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The insecticidal potential of botanical extracts for management of Peach fruit fly, *Bactrocera zonata* Saunders, 1842 (Diptera: Tephritidae)

Şeftali meyve sineği, *Bactrocera zonata* Saunders, 1842 (Diptera: Tephritidae) mücadelesinde bitkisel ekstraktların insektisidal potansiyeli

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Summary

Methanolic extracts of *Isodon rugosus* Wall. ex Benth (Labiatae), *Boenninghausenia albiflora* (Hook.) Rchb. ex Meisn. (Rutaceae), *Calotropis procera* Aiton (Dryand) (Apocynaceae), *Daphne mucronata* Royle (Thymelaeaceae), *Tagetes minuta* L. (Asteraceae), *Cinnamomum camphora* (L.) J. Presl (Lauraceae) and *Eucalyptus sideroxylon* A. Cunn. ex Woolls (Myrtaceae), grown in lower Himalayan regions of Pakistan were evaluated at 2% concentration against Peach fruit fly *Bactrocera zonata* Saunders, 1842 (Diptera: Tephritidae). *Tagetes minuta* extract showed maximum of 73% mortality against male fruit flies and in case of female maximum mortality shown by *C. camphora* and *I. rugosus* was only 16.6%. *Boenninghausenia albiflora* extract had maximum repellence of 44.4% followed by 42% by *D. mucronata* extract and the minimum number of flies were settled on these two plant extracts as compared to the others. The lowest number of pupae 3.3% as collected from guavas treated with *T. minuta* extract; this was significantly lower than the 62.6% pupae recovered from untreated guavas. The lowest numbers of adults (0.33%) were recovered from guavas treated with *T. minuta* extract compared to 45.3% adults from untreated guavas. The pupae inhibition was highest (94.6%) for *T. minuta* extract. Inhibition of adult emergence was highest i.e. 99.2% for *T. minuta* extract. *Tagetes minuta* can be exploited as a potent source of pesticide against fruit fly, *B. zonata*, due to maximum pesticidal potential as compared to all other plant extracts applied. The results are discussed in relation with potential benefits of incorporating plant based insecticides in integrated pest management strategies against *B. zonata*.

Keywords: *Bactrocera zonata*, botanical insecticides, insecticidal activity

Özet

Pakistan'da Himalaya'nın düşük rakımlı bölgelerinde yetişen, *Isodon rugosus* Wall. ex Benth (Labiatae), *Boenninghausenia albiflora* (Hook.) Rchb. ex Meisn.(Rutaceae), *Calotropis procera* Aiton (Dryand) (Apocynaceae), *Daphne mucronata* Royle (Thymelaeaceae), *Tagetes minuta* L. (Asteraceae), *Cinnamomum camphora* (L.) J. Presl (Lauraceae) ve *Eucalyptus sideroxylon* A. Cunn. ex Woolls (Myrtaceae) bitkilerinin metanolik ekstraktlarının %2'lik konsantrasyonlarının Şeftali meyve sineği *Bactrocera zonata* Saunders, 1842 (Diptera: Tephritidae)'ne karşı etkileri değerlendirilmiştir. *Tagetes minuta* ekstraktı, erkek meyve sineklerine karşı maksimum %73 ölüm oranı gösterirken, dişilerde en fazla ölüm oranı sadece %16.6 ile *C. camphora* ve *I. rugosus* ekstraktlarından elde edilmiştir. Maksimum uzaklaştırıcı etkiye %44.4 ile *B. albiflora*, bunu takiben %42 ile *D. mucronata* ekstraktları sahip olurken en az sayıda sinek diğerlerine kıyasla bu iki bitki ekstraktında saptanmıştır. En düşük pupa sayısı %3.3 ile *T. minuta* ekstraktı uygulanan guava meyvelerinden toplanırken; bu oran uygulama yapılmamış olanlardan elde edilen %62.6'lık pupa oranına göre önemli derecede düşük olarak değerlendirilmiştir. Erginlerin en düşük sayıları (%0.33), *T. minuta* ekstraktı uygulanan guava meyvelerinden elde edilirken, uygulama yapılmamış olanlardan %45.3 oranında ergin elde edilmiştir. Pupaların engellenmesi oranı, en yüksek (%94.6) *T. minuta* ekstraktından elde edilmiştir. Erginlerin ortaya çıkışının engellenmesi %99.2 ile *T. minuta* ekstraktı için en yüksek olmuştur. Uygulanan diğer bitki ekstraktlarına kıyasla maksimum insektisit potansiyeline sahip olduğu için, *T. minuta* meyve sineği *B. zonata*' ya karşı etkili bir pestisit kaynağı olarak kullanılabilir. Sonuçlar bitki bazlı insektisitlerin *B. zonata*'ya karşı entegre zararlı mücadele stratejilerine dahil edilmesinin potansiyel faydaları ile ilişkili olarak tartışılmıştır.

Anahtar sözcükler: *Bactrocera zonata*, bitkisel insektisitler, insektisidal aktivitesi

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Introduction

The Peach fruit fly, *Bactrocera zonata* Saunders, 1842 (Diptera: Tephritidae), is one of the most economically important insect pests that causes economic loss by damaging fruit and by interfering international horticultural trade (Shehata et al., 2008). It is native to Asia where it causes severe damage to over 50 species of fruit crop. Its most preferred host is guava, in which losses may reach 50%, if effective control measures are not adopted (Awad et al., 2014)

Like many other species in the genus *Bactrocera*, males of *B. zonata* show strong attraction to a phenylpropanoid compound, methyl eugenol (ME; 1,2-dimethoxy-4-(2-propenyl) benzene), which occurs naturally in many plant species (Tan & Nishida, 2012). ME is used to monitor and suppress populations of fruit flies by a male lure and kill approach (male annihilation technique; MAT) (Steiner et al., 1970). The MAT relies on attracting males from the field population in devices containing ME and insecticides (Vargas et al., 2010). To suppress female populations in conjunction with MAT, sprays of protein baits containing insecticides (bait application technique; BAT) can also be used. As components of integrated pest management, both MAT and BAT are more efficient when used on an area-wide basis (AW-IMP). In addition to these control measures, farmers routinely apply synthetic insecticides. However, synthetic insecticide application is undesirable because of adverse effects to the environment, poisonous residues in fruit and issues for international trade (El-Aw et al., 2008).

Due to the limitations of each control strategy as a stand-alone technique, it is recommended that an IPM approach be adopted (Vargas et al., 2015). Although MAT and BAT are components of IPM and these are environmentally benign techniques, overuse of baits with synthetic insecticides deposits a huge quantity of insecticides into the environment, so to protect the environment safer insecticides i.e. Spinosad are incorporated into baits. Therefore, exploring plant based insecticides may lead to the discovery of safer insecticides either for direct application or for incorporation into the baits. Plant extracts can potentially be eco-friendly alternatives to synthetic insecticides in IPM of fruit fly populations.

Different plant extracts have been used effectively against a wide range of insect pests (Isman, 2006). Extracts from some plants, e.g. *Acorus calamus* L., *Azadirachta indica* A. Juss, *Curcuma longa* L., *Peganum harmala* L., *Saussurea lappa* (Decne.) C. B. Clarke and *Valeriana jatamansi* Jones, have shown repellence and growth inhibition of *B. zonata* (Akhtar et al., 2004). More recently, Siddiqi et al. (2006) analyzed turmeric plant extracts in solvents such as petroleum ether, acetone and ethanol, and found that acetone extract gave the highest repellence and growth inhibition of *B. zonata*. An advantage of plant based insecticides is that they contain many substances and are capable of showing higher efficacy against target pests. For example, *A. indica* extract shows anti-feeding effects, repellence, toxicity and anti-oviposition effects on the oriental fruit fly, *Bactrocera dorsalis* Hendel, 1912 and melon fly, *Bactrocera cucurbitae* Coquillett, 1849 (Shivendra & Singh, 1998).

The main objective of this study was to evaluate the insecticidal potential of seven plant species for mortality, repellence and oviposition deterrence of *B. zonata*. This was achieved by analyzing the toxic effects of crude methanolic extracts of the plants on *B. zonata* in laboratory bioassays. The selected plants have been long known for their folk or ethnobotanical uses in northern Pakistan. Exploring the scientific base of traditional use in order to transform local knowledge into commercial use was the major and long term goal of this study.

Materials and Methods

Plant material

The leaves of *Cinnamomum camphora* (L.) J. Presl (Lauraceae) and *Eucalyptus sideroxylon* A. Cunn. ex Woolls (Myrtaceae), and aerial parts of *Isodon rugosus* Wall. ex Benth (Labiatae), *Boenninghausenia albiflora* (Hook.) Rchb. ex Meisn. (Rutaceae), *Calotropis procera* Aiton (Dryand) (Apocynaceae), *Daphne mucronata* Royle (Thymelaeaceae) and *Tagetes minuta* L. (Asteraceae) were collected from northern Pakistan (34.1558° N, 73.2194° E). These plants are commonly grown in Pakistan and their taxonomic identification was done by Dr. Zafar Jamal, Chairman Botany Department, Abbottabad Government College, Pakistan.

Rearing of fruit fly, *Bactrocera zonata*

Pupae of *B. zonata* were collected from the laboratory colony maintained on an artificial larval diet at the Nuclear Institute of Agriculture, Tandojam, Pakistan. Three to 4 d before eclosion of pupae, the pupae were received at the Insect Pest Management Program, National Agricultural Research Centre Islamabad, Pakistan. After emergence, the flies were kept in 30x30x45 cm screened cages and maintained at 26±1°C and 60±5% RH with a 10L:14Dh photoperiod, and fed *ad libitum* with a protein diet containing hydrolyzed yeast (MP Biomedicals Inc.; www.mpbio.com) and sugar in a 1:3 ratio by weight, and water. On the first day of emergence flies were sexed and kept in separated cages having different food regimes. Male and female flies were identified on the basis of morphological characteristics; the female flies have long pointed ovipositor at the end of their abdomen. Female flies were separated into two groups. One group of females was reared on protein diet that contained both yeast and sugar and the other group was reared on sugar only until they were transferred to experimental cages. Females reared on protein diet were used to assess oviposition deterrence and those on sugar only to assess toxicity. The reason for keeping flies deprived of protein was that protein is critical for producing fertile eggs and protein deprived females will show attraction to protein. Therefore, for assessment of toxicity, females were initially maintained on sugar only and switched to a diet containing protein at the onset of their sexual maturity. Males were in one group and reared on protein diet from emergence onwards because males showed strong attraction to methyl eugenol and there was no need to have different food regimes for their attraction purpose.

Preparation of plant extracts

Plants were dried in the shade for three months. Dried plant material was ground to powder using an electric grinder. Metabolites were extracted by a maceration method using organic solvent methanol at room temperature (Padin et al., 2013). After 2 d the solvent layer was filtered with Watman No.1 filter paper and the process repeated three times. The filtrate was concentrated using a rotary evaporator at 35°C and resulting extracts stored at 4°C.

Bioassays

Adult male fruit fly toxicity bioassay

After 14 d of emergence *B. zonata* males from large cage were shifted to experimental screened cages 15x15x20 cm and kept them for 1 h before bioassay. Laboratory adopted males reached sexual maturity at 14 d. Studies of *B. zonata* male age response to ME have not been undertaken, however, Shelly et al. (2010) reported that many of the ME responsive males are responsive at the beginning of their sexual maturity. Therefore, sexually mature males were selected for toxicity bioassay by mixing plant extracts with ME. For this bioassay, crude methanolic plant extracts were tested against adult male fruit fly. Four mg each of plant extract was mixed with 200 µL ME and 50 µL added to single filter papers in three replicate Petri dishes. These Petri dishes (without lids) were then placed in experimental cages having male flies at 10:00 h and removed after 24 h. Thirty males (10 males in each replication) were exposed to each treatment. Three controls were included; ME (a negative control), untreated filter paper (a negative control) and organophosphate synthetic insecticide i.e. 2,2-dichlorovinyl dimethyl phosphate (DDVP; the positive control). In the positive control,4 µL of DDVP was mixed with 200 µL of ME. Mortality was observed after 24 and 48 h.

Adult female fruit fly toxicity bioassay

From emergence, female flies were provided with sugar only and after 14 d they were transferred to experimental cages, starved for 8 h and switched to protein diet. Feeding toxicity bioassay was used to analyze the toxicity of plant extracts by mixing each plant extract into the diet of the flies (Shakunthala & Thomas, 2001b). For this purpose, each crude methanolic plant extract was mixed at 2% into the adult diet and placed on filter paper in Petri dishes. Four mg of each plant extract was first dissolved in 200 µL of methanol and then mixed with diet containing 2 mg of sugar and 2 mg of hydrolyzed yeast and placed in experimental cages. In each treatment total of 30 flies were exposed in three replicates of 10 flies. Three control treatments were included; methanol only (a negative control), food containing hydrolyzed yeast and sugar (a negative control) and commercial protein bait containing Spinosad (GF 120; positive control). In the positive control, 12 mg of GF 120 was mixed with 90 µL of water as recommended by the manufacture. Mortality was observed after 24 and 48 h.

Repellence and oviposition deterrence bioassay

Fifty each of virgin male and female fruit flies, maintained on protein diet for 14 d, were combined for copulation 90 min before sunset in a 45x45x45 cm plexiglass screen cage. Fruit fly couples were collected in plastic vials, transferred to separate cages and left to continue copulation. Next morning female flies were transferred to experimental cages and provide a protein diet and water *ad libitum*. Fifteen flies (five per replicate) were taken for each treatment for evaluation. Next day at 10:00 h, the females were provided access to guava fruit treated with 2% crude methanolic plant extracts. The fruit used were of uniform size, cold treated at 4°C for 22 d in order to eliminate any larvae from wild flies and kept at room temperature for 24 h before exposure for oviposition. In each treatment, 60 mg of plant extract was mixed with 3 mL of methanol. An aliquot of 1 mL was applied on each guava by pipette while continuously rotating the guava to ensure uniform distribution over the fruit. Three guavas were used for each treatment (1 guava per replication). After treatment, the guavas were allowed to dry for 2 h, then exposed to flies for 48 h. For repellence bioassay, settled or repelled females from treated guava in each treatment were counted every 2 h. For oviposition, deterrent effect bioassay, female flies were removed after 48 h and guavas were placed in sawdust for 15 d so that larvae could pupate in sawdust. Number of pupae and emerged adults were counted. Two negative control treatments were included; methanol only and untreated guavas.

Data analysis

Percent repellence was calculated by using the formula (Rehman et al., 2009):

$\%R = [1/2\ (A\text{-}B)/A] \times 100$

Where R represents repellence, a represent half of the number of flies settled on both treated and untreated guavas and B represents number of flies settled on treated guava. Differences in mortality, repellence and oviposition deterrence caused by different plant extracts were analyzed by one-way analysis of variance (ANOVA) Complementary pairwise comparisons of means were performed by Tukey's test. All analyses were performed with SPSS version 16.

Results

Adult mortality

Male mortality between treatments was significantly different (F= 5.79, *P*>0.001). Five treatments, DDVP and *T. minuta*, *I. rugosus*, *E. sideroxylon* and *D. mucronata* extracts, gave similar but significantly higher mortality than all other treatments (Table1). Female mortality was significantly higher with GF 120 treatment than all other treatments (Table1).

Table1. Mean percentage mortality of males and females of *Bactrocera zonata* exposed tomethanolic extracts of different plants in female protein baits and male lures under laboratory conditions

Pesticide/Plant extracts	Mortality (female)	Mortality (male)
GF 120*	100% a	-
DDVP*	-	100% a
Tagetes minuta	6.6% b	73.3% a
Isodon rugosus	16.6% b	53.3% a
Daphne mucronata	13.3% b	50% a
Eucalyptus sideroxylon	3.3% b	50% a
Calotropis procera	0% b	43.3% b
Cinnamonum camphora	16.6% b	40% b
Boenninghausenia albiflora	10% b	26.6% b
Methanol*	0% b	-
Methyl eugenol*	-	13.3% b
Untreated	0%b	20% b

*GF 120, positive control for female toxicity bioassay; DDVP, positive control for male toxicity bioassay; methanol, negative control for female toxicity bioassay; methyl eugenol, negative control for male toxicity bioassay. Means followed by the same letter within a column are not significantly different (Tukey's test, P< 0.05).

Effect of treatments on settlement and repellence behavior of females

Mean number of females settling on untreated guavas was greater than on treated guavas (F= 5.79, *P*>0.001). The minimum number of females (3.3 of 15) that settled on any treated guavas was on those treated with *D. mucronata* extract, followed by those treated with *B. albiflora* (3.7 of 15), *I. rugosus* (4 of 15) and *C. camphora* (4.7 of 15) extracts. Both methanol and *C. procera* extract treated guavas had 5 of 15 females settle. With *E. sideroxylon* and *T. minuta* extracts 5.3 and 6.3 of the 15 females settled, which was also less than the 8.3 females that settled on untreated guavas.

In the repellence bioassay, different treatments showed significantly different repellence (F= 3.06, *P*>0.023). *Boenninghausenia albiflora, D. mucronata, I. rugosus* and *C. camphora* extracts gave similar but significantly higher repellence than *C. procera and E. sideroxylon* extracts, and methanol. *Tagetes minuta* extract showed the least repellence, which was similar to that of untreated guavas (Figure1).

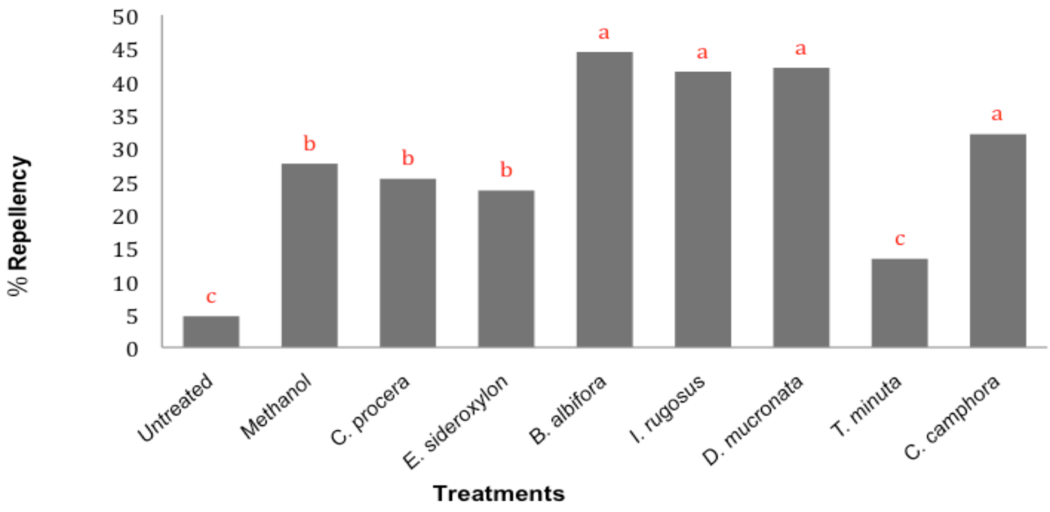

Figure1. Mean percent repellence (%) caused by selected plant extracts at 2% concentration against fruit flies, *Bactrocera zonata,* under laboratory conditions. Means followed by the same letter within a column are not significantly different (Tukey's test, P< 0.05).

Oviposition deterrence

Effect of plant extracts on recovery of pupae

There were significant treatment effects on number of pupae recovered from guavas. (F= 5.15, *P*= 0.001) (Table 2). The lowest number of pupae (3.3) was obtained from guavas treated with *T. minuta* extract*,* followed by those treated with *D. mucronata* and *E. sideroxylon*. Treatment with *B. albiflora*, *C. camphora*, *C. procera* and *I. rugosus* extracts resulted in recovery of about 30 to 40 pupae. The highest number of pupae were recovered from untreated guavas.

Effect of plant extracts on adult emergence

Paralleling the results for pupae, the lowest number of adults emerged was for guavas treated with *T. minuta* extract, followed those treated with *D. mucronata* and *E. sideroxylon* extracts (Table 2).Guavas treated with *B. albiflora*, *C. camphora, C. procera* and *I. rugosus* extracts had greater numbers, but the highest number of adults that emerged was for untreated guavas.

Table 2. Mean number of *Bactrocera zonata* pupae recovered and adults emerging for guava fruit treated with various plant extracts and exposed for oviposition for 48 h

Plants Extracts	Pupae Count	Adult Emergence
Tagetes minuta	3.3 ± 6.16 a*	0.3 ± 5.39 a
Daphne mucronata	26.7 ± 4.55 b	7.0 ± 4.95 a
Eucalyptus sideroxylon	28.3 ± 4.62 b	13.0 ± 4.71 b
Boenninghausenia albiflora	31.7 ± 4.69 c	13.3 ± 4.73 b
Cinnamomum camphora	35.0 ± 8.38 c	16.7± 4.44 b
Calotropis procera	35.7 ± 9.58 c	27.3 ± 3.67 c
Isodon rugosus	39.7 ± 9.78 c	29.7 ± 4.16 c
Untreated	62.70 d	45.30 d

*Means followed by the same letters within each column are not significantly different (Tukey's test, *P*< 0.05).

Discussion

The plant species selected for study are common in Pakistan, so are readily available. Apart from *I. rugosus* and *D. mucronata,* the other species have all been reported to have insecticidal effects on a range of insect pests, so this is the first report of the insecticidal potential of *I. rugosus* and *D. mucronata*.

The plant extracts were assayed for their effect as toxicants against *B. zonata* males and females, their repellence effect on females and oviposition deterrence effect. Extracts of *T. minuta* were found to give the highest male mortality and oviposition deterrence, whereas *B. albiflora* extract showed the strongest repellence. Female mortality with these plant extracts was less than for males, which may have been due to differences in the mode of application for males and females. The highest mortality of males with *T. minuta* extract was about 73% (Table 1), but against females, the highest mortality was only 16% with *I. rugosus* extract, which was not significantly different from the results with the other plant extracts. The lower mortality in females compared to males may have been due to the plant extracts being mixed into the diet and females may have consumed less toxicants by restricting their intake, whereas the males may have been unable to restrict their intake of plant extracts mixed with ME (Haq et al. 2014).

Currently farmers use synthetic insecticides to control fruit flies. In a summer crop of guava, 5 to 7 insecticide sprays are applied, in mango, 2 to 3 sprays and in plums, peaches, apricot and pear, sprays are applied every 10 to 15 days. Ten percent of total synthetic insecticides applied in Pakistan are for fruit flies (Stonehouse et al., 1998; Siddiqi et al., 2006). Due to unacceptable levels of insecticide residues in fruit and vegetables, exports of these are adversely affected.

As plants contain a rich source of bioactive compounds, they may give an alternative solution to synthetic insecticides for control plant pests and diseases (Ghosh et al., 2012; Pino et al., 2013). According to different reports, plant extracts showed strong pesticidal properties and have additional advantages as these chemicals can be specific for targeted pests, biodegradable to nontoxic products and therefore, considered as appropriate to apply in integrated pest management programs (Tare et al., 2004).

In this study, we assayed the insecticidal effects of different plants by applying extracts in ME and protein baits that ensured the ingestion of these extracts. Among the plant extracts assayed, *T. minuta* extracts had the highest toxicity for males. The studies of Shivendra & Singh (1998), Shakunthala & Thomas (2001a) and Tewari (2001) revealed the insecticidal properties of *A. calamus* and *A. indica* against fruit flies. Assessing the efficacy of neem extracts by applying them along with food, Van Randen & Roitberg (1998) reported an inverse effect on adult survival and on the development of eggs of Western fruit fly. Van Randen & Roitberg (1998) also reported that the artificial diet containing neem based insecticides has negative effect on pupae formation and adult emergence of Western fruit fly. Shakunthala & Thomas (2001b) indicated the significant changes in the appearance of reproductive organs of adult *B. cucurbitae* when the flies were fed with a diet containing *A. calamus* extract. However, the plant extracts assayed in this study did not cause high female mortality. In addition to plant extracts causing mortality of adult fruit flies, they can repel fruit fly females and deter their oviposition. These effects encourage their incorporation in integrated pest management strategies against fruit flies. This study recorded the highest repellence with *B. albiflora* and *D. mucronata* extracts and in was accordance with Walter (1999) and Jimenez et al. (2000), who reported repellence of a number of botanical pesticides against *B. zonata* on guava. Similarly, Singh et al. (2007) reported the repellent effect of neem products as biopesticide against *B. zonata* and *B. dorsalis*. Later studies by Rehman et al. (2009) indicated the effectiveness of petroleum ether, ethanol and acetone extracts of *A. calamus, Citrullus colocynthis* (L.) Schrad.*, C. longa, P. harmala, S. lappa, V. jatamansi* and for repellence and oviposition deterrence of *B. zonata* and reported promising effects of these plant based pesticides. Solangi et al. (2011) reported that botanical pesticides, such as neem oil, neem seed powder solution, tobacco leaf solution and solution prepared from Eucalyptus leaves, have repellent effects on *B. zonata.*

Khattak et al. (2006) demonstrated the repellence and growth inhibition caused by petroleum ether, acetone and ethanol extracts of *P. harmala, S. lappa* and *Valariana officinalis* L. of *B. zonata*. These results are in concurrence with the studies of Akhtar et al. (2004), reporting that three plants, sweet flag, neem seed and turmeric rhizomes had repellent effects on *B. zonata* and that turmeric extract had pronounced effect on suppression of egg laying and emergence of pupae and adults. Siddiqi et al. (2006) indicated the pesticidal effect of acetone, petroleum ether and ethanol extracts of turmeric on *B. zonata* settling response and fecundity, and reported promising results. Studies on foraging and oviposition behavior of different *Bactrocera* spp. found that these species have a non-resource based mating system (Kuba & Koyama, 1985; Iwahashi & Majima, 1986) and adult flies engaged in mating during dusk time at the surrounding vegetation of the main host fruits. This behavior of flies suggests that such control strategies should be useful as an area-wide integrated pest management (AW-IPM) approach. The systemic insecticides are not the preferred choice for fruit flies control in guava fruit and, the insecticides having contact action remained insufficient to give successful control of fruit flies, unless targeting the fruit fly adults in abandoned areas and vegetation. Therefore, plant extract formulations giving oviposition deterrence effects have an added advantage over synthetic insecticides and can be included in integrated pest management programs for the control of fruit flies (Khattak et al., 2006).

Conclusion

The results of this study demonstrated broad-spectrum toxic effects of the tested plant extracts against *B. zonata*. The noteworthy results are the efficiency of the extracts against fruit fly as toxicants, repellents and oviposition deterrents. These actions can be exploited for the control of *B. zonata* by developing proper delivery strategies. Further investigations are required to separate and identify active compounds present in these active extracts through chromatographic techniques and by different spectroscopic analysis. Such compounds may also have profound effects on hormonal balance and reproductive physiology of other insect pests. This information can be helpful in developing some competent formulations for commercial use against *B. zonata*.

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