

Laboratory studies and field evaluation of response of *Bactrocera cucurbitae* coq. and *Bactrocera dorsalis* Hendel to different doses of Cuelure and methyl eugenol

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ABSTRACT: Laboratory and field studies were conducted to evaluate responses of melon flies, *Bactrocera cucurbitae* and Oriental fruit flies, *B. dorsalis* to different doses (0.5 g, 1 g, 2 g and 3 g) of para-pheromones Cuelure and Methyl eugenol respectively. Electroantennogram (EAG) response was recorded in mV with male antennae of *B. cucurbitae* and *B. dorsalis* whereas field evaluation was carried out under farmers' field condition with dispensers baited with different doses of cuelure and methyl eugenol and numbers of flies trapped were compared. *B. dorsalis* has shown an increased response with increase in quantity of methyl eugenol both in lab and field studies. In EAG study, *B. cucurbitae* response in mV was increased as the cuelure dosage was increased up to 2 g whereas in field, the response was increased up to 1 g and reduced as the dosage further increased to 3 g. These studies showed that 1 g of cuelure and 2 g of methyl eugenol impregnated on wooden block dispensers attract and trap high numbers of *B. cucurbitae* and *B. dorsalis* flies respectively. Further comparative study was done with different sized wooden block dispensers 38 x 27 x 7 mm (big) and 35 x 14 x 8 mm (small) with 1 g loading of methyl eugenol and found small sized wooden block with 1 g methyl eugenol attracted and trapped more no. of *B. dorsalis*.

Keywords: *Bactrocera cucurbitae*, *Bactrocera dorsalis*, para-pheromone, dosage, EAG, field studies

INTRODUCTION

The *Bactrocera cucurbitae* coq. and *Bactrocera dorsalis* Hendel commonly called as melon fly and oriental fruit fly respectively, belong to family Tephritidae which contains >500 species and are severe pests of fruits and vegetables (Drew, 2004; Clarke *et al.* 2005; Michael san jose, *et al.* 2013). They are widely distributed in Southeast Asian countries, Pacific islands, African countries and many species are widespread to other continents except Antarctica (Drew, 2004; Michael san jose, *et al.* 2013). A single female belonging to genus *Bactrocera* lays around 1000 eggs in a lifetime. Eggs are laid below the skin of host ripe fruit / vegetable and the hatched larva feed on them causing damage and yield loss (Weems *et al.* 1999; Weems Jr. *et al.* 2001). The widely used early detection control systems for *B. cucurbitae* and *B. dorsalis* are by baiting traps with cuelure [4-(p-acetoxyphenyl)-2-butanone] and methyl eugenol [4-allyl-1,2-dimethoxybenzene-carboxylate] respectively (Luc Leblanc *et al.* 2011). The "Lure and Kill" technology also known as "Attract and kill" is one of the integrated pest management method used for

many invasive pests (Jones, 1998; El-Sayed *et al.* 2009) including *Bactrocera* species (Mazomenos *et al.* 2002) in which the insect gets attracted towards the source and is not entrapped but instead subjected to killing agent (insecticides), which indeed results in reduction in insect population. The lure formulation, density and release rate should be such that insect is more likely to contact the lure along which adequate insecticide dosage formulations would be effective to decrease the population of target pest (Ryan Pachta *et al.* 2018). The Response variations for increased doses of these two para pheromones to fruit flies were not clearly given in literature. In order to get constructive information on lure formulation dosage the responses of oriental fruit flies and melon flies with increased doses of these attractants, the present study was designed.

This study compares the responses of *B. cucurbitae* and *B. dorsalis* to different doses of cuelure and methyl eugenol respectively, both at lab and field levels. The electroantennogram (EAG) study was carried to measure the *B. cucurbitae* and *B. dorsalis* antennal response/sensitivity to increased doses of cuelure and methyl

Table 1. Mean number of trapped *B. cucurbitae* to different doses of Cuelure impregnated on 38 x 27 x 7 mm wooden block dispenser

Treatment (Different doses of Cuelure)	Mean catches of <i>Bactrocera cucurbitae</i> per trap/day \pm SEM
T1 – 0.5g loading	7.44 \pm 1.255 ^a
T2 – 1g loading	8.68 \pm 1.455 ^a
T3 – 2g loading	7.65 \pm 1.126 ^a
T4 – 3g loading	7.18 \pm 1.086 ^a
F Value	0.281
p Value	0.839

The mean difference is non-significant at the 0.05 level. Values followed by same letters are not significantly different at the 0.05 level by DMRT, IBM SPSS Software 25v.

Table 2. Mean catches of *B. dorsalis* to different doses of Methyl eugenol impregnated on 38 x 27 x 7 mm wooden block dispenser

Treatment (Different doses of Methyl eugenol)	Mean Catches of <i>Bactrocera dorsalis</i> per trap/day \pm SEM
T1 – 0.5g loading	54.86 \pm 6.922 ^c
T2 – 1g loading	170.6 \pm 18.595 ^b
T3 – 2g loading	271.59 \pm 29.917 ^a
T4 – 3g loading	321.15 \pm 24.601 ^a
F Value	29.322
p Value	0.000*

* The mean difference is significant at the 0.05 level. Values followed by same letters are not significantly different at the 0.05 level by DMRT, IBM SPSS Software 25v.

eugenol respectively. Field studies were conducted to evaluate the best dosage of cuelure and methyl eugenol in attracting and trapping highest number of *B. cucurbitae* and *B. dorsalis* respectively.

MATERIALS AND METHODS

Electroantennogram study with different doses of Attractants on *B. cucurbitae* and *B. dorsalis*:

EAG was carried out with field collected *B. cucurbitae* and *B. dorsalis* flies. The insect antenna was carefully excised and mounted on Syntech EAG Combiprobe antenna holder to record the responses. The Pasteur pipette with filter paper impregnated with different doses

of 0.4 mg, 0.8 mg, 1.6 mg and 2.4 mg of cuelure and methyl eugenol (Doses corresponds to field study dosage with 0.5g, 1g, 2g, 3g in 1, 2, 4 and 6 times) were used as stimuli to puff over the *B. cucurbitae* and *B. dorsalis* male antenna using Syntech Stimulus controller CS-55 (with built in air pumps, activated carbon inlet filter to deliver purified continuous air flow). The Antennal response elicited to the stimulus was recorded using Syntech IDAC-2 (Intelligent Data Acquisition Controller) with software (EAGpro) controlled signal acquisition with built-in amplifier. The Insect antennal responses in millivolts to different doses of attractant were statistically analyzed using one way ANOVA by DMRT method

(IBM SPSS Statistics Software Version 25).

Field study with different doses of attractants for trapping *B. cucurbitae* and *B. dorsalis*

Field studies were conducted under farmers' field condition to compare 0.5 g, 1 g, 2 g and 3 g doses of cuelure and methyl eugenol impregnated in 38 x 27 x 7 mm wooden block dispenser using commercial Mcphail traps named Fligh-T™ trap to attract and trap *B. cucurbitae* and *B. dorsalis* flies respectively. Trial for *B. cucurbitae* was carried out in ridge gourd field (crop age of 45days) in Koluvarayanahalli, Rajankunte outskirts of Bengaluru city (13.1651° N, 77.5225° E) for 60 days (27th June 2017 to 24th August 2017) using 4 replications of each treatment traps installed in total 2 acres with traps placed at a height of 6 feet above the ground level. The *B. dorsalis* field trial was carried out in Guava field (crop age of 7 years) in Budumanahalli, Rajankunte outskirts of Bangaluru city (13.1833° N, 77.5168° E) for 60 days (1st June 2017 to 31st July 2017) using 5 traps per treatment installed in total 2.5 acres with traps placed at a height of 4 feet above the ground surface. The observation of flies trapped in different treatments was recorded twice a week during the trial period. The recorded data of number of flies trapped in different treatments were statistically analyzed using one way ANOVA by DMRT method (IBM SPSS Statistics Software Version 25).

Field study with different sizes of wooden blocks in Attracting and trapping *B. dorsalis*

A comparative study of different size of wooden block dispensers was conducted under farmers field conditions between 38 x 27 x 7 mm (Big) and 35 x 14 x 8 mm (Small) wooden block dispenser loaded with 1 g methyl Eugenol along with Fligh-T™ trap mainly to improve the

release rate and reduce the cost of production. The study was carried out in Budumanahalli village (13.1833° N, 77.5168° E) in 2.5 acres of Guava field with crop age of 7 years using 3 replications per treatment for 104 days (15 weeks). The No. of flies trapped in different treatments observation was recorded twice a week. To assess the statistical difference between the numbers of flies caught in trap with different sizes of wooden block was analyzed using two sample independent t-test. (IBM SPSS Statistics Software Version 25).

RESULTS

Electroantennogram Study with different doses of Attractants for *B. cucurbitae* and *B. dorsalis*

The treatments for EAG Study with *B. cucurbitae* and *B. dorsalis* antennae using different doses of cuelure and methyl eugenol were prepared corresponding to increased ratio (1 : 2 : 4 : 6) as in field lure study 0.5 g, 1 g, 2 g and 3 g (1 : 2 : 4 : 6) to obtain the insect antennal response for comparison. The EAG response of *B. cucurbitae* male antenna increased as the cuelure dosage / concentration was increased from 0.4 mg (0.303 mV) to 1.6 mg (1.261mV) and the response reduced when dosage further increased to 2.4 mg (0.797mV) as in Fig 1. The EAG response of *B. cucurbitae* statistical analysis using one way ANOVA by DMRT method was non-significant with p value > 0.05 (i.e., 0.370). EAG response of *B. dorsalis* male antennae was increased as methyl eugenol dosage / concentration was increased. Treatment with 0.4 mg elicited 0.158 mV response, 0.8 mg shown 0.387 mV, 1.6 mg shown 0.725 mV and 2.4 mg shown 0.903 mV (Fig 2). The Results of EAG response of *B. dorsalis* subjected to one way ANOVA by DMRT method was also non-significant with p value > 0.05 (ie., 0.191).

Table 3. Mean catches of *B. dorsalis* using Methyl eugenol as attractant impregnated on different sizes of wooden block dispensers

Treatments (Different sizes of dispensers with 1g loading of Cuelure)	Mean Catches of <i>Bactrocera cucurbitae</i> per trap/day ± SEM
T1 – 35 x 14 x 8 mm (small) wooden block dispenser	20.90 ± 3.460 ^a
T2 – 38 x 27 x 7 mm (big) wooden block dispenser	15.32 ± 2.196 ^b
F Value	7.442
t Value	1.361
p Value	0.007*

The mean difference is significant at the 0.05 level. Values followed by same letters are not significantly different at the 0.05 level by two sample independent t-test, IBM SPSS Software 25v.

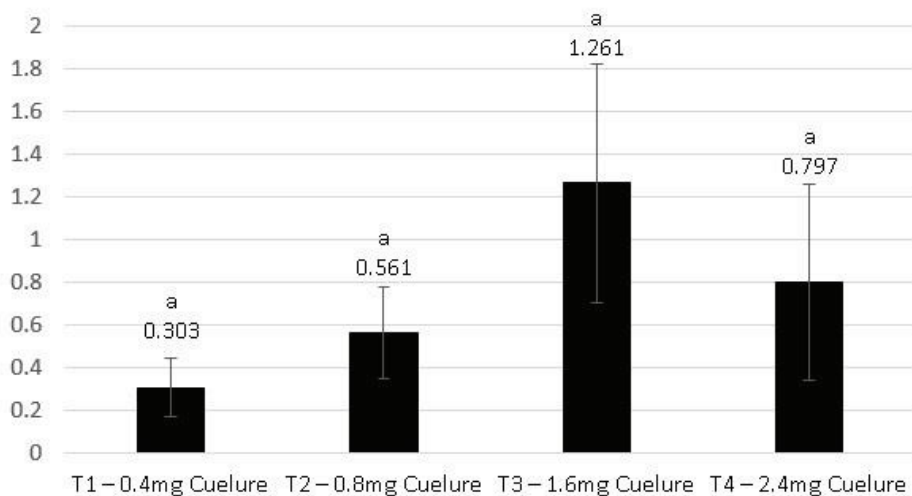
***B. cucurbitae* - EAG Response in mV**

Fig 1. Antennal responses (in mV) of *B. cucurbitae* to different doses of cuelure on EAG

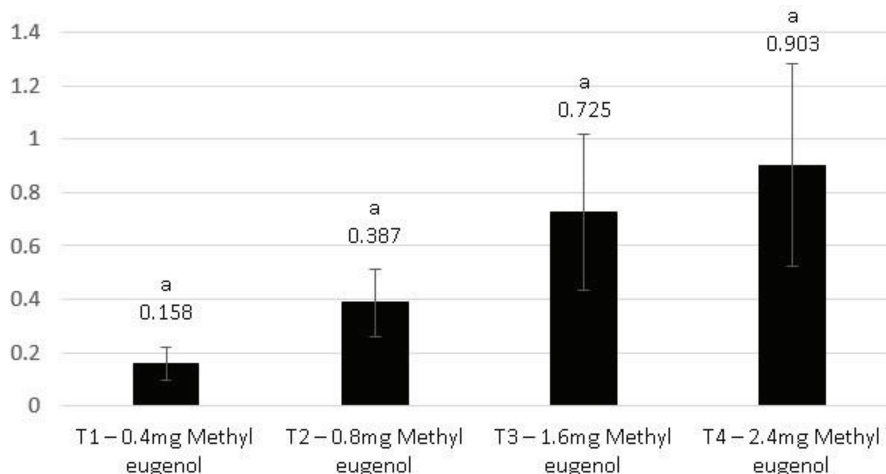
***B. dorsalis* - EAG Response in mV**

Fig 2. Antennal responses (in mV) of *B. dorsalis* to different doses of methyl eugenol on EAG

Field Study with different dosage of attractants for *B. cucurbitae* and *B. dorsalis*

The comparative study was carried out to find out the best dosage among 0.5 g, 1 g, 2 g and 3 g of cuelure and methyl eugenol in attracting and trapping more number of *B. cucurbitae* and *B. dorsalis* respectively. In the dosage study of cuelure, the number of *B. cucurbitae* attracted and trapped in Fligh-T™ traps increased as the dosage was increased from 0.5 to 1 g and reduced when the dosage was further increased to 2 g and 3 g. The 1 g cuelure loaded in wooden block dispenser trapped highest number of flies 8.68 flies caught / trap / day followed by 2 g with 7.65 flies caught / trap / day, 0.5

g with 7.44 flies caught / trap / day and least number of flies were trapped in 3 g loading with 7.18 flies caught / trap / day. The One way ANOVA by DMRT method showed statistically non-significant results with p value > 0.05 (ie., 0.839) (Table 1). In case of dosage study with methyl eugenol, the number of *B. dorsalis* attracted and trapped in Fligh-T™ traps increased as dosage increased to 0.5 g, 1 g, 2 g and 3 g. The 3 g methyl eugenol loaded in wooden block dispenser having dimensions as 38 x 27 x 7 mm (Big) with Fligh-T™ trap caught highest number of flies with 321.15 flies caught / trap / day followed by 2 g with 271.59 flies caught / trap / day, 1 g with 170.60 flies caught / trap / day and least trapped in 0.5 g with 54.86 flies caught / trap / day. The One way ANOVA by

DMRT method showed statistically significant result for *B. dorsalis* with p value < 0.05 (ie., 0.000). The 2 g and 3 g loading of Methyl eugenol were on par with each other and statistically differed from 1 g and 0.5 g loading (Table 2).

Field study with different sizes of wooden blocks in attracting and trapping *B. dorsalis*

In a comparative study of different sizes of wooden block dispensers loaded with 1 g methyl eugenol along with Fligh-T™ trap, field results shown that 35 x 14 x 8 mm (Small) wooden block attracted and trapped highest number of flies compared to 38 x 27 x 7 mm (Big) with 20.9 flies caught / trap / day and 15.32 flies caught / trap / day respectively (Table 3). Statistical significance revealed by two sample independent t-test with p value of 0.07 (t=1.361, df=178).

DISCUSSION

Fruit flies *B. cucurbitae* and *B. dorsalis* are serious pests of melon, gourds, guava, and mango fields causing severe damage to the farm – level productivity. Lot of lab and field work was carried out between 1995 to 2005 in India and Pakistan based on bait application technique (BAT) and male annihilation technique (MAT) using food baits, attractants and insecticides as ‘killing points’ of food bait spots, parapheromone traps, or parapheromone lure blocks impregnated with insecticides having collection devices for collecting dead insects etc., (Stonehouse *et al.* 1995, 2002a-c, 2005a-g, 2007). These techniques have seen the success of the technology in managing fruit flies in some extent but have not given clarity on effect of quantity of attractant in attracting fruit flies. The present studies gave us insight about the antennal responses of fruit flies to different doses of their corresponding parapheromones and also field studies were carried out to evaluate the effect of dosages on *B. cucurbitae* and *B. dorsalis* for effective treatments of parapheromones in managing their infestations.

Based on our studies, the number of *B. cucurbitae* flies trapped increased with the dosage up to 1 g and trapped flies count reduced when the dosage was further increased to 2 g and 3 g. While the *B. cucurbitae* EAG response was increased as the dosage increased up to 2g and reduced when dosage was further increased to 3 g. However, these results clearly indicated that the *B. cucurbitae* field and EAG study results were statistically non-significant. Hence considering the field study results and cost factor for commercialization, it is suggested that 1 g cuelure can be impregnated on wooden blocks for attracting and trapping more number of *B. cucurbitae* flies in 60 days. The EAG and field studies of *B. dorsalis* have showed the results in a similar pattern. The *B.*

dorsalis EAG response and number of adults trapped in the field were increased with the increased dose of methyl eugenol. The field study gave statistically significant results while the EAG study gave non-significant results. In case of *B. dorsalis* with 2 g and 3 g loaded wooden blocks were statistically on par with each other in attracting and trapping highest number of adult flies in 60 days. Another comparative study with different size wooden block dispensers impregnated with 1 g methyl eugenol has resulted 35 x 14 x 8 mm (small) wooden block dispenser attracted and trapped statistically high number of flies compared to 38 x 27 x 7 mm (big) wooden block dispenser.

Hence, considering the field and cost effectiveness, it is recommended to use 1 g cuelure and 1 g methyl eugenol respectively impregnated on 35 x 14 x 8 mm (Small) wooden block in attracting and trapping more number of *B. cucurbitae* and *B. dorsalis* flies.

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