Research Article

# Biocidal Activity of Some Selected Phytoextracts and Fruits of Different Citrus Cultivars against Fruit Fly *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae)

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**Abstract** | *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) is one of the economic pests of horticultural crops. This invasive species causes substantial economic loss to citrus produce each year in Pakistan. Farmers rely on persistent synthetic insecticides for fruit fly control. Insecticidal phytoextracts are biorational alternates to hazardous synthetic insecticides. This study evaluated the efficacy of aqueous extracts of neem (*Azadirachta indica* A. Juss), garlic (*Allium sativum* L.), ginger (*Zingiber officinale* Roscoe) and lime citrus (*Citrus aurantifolia* (Christm.) Swingle) on the fruits of five citrus cultivars (*i.e.* bitter orange (*Citrus aurantium* L.), grapefruit (*Citrus paradisi* Macfad), lime (*Citrus aurantifolia* Christm), mandarin (*Citrus reticulata* Blanco) and sweet orange (*Citrus sinensis* (L.) Osbeck) against *B. dorsalis* using choice and no-choice fruit-dip bioassays. Results revealed a significant reduction of pupal weight and adult emergence of *B. dorsalis* by 4% extracts of *A. indica*, *A. sativum* and *C. aurantifolia*. Moreover, a significantly higher male to female adult sex ratio was observed in case of *A. indica* treatment. It is concluded from overall study results that the extracts of *A. indica* and *C. aurantifolia* exhibited significant anti-insect effects on the pupal recovery, pupal weight, adult emergence and male to female ratio of *B. dorsalis* on all five citrus host fruits under laboratory conditions, and hence are recommended to be further evaluated under field conditions and to consider their potential incorporation in IPM programs against fruit fly infestations on citrus crop.

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## Introduction

Oriental fruit fly *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) is one of the world's most damaging and economic pests of horticultural crops. It is an invasive polyphagous species with more than 250 host plants including vegetables and fruits (Shi, 2017; Qin, 2018). This species causes damage through ovipositioning and subsequent larval development within the infested fruits resulting in considerable economic loss (Hsu, 2015). Female flies lay eggs inside the fruits, thereby rendering the maggots inaccessible to contact insecticides. Once eggs are laid, no effective control is possible except the removal and destruction of infested fruits (Vargas, 2007).

Primarily conventional synthetic insecticides are being used by the local farmers to combat fruit fly infestations. Frequent and intensive use of such chemicals cause many non-target effects including eradication of beneficial fauna, insecticide resistance in insect pests, contamination of environment and human health hazards due to persistent insecticidal residues (Desneux *et al.*, 2007; Naeem *et al.*, 2016; Deng *et al.*, 2020; Dhananjayan *et al.*, 2020; Haddi *et al.*, 2020). These ecological consequences of synthetic chemical insecticides necessitate seeking alternative fruit fly management practices such as botanical insecticides (Umeh, 2016; Isman, 2020).

Botanical insecticides are one of the biorational tools to control insect pests and are usually safer for the environment, non-target species and for human health than the synthetic insecticides (Isman, 2020). Some plant species are rich source of bioactive compounds such as plant secondary metabolites that can defend plants against insect pests (Prakash and Rao, 2018). Many plant-based compounds and botanical extracts have been demonstrated to exhibit growth inhibition, feeding deterrence and repellency against a wide array of insect pests including fruit flies (Campos *et al.*, 2019; Isman, 2020).

Moreover, the advantages of insecticidal botanical extracts include their quick and easy preparation by local farmers, cost-effectiveness and lower mammalian toxicity (Turek and Stintzing, 2013; Isman, 2020). Besides, whereas synthetic insecticides are based on a particular active ingredient, plant-derived insecticides are composed of various compounds that work on both behavioural and biochemical processes of insect pests. Therefore, it is less feasible that pests gain resistance to these substances (Marrone, 2019). Similarly, some studies have demonstrated that different host plants exert differential impact on fruit fly ovipositioning preference, egg and maggot development and pupal and adult life parameters (Brévault and Quilici, 2007; Muthuthantri and Clarke, 2012).

Keeping in view the aforementioned background, this laboratory study was aimed to determine the bioactivity of aqueous extracts of four local plant species on the ovipositional preference and development of fruit fly *B. dorsalis* on the fruits of five locally available citrus cultivars.

## Materials and Methods

*Insect rearing*

The experiment was conducted in laboratory of the Department of Entomology, University of Sargodha. Culture of fruit fly *B. dorsalis* was obtained from the fruit fly rearing laboratory and was maintained at 27 ± 1°C temperature, 60 ± 5% relative humidity and at 16 h:10 h light and dark photoperiod. Adults were fed on a banana-based artificial diet with ingredients including egg yolk, sugar, honey, yeast, syrup and vitamin B complex blended in a ratio of 2:4:8:2:1, respectively, and was placed in a freezer for subsequent use (Ahmad *et al.*, 2010). Fifty newly emerged pairs of *B. dorsalis* adults from stock culture were sexed and transferred into a new adult rearing cage (40 × 45 × 45 cm). Twenty-one day old female flies were used in the bioassays as this is the optimum age for them to oviposit. Moreover, cleaning of rearing cages and adjustment of diet slides were done daily to avoid any microbial contamination (Rattanapun *et al.*, 2009).

**Table 1:** *Detail of plants used in the study.*

| Common / Vernacular Name | Botanical name | Family | Parts used |
|---|---|---|---|
| Neem | *Azadirachta indica* A. Juss | Meliaceae | leaves |
| Ginger | *Zingiber officinale* Roscoe | Zingiberaceae | rhizomes |
| Garlic | *Allium sativum* L. | Liliaceae | tubers |
| Citrus lime | *Citrus aurantifolia* (Christm.) Swingle | Rutaceae | peel |

*Botanical extracts preparation*

Extracts of four local plant species were used in this research for assessing their effectiveness against *B. dorsalis*. The detail of these plants is mentioned in Table 1. Different parts of these plants, as mentioned in Table 1, were rinsed thoroughly with tap water and were let to dry under sunshine for one day, followed by an oven drying at 50 °C for two days. The dry samples were grinded into powder by an electric blender. For extraction, a 200 mL conical flask was loaded with 20 g of each plant powder along with 100 mL of distilled water. The conical flasks were wrapped with aluminum foil and were kept on an electric shaker set at 150 rpm for 24 h. The samples were then sieved through a fine mesh muslin cloth followed by filtering through Whatman No 1 filter paper sheets.

**Table 2:** *Recovered pupae (number/fruit) of Bactocera dorsalis from fruits of different citrus cultivars treated with different botanical extracts under choice test.*

| Host Plants | Concentration (%) | *Azadirachta indica* | *Allium sativum* | *Zingiber officinale* | *Citrus aurantifolia* |
|---|---|---|---|---|---|
| Mandrins | 1.0 | 4.00±0.342b | 5.00±0.233b | 4.70±0.321b | 3.30±0.345b |
| | 2.0 | 3.00±0.284bc | 3.00±0.523c | 3.30±0.123bc | 2.30±0.034b |
| | 4.0 | 1.70±0.183c | 1.30±0.084d | 2.30±0.241c | 1.30±0.075b |
| | Control | 7.70±0.428a | 8.00±0.992a | 8.30±0.923a | 6.00±0.783a |
| | Sign. | 29.8*** | 42.3*** | 29.7*** | 9.67** |
| Sweet orange | 1.0 | 3.33±0.347b | 3.67±0.157b | 4.33±0.231b | 3.33±0.083b |
| | 2.0 | 2.33±0.238c | 3.67±0.154b | 3.00±0.342b | 2.33±0.783bc |
| | 4.0 | 1.00±0.289d | 1.00±0.092c | 1.33±0.082c | 1.00±0.093c |
| | Control | 7.67±0.634a | 8.00±0.923a | 8.33±0.923a | 6.00±0.345a |
| | Sign. | 99.9*** | 60.5*** | 35.7*** | 11.5** |
| Grapefruit | 1.0 | 2.33±0.237b | 3.33±0.183b | 3.33±0.042b | 2.67±0.893b |
| | 2.0 | 1.67±0.428bc | 3.33±0.132b | 2.67±0.023b | 2.00±0.985b |
| | 4.0 | 0.67±0.087c | 0.67±0.023c | 0.33±0.002c | 2.33±0.991b |
| | Control | 7.67±0.723a | 8.00±0.989a | 8.33±0.941a | 6.00±0.898a |
| | Sign. | 88.3*** | 55.8*** | 102.0*** | 14.8** |
| Lime | 1.0 | 1.33±0.182b | 1.33±0.097b | 1.67±0.023b | 0.67±0.072b |
| | 2.0 | 0.67±0.094bc | 0.67±0.082bc | 1.33±0.042b | 0.67±0.066b |
| | 4.0 | 0.00c | 0.00c | 0.00c | 0.00b |
| | Control | 7.67±0.742a | 8.00±0.923a | 8.33±0.892a | 6.00±0.872a |
| | Sign. | 151.0*** | 247.0*** | 168.0*** | 20.1*** |
| Bitter orange | 1.0 | 2.33±0.238b | 3.00±0.384bc | 3.00±0.231b | 2.00±0.034b |
| | 2.0 | 1.67±0.271b | 3.33±0.281b | 2.33±0.324b | 1.67±0.038b |
| | 4.0 | 0.00c | 1.67±0.128c | 0.33±0.039c | 0.00b |
| | Control | 7.67±0.548a | 8.00±0.923a | 8.33±1.023a | 6.00±0.823a |
| | Sign. | 65.9*** | 34.3*** | 70.0*** | 14.6** |

***: *significant at P < 0.001;* **: *significant at P < 0.05*

The solvent was evaporated by a rotary vacuum evaporator (Laborota 4001, Heidolph, U.S.A.) set a temperature of 50 ˚C in the water bath. Three concentrations of each botanical extract *i.e.* 1.0, 2.0 and 4.0% were made through serial dilution process.

*Fruit-dip bioassays*

Fruits of almost similar size and color were picked from the orchard trees of five locally available citrus cultivars (*i.e.* bitter orange (*C. aurantium*), grapefruit (*C. paradisi*), lime (*C. aurantifolia*, mandarin (*C. reticulata*) and sweet orange (*C. sinensis*) with no previous application of any insecticide during the season. Fruits were thoroughly washed with tap water and were air-dried at room temperature (28 ˚C). These fruits from each cultivar were used as an oviposition medium. The fruits were then dipped in each concentration of each botanical for 60 sec, and were air-dried and labelled. Oviposition preference of *B. dorsalis* adults was tested by performing following two types of bioassays.

*Choice test*

For oviposition, three fruits of each citrus cultivar were offered collectively as free choice. Twenty pairs of *B. dorsalis* adults were placed in an insect rearing cage (40 × 45 × 45 cm). After 24 h of egg deposition, the fruits were gathered individually in plastic jars (25 × 12 cm) lined with sterilized sand and sawdust at the bottom for pupation. Pupae were obtained after 6–8 days by sieving the pupation medium. The experiment was replicated independently three times. Fruit-wise observations were recorded regarding the ovipositional preference and biological parameters such as pupal recovery, pupal weight, pupal deformity, adult emergence and adult sex ratio.

*No-choice test*

In this test, oviposition preference was determined with three fruits of each cultivar placed separately in a separate cage. Fruits of each cultivar were treated with each botanical extract individually. Ten pairs of *B. dorsalis* adult flies were placed in each cage and were permitted for 24 h for ovipositioning. A similar series of experiments were carried out using each cultivar's fresh fruits as controls. In case of no-choice test, same observations were recorded as described above for choice test. The experiment was replicated three times.

*Statistical analysis*

A factorial analysis of variance (ANOVA) was performed to assess the impact of different concentrations of botanical extracts on *B. dorsalis* performance. Means were compared using Fisher's least significant difference (LSD) test at 5% probability level. Statistical interpretations were done using Minitab 17.0 software.

## Results and Discussion

*Choice test*

As compared to control, all botanical extracts particularly at higher (4%) concertations exhibited significant effect ($P < 0.05$) on *B. dorsalis* pupal recovery under choice test and this effect was found concentration-dependent. Control treatment showed maximum pupal recovery (6.0–8.3 pupae/fruit) in all citrus host fruits (Table 2). No pupae were recovered at 4% concentration of all botanicals from the fruits of lime and bitter orange cultivars. In general, *A. indica* and *C. aurantifolia* extracts showed significantly reduced ovipositional preference on each citrus cultivar than the extracts of *A. sativum* and *Z. officinale*.

A similar concentration-dependent response was observed in case of pupal weight (mg) for all botanicals. Pupal weight varied less significantly with mandarin

**Table 3:** *Pupal weight (mg) of Bactocera dorsalis from fruits of different citrus cultivars treated with different botanical extracts under choice test.*

| Host Plants | Concentration (%) | *Azadirachta indica* | *Allium sativum* | *Zingiber officinale* | *Citrus aurantifolia* |
|---|---|---|---|---|---|
| Mandrins | 1.0 | 4.32±0.573a | 4.10±0.304a | 4.32±0.452ab | 3.53±0.653ab |
| | 2.0 | 4.10±0.734ab | 3.10±0.623a | 4.10±0.561ab | 3.51±0.783ab |
| | 4.0 | 4.32±0.823b | 4.10±0.642a | 2.74±0.358b | 2.53±0.732b |
| | Control | 3.53±0.572a | 3.50±0.452a | 4.67±0.562a | 4.85±0.653a |
| | Sign. | 2.94** | 2.17NS | 2.95** | 5.69** |
| Sweet orange | 1.0 | 4.00±0.532a | 3.68±0.303ab | 3.69±0.542ab | 3.53±0.733bc |
| | 2.0 | 4.10±0.653a | 3.03±0.360b | 3.03±0.234b | 4.18±0.743ab |
| | 4.0 | 2.35±0.632b | 2.39±0.624b | 2.39±0.236b | 3.07±0.465c |
| | Control | 4.60±0.345a | 4.50±0.632a | 4.67±0.526a | 4.85±0.743a |
| | Sign. | 6.95** | 4.68** | 5.27** | 7.67** |
| Grapefruit | 1.0 | 4.00±0.632a | 3.69±0.673ab | 3.89±0.456a | 3.78±0.456a |
| | 2.0 | 4.10±0.636a | 2.55±0.562bc | 3.35±0.563a | 3.39±0.245ab |
| | 4.0 | 1.73±0.085b | 1.72±0.636c | 0.81±0.034b | 1.73±0.254b |
| | Control | 4.60±0.587a | 4.50±0.632a | 1.73±0.453a | 4.85±0.657a |
| | Sign. | 5.27** | 5.31** | 10.3** | 5.42** |
| Lime | 1.0 | 3.50±0.452a | 2.77±0.542b | 2.77±0.732b | 3.08±0.653ab |
| | 2.0 | 2.87±0.146a | 1.37±0.073bc | 2.27±0.562b | 2.87±0.034ab |
| | 4.0 | 0.59±0.037b | 0.47±0.027c | 0.56±0.028c | 0.71±0.005b |
| | Control | 4.60±0.632a | 4.50±0.532a | 4.67±0.734a | 4.86±0.633a |
| | Sign. | 6.15** | 19.2*** | 39.2*** | 4.10** |
| Bitter orange | 1.0 | 3.43±0.353b | 3.77±0.632ab | 3.77±0.453a | 2.67±0.234b |
| | 2.0 | 2.70±0.653b | 3.57±0.435b | 3.23±0.457a | 2.70±0.456b |
| | 4.0 | 0.48±0.037c | 3.23±0.652ab | 0.97±0.045b | 0.58±0.082c |
| | Control | 4.60±0.653a | 4.50±0.532a | 4.67±0.653a | 4.85±0.654a |
| | Sign. | 45.5*** | 2.85** | 7.76** | 60.2*** |

******:*** *significant at* $P < 0.001$; ****:** *significant at* $P < 0.05$; **NS:** *non-significant.*

**Table 4:** *Adult emergence (%) of Bactocera dorsalis from fruits of different citrus cultivars treated with different botanical extracts under choice test.*

| Host Plants | Concentration (%) | *Azadirachta indica* | *Allium sativum* | *Zingiber officinale* | *Citrus aurantifolia* |
|---|---|---|---|---|---|
| Mandrins | 1.0 | 85.0±5.523ab | 46.7±3.863b | 77.8±4.736ab | 69.4±7.743a |
| | 2.0 | 55.6±5.363b | 55.5±5.565b | 58.3±7.343bc | 72.2±7.342a |
| | 4.0 | 50.0±5.562b | 100a | 50.0±4.746c | 66.8±4.764a |
| | Control | 100a | 96.8±2.545a | 100a | 77.8±7.343a |
| | Sign. | 2.47** | 50.3*** | 6.95** | 0.743NS |
| Sweet ornage | 1.0 | 69.4±6.338a | 68.9±7.344a | 71.1±5.672a | 69.4±7.343a |
| | 2.0 | 72.2±6.238a | 72.2±6.348a | 72.2±5.433a | 44.4±4.733ab |
| | 4.0 | 66.7±6.344a | 71.2±7.638a | 0.00b | 16.7±2.346b |
| | Control | 82.1±7.363a | 84.3±6.344a | 87.5±4.566a | 86.1±6.734a |
| | Sign. | 1.75NS | 0.17NS | 21.3*** | 4.96** |
| Grapefruit | 1.0 | 66.7±7.384ab | 69.4±5.346a | 83.3±6.344a | 100a |
| | 2.0 | 33.3±5.635bc | 66.7±5.324a | 41.7±6.342b | 83.3±7.342a |
| | 4.0 | 0.00c | 0.00b | 16.7±3.634b | 11.1±1.346b |
| | Control | 100a | 100a | 100a | 77.8±7.345a |
| | Sign. | 13.3** | 9.49** | 9.30** | 4.22** |
| Lime | 1.0 | 16.7±2.344a | 66.7±6.348a | 50.0±3.643ab | 83.3±6.342a |
| | 2.0 | 0.00b | 16.7±2.563b | 66.7±4.734ab | 33.3±3.264ab |
| | 4.0 | 0.00b | 0.00b | 0.00b | 0.00b |
| | Control | 100a | 100a | 95.8±4.743a | 94.4±6.345a |
| | Sign. | 33.0*** | 15.2** | 3.29** | 5.48** |
| Bitter orange | 1.0 | 50.0±3.677ab | 38.9±3.533bc | 72.2±5.743a | 33.3±1.734b |
| | 2.0 | 44.4±5.384ab | 61.1±5.352ab | 27.8±6.342b | 11.1±1.435b |
| | 4.0 | 0.00b | 0.00c | 0.00b | 0.00b |
| | Control | 100a | 100a | 95.8±4.732a | 77.8±3.634a |
| | Sign. | 5.05** | 8.72** | 16.6*** | 7.67** |

*****:** *significant at P < 0.001;* ****:** *significant at P < 0.05;* **NS:** *non-significant.*

showing the highest pupal weight 2.5–4.4 mg at 4% concentration application of each botanical, while grapefruit resulted into the least pupal weight of 0.81 mg when treated with a 4% *Z. officinale* extract as compared to other treatments (Table 3). Among the host citrus fruits, the least pupal weight was recorded for the fruits of lime and bitter orange fruits.

Similarly, the effect of all botanical extracts on *B. dorsalis* adult emergence was dose-dependent. Higher concentrations of all botanicals showed fewer adults' emergence. Particularly, no emergence of adults was observed from the pupae recovered from the fruits of lime, grapefruit and bitter orange treated with 4% extracts of *A. indica* and *A. sativum* (Table 4). Among host citrus cultivars, lime and bitter orange fruits revealed the minimum adult emergence of *B. dorsalis*.

In the case of adult sex ratio, *A. indica*, *A. sativum*, *Z. officinale*, and *C. aurantifolia* treatments significantly altered the adult sex ratio in all citrus cultivars under the choice test. Male to female adult sex ratio was close to one for the lowest concentration of *A. indica* extract. However, it was consistently higher with 1.0, 2.0 and 4.0% concentration of each extract. Among the different host fruits, the female ratio was higher in grapefruit, lime and bitter orange than in mandarin and sweet orange (Figure 1).

*No-choice test*

All four botanical extracts under no-choice test with each citrus cultivar showed a concentration-dependent response regarding the pupal recovery as the number of recovered pupae decreased with the increase in botanical concentration (Table 5). Highest concentration of all tested extracts adversely affected the oviposition of *B. dorsalis*. Minimum recovery (0.0–0.33 pupae/fruit) was from the fruits of lime

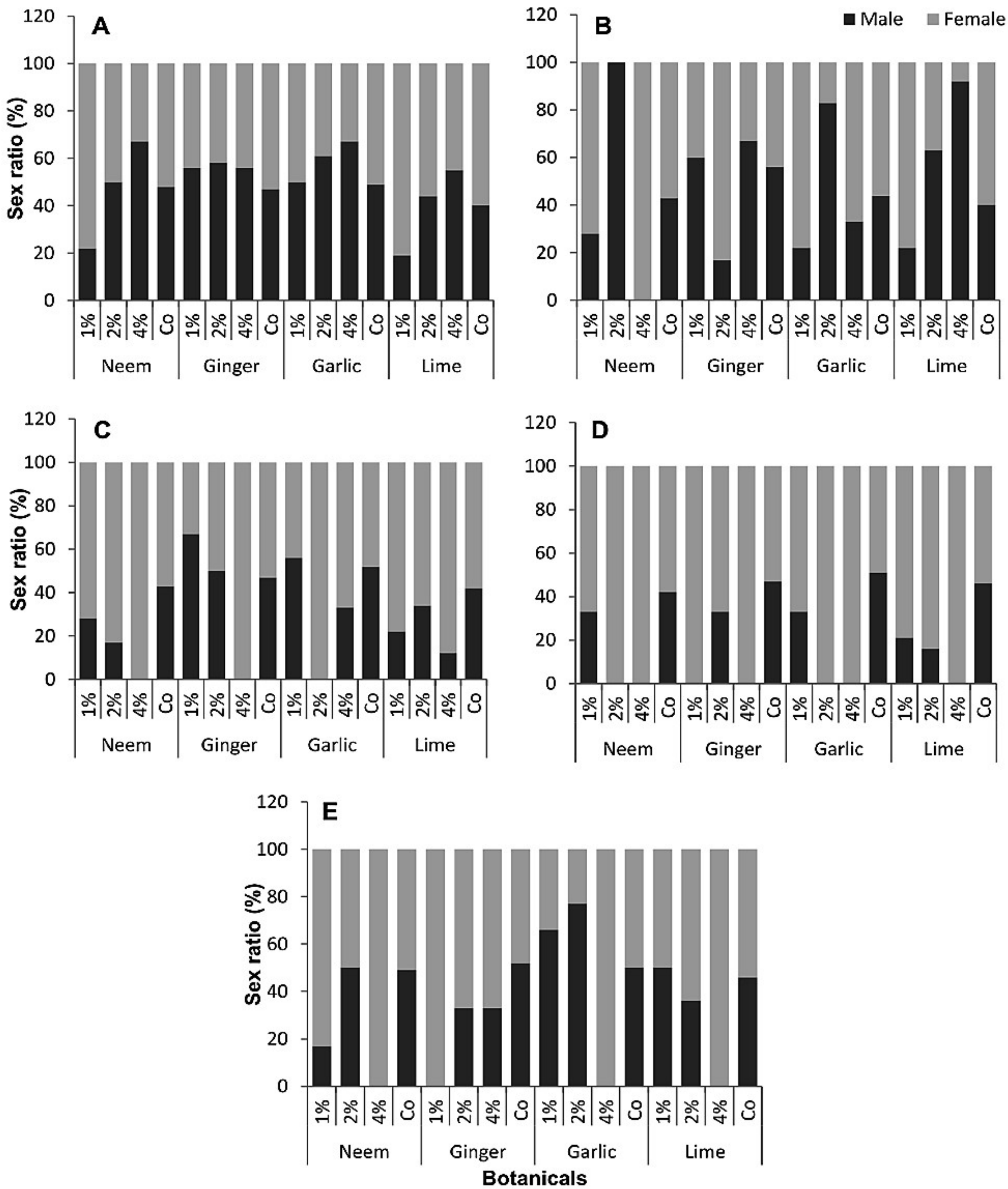


**Figure 1:** *Sex ratio of Bactrocera dorsalis adults resulted due to different botanical treatments on citrus cultivars under choice test. Means followed by the same letters are not statistically different (factorial ANOVA; LSD at P = 0.05). Co = control.*

and bitter orange, and maximum (1.00–1.67 pupae/fruit) from the fruits of mandarins and sweet orange cultivar which were significantly lower than the pupae in their respective controls (Table 5).

Similar trend was observed regarding the impact of different botanical extracts and citrus cultivars on the pupal weight of *B. dorsalis* under the no-choice test. Mandarins and lime showed highest (1.95–2.97 mg) and lowest (0.33–0.49 mg) pupal weight, respectively.


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**Table 5:** *Recovered pupae (number/fruit) of Bactocera dorsalis from fruits of different citrus cultivars treated with different botanical extracts under no-choice test.*

| Host Plants | Concentration (%) | *Azadirachta indica* | *Allium sativum* | *Zingiber officinale* | *Citrus aurantifolia* |
|---|---|---|---|---|---|
| Mandrins | 1.0 | 3.33±0.523b | 3.33±0.927b | 4.00±0.592b | 3.00±0.348b |
| | 2.0 | 1.67±0.047c | 3.00±0.782b | 3.33±0.382b | 2.00±0.583bc |
| | 4.0 | 1.00±0.034c | 1.33±0.382c | 1.67±0.328c | 1.33±0.034c |
| | Control | 7.33±0.623a | 7.67±0.993a | 7.00±0.943a | 7.00±1.082a |
| | Sign. | 96.9*** | 87.6*** | 35.7*** | 33.1*** |
| Sweet ornage | 1.0 | 3.67±0.532b | 3.33±0.347b | 4.00±0.348b | 3.00±0.634b |
| | 2.0 | 2.67±0.623c | 2.67±0.724bc | 2.67±0.682c | 2.67±0.047b |
| | 4.0 | 1.00±0.348d | 1.00±0.084c | 1.33±0.238d | 1.67±0.073b |
| | Control | 7.33±0.823a | 7.67±0.989a | 7.00±0.996a | 7.00±0.943a |
| | Sign. | 86.2*** | 24.2*** | 42.3*** | 18.0*** |
| Grapefruit | 1.0 | 4.00±0.237b | 4.67±0.082b | 4.33±0.348b | 3.67±0.634b |
| | 2.0 | 3.00±0.348b | 2.67±0.194c | 3.33±0.634bc | 3.33±0.348b |
| | 4.0 | 0.67±0.007c | 1.33±0.083c | 1.33±0.234c | 1.33±0.238c |
| | Control | 7.33±0.932a | 7.00±0.974a | 7.00±0.893a | 7.00±0.834a |
| | Sign. | 34.5*** | 27.3*** | 13.3** | 22.1*** |
| Lime | 1.0 | 1.00±0.093b | 1.67±0.189b | 1.67±0.238b | 0.67±0.009b |
| | 2.0 | 0.33±0.006b | 1.33±0.037b | 0.67±0.007bc | 0.67±0.006b |
| | 4.0 | 0.00b | 0.00c | 0.00c | 0.33±0.003b |
| | Control | 7.33±0.728a | 7.67±0.892a | 7.00±0.348a | 7.00±0.993a |
| | Sign. | 86.7*** | 140.0*** | 73.1*** | 62.4*** |
| Bitter orange | 1.0 | 2.67±0.238b | 2.00±0.238b | 3.00±0.374b | 2.67±0.539b |
| | 2.0 | 1.00±0.093c | 1.00±0.072bc | 2.33±0.238b | 1.00±0.348c |
| | 4.0 | 0.00d | 0.33±0.002c | 0.00c | 0.00c |
| | Control | 7.33±0.998a | 7.67±0.992a | 7.00±0.638a | 7.00±1.093a |
| | Sign. | 190.0*** | 80.7*** | 43.6*** | 86.0*** |

*****:** *significant at P < 0.001;* ****:** *significant at P < 0.05.*

*A. indica* and *C. aurantifolia* extracts exhibited maximum and significant reduction of pupal weight in all citrus cultivars (Table 6).

Similarly, highest adult *B. dorsalis* emergence was recorded for untreated fruits of all cultivars as compared to those treated with different botanical extracts. Higher concentration of botanicals results exhibited statistically a fewer number of adult emergence. The emergence rate was higher in the mandarin fruits as compared to other cultivars. *A. indica*, *A. sativum* and *C. aurantifolia* extracts at 4% concentration resulted in zero adult emergence in fruits of lime and bitter orange cultivars (Table 7).

Male to female adult sex ratio of *B. dorsalis* was also affected by different concentrations of *C. aurantifolia, A. indica*, *A. sativum* and *Z. officinale.* It was close to one in the lowest concentration of *A. indica* extract. However, it was consistently higher with 1.0, 2.0 and 4.0% concentration of each extract (Figure 2).

Fruit fly infestations are one of the detrimental factors for fruit production in Pakistan. *B. dorsalis* is an economic pest of citrus and other horticultural crops in Pakistan. Obscured feeding nature of its maggots renders this pest very difficult to control with conventional synthetic insecticides. This laboratory study assessed the biocidal activity of four local botanical extracts (*i.e.* of *A. indica, A. sativum*, *Z. officinale* and *C. aurantifolia*) against *B. dorsalis* on the fruits of five commonly grown citrus cultivars.

Results demonstrated a differential and significant effect of all four botanical extracts on the pupal recovery, pupal weight, adult emergence and male to female adult sex ratio of *B. dorsalis* on the fruits of all citrus hosts. Among these botanicals, extracts of

**Table 6:** *Pupal weight (mg) of Bactocera dorsalis from fruits of different citrus cultivars treated with different botanical extracts under no-choice test.*

| Host Plants | Concentration (%) | *Azadirachta indica* | *Allium sativum* | *Zingiber officinale* | *Citrus aurantifolia* |
|---|---|---|---|---|---|
| Mandrins | 1.0 | 4.14±0.572a | 3.43±0.583a | 4.33±0.683ab | 4.14±0.682a |
| | 2.0 | 3.40±0.672ab | 3.26±0.587ab | 3.50±0.632bc | 2.77±0.298b |
| | 4.0 | 2.73±0.582b | 1.95±0.036b | 2.97±0.387c | 2.73±0.295b |
| | Control | 3.20±0.327ab | 3.23±0.587ab | 4.68±0.523a | 3.36±0.634ab |
| | Sign. | 2.01** | 2.63** | 5.1** | 3.08** |
| Sweet ornage | 1.0 | 3.43±0.347a | 3.24±0.637a | 3.70±0.378a | 3.71±0.678a |
| | 2.0 | 2.73±0.348ab | 3.00±0.683ab | 3.60±0.582a | 2.73±0.298ab |
| | 4.0 | 1.73±0.118b | 1.21±0.587b | 2.70±0.183a | 2.20±0.284b |
| | Control | 3.26±0.732ab | 3.23±0.583a | 4.60±0.658a | 3.36±0.683a |
| | Sign. | 2.43** | 2.67** | 2.1NS | 3.87** |
| Grapefruit | 1.0 | 2.80±0.285a | 3.19±0.673a | 2.76±0.283b | 3.20±0.099a |
| | 2.0 | 2.63±0.198a | 1.87±0.683b | 1.41±0.093c | 2.41±0.048ab |
| | 4.0 | 0.98±0.073b | 2.21±0.298ab | 1.54±0.285bc | 1.36±0.039b |
| | Control | 3.20±0.285a | 3.23±0.386a | 4.68±0.387a | 3.36±0.386a |
| | Sign. | 4.99** | 415** | 15.7*** | 3.29** |
| Lime | 1.0 | 1.40±0.089ab | 2.12±0.376b | 2.12±0.284b | 1.40±0.007b |
| | 2.0 | 0.81±0.005b | 2.76±0.218ab | 0.96±0.009c | 1.14±0.003b |
| | 4.0 | 0.37±0.023b | 0.49±0.037c | 0.45±0.032d | 0.33±0.083b |
| | Control | 3.19±0.237a | 3.23±0.568a | 4.68±0.736a | 3.36±0.593a |
| | Sign. | 5.64** | 19.7*** | 55.7*** | 5.96** |
| Bitter orange | 1.0 | 2.77±0.385ab | 2.57±0.835ab | 2.71±0.285b | 2.52±0.275b |
| | 2.0 | 2.34±0.285b | 2.74±0.285a | 2.50±0.382b | 2.20±0.386b |
| | 4.0 | 0.34±0.037c | 0.96±0.073b | 0.67±0.057c | 0.41±0.087c |
| | Control | 3.17±0.398a | 3.23±0.683a | 4.68±0.682a | 3.36±0.285a |
| | Sign. | 45.1*** | 3.55** | 68.1*** | 61.0*** |

*****: *significant at P < 0.001;* ****: *significant at P < 0.05;* **NS:** *non-significant.*

*A. indica* (neem) and *C. aurantifolia* (citrus lime) exhibited a significant and maximum suppression of *B. dorsalis* pupal recovery, average pupal weight and adult emergence percentage and male to female adult sex ratio as compared to other extracts and control treatments. Many studies reported the deterrence of ovipositioning and suppression of ovary development in different *Bactrocera* species by *A. indica* extracts (Ignacimuthu and Vendan, 2008; Khan et al., 2016; Ilyas et al., 2017).

Similarly, some previous research reported negative and suppressive effects of neem seeds extract on the landing, preference and oviposition by *B. dorsalis* fruit flies on their guava fruits in both choice and no-choice tests (Sandeep and Desraj, 2016; Singh and Singh, 1998). Another study by Stark *et al.* (1990) showed adverse effects of *A. indica* extract based diets on the pupae formation and adult emergence of three Tephritid flies. Moreover, our results regarding the ovipositional deterrence by extracts of *A. indica* and *C. aurantifolia* are consistent with those of previous studies on *B. zonata* (Mahmoud and Shoeib, 2008; Papachristos *et al.*, 2008). Later study has demonstrated that lime peel extract confers resistance to citrus fruits against *B. dorsalis*. Essential oil and extract of this citrus species have been shown ovicidal, larvicidal and adulticidal effects against *Aedes* mosquitoes (Sarma *et al.*, 2019).

Among five citrus cultivars evaluated in this study, fruits of Lime and bitter orange were least preferred and most suppressive against *B. dorsalis* adults followed by the fruits of grapefruit, while fruits of sweet orange and kinnow mandarin were found most preferred and susceptible for fruit fly in both choice and no-choice tests. This differential oviposition preference and development of fruit flies could be due to

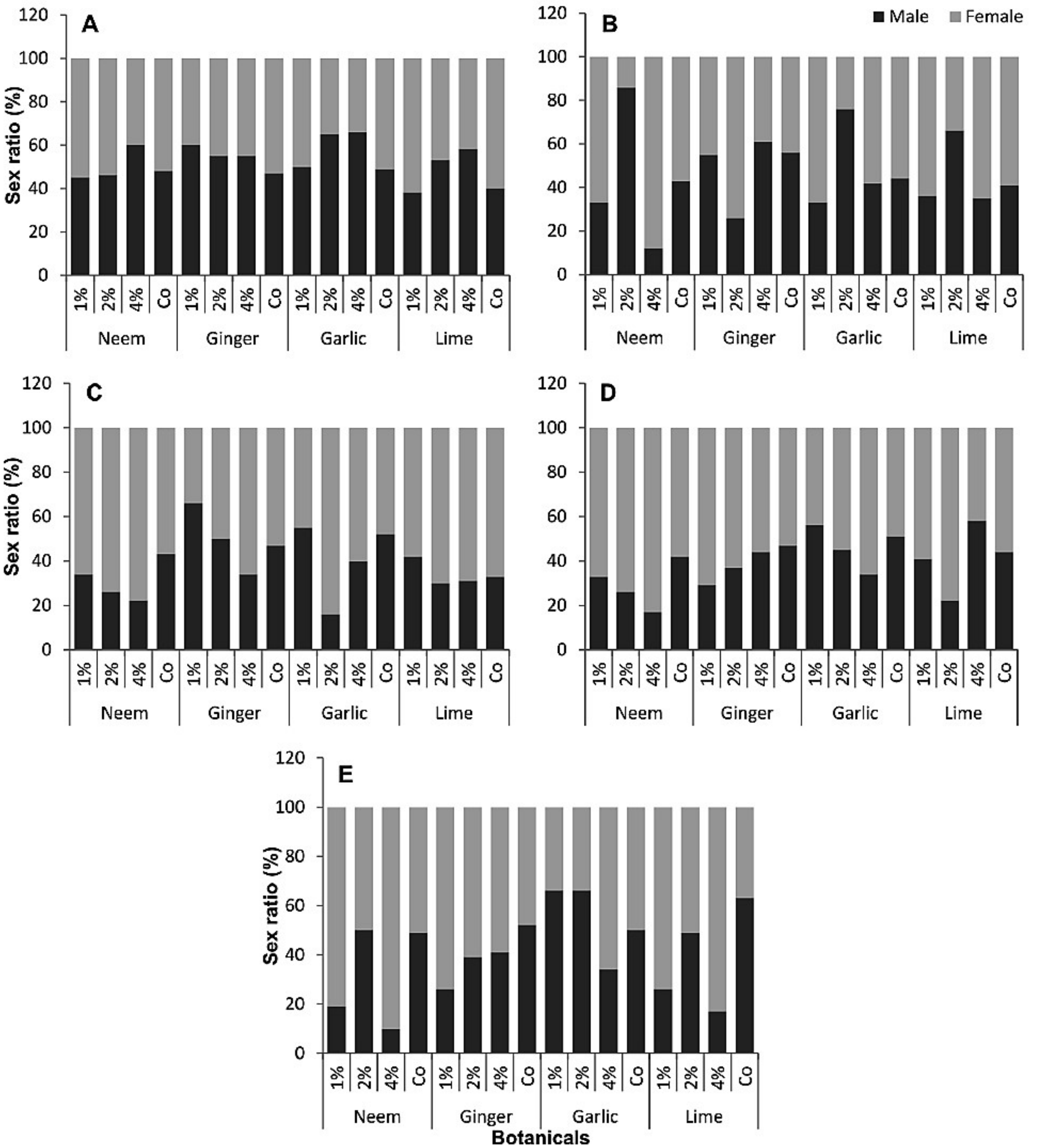


**Figure 2:** *The sex ratio of Bactrocera dorsalis adults resulted due to different botanical treatments on citrus cultivars under the no-choice test. Means followed by the same letters are not statistically different (factorial ANOVA; LSD at P = 0.05). Co = control.*

the differential olfactory bio-constitutions (volatile oils) of different citrus cultivars which would trigger and affect the fruit fly olfactory response towards these cultivar fruits (Papachristos and Papadopoulos, 2009; Liu and Zhou, 2016). Our results are in line with the findings of Diatta et al. (2013) showing that fruits of lime (*C. aurantifolia*) were not preferred at all by the adults of fruit fly *B. invadens*. Similarly, disturbance in the sex ratio due to botanical treatments and due to different citrus cultivars are sub-lethal effects as reviewed by Isman (2020).

## Conclusions and Recommendations

It is concluded that the fruits of citrus cultivars kinnow mandarin and sweet orange appeared as most

**Table 7:** *Adult emergence (%) of Bactocera dorsalis from fruits of different citrus cultivars treated with different botanical extracts under no-choice test.*

| Host Plants | Concentration (%) | *Azadirachta indica* | *Allium sativum* | *Zingiber officinale* | *Citrus aurantifolia* |
|---|---|---|---|---|---|
| Mandrins | 1.0 | 80.2±5.233a | 83.3±8.453a | 58.3±3.587ab | 88.9±8.452a |
| | 2.0 | 83.3±5.236a | 61.1±6.458ab | 44.4±5.734bc | 61.1±4.684ab |
| | 4.0 | 69.6±6.234a | 33.3±4.583b | 16.7±2.474c | 16.7±3.458b |
| | Control | 86.9±6.634a | 90.5±7.453a | 95.8±7.453a | 95.2±7.347a |
| | Sign. | 2.34NS | 3.16** | 7.47** | 6.20** |
| Sweet ornage | 1.0 | 72.2±7.342ab | 83.3±8.457a | 59.1±5.874b | 80.6±7.894a |
| | 2.0 | 33.3±2.734bc | 38.9±5.784b | 50.0±4.684b | 33.3±3.458b |
| | 4.0 | 16.7±3.734c | 16.7±3.657b | 11.1±2.347c | 11.1±3.465b |
| | Control | 95.2±8.673a | 90.5±5.845a | 95.8±7.874a | 94.4±7.457a |
| | Sign. | 7.57** | 10.7** | 29.5*** | 9.82** |
| Grapefruit | 1.0 | 76.7±4.734ab | 83.3±5.843a | 71.7±6.643a | 88.9±8.754a |
| | 2.0 | 55.6±5.734b | 69.4±5.784a | 72.2±7.453a | 77.8±8.544ab |
| | 4.0 | 0.00c | 33.3±3.458b | 16.7±2.547b | 33.3±3.856b |
| | Control | 95.3±7.453a | 95.2±6.747a | 95.8±7.458a | 100a |
| | Sign. | 25.7*** | 7.62** | 8.23** | 3.82** |
| Lime | 1.0 | 66.7±7.452a | 83.3±7.457ab | 83.3±8.845a | 83.3±6.854ab |
| | 2.0 | 0.00b | 33.3±3.488bc | 50.0±5.856ab | 33.3±2.785bc |
| | 4.0 | 0.00b | 0.00c | 0.00b | 0.00c |
| | Control | 96.2±6.236a | 100a | 95.8±8.453a | 97.3±8.453a |
| | Sign. | 8.19*** | 6.07** | 6.50** | 5.57** |
| Bitter orange | 1.0 | 100a | 77.8±6.783a | 88.9±6.845a | 95.3±7.453a |
| | 2.0 | 66.7±6.348a | 69.4±6.348a | 66.7±6.458ab | 88.9±7.458a |
| | 4.0 | 0.00b | 0.00b | 16.7±3.456b | 0.00b |
| | Control | 100a | 90.2±5.346a | 95.8±8.845a | 100a |
| | Sign. | 8.2** | 6.83** | 5.5** | 61.9*** |

***: *significant at P < 0.001;* **: *significant at P < 0.05;* **NS:** *non-significant.*

preferred by *B. dorsalis* and favored its biology as compared to grapefruit, lime and bitter orange. Moreover, the aqueous extracts of *A. indica* (neem) and *C. aurantifolia* (citrus lime) were the most effective and exerted significant reduction of pupal development and adult emergence and sex ratio of *B. dorsalis* flies on the fruits of all five citrus cultivars tested under the laboratory conditions. Hence, further studies should be conducted to incorporate these botanicals extracts into IPM programs for managing *B. dorsalis* on the citrus crop. Moreover, these effective botanical extracts can be applied in combination with other pest control strategies to control fruit fly infestations as demonstrated by Mahmoud (2007) and Ismail *et al.* (2016) which have shown compatibility of entomopathogenic nematodes and fungi, respectively along with different botanical extracts against different fruit fly species.

## Novelty Statement

This laboratory study has demonstrated a differential effect of the fruits of commonly grown citrus cultivars on the oviposition preference and development parameters of fruit fly *B. dorsalis*. Moreover, the aqueous extracts of *Azadirachta indica* (neem) and *Citrus aurantifolia* (citrus lime) can be effectively used against fruit fly infestations in citrus crop.

## Authors' Contribution

**Muhammad Ismail** : Conducted the bioassays, performed statistical analysis and prepared results.
**Abu Bakar Muhammad Raza**: Conceived the idea, planned the experiment and technically revised the manuscript.
**Muhammad Zeeshan Majeed**: Wrote first draft of the manuscript.
**Umair Abbas**: Conducted the bioassays, performed

statistical analysis and prepared results.
**Riaz Hussain**: Helped in format setting and proof-reading

*Conflict of interest*
The authors have declared no conflict of interest.

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