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% LC₅₀), 34.7 (0.50% LC₅₀), 30.9 (0.50% LC₅₀), 25.1 (0.97% LC₅₀) and 22.5% (1.05% LC₅₀) 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; Urbanjea 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; Urbanjea 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 (Klugness 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, imperative 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, Pyriproxifen 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. Papae 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.

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

The quantity of formulated 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 cm³ 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 °C 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 (C₃H₆O₁₀⁻₅)] 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 Neubauer 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:

\[
\text{Mean counts (spers) /large square} = \frac{\text{Total sperm counts}}{9}
\]

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 38.0, 34.7, 31.1, 25.0 and 23.5% less sperm concentration liberated from spermatheca over control treatment (Fig. 1f). The overall results confirm that Methoxyfenozide 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 $df = 84$).

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

$P < 0.05$
Toxicity and chemosterility impact of Insect Growth Regulators baited diet on Peach Fruit Fly

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 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.
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

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.
significant 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 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 2016.5 eggs 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 Methoxyfenozide (0.44±0.04 and 2.37±0.42%), Fenoxycarb (0.50±0.05 and 2.60±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.

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