days of exposure were counted and percent mortality was calculated. An egg-receptacle (small perforated plastic cups coated with guava-pulp as oviposition attractant on their internal surface) was placed inside each experimental unit for the collection of eggs. The eggs were collected on every 3rd alternate day till the death of all the *B. zonata* flies and then average eggs per female were calculated by the following formula:

$$\text{Eggs/female} = \frac{\text{Sum of eggs collected for whole period}}{\text{Total female flies}}$$

Similarly, the eggs collected on every 3rd alternate day were counted and washed with tap water. These washed eggs were spread on wet black cloth and incubated inside the incubator maintained at 25 ± 2 ºC and 60±5% R.H for three days. After incubation for three days, the number of hatched and unhatched eggs was counted, and egg-hatching percentage was calculated by the following formula:

$$\text{Eggs hatching (\%)} = \frac{\text{Number of hatched eggs}}{\text{Sum of hatched and unhatched eggs}} \times 100$$

Randomly three male and three female flies were taken from each of treated and untreated (control) lots. The ovary and spermatheca of female flies and testes of male flies of these lots were dissected out under stereomicroscope and placed separately in Petri dishes. These isolated organs were then put in separate Eppendorf and 50 ml of double-sterilized saline water solution containing 0.1% of the surfactant Triton® [alkylaryl polyether alcohols ($C_{33}H_{60}O_{10.5}$)] was added. Then these organs were gently crushed separately with the help of fused end of the capillary tube in Eppendorf which was later on vortexed for five minutes to liberate the sperms and eggs from the organs into the solution. A volume of 10 µl was taken immediately from the middle of the suspension with micropipette and loaded on the hemocytometer. The loaded sample was allowed to settle for 2-3 minutes and left to dry at room temperature. After drying the sample, the hemocytometer was placed on the microscope and number of the sperms (in testes and spermatheca) and eggs (in ovaries) were counted in 9 large squares of the Neubauser hemocytometer (along the diagonals of squares). Total counts of sperm and eggs for each respective organ were converted into average/mean counts per large square of hemocytometer by the following formula:

$$\text{Mean counts (sperms) /large square} = \frac{\text{Total sperm counts}}{9}$$

$$\text{Mean counts (eggs) per large square} = \frac{\text{Total egg counts}}{9}$$

The concentration of sperms or eggs per ml was calculated by the following formula:

$$\text{Concentration/mL} = \text{Dilution Factor} \times \text{Mean counts}$$

***Statistical analysis*:** The data regarding *B. zonata* mortality, fecundity, percent egg-hatching, sperms concentration in testes or spermatheca and eggs concentration in ovary were subjected to ANOVA technique to determine the parameters of significance while mean values for different treatments were compared with Tukey's honestly significant difference test, as performed by Danho *et al.* (2002) using statistical software of STATISTICA-10. The data on *B. zonata*

mortality, reduction in fecundity, sperm-reduction (in male testes and female spermatheca) and egg-reduction in the female ovary were subjected to probit analysis to determine LC_{50} and LC_{90} using Minitab as statistical software (Finney, 1971). The chemosterilants demonstrating higher mortality and least fecundity as well as sperm and egg production were considered as highly effective chemosterilants.

RESULTS

***Effect on mortality, fecundity, egg hatchability, ovarian egg concentration, testes sperm concentration and spermathecal sperm concentration of Bactrocera zonata*:** ANOVA parameters demonstrate that IGRs, their concentrations and first level interaction between these had highly significant effects on the variation in mortality, fecundity, egg hatching, egg concentration in the ovary, sperm concentration in testes, and sperm concentration in the spermatheca of *B. zonata* (p <0.05) (Table 2).

The mortality results indicate that Methoxyfenozide, Fenoxycarb, Lufenuron, Pyriproxyfen, and Buprofezin explained 37.5, 34.7, 30.9, 25.1 and 22.5% mortality in adult *B. zonata*, respectively (Fig. 1a). The female *B. zonata* fed on Methoxyfenozide treated diet deposited minimum eggs (154.9 eggs/female) followed by Fenoxycarb (161.7 eggs/female), Lufenuron (171.2 eggs/female), Pyriproxyfen (185.8 eggs/female), Buprofezin (192.4 eggs/female) and control treatment (248.1 eggs/female). These results reveal that Methoxyfenozide, Fenoxycarb, Lufenuron, Pyriproxyfen, and Buprofezin demonstrated approximately 37.5, 34.8, 30.9, 25.1 and 22.4% reduction in fecundity over control treatment, respectively (Fig. 1b). The results regarding egg hatching percentage indicate that eggs deposited by Methoxyfenozide treated female *B. zonata* exhibited 65.9% hatchability. The eggs deposited by Fenoxycarb, Lufenuron, Pyriproxyfen, and Buprofezin treated female *B. zonata* exhibited 67.3, 67.8, 72.3, and 72.9% hatchability, respectively as compared to 97.1% hatchability in control treatment. These results explained that Methoxyfenozide, Fenoxycarb, Lufenuron, Pyriproxyfen, and Buprofezin demonstrated 32.0, 30.7, 30.1, 25.6 and 24.9% less hatchability over control treatment (Fig. 1c). The concentration of eggs liberated from the ovaries of female *B. zonata* were recorded in the range of 131.7-166.1 eggs mL^{-1} in IGRs treated female *B. zonata*, as compared to control treatment (206.5 eggs mL^{-1}), being 131.7, 139.9, 149.1, 163.6 and 166.1 eggs mL^{-1} in Methoxyfenozide, Fenoxycarb, Lufenuron, Pyriproxyfen and Buprofezin treated female *B. zonata*, respectively. These results indicate that Methoxyfenozide, Fenoxycarb, Lufenuron, Pyriproxyfen, and Buprofezin demonstrated 36.2, 32.2, 27.8, 20.8 and 19.6% less egg concentration over control treatment, respectively (Fig. 1d). The concentration of sperm liberated from testes ranged from 1819.1 to 2163.2 sperms mL^{-1} in IGRs treated male *B. zonata*, as compared to control treatment (2577.6 sperms mL^{-1}), being 1819.1, 1911.6, 2004.1, 2122.5 and 2163.2 sperms mL^{-1} in Methoxyfenozide, Fenoxycarb, Lufenuron, Pyriproxyfen and Buprofezin treated male *B. zonata*, respectively. These results indicate that Methoxyfenozide, Fenoxycarb, Lufenuron, Pyriproxyfen, and Buprofezin demonstrated 29.4, 25.8, 22.2, 17.6 and 16.1% less sperm concentration liberated from testes over control treatment (Fig. 1e). Similarly concentration of sperm liberated from spermatheca ranged from 673.5 to 807.4 sperms mL^{-1} in IGRs treated female *B. zonata*, as compared to control treatment (978.99 sperms mL^{-1}), being 673.5, 701.3, 725.8, 791.1 and 807.4 sperms mL^{-1} in Methoxyfenozide, Fenoxycarb, Lufenuron, Pyriproxyfen and Buprofezin treated female *B. zonata*, respectively. These results indicate that Methoxyfenozide, Fenoxycarb, Lufenuron, Pyriproxyfen, and Buprofezin demonstrated 31.2, 28.4, 25.9, 19.2 and 17.5% less sperm concentration liberated from spermatheca over control treatment (Fig. 1f). The overall results confirm that Methoxy fenozide demonstrated higher chemosterility impacts on both male and female *B. zonata* followed by Fenoxycarb, Lufenuron, Pyriproxyfen and Buprofezin (Fig. 1).

The results of first-level interaction show that mortality of *B. zonata* ranged from 13.1-81.2, 11.2-77.3, 9.1-75.2, 2.2-54.3, and 2.1-52.2% when treated with Methoxyfenozide, Fenoxycarb, Lufenuron, Pyriproxyfen, and Buprofezin, respectively, being less at lower concentration (0.02%) and greater at higher concentration (1.28%) of each IGR. Methoxy fenozide, Fenoxycarb, and Lufenuron demonstrated

Table 2. ANOVA parameters regarding percent mortality, fecundity, percent egg hatching, egg concentration in the ovary, sperm concentration in testes and sperm concentration in the spermatheca of *Bactrocera zonata* treated with different concentration of Insect Growth Regulators (IGRs) (Total *df* = 125; Error *df* = 84).

Source of variation	*df*	*F* values for all dependent parameters						*P* values for all dependent parameters
		Percent mortality	Fecundity (Eggs/Female)	Percent egg hatching	Egg concentration in the ovary	Sperm concentration		
						In testes	In spermatheca	
IGRs	5	5979.54	132.25	1160.8	509.3	3930	805.7	<0.01
Concentrations (C)	6	52628.99	202.98	832.5	309.4	2841	714.9	<0.01
IGRs × C	30	2799.55	8.31	36.1	13.4	126	33.5	<0.01

$P < 0.05$

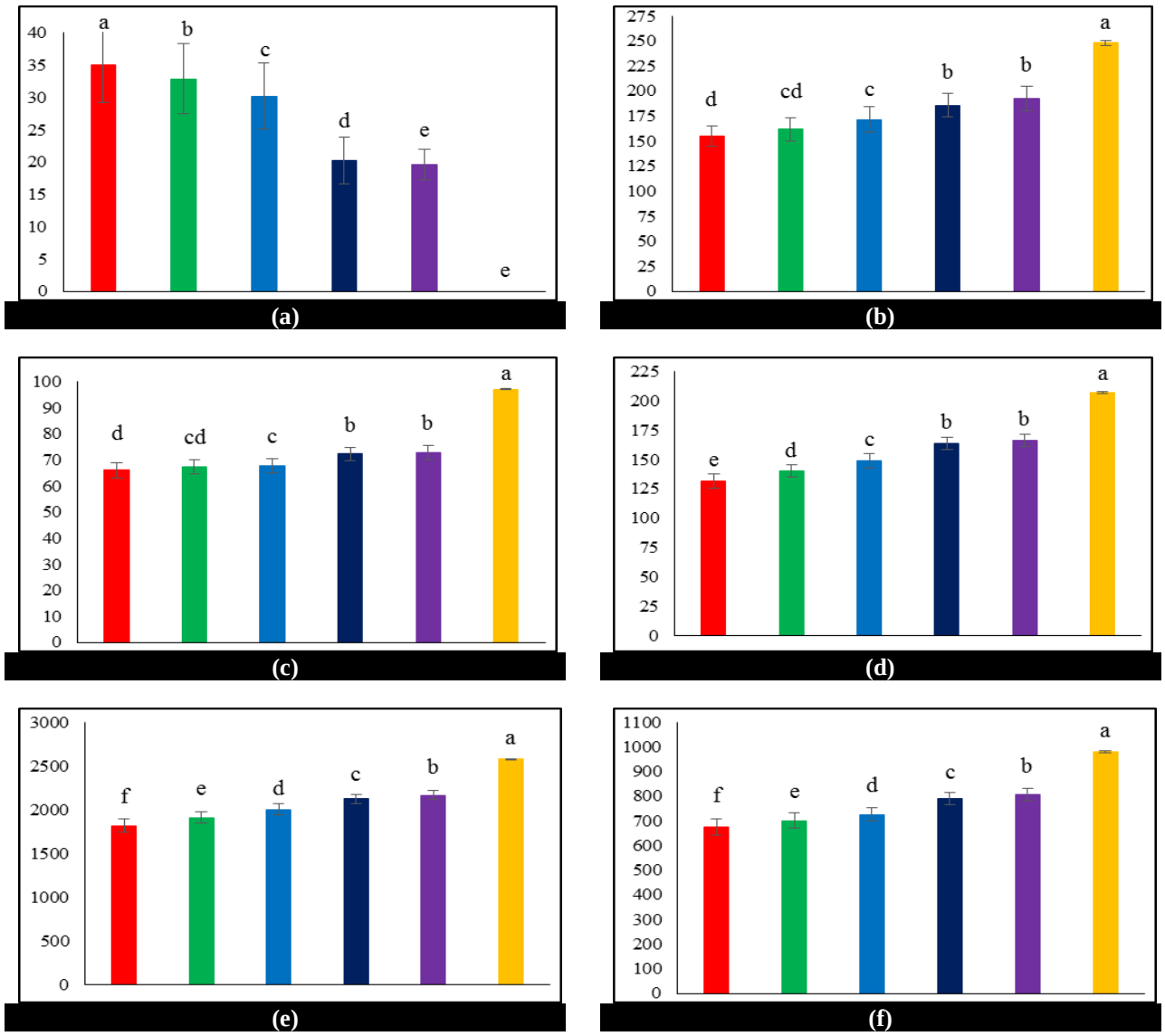

Figure 1. Means (±SE, *n* = 3) of (a) percent mortality, (b) fecundity (eggs/female), (c) percent egg hatching, (d) egg concentration in ovary (eggs mL^{-1}), (e) sperm concentration in testes (sperms mL^{-1}) and (f) sperm concentration in spermatheca (sperms mL^{-1}) of *Bactrocera zonata* treated with different Insect Growth Regulators (IGRs) irrespective of various concentrations. Colored bars depict the different treatment; Methoxyfenozide, Fenoxycarb, Lufenuron, Pyriproxyfen, Buprofezin, Control. Bars indicate standard errors, Means sharing similar style letters don't differ significantly at the probability level of 5%. Treatments (IGRs) are on *x-axis* and different dependent parameters are on *y-axis*.

statistically similar but higher mortality while Pyriproxyfen and Buprofezin explained statistically similar but lower mortality at each concentration (Fig. 2a).

Treatment of female *B. zonata* with IGRs demonstrated fecundity in the range of 46.0-203.2, 61.1-209.6, 66.3-222.4, 79.1-230.1 and 84.2-235.2 eggs/female when treated with Methoxyfenozide, Fenoxycarb, Lufenuron, Pyriproxyfen and Buprofezin, respectively, being higher fecundity at lower concentration (0.02%) and lower fecundity at higher concentration (1.28%). These fecundity results show that treatment of *B. zonata* with Methoxyfenozide, Fenoxycarb, Lufenuron, Pyriproxyfen, and Buprofezin resulted in 18.1-74.1, 15.5-75.3, 10.3-73.3, 7.2-68.1 and 5.2-66.1% less fecundity, respectively over control (248.1 eggs/female). This reduction in fecundity was found higher at higher concentrations and lower at lower concentrations (Fig. 2b).

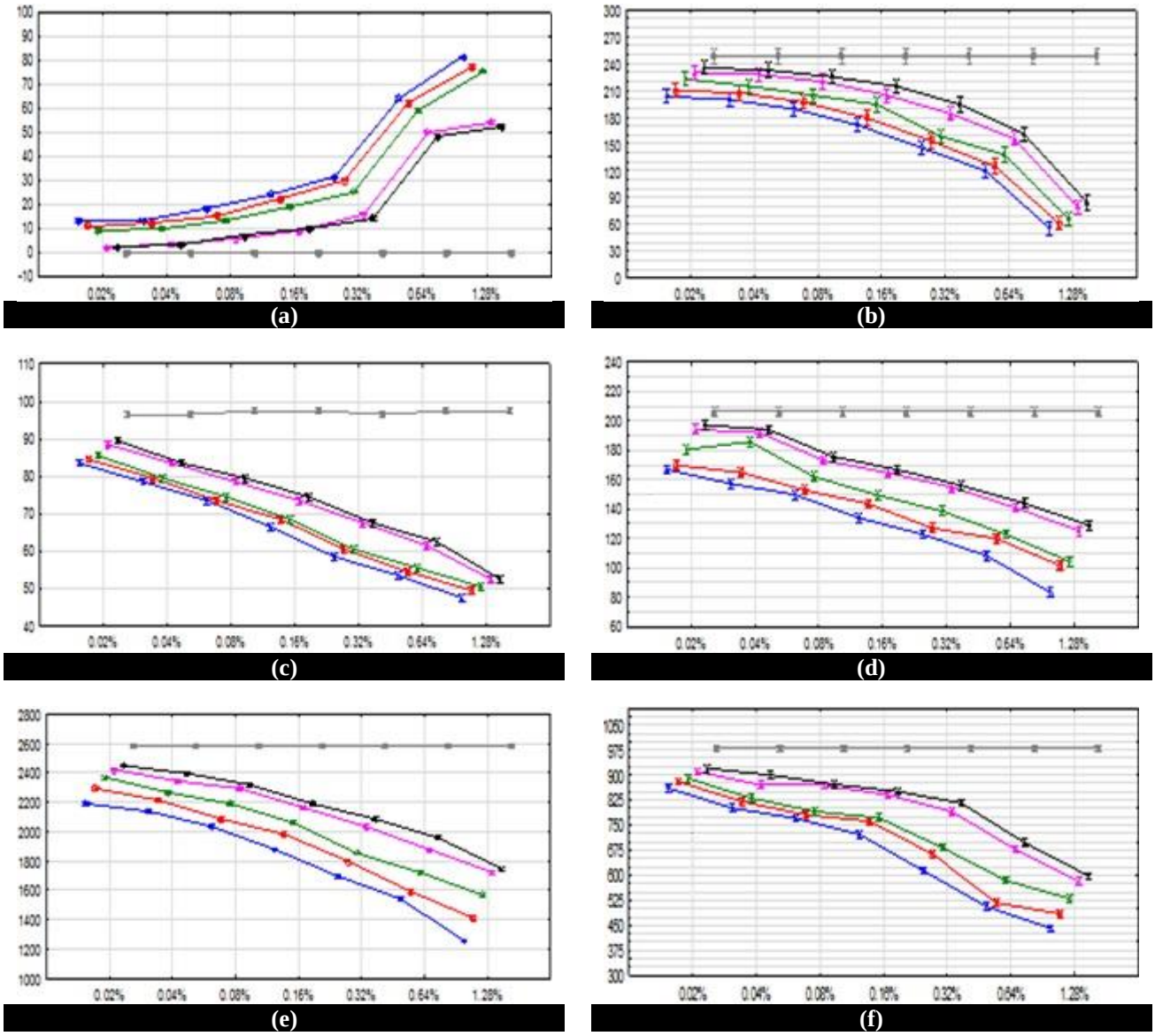

Figure 2. Means (±SE, *n* = 3) of (a) percent mortality, (b) fecundity (eggs/female), (c) percent egg hatching, (d) egg concentration in ovary (eggs mL^{-1}), (e) sperm concentration in testes (sperms mL^{-1}) and (f) sperm concentration in spermatheca (sperms mL^{-1}) of *Bactrocera zonata* treated with different Insect Growth Regulators (IGRs) at various concentrations. Colored lines depict the different treatment; Methoxyfenozide, Fenoxycarb, Lufenuron, Pyriproxyfen, Buprofezin, Control. Means sharing similar style letters don't differ significantly at the probability level of 5%. Concentrations of IGRs are on *x-axis* and different dependent parameters are on *y-axis*.

The results of egg hatching reveal that eggs deposited by Methoxyfenozide, Fenoxycarb, Lufenuron, Pyriproxyfen, and Buprofezin treated *B. zonata* flies exhibited 47.5-83.5, 49.4-84.3, 50.3-85.5, 52.5-88.6 and 52.5-89.5% hatchability, respectively, being higher hatchability at lower concentration (0.02%) and lower hatchability at higher concentration (1.28%). In control treatment, egg-hatchability was recorded in the range of 96.3-97.5% (Fig. 2c).

Eggs concentration liberated from the ovary of female *B. zonata* ranged from 83.1-166.9, 101.5-169.8, 104.3-180.6, 125.2-194.2 and 129.1-197.1 eggs mL^{-1} when fed on diet treated with Methoxyfenozide, Fenoxycarb, Lufenuron, Pyriproxyfen, and Buprofezin, respectively, being significantly higher egg-concentration at lower IGR concentration and lower egg concentration at higher IGR concentration. However, egg concentration liberated from

ovary of female *B. zonata* was found in the range of 205.1-2016.5 eggs mL^{-1} in control treatment (Fig. 2d). Similarly, the sperm concentration liberated from testes of male *B. zonata* was found in the range of 1256.7-2189.1, 1412.1-2292.7, 1567.5-2370.4, 1722.9-2422.2 and 1748.8-2448.1 sperms mL^{-1} when fed on diet treated with Methoxyfenozide, Fenoxycarb, Lufenuron, Pyriproxyfen and Buprofezin, respectively, being higher sperm concentration at lower IGR concentration and lower sperm-concentration at higher IGR concentration. Sperm concentration liberated from testes of male *B. zonata* was found in the range of 2576.9-2577.6 sperms mL^{-1} in control treatment (Fig. 2e). Likewise, The sperm concentration liberated from spermatheca of female *B. zonata* was found in the range of 441.4-860.2, 484.5-879.9, 530.5-889.9, 581.0-909.7 and 597.8-919.6 sperms mL^{-1} when fed on diet treated with Methoxyfenozide, Fenoxycarb, Lufenuron, Pyriproxyfen and Buprofezin, respectively, being significantly higher sperm concentration at lower IGR concentration and lower sperm concentration at higher IGR concentration. However, sperm concentration liberated from spermatheca of female *B. zonata* was found in the range of 977.8-978.9 sperms mL^{-1} in control treatment (Fig. 2f). These interaction results also depict that Methoxyfenozide demonstrated higher chemosterility impacts in *B. zonata* at higher concentrations followed by Fenoxycarb, Lufenuron, Pyriproxyfen and Buprofezin (Fig. 2).

***LC_{50} and LC_{90} values for mortality and reduction in fecundity, ovarian egg concentration, testes sperm concentration, spermathecal sperm concentration of Bactrocera zonata*:** Lethal concentration values for fifty and ninety percent mortality and reduction in fecundity, testes sperm concentration, spermathecal sperm concentration and ovarian egg concentration in *B. zonata* varied significantly for different IGRs as 95% Fudicial CI values against said parameters did not overlap. The LC_{50} and LC_{90} values of mortality for Methoxy fenozide (0.44±0.04 and 2.37±0.42%), Fenoxycarb (0.50±0.05 and 2.6±0.47%), Lufenuron (0.57±0.05 and 2.7±0.48%), Pyriproxyfen (0.97±0.10 and 3.3±0.47%) and Buprofezin (1.05±0.11 and 3.76±0.76%) demonstrated that Methoxyfenozide proved more toxic for *B. zonata* followed by Fenoxycarb, Lufenuron, Pyriproxyfen, and Buprofezin. Methoxyfenozide demonstrated lower LC_{50}

Table 3. Different probit analysis parameters and lethal concentration values for fifty and ninety percent mortality and reduction in fecundity, ovarian egg concentration, testes sperm concentration and spermathecal sperm concentration in *Bactrocera zonata* due to different Insect Growth Regulators (IGRs) at various concentrations.

Probit analysis parameters	Lethal concentration	Methoxyfenozide	Fenoxycarb	Lufenuron	Pyriproxyfen	Buprofezin
Mortality	Chi Square (df ; p)	12.8 (5; 0.03)	10.1 (5; 0.07)	11.3 (5; 0.05)	10.7 (5; 0.06)	10.7 (5; 0.06)
	LC_{50} (FL 95%)	0.44±0.04 (0.37-0.53)	0.50±0.05 (0.42-0.61)	0.57±0.05 (0.48-0.70)	0.97±0.10 (0.81-1.21)	1.05±0.11 (0.85-1.33)
	LC_{90} (FL 95%)	2.37±0.42 (1.74-3.56)	2.6±0.47 (1.88-3.90)	2.7±0.48 (1.95-3.99)	3.3±0.47 (1.88-3.90)	3.76±0.76 (2.67-6.07)
Reduction in fecundity	Chi Square (df ; p)	6.4 (5; 0.27)	5.5 (5; 0.36)	5.3 (5; 0.37)	5.9 (5; 0.32)	4.9 (5; 0.42)
	LC_{50} (FL 95%)	0.43±0.05 (0.34-0.57)	0.51±0.06 (0.40-0.66)	0.62±0.07 (0.49-0.80)	0.83±0.11 (0.67-1.08)	0.91±0.10 (0.73-1.17)
	LC_{90} (FL 95%)	3.90±0.86 (2.68-6.55)	4.10±1.04 (2.69-7.52)	4.20±1.02 (2.81-7.47)	4.40±1.26 (2.76-8.72)	4.60±1.38 (2.77-9.43)
Ovarian egg concentration	Chi Square (df ; p)	0.5 (5; 1.00)	0.5 (5; 1.00)	3.2 (5; 0.70)	2.2 (5; 0.82)	2.7 (5; 0.75)
	LC_{50} (FL 95%)	0.68±0.15 (0.45-1.13)	1.19±0.39 (0.70-2.83)	1.25±0.32 (0.81-2.38)	2.26±0.72 (1.35-5.18)	2.38±0.76 (1.42-5.45)
	LC_{90} (FL 95%)	20.60±13.10 (7.66-112.20)	22.30±13.50 (8.62-109.90)	26.90±17.10 (10.06-146.60)	28.40±18.50 (10.39-161.90)	53.60±47.40 (14.13-658.20)
Testes sperm concentration	Chi Square (df ; p)	0.3 (5; 1.00)	0.1 (5; 1.00)	0.6 (5; 1.00)	0.2 (5; 1.00)	0.2 (5; 1.00)
	LC_{50} (FL 95%)	1.31±0.39 (0.81-2.80)	1.75±0.57 (1.05-4.10)	2.52±0.95 (1.39-6.88)	4.11±1.90 (2.11-14.96)	4.33±1.98 (2.14-15.46)
	LC_{90} (FL 95%)	33.60±24.40 (10.96-243.80)	36.40±26.70 (11.82-268.50)	46.10±36.40 (13.90-410.20)	63.70±56.50 (16.86-805.80)	55.70±47.30 (15.56-626.10)
Spermathecal sperm concentration	Chi Square (df ; p)	0.8 (5; 0.98)	2.0 (5; 0.85)	1.0 (5; 0.97)	2.2 (5; 0.83)	1.3 (5; 0.94)
	LC_{50} (FL 95%)	0.85±0.17 (0.60-1.37)	1.06±0.23 (0.73-1.79)	1.54±0.44 (0.97-3.17)	2.52±0.85 (1.48-6.05)	2.68±0.89 (1.58-6.40)
	LC_{90} (FL 95%)	13.00±6.30 (5.98-44.90)	14.70±26.70 (7.39-53.30)	25.70±16.40 (9.54-139.70)	28.90±19.00 (10.43-170.60)	26.40±16.80 (9.89-145.40)

and LC_{90} values of reduction in fecundity (0.43±0.05 and 3.9±0.86%) for *B. zonata* followed by Fenoxycarb (0.51±0.06 and 4.1±1.04%), Lufenuron (0.62±0.07 and 4.2±1.02%), Pyriproxyfen (0.83±0.11 and 4.4±1.26%) and Buprofezin (0.91±0.10 and 4.6±1.38%). Methoxyfenozide demonstrated lower LC_{50} and LC_{90} values of reduction in sperm concentration in testes (1.31±0.39 and 33.6±24.4%) for *B. zonata* followed by Fenoxycarb (1.75±0.57 and 36.4±26.7%), Lufenuron (2.52±0.95 and 46.1±36.4%), Pyriproxyfen (4.11±1.90 and 63.7±56.5%) and Buprofezin (4.33±1.98 and 55.7±47.3%). Methoxyfenozide demonstrated lower LC_{50} and LC_{90} values of reduction in sperm concentration in spermatheca (0.854±0.17 and 13.0±6.3%) for *B. zonata* followed by Fenoxycarb (1.06±0.23 and 14.7±26.7%), Lufenuron (1.54±0.44 and 25.7±16.4%), Pyriproxyfen (2.52±0.85 and 28.9±19.0%) and Buprofezin (2.68±0.89 and 26.4±16.8%). Methoxyfenozide demonstrated lower LC_{50} and LC_{90} values of reduction in egg concentration in the ovary (0.675±0.15 and 20.6±13.1%) for *B. zonata* followed by Fenoxycarb (1.189±0.39 and 22.3±13.5%), Lufenuron (1.25±0.32 and 26.9±17.1%), Pyriproxyfen (2.26±0.72 and 28.4±18.5%) and Buprofezin (2.38±0.76 and 53.6±47.4%). These results also depict that Methoxyfenozide proved more toxic and had higher chemosterility impacts on *B. zonata* followed by Fenoxycarb, Lufenuron, Pyriproxyfen and Buprofezin (Table 3).

DISCUSSION

Insect growth regulators (IGRs) have been reported for use against various insects specially tephritid pests (fruit flies) as chemosterilants that suppress the fertility and fecundity of their adult stages (Navarro-Llopis *et al.*, 2010; Zhou *et al.*, 2016). The present study was carried out to assess the chemosterility of Methoxyfenozide, Fenoxycarb, Lufenuron, Pyriproxyfen and Buprofezin against *B. zonata* fed on IGR-baited adult diet. In this study, all the life parameters of *B. zonata* were observed that would demonstrate adequate justification for IGRs being evaluated as chemosterilants. The results exhibited that all five IGRs induced different levels of sterility and reproduction inhibition impacts in *B. zonata*. The difference in sterility impacts is due to their different modes of action as described by Zhou *et al.* (2016). The first level interaction between IGRs and their concentrations demonstrated that mortality increased while fecundity, hatchability, sperm concentration liberated from testes and spermatheca and egg concentration liberated from ovaries decreased with an increase in concentration in *B. zonata*. The overall results demonstrate that Methoxyfenozide demonstrated higher chemosterility impacts on both male and female flies of *B. zonata* followed by Fenoxycarb, Lufenuron, Pyriproxyfen, and Buprofezin. Similarly, lethal concentration (LC) values for fifty and ninety percent mortality and reduction in fecundity, testes sperm concentration, spermathecal sperm concentration and ovarian egg concentration in *B. zonata* also demonstrated that Methoxyfenozide proved more toxic and had higher chemosterility impacts on *B. zonata* followed by Fenoxycarb, Lufenuron, Pyriproxyfen, and Buprofezin. The results of present study regarding moderate sterility impacts of lufenuron on *B. zonata* are contradictory with those of Navarro-Llopis *et al.* (2004) who reported high sterility impact of lufenuron in wild medfly *Ceratitis capitata* (Wiedemann) populations when sprayed as its emulsion in a protein bait, and applied as its solid-bait (proteinaceous gel with lufenuron) in delta traps. Sterility impacts of lufenuron have also been endorsed by Katsoyannos *et al.* (1999) and Casana-Giner *et al.* (1999) who reported inhibition of egg hatching laid by *C. capitata* female fed on lufenuron-baited bait and production of non-viable eggs by *C. capitata* females mated with lufenuron treated males. The results of present study were also endorsed by Navarro-Llopis *et al.* (2004) who reported drastic reduction in *C. capitata* population and least stung-fruits in Lufenuron treated orchards due its severe chemosterility impacts in fruit flies. Chemosterility impacts of lufenuron on *B. dorsalis* have also been reported by Chang *et al.* (2012) that also endorse its sterility impacts on tephritid flies as demonstrated in present study. In present study, significant mortality and sterility were induced by lufenuron in adults of *B. zonata*. These results are partially endorsed by the results of Zhou *et al.* (2016) who reported fertility inhibition of adult *Delia antiqua* (onion flies) (Meigen) (Diptera: Anthomyiidae) by lufenuron without affecting survival of newly emerged adults. This partial variation in results may be attributed to variation in the target insect species as chemosterility and toxicity impact of insecticides varies from species to species. Pyriproxyfen was the fourth potential IGR with chemosterility effects in present study. Similar results were also demonstrated by Zhou *et al.* (2016) who reported less sterility impact of pyriproxyfen on the adult of *D. antiqua.*

In the present study, Methoxyfenozide demonstrated higher mortality and sterility in the adult of *B. zonata*. These results are partially in agreement with those of Sun *et al.* (2000) who reported less mortality, but high sterility impacts of Methoxyfenozide on insects especially Lepidopterous insect pests. Fewer eggs and sperm concentration in respective male and female reproductive system of *B. zonata* were observed in present study in treated flies which may be due to the disruption of oogenesis and spermatogenesis in female and male flies, respectively as reported and explained by Dhadialla (1998), Sun *et al.* (2000) and Hoelscher and Barrett (2003). Less eggs-viability as observed in Methoxyfenozide treatments in present study may be due to the fact that Methoxyfenozide retards the locomotory ability of both the sexes, especially male insect, for locating their counterpart and make the male incapable of transferring its sperms during mating with female counterpart (Hoelscher and Barrett,

2003). This reason also explains the justification for less sperm concentration in the spermatheca of female flies of both species as observed in present studies. The sterility potentials of this molecules have been reported by Hagedorn (1985) and Sun *et al.* (2000) in adult codling moth (*Cydia pomonella*), Carlson *et al.* (2001) in Diptera (*Drosophila* species) and Pineda *et al.* (2007) in Lepidopterans and Dipterans which endorse and confirm the finding of present study regarding Methoxyfenozide as chemosterilant. The sterilant activity of Buprofezin was observed very low in present study. Similar results were also reported by Casana-Giner *et al.* (1999) who reported low chemosterilant activity of Buprofezin against *C. capitata.* However the results of Fenoxycarb in present study are highly in contradictory with those of Casana-Giner *et al.* (1999) who reported low level of sterilant activity by Fenoxycarb in *C. capitata* against the sterility impact of Fenoxycarb on *B. zonata* in present study. This variation may due to differences in the formulation of IGRs and target species used in both studies. Overall, Methoxyfenozide, Fenoxycarb, and Lufenuron can be used as chemosterilants against both male and female flies of *B. zonata* but it needs to be investigated in the field conditions by different application techniques.

***Conclusions*:** Mostly, farmers of developing countries used the cover spray of synthetic insecticides for the management of tephritid fruit flies which ultimately leaves the toxic residues in fruits and is not only hazardous to human health but also became the reason for environmental pollution. IGR based chemosterilants are an alternative approach to minimize the *B. zonata* infestation without any harmful impact on both humans and the environment. In present research, Methoxyfenozide baited diet was found toxic and had chemosterilant impacts on both male and female flies of *B. zonata*. The study is indicating the base line information for its future efficacy test under the field conditions. This study will also be helpful for other researchers and farmers to develop an integrated pest management model to reduce the direct and indirect losses to fruit crops by *B. zonata*.

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REFERENCES

Ahmad, U. and U. Begum. 2017. A weekly study on *Bactrocera zonata* S. and *Bactrocera dorsalis* H. (Diptera: Tephritidae) against methyl eugenol, raspberry essence and GF-120 in persimmon orchards from Kohat, Pakistan. Indian J. Agric. Res. 51:176-179.

Alam, S.M., R. Ansari and M.A. Khan. 2001. Application of radioisotopes and radiation in the field of agriculture. Online J. Biol. Sci. 1:82-86.

Alemany, A., A. Gonzalez, A. Juan and C. Tur. 2008. Evaluation of a chemosterilization strategy against *Ceratitis capitata* (Diptera: Tephritidae) in Mallorca island (Spain). J. Appl. Entomol. 132:746-752.

Atta, B., M.D. Gogi, M.J. Arif, F. Mustafa, M.F. Raza, M.J. Hussain, M.A. Farooq, M.J. Nisar and M. Iqbal. 2015. Toxicity of some Insect Growth Regulators (IGRs) against different life stages of dusky cotton bugs *Oxycarenus hyalinipennis* Costa (Hemiptera: Lygaeidae: Oxycareninae). Bulg. J. Agric. Sci. 21:367-371.

Bachrouch, O., J.J. Mediouni-Ben, E. Alimi, S. Skillman, T. Kabadou and E. Kerber. 2008. Efficacy of the lufenuron bait station technique to control Mediterranean fruit fly (medfly) *Ceratitis capitata* in citrus orchards in Northern Tunisia. Tunis. J. Plant Prot. 3:35-45.

Böckmann, E., K. Köppler, E. Hummel and H. Vogt. 2014. Bait spray for control of European cherry fruit fly: an appraisal based on semi-field and field studies. Pest Manag. Sci. 70:502-509.

Carlson, G.R., T.S. Dhadialla, R. Hunter, R.K. Jansson, C.S. Jany, Z. Lidert and R.A. Slawecki. 2001. The chemical and biological properties of methoxyfenozide, a new insecticidal ecdysteroid agonist. Pest Manag. Sci. 57:115-119.

Casana-Giner, V., A. Gangia-Balaguer, C. Mengod-Puerta, J. Primo-Millo and E. Primo-Yufera. 1999. Insect growth regulators as chemosterilants for *Ceratitis capitata* (Diptera: Tephritidae). J. Econ. Entomol. 92:303-308.

Chance, L.E., M. Degrugillier and A.P. Leverich. 1969. Comparative effects of chemosterilants on spermatogenic stages in the house fly I. Induction of dominant lethal mutations in mature sperm and gonial cell death. Mutation Res. 7:63-74.

Chang, C.L., I.K. Cho and Q.X. Li. 2012. Laboratory evaluation of the chemosterilant lufenuron against the fruit flies *Ceratitis capitata*, *Bactrocera dorsalis*, *B. cucurbitae*, and *B. latifrons*. J. Asia-Pac. Entomol. 15:13-16.

Chang, S.C., P.H. Terry and A.B. Borkovec. 1964. Insect chemosterilants with low toxicity for mammals. Sci. 144:57-58.

Danho, M., C. Gaspar and E. Haubruge. 2002. The impact of grain quality on the biology of *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) oviposition, distribution of eggs, adult emergence, body weight and sex ratio. J. Stored Prod. Res. 38:259-266.

De Bon, H., J. Huat, L. Parrot, A. Sinzogan, T. Martin, E. Malézieux and J.F. Vayssières. 2014. Pesticide risks

from fruit and vegetable pest management by small farmers in sub-Saharan Africa: A review. Agron. Sust. Develop. 34:723-736.

Dhadialla, T.S. 1998. New insecticides with ecdysteroidal and juvenile hormone activity. Ann. Rev. Entomol. 43:545-569.

Dhami, M.K., D.N. Gunawardana, D. Voice and L. Kumarasinghe. 2016. A real-time PCR toolbox for accurate identification of invasive fruit fly species. J. Appl. Entomol. 140:536-552.

Dias, N.P., M.J. Zotti, P. Montoya, I.R. Carvalho and D.E. Nava. 2018. Fruit fly management research: A systematic review of monitoring and control tactics in the world. Crop Prot. 112:187-200.

El-Minshawy, A.M., S.A.M. Abdelgaleil, G.G. Gadelhak, M.A. Al-Eryan and R.A. Rabab. 2018. Effects of monoterpenes on mortality, growth, fecundity, and ovarian development of *Bactrocera zonata* (Saunders) (Diptera: Tephritidae). Environ. Sci. Pollution Res. 25:15671-15679.

Finney, D.J. 1971. Probit Analysis, 3rd edition, Cambridge University Press, Cambridge, UK.

Gogi, M.D., M. Ashfaq, M.J. Arif, R.M. Sarfraz and N.N. Nawab. 2010. Investigating phenotypic structures and allelochemical compounds of the fruits of *Momordica charantia* L. genotypes as sources of resistance against *Bactrocera cucurbitae* (Coquillett) (Diptera: Tephritidae). Crop Prot. 29:884-890.

Hagedorn, H.H. 1985. The role of ecdysteroids in reproduction. In: G. Kerkut and L. Gilbert (eds.), Comprehensive Insect Physiology, Biochemistry and Pharmacology. Pergamon, Oxford, 8:205-261.

Hoelscher, J.A. and B.A. Barrett. 2003. Effects of methoxyfenozide treated surfaces on the attractiveness and responsiveness of adult codling moth. J. Econ. Entomol. 93:623-629.

Hossain, M.A., M. Momen, M.S. Uddin, S.A. Khan and A.J. Howlader. 2017. Abundance of peach fruit fly, *Bactrocera zonata* (Saunders) in mango orchard. Bangladesh J. Entomol. 27:25-34.

Jemaa. J.M. and E. Boushih. 2010. Cyromazine induced effects on larvae and adults of laboratory Tunisian strain of the Mediterranean fruit fly *Ceratitis capitata*. Tunis. J. Plant Prot. 5:213-222.

Katsoyannos, B.I., N.T. Papadopoulos, R.R. Heath, J. Hendrichs and N.A. Kouloussis. 1999. Evaluation of synthetic food-based attractants for female Mediterranean fruit flies (Diptera: Tephritidae) in McPhail type traps. J. Appl. Entomol. 123:607-612.

Klungness, L.M., E.B. Jang, R.F.L. Mau, R.I. Vargas, J.S. Sugano and E. Fujitani. 2005. New sanitation techniques for controlling tephritid fruit flies (Diptera: Tephritidae) in Hawaii. J. Appl. Sci. Environ. Manag. 9:5-14.

Lanzavecchia, S.B., M. Juri, A. Bonomi, L. Gomulski, A.C. Scannapieco, D.F. Segura, A. Malacrida, J.L. Cladera and G. Gasperi. 2014. Microsatellite markers from the "South American fruit fly" *Anastrepha fraterculus*: a valuable tool for population genetic analysis and SIT applications. BMC Genet. 15(Suppl. 2):S13.

Li, L., J.N. Westgate, L. Hughes, X. Zhang, B. Givehchi, L. Toose, J.M. Armitage, F. Wania, P. Egeghy and J.A. Arnot. 2018. A model for risk-based screening and prioritization of human exposure to chemicals from near-field sources. Environ. Sci. Technol. 52:14235-14244.

Magoc, L., J.L. Yen, A. Hill-Williams, J.A. McKenzie, P. Batterham and P.J. Daborn. 2005. Cross-resistance to dicyclanil in cyromazine-resistant mutants of *Drosophila melanogaster* and *Lucilia cuprina*. Pestic. Biochem. Physiol. 81:129-135.

Mangan, R.L. and D.S. Moreno. 2007. Development of bait stations for fruit fly population suppression. J. Econ. Entomol. 100:440-450.

Mostafalou, S. and M. Abdollahi. 2013. Pesticides and human chronic diseases: evidences, mechanisms and perspectives. Toxic. App. Pharmacol. 268:157-177.

Navarro-Llopis, V., J. Domínguez-Ruiz, M. Zarzo, C. Alfaro and J. Primo. 2010. Mediterranean fruit fly suppression using chemosterilants for area-wide integrated pest management. Pest Manage. Sci. Form. Pest Sci. 66:511-519.

Navarro-Llopis, V., J. Sanchis-Cabanes, I. Ayala, V. Casaña-Giner and E. Primo-Yúfera. 2004. Efficacy of lufenuron as chemosterilant against *Ceratitis capitata* in field trials. Pest Manag. Sci. 60:914-920.

Nicholson, G.M. 2007. Fighting the global pest problem: preface to the special Toxicon issue on insecticidal toxins and their potential for insect pest control. Toxicon. 49:413-422.

Oerke, E.C. 2006. Crop losses to pests. J. Agric. Sci. 144:31-43.

Pineda, S., M. Schneider, G. Smagghe, A. Martínez, P. Del Estal, E. Viñuela, J. Valle and F. Budia. 2007. Lethal and sublethal effects of methoxyfenozide and spinosad on *Spodoptera littoralis* (Lepidoptera: Noctuidae). J. Econ. Entomol. 100:773-780.

Riddiford, L.M. and J.W. Truman. 1978. Biochemistry of insect hormones and insect growth regulators. In: M. Rockstein (ed.), Biochemistry of Insects. Acad Press, New York. pp. 307-357.

Robert, R.H., S.G. Lavallee, E. Schnell, D.G. Midgarden and N.D. Epsky. 2009. Laboratory and field cage studies on female-targeted attract-and-kill bait stations for *Anastrepha suspensa* (Diptera: Tephritidae). Pest Manag. Sci. 65:672-677.

Sun, X., B.A. Barrett and D.J. Biddinger. 2000. Fecundity and fertility reductions in adult leafroller exposed to surfaces

treated with the ecdysteroid agonists tebufenozide and methoxyfenozide. Entomol. Exper. Appl. 94:75-83.

Urbaneja, A., H. Montón and O. Molla. 2009. Suitability of the tomato borer *Tuta absoluta* as prey for *Macrolophus pygmaeus* and *Nesidiocoris tenuis*. J. App. Entomol. 133:292-296.

Wendell, W.K. and R.P. Ruth. 1964. Effect of the chemosterilant Apholate on the synthesis of cellular components in developing house fly eggs. Bioch. J. 92:353-357.

Williams, T., J. Valle and E. Vinuela. 2003. Is the naturally derived insecticide spinosad® compatible with insect natural enemies biocontrol? Sci. Technol. 13:459-475.

Yee, W.L. 2007. Attraction, feeding and control of *Rhagoletis pomonella* (Diptera: Tephritidae) with GF-120 and added ammonia in Washington State. Fla. Entomol. 90:665-673.

Zhou, F., G. Zhu, H. Zhao, Z. Wang, M. Xue, X. Li, H. Xu, X. Ma and Y. Liu. 2016. Sterilization effects of adult-targeted baits containing insect growth regulators on *Delia antiqua*. Sci. Rep. 6:32855.

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