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Evaluation of Methyl Eugenol Doses against *Bactrocera zonata* S. (Diptera: Tephritidae) in Ber fruit Orchard in Bahawalpur, Pakistan

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Abstract

Among the fruit flies, *Bactrocera zonata* S. (Diptera: Tephritidae) is native to South and Southeast Asia. It is commonly found in India and Pakistan but it is also found in more than 20 countries in Asian and African regions. In this study, fruit fly male annihilation traps containing different doses of methyl eugenol (ME) attractant, ranging from 0.1, 0.2, 0.3, 0.4, 0.5, 0.75, 1.0 ml dose, and a control treatment (water only) were analyzed. Additionally, a uniform application of one milligram of toxicant Trichlorfon applied in all traps was installed in a Ber orchard in Bahawalpur, Pakistan. The prime purpose of this study was to evaluate the relative efficacy of ME doses against *B. zonata* in this region. Apart from this, knowledge of seasonal population change could help in controlling these pest insects. Therefore, experiment data for the population was recorded on fortnightly basis from Feb 7, 2018-April 04, 2018 and onward on a weekly basis till May16, 2021 after which the population started to decline. No fruit fly adults were attracted to traps containing water only during all observed dates. Maximum population of *B. zonata* on Feb 7, 2018, was 3 at ME concentrations of 0.2 ml, 0.75 ml, and 1.0 ml, on Feb 21, 2018, its population was 0.667 at 0.2 ml concentration, on March 07, 2018 the population was 14.667 at 1.0 ml concentration, on March 21, 2018, the population was 3.667 at 0.5 ml concentration, on April 04, 2018, the population was 60.333, on April 11, 2018, the population was 69.000 at 1.0 ml concentration, on April 18, 2018, the population was 94.667 at 0.5ml concentration, on April 26, 2018, the population was 400.670, on May 02,2018, the population was 247.333, on May 9, 2018, the population was 181.667, and on May16, 2018, population was 20.667 at 1.0 ml concentration of methyl eugenol. Thus, more concentrations of ME usually attracted more flies. *B. zonata* population remained low in February and March. In April the population started to build up and reached its peak at the end of April. In May, the population started to decrease and it declined after mid-May. Correlation with

weather factors showed that temperature had a positive while relative humidity showed a negative effect on *B. zonata* population. This above collected data gave useful information about the population dynamics of fruit flies in the Asian region and an effective attractant dose for the better management of these fruit flies.

1. Introduction

Insect pests attack crops and fruit trees, which could cause a significant reduction in yield. Insect pests having economic importance receive a special attention for their control. In fruit crops and orchards, there are several insect pests of significant importance; among those, fruit flies have received significant importance. Adult fruit flies' are quite mobile and dangerous, they attack and oviposit in the mature and ripe fruit. They also oviposit on a fallen fruit which is fully mature and ripped. After hatching from eggs, its larvae, also known as maggots, feed on pulp inside a fruit. Larvae complete their development inside the fruit and just before pupation, they eject from the fruit, which had fallen on the ground, and pupate in the soil to complete their life cycle. After the pupal period is completed in the soil, adults emerge to start their life cycle again. They are multivoltine, which means that they complete several generations in a year. Adults damage fruit by feeding on fruit surface and oviposit into fruit pulp.

Jujube is a paramount and delicious fruit of tropical and subtropical places in the world. It belongs to the family Rhamnaceae of the genus *Zizyphus*. In Pakistan, it is grown in warm subtropical districts. It is developed as a windbreak or it is grown on the margins of the field crops while in the warm territory of Punjab and Northern Sindh as a single plant in cultivated crops and in a garden. In Pakistan, Ber fruits are mainly grown by Sindh province as for as yield and other areas are concerned. Numerous seedlings and grafted types are available within Pakistan, and more than a few species of *zizyphus* are safe to eat fruit, but two species namely *Zizyphus jujube* and *Zizyphus mauritiana* have commercial significance. It has an extended history of being used as important food or medicine. Many portions, including leaves, flowers, roots, and fruits are utilized as medicinal means however, the fruit part comprising the peel and the pulp are regarded as key parts and have maximum bioactive compounds [1]. The scientific evidence has shown that jujube fruits contain various bioactive compounds, including ascorbic acid, triterpenic acids, phenolic acids, amino acids, saponins, cerebrosides, flavonoids, polysaccharides, and mineral constituents. These phytoconstituents play an important role to suppress different diseases, exerting antioxidant, anti-inflammatory, antiobesity, anti-cardiovascular disease, hepatoprotective, antidiabetic, anti-microbial, anti-cancer, and gastrointestinal-protective effects [2].

B. zonata is one of the most significant polyphagous fruit flies, which are distributed widely in several regions of Pakistan [3]. The estimated annual loss caused by fruit flies in Pakistan is around US$ 200 million [4]. Hence, the integrated pest management (IPM) is a well-recognized technique that has been recently used for managing fruit flies worldwide. [5].

A chemical that causes the insect to make an oriented movement toward its source is known as an attractant. The most popular use of attractants is to lure pests to traps for control via the mass trapping strategy. Trapping networks in fruit fly control programs are composed of traps that use para-pheromone attractants that are male-specific (e.g., trimedlure and methyl eugenol), as well as traps that use protein-based attractants that capture both males and females (e.g., Biolure, and Nulure). Protein-based attractants are female-biased, capturing, on average 60% females and 40% males. The parapheromone methyl eugenol (ME) captures many species of the genus *Bactrocera* including *B. dorsalis, B. zonata, B. carambolae, B. correcta and B. musae* [6]. Insect pheromones are not true insecticides because they do not kill insects. They influence insects' behaviour through the olfactory system. Mass trapping in the absence of insecticide sprays has been less successful, many traps are required. Alternatively, pheromones could be used to attract insects to a surface treated with insecticide, the "lure and kill" technique [7].

Male Annihilation Technique (MAT) is a key tool to suppress or eradicate pestiferous tephritid fruit flies for which there exist a powerful male lure. Current implementations of this technique utilized a combination of the male attractant methyl eugenol and a toxicant applied at a high density with the objective of attracting and killing an extremely high proportion of males [8]. In this current study this technique, for *Bactrocera* species several toxicants including spinosad for *B. dorsalis* [8] while Malathion and Spinosad for *B. zonata* have been used [9]. Intensive use of this technique is very common these days because this is the only available option for fruit flies' successful management. Recently, there was an outbreak of the peach fruit fly *B. zonata* in the metropolitan area of Tel Aviv [10]. The development of insecticide resistance and pest outbreak against these intensively used toxicants has been significantly, reported [9].

Additionally, it has been reported that the attracted males fruit flies within the trap were unable to escape and died because of the toxicant in traps [11] while the escaped adults after exposure to sub lethal dose of toxicant resulted in resistant populations because mating success of males having fed some methyl eugenol is much more than those who did not consume methyl eugenol [12].

Owing to the obligatory role of male annihilation technique involving use of methyl eugenol along with toxicants for the control of fruit flies in countries like Pakistan, this current study was designed to cover two main objectives:

a. Population dynamics of fruit flies in Ber orchard under agroecological conditions of Bahawalpur, Pakistan
b. Evaluation of efficacy of methyl eugenol doses to attract adult fruit flies

2. Materials and methods

2.1 Study Site

The current study was conducted in the experimental area of Regional Agriculture Research Institute (RARI) on the population dynamics of fruit flies on Ber plants (Jujube variety) in Bahawalpur starting from February, 2018-May, 2018. Plants of Jujube varieties of Ber namely Ajooba, Anokhi, Delhi sufaid, Dilbahar, Karela, Mehmoodwali, and Pakwhite were cultivated in the RARI, experimental farm. The orchard area was approximately of two hectares.

2.2 Design of the Fruit Fly Trap and Installation

Fruit fly traps were prepared from transparent plastic bottles having 1.5-liter capacity. Two holes (2.5 cm diameter) were made, exactly opposite to each other below the neck of bottle to facilitate the entry of male fruit flies. The lure (Methyl Eugenol) was applied in the form of droplets with the help of a needle and syringe onto cotton (2 cm^2) [13] placed in the bottom of traps. One-milligram pesticide powder (Trichlorfon) was also applied around cotton containing methyl eugenol in all traps to kill the attracted fruit flies. 24 such traps were attached with the branch of the Ber trees, 2.0 meters above the soil surface. All the traps were distributed among Ber plants by maintaining the distance of 10 meters in every two traps.

Methyl eugenol functioned as a precursor for the male sex pheromone in *B. dorsalis* [14]. Commercial formulation of methyl eugenol, a sex pheromone used specifically for capturing male fruit flies was used in the experimental study. Using a 1.0 ml syringe for different treatments (concentrations) of methyl eugenol were applied to cotton swab and then placed in bottom of the bottle. The cotton swabs were replaced every week with the fresh ones.

2.3 Treatments and replications

This experiment was conducted during February-May, 2018 in Ber orchard. Eight treatments were included to apply the traps with different concentrations of methyl eugenol and a trap prepared. The trap consisted of the following treatments:

T1 – Trap charged with a 0.1 ml methyl eugenol + 1 mg trichlorfon

T2 – Trap charged with a 0.2 ml methyl eugenol + 1 mg trichlorfon

T3 – Trap charged with a 0.3 ml methyl eugenol + 1 mg trichlorfon

T4 – Trap charged with a 0.4 ml methyl eugenol + 1 mg trichlorfon

T5 – Trap charged with a 0.5 ml methyl eugenol + 1 mg trichlorfon

T6 – Trap charged with a 0.75 ml methyl eugenol + 1 mg trichlorfon

T7 – Trap charged with a 1 ml methyl eugenol + 1 mg trichlorfon

T8 –Trap water only (control)

2.4 Treatment layout

Row 1	Row 2	Row 3
R1T1	R2T1	R3T1
R1T2	R2T2	R3T2
R1T3	R2T3	R3T3
R1T4	R2T4	R3T4
R1T5	R2T5	R3T5
R1T6	R2T6	R3T6
R1T7	R2T7	R3T7
R1T8	R2T8	R3T8

2.5 Data Recording

Data regarding fruit flies in traps was recorded every week starting from Feb 07, 2018. The number of fruit flies (dead) present inside all trap bottles were taken out and spread on white paper for counting. After that methyl eugenol concentrations were applied to all traps except the control, which had water only for the subsequent data recordings. Data was recorded till May16,2018, after which the fruit flies' population began to decline.

2.6 Identification of Fruit Flies

Fruit flies sample was taken from each date of the recorded dates. Identification

features like wing, scutellum of the thorax, and abdomen were used following the pictorial key [15]. The majority of the population samples recorded on Ber plants on different observation dates belonged to *Bactrocera zonata* S. (Diptera: Tephritidae) species.

2.7 Data analysis

The current study gathered data of experiment regarding fruit flies population on different observed dates. This data was further analyzed statistically using the variance analysis (ANOVA) technique. The obtained means were separated post hoc by the Tukey HSD test at a 5 % level of probability. The population of fruit flies was also correlated with the weather factor *for example,* temperature and relative humidity to know the effect of these factors on fruit flies' population dynamics. Bivariate correlations (Pearson) were performed taking temperature and relative humidity data of respective dates for the Bahawalpur region when fruit fly population data was being recorded. All the data was analyzed in SPSS (SPSS 2016) software for windows.

3. Results

Figure 1 shows population of *B. zonata* on different dates of observation under the effect of different concentrations of methyl eugenol compared with control (water only). On Feb 07, 2018, the population of adults in traps was recorded as maximum (3.000) containing 0.2, 0.75-, and 1.0-ml methyl eugenol. Minimum population was recorded in control (0.000 ± 0.000) ($P > 0.05$). On Feb 21st, 2018 the maximum population was present (.667) in a trap containing 0.2 ml methyl eugenol. Minimum population was recorded in control (0.000 ± 0.000) ($P > 0.05$). On March 07, 2018, the population of *B. zonata* varied significantly in control versus methyl eugenol concentrations in traps ($P < 0.05$). The maximum population of fruit flies was (14.667) in a trap containing 1 ml methyl eugenol. Therefore, minimum population was recorded in the control (0.000 ± 0.000). On March 21,2018, the maximum population of fruit flies was recorded (3.667 ± .882) in traps of 0.5 ml concentration and the minimum population was recorded (0.000 ± 0.000) in control treatments ($P > 0.05$). On April 04, 2018, the maximum population was recorded (60.333 ± 7.965) in traps with a1 ml concentration of methyl eugenol and the minimum was in control-related traps ($P < 0.05$). On April 11, 2018, the maximum population was recorded (69.000 ± 5.568) in traps with 0.75 ml concentration and the minimum was (0.000 ± 0.000) recorded in control ($P > 0.05$). On April 18, 2018, the maximum population was recorded (94.667 ± 13.980) in 0.5 ml concentration and minimum was recorded in control (0.000 ± 0.000) ($P > 0.05$). On April 26, 2018, the maximum population was recorded (400.670 ± 102.610) in 1 ml concentration of methyl eugenol while the minimum population was recorded (0.000 ± 0.000) in traps for the control treatment ($P < 0.05$). On May 02, 2018, the maximum population was recorded (247.333 ± 6.438) in a 1 ml concentration while the minimum population was recorded (0.000 ± 0.000) in traps for the control treatment ($P < 0.05$). On May 09, 2018, the maximum population was recorded (181.667 ± 53.095) in a 1 ml concentration and the minimum was recorded (0.000 ± 0.000) for the control treatment traps ($P > 0.05$). On May16, 2018, the maximum population was recorded (20.667 ± 1.856) in a 1 ml concentration and minimum population was recorded in the control treatment traps (0.000 ± 0.000) ($P < 0.05$).

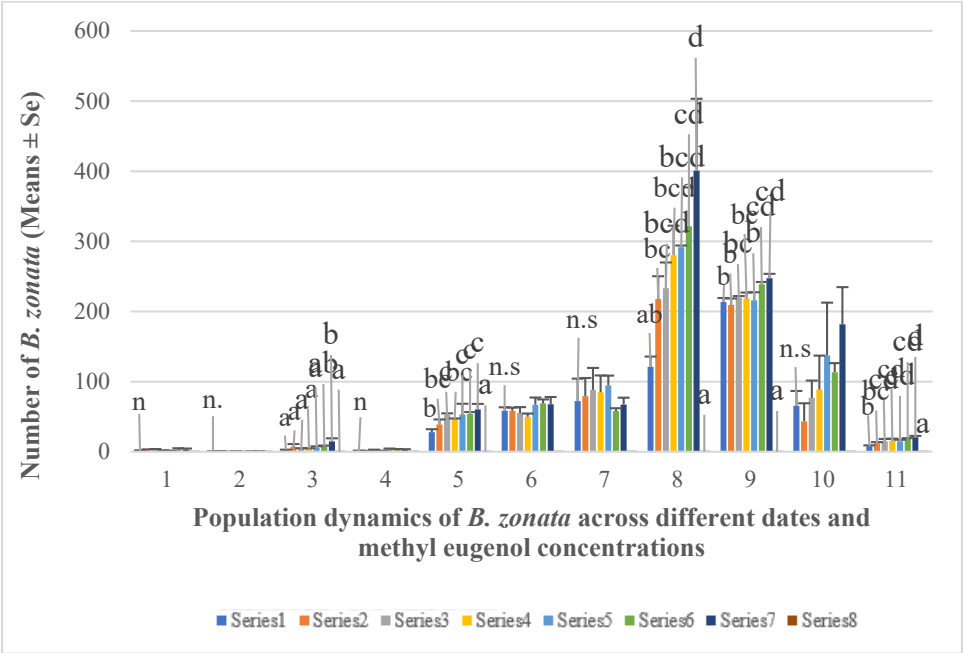

Figure 1. Fruit flies' population fluctuation during 2018: 1: 7th Feb; 2: 21st Feb; 3: 7th March; 4: 21st March; 5: 4th April; 6: 11th April; 7: 18th April; 8: 26th April; 9: 2nd May; 10: 9th May; 11: 16th May. On each date treatments (Methyl eugenol concentrations) included: Series 1: 0.1 ml; Series 2: 0.2 ml; Series 3: 0.3 ml; Series 4: 0.4 ml; Series 5: 0.5ml; Series 6: 0.75 ml; Series 7: 1.0 ml; Series 8: water only; different letters on error bars denote significant difference among treatments at *P*: 0.05; n.s: non-significant

Although, these results show a bit of variation along different dates of observation with respect to different methyl eugenol concentrations, however, it has been observed that adult fruit flies were never attracted towards traps containing no methyl eugenol or traps containing water drops on cotton. While overall, results showed increased concentrations of methyl eugenol, which attracted more males in traps relative to lower concentrations of methyl eugenol.

The seasonal population of *B. zonata* showed its population started to appear in small numbers in the month of February. The population counts also remained relatively low in the month of March. There was an obvious increase in fruit fly populations at the beginning of April. Hence, the population remained higher in all months but it reached at a peak in the end of April. In May population began to decrease again and there was a significant decline in B. *zonata* in mid of May after which no population was recorded in all traps (Fig 1).

Correlation of weather factors e.g., temperature (min, max, and average) and relative humidity showed that there was a positive effect of temperature on the *B. zonata* population while the relative humidity present in the atmosphere had a negative effect on *B. zonata* population (Table 1).

Table 1. Correlation of Weather Factors (Temperature and Relative Humidity) with Population Dynamics of *B. zonata* Under Field Conditions

		Population	RH	Average Temp	Low Temp	High Temp
Population	**Pearson Correlation**	1	-.586*	.564	.515	.569
	Sig. (2-tailed)		.045	.056	.086	.053
	N	12	12	12	12	12

*Correlation is significant at the 0.05 level (2-tailed).
Weather data source Bahawalpur, Punjab Pakistan:
(https://www.timeanddate.com/weather/pakistan/bahawalpur/climate)

4. Discussion

Experiment 1 results showed that fruit fly *B. zonata* males were attracted only to traps where methyl eugenol was applied, hence, no fruit fly adults were recorded at all. In contrast usually, more males were recorded in traps with an increased dose of methyl eugenol. These results are related to previous studies which stated that for fruit fly management, an effective and environmentally friendly technique could be applied to control this pest by the use of methyl eugenol as an attractant. These results showed that more fruit flies were captured due to the increased doses of methyl eugenol which was 1.5 ml sper trap compared to its less dose of 0.5 ml and this factor had a significant effect on fruit fly capturers [16]. Therefore, the current study results are also comparable to another study which reported that more doses of methyl eugenol comprising 0.5 ml captured more adult fruit flies as compared with its lesser doses which were 0.1, 0.2, 0.3, and 0.4 ml [17].

In this study it was found that *B. zonata* was the predominant species in traps containing methyl eugenol attractant. Their traps were installed in mango orchards so the peak population was recorded in June and July when mango was at the ripe stage. Contrarily, this study analyzed the *B. zonata* population which reached at its peak in the end of April when more Ber fruit was available. Ber fruit is usually harvested in April in South Punjab region. Therefore, after the month of April fruit fly population began to decline on Ber which could have started to shift to alternate hosts.

Correlation of weather factors e.g., temperature and humidity in atmosphere showed that temperature (minimum, maximum, and average) had a positive effect on fruit fly populations. Adults of the last season usually emerge in the spring next year from pupae hidden in soils. Therefore, the temperature had a significant positive effect on the population buildup of fruit flies in the previous studies. It has also been stated that temperature had a positive effect on *B. zonata* population buildup infesting mango fruit trees in Multan, Pakistan [11]. Whereas the colder month, February has more relative humidity in the atmosphere in which its population remained low which clearly indicated a negative effect of relative humidity on the population buildup of *B. zonata* in the

current studies.

Concerning the use of toxicant along with methyl eugenol, comparative toxicity studies were performed while following the laboratory conditions in this case. Hence, it was observed that the insecticide which generated maximum efficacy as compared to other chemicals and even after 24 hours' time period was trichlorfon [18]. These results are also in agreement with previous studies, performed under field conditions in male annihilation technique against fruit flies showing relatively more efficacy of trichlorfon belonging to organophosphate group over other insecticides belonging to carbamates and spinosyns [19]. Such insecticides which were less effective early in controlling the majority of the pest population these might result in individuals with sub lethal exposure history to a toxicant. Therefore, these fruit flies, later in turn gave rise to resistant population. These surviving individuals have a chance to attack alternate hosts in the area after approaching of harvesting period of first fruit for example Ber and these migrate to other hosts for example mangoes and others fruit available in this area. Therefore, for the complete eradication of pest fruit flies an insecticide with quick knock down effect was followed along with the attractant methyl eugenol to avoid the individuals.

4.1 Conclusion

This study highlighted the population dynamics of *B. zonata* in small population for the months of February and March, 2018. Its peak population was recorded at the end of April 2018. Several factors including temperature, availability of preferred host, and alternate host could affect the population growth of fruit flies, especially *B. zonata,* in Southern Punjab, Pakistan. Therefore, based on the above findings, we suggest using the toxicant trichlorfon and methyl eugenol attractant administered at a minimum dose of 1.0 ml in traps over 2.0 cm^2 cotton swab in male annihilation technique for fruit flies especially *B. zonata* in Ber and other fruit tree orchards. In Ber, fruit flies especially *B. zonata* are active from February- April. During this period maximum traps loaded with methyl eugenol attractant can be installed along with triclorfon insecticide.

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