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Research Article

Efficacy of methyl eugenol bait traps for controlling the mango fruit fly *Bactrocera zonata* (Diptera: Tephritidae)

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Abstract: *Bactrocera zonata* (Saunders) (Diptera: Tephritidae), an extremely aggressive species infesting some of the main commercial fruit and vegetables, has been subjected to a diverse array of control approaches. In the current study, performance of various Methyl Eugenol (ME) dispensers in combination with an organophosphate insecticide, Malathion, were evaluated against *B. zonata* in two consecutive years (2006-2007). Treatments efficacy was studied in various months using the criterion of trapped *B. zonata* flies. ANOVA revealed a significant difference among various application methods in both fruit infestation rate and capture of *B. zonata* flies. So that impregnation of chipboard block in ME caused the lowest *B. zonata* infestation and the highest *B. zonata* capturing values in both years. Population fluctuation study revealed a major peak for *B. zonata* in September *i.e.*, mango ripening time. According to our results, it could be suggested that chipboard dispenser is the best application method as attractant in bait trap which exhibits more potent and longer lasting activity.

Keywords: *Bactrocera zonata*, methyl eugenol, malathion, dispenser, chipboard block

Introduction

Tephritid flies with over 500 genera and around 4000 species, attack many host plants from various families and cause serious losses in agricultural products by direct and indirect injuries (Rattanapun, 2009; Khalil *et al.*, 2010; Mosleh *et al.*, 2011; El-Gendy, 2012; Draz, 2016). *Bactrocera* as a main genus in Tephritid has a wide host range and several aggressive species like *Bactrocera zonata*. *B. zonata* is an extremely aggressive pest infesting more than 50 wild and agricultural host plant species (White and Elson-Hariss, 1992; Amin, 2003). It is a well-known pest of tropical and subtropical fruits which has been introduced from South and Southeast Asia to the currently distributed regions (Draz, 2016).

B. zonata can affect both fruit yield and quality (Shinwari *et al.*, 2015). The females insert their eggs into the fruit skin and the larvae emerge within 1.5 to 3 days. Larvae upon hatching start eating and caving on the

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fruit and might remain close together in feeding until nearly full grown. The larval period last 6 to 15 days depending on the temperature. By passing this period of time, the larvae leave fruits and preferably pupate in the soil. The pupal stage terminates in 6-19 days, depending on the temperature and finally adults emerge. The adult emergence occurs profusely early in the morning and more infrequently during cool weather (Shehata *et al.,* 2008).

Given that by the current management *B. zonata* may be in the process of undergoing resistance (Hawkes et al., 2005; Ahmad et al., 2010). Then excessive application of chemical pesticides may exacerbate this process. By considering high injury level of this pest, we should confess that practicing non-chemical control is inevitable. Various eco-friendly control approaches such as releasing sterile insect (Enkerlin *et al.,* 2003), cultural practices (Ali *et al.,* 2010) and male annihilation technique (Leblanc *et al.,* 2011) have been suggested to control *B. zonata* in which lure trap-mediated approaches have been considered substantially more practical. Methyl Eugenol (ME), as a main lure trap used either in solid or in liquid form, has been applied successfully solely or in combination with insecticides (Vargas *et al.,* 2000; Leblanc *et al.,* 2011). There are many documents dealing with comparison of various dispensers to determine superior traps. In the current study, we aimed: to investigate efficiency of different application methods of ME alone and in combination with an organophosphate insecticide (Malathion) against *B. zonata*. To study the efficacy of the traps in different application methods, both infestation rate and the number of captured flies have been taken into account as the two main criteria of control for this pest.

Materials and Methods

Six application methods of ME solely and in combination with Malathion (Ma) technical 95% Min were studied in a Randomized Complete Block design (RCB) experiment with five replications (each mango tree was a replicate) in a mango orchard in the Minab Agricultural Research Station. The treatments were including coded as A (Ma + Me trunk painting; MeMaTP), B (Ma + Me hanging impregnated jute bag; MaMeIJB), C (Bucket with Me impregnated wood block; BINB), D (Me trunk painting; MTP), E (Diluted Ma + Me in hanged bucket; DMM) and F (Control) described in Table 1 (also see Fig. 1). A chipboard block (with 6 × 2 × 2 cm dimensions) impregnated by dipping in ME, was used as dispenser in treatment C. It was suspended diagonally above a plastic bucket using metal wire in such a way as to avoid any contact with the plastic bucket and its content. Plastic buckets were filled with 2 liters of water and a detergent liquid (in the rate of 2% detergent liquid in 98% water). Plastic bucket and chipboard block were refreshed at 14 and 30 days interval, respectively, after installation. The other treatment dispensers (A, B, D and E) were refreshed at 10 days interval up to the end of experiment. In the control, only water was sprayed on trunk and main branches.

At the picking time, all fruits were checked precisely for *B. zonata* infestation by counting the number of black points on the infested fruits where the flies had laid eggs and the larvae caving in the fruit by cutting and feeding the fruit tissue. These data were used as the infestation criterion to evaluate the efficacy of the treatments. Statistical analyses was done by converting raw data to its respective percentage values for the infestation rate.

Efficacy of various ME applications in capturing the male flies was tested by analyzing split-plot data for the number of captured *B. zonata* males throughout a year. This data was also analyzed to manifest *B. zonata* fluctuations. All data were analyzed using SAS and mean comparison analysis was done using LSD test. The graphs were prepared using Excel.

Table 1 Description of different treatments according to the dispenser type and fluid mixture, methyl eugenol and malathion used in the traps.

Treatment code	Application method	MEM Proportion
A	MEM mixing spray on the tree trunk	7:7:86 for methyl eugenol, malathion and water, respectively
B	Application of MEM mixing by cotton sacks located inside tree canopy	7:7:86 for methyl eugenol, malathion and water, respectively
C	Dipping of chipboard block ($6 \times 2 \times 2$ cm dimensions) in ME	-
D	Spray of technical ME on tree trunk	-
E	Application of MEM solution inside a plastic bucket	6:6:88 for methyl eugenol, malathion and water, respectively
F (CONTROL)	-	-

MEM: mixture of methyl eugenol and malathion.

Figure 1 Various treatments of MEM application; a: Application of ME and MEM mixing on the mango trunk (treatments A and D); b: MEM mixing in water in plastic bucket (treatment E); c: Application of ME using a chipboard block as dispenser (treatment C) and d: Application of MEM using cotton sacks inside the mango canopy.

Results

By analyzing split-plot data, we found a significant variation in the number of captured *B. zonata* in different treatments ($F = 5355.1$; df = 4; $P < 0.01$) which varied in different months also ($F = 1023.7$; $P < 0.01$). There was also a significant interaction between treatments and the month of the year ($F = 1136.8$; $P < 0.01$) (Table 2). Mean comparison analysis revealed that the highest numbers of *B. zonata* flies were captured by treatments C followed by B and A treatments, respectively. Statistically, treatments D and A were categorized in the same group with no significant difference. The lowest number of captured *B. zonata* was recorded in treatment E (Fig. 2). Population fluctuations revealed an increase in *B. zonata* population starting from June and July *i.e.*, coinciding with the beginning of mango fruit ripening which continued until September and then decreased again. A major peak of *B. zonata* population occurred in September which coincided with the physiologically ripening of mango (Fig. 3).

The results of combined analysis showed that there was a significant interaction between year and treatment at 1% level in terms of mango infestation percentage ($F = 20$; $P < 0.01$) (Table 3).

Mean comparison of the infested mango percentage in the various ME or MEM application treatments revealed the lowest infestation rate in treatment C in 2006 followed by treatments D, B and A (with no significant difference) and treatment E, respectively. The highest infestation rate was observed in treatment F (control) which was to be expected (Table 4).

In 2007, except for treatments D and control with the highest percent of infested mango but no significant difference; the other treatments (A, B, C and E) ranked within one and the same group (Table 4). The ANOVA also demonstrated significant variation of the treatments by year ($F = 101.8$; $P < 0.01$).

Table 2 Split plot analysis of trapping *Bactrocera zonata* by various application methods in the different months.

Source of variation	df	Sum of square	Mean square	F	C.V
Rep. (R)	4	67458.4	16864.6	1.14^{ns}	
Treatment (A)	4	316981278.2	79245319.5	5355.1**	
Error a	16	236769.2	14798.1	-	
Time (B)	11	202679543.5	18425413.0	1023.7**	20.6%
A × B	44	900316545.5	20461739.7	1136.8**	
R × B	44	803081.7	18251.9	1.01^{ns}	
Error b	176	3167757.0	17999.0	-	
Total	299	1424252433.5	-	-	

** significant at 1%; ^{ns} non-significant.

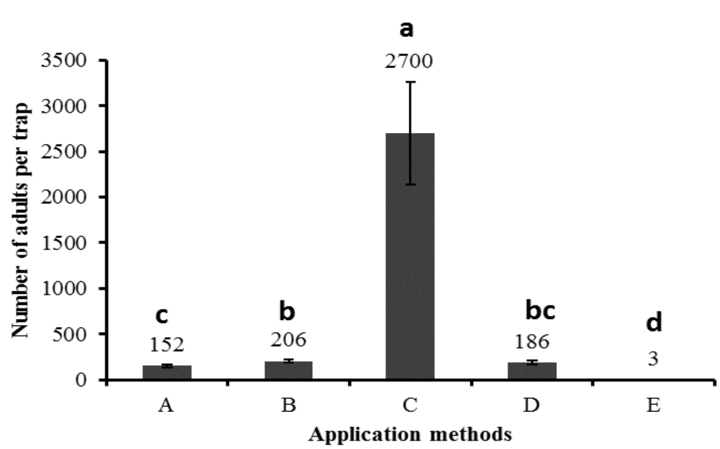

Figure 2 Mean comparison of the captured *Bactrocera zonata* in the various application methods in 2006. For the methods refer to table 1. Means followed by the same letters are not significantly different (LSD test, $P < 0.05$).

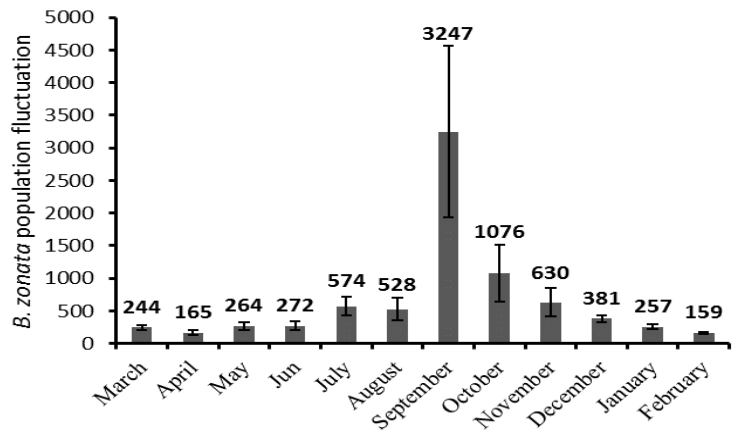

Figure 3 *Bactrocera zonata* population fluctuation in the various months of 2006, showing population peak in September.

Table 3 Combined analysis of the infested *Bactrocera zonata* percentage in the various MEM application methods.

Source of variation	df	Sum of square	Mean square	F	C.V
Block	4	26.5	6.6	0.6ns	
Year	1	1089.6	1089.6	101.8**	
Error	4	42.8	10.7	-	
Treatment	5	2144.5	428.9	64.5*	28.4%
Y × T	5	666.6	133.3	20.0**	
b	40	266.0	6.6	-	
Total	59	4236.0	-		

MEM: mixture of methyl eugenol and malathion.

Table 4 Comparison of the percentage of infested mango to *Bactrocera zonata* in various ME or MEM application methods in 2006 and 2007.

Application methods[1]	Infested mango (%)	
	Year (2006)[2]	Year (2007)[2]
A	11.60c	1.12b
B	9.41c	1.15b
C	3.96d	1.83b
D	9.40c	10.97a
E	15.90b	1.19b
F	30.21a	12.55a

MEM: mixture of methyl eugenol and malathion, ME: MEM: methyl eugenol.

[1.] For the methods refer to Table 1.

[2.] Means followed by the same letters in each columns are not significantly different (LSD test, $P < 0.05$).

Discussion

ME is a highly potent male-kairomone lure for many Bactrocera species which has been used extensively in many male annihilation programs (Ghanim, 2014). It was used either solely or in combination with different insecticides in different traps (Vargas *et al.*, 2000; Leblanc *et al.*, 2011). In the current study, we investigated various ME application methods to determine the most effective approaches in controlling *B. zonata*.

The ANOVA revealed significant variation among MEM application methods in both years of study. Based on mean comparison of the infested fruit percentage, treatments C and F (control), with 3.96 and 30.21 infested mango percentage, showed the lowest and the highest infestation rate, respectively, in 2006. The lowest percent of infested fruit for treatment C (1.83) was also confirmed in 2007 and supported high efficiency of treatment C in controlling *B. zonata* invasion which can be stemming from its dispenser type. We used a chipboard block as ME dispenser in treatment C which allows release of ME incrementally with long lasting effect and decreasing number of refreshing times due to fading adverse impact of weather. Role of dispenser type on increasing ME efficacy and decreasing weather impact has been evidenced by Vargas *et al.* (2000) and Leblanc *et al.* (2011). In addition, our results are in line with Sadeghi (2011) outcomes in which chipboard block dispenser decreased successfully *B. zonata* infestation rate of the fruits in mango orchards.

What we observed for lower infestation rate to *B. zonata* in the second year by mean comparison in all treatments can be reasonably argued that declining of the pest population in the first year might have resulted in its low infestation rate in the second year. There are many instances dealing with decreasing of a pest injury level due to pest control practices continuously in consecutive years (Chowanski *et al.*, 2016; Zuhara *et al.*, 2016). The alternate bearing nature of mango (Sharma *et al.*, 2001; Sharma *et al.*, 2015) is another factor which could have influenced *B. zonata* population density and brought about the lower infestation rate. However, more research on this issue needs to be undertaken. The most striking results to emerge from this data is a strong and stable effect of treatment C in the two consecutive years. Apart from this strong and repeated result, grouping of the three treatments, B, A and D in the same category in 2006 and their separation in 2007, is a fluctuation in results which can be explained to some extent. First, discrepancy in the results of close treatments of pest control methods when the evaluation criteria are infestation rate or pest population may change by the climate variation in the consecutive years and this is the reason why it is required to repeat such experiments at least in two consecutive years (Eriksson, 2008). Second, population of the fruit pest are closely related to the number of fruit on the tree, then alternate bearing is a powerful factor to cause fluctuation in the results for such experiments.

We found also significant variation in the number of captured *B. zonata* flies in different ME and MEM application methods. The number of *B. zonata* capturing was also significantly different in different months too. Mean comparison studies showed that the most *B. zonata* trapping was taken in treatment C. The above-mentioned result for high efficacy of treatment C also is demonstrated here by more flies trapped in this treatment. The most *B. zonata* trapping occurred in September which is an expected result due to its synchronization with mango ripening time. Vargas *et al.* (2000) findings revealed variation among ME lure traps and seasons which is supported the current results.

In the current study, the mango fruits were checked both symptomatically (by inspecting fruit skin for black spots dealing with larvae feeding channel) and anatomically (by cutting suspected fruits and checking *B. zonata* infestation). Only reliance on symptomatically checking may fail to realize *B. zonata* infestation in the primary infestation stages in which the fly egg-laying symptoms are not distinct or too small to recognize correctly. By considering that infested fruits are susceptible for infecting by secondary pathogens, this checking approach is also necessary to perceive for suspected mango fruits packed for transporting and marketing. Because these symptomless infested fruits can induce infection in the marketing boxes and should be discarded.

Conclusion and Suggestions

Results of the current study revealed high efficacy of the chipboard block by capturing more flies and reducing *B. zonata* infestation rate in the fruits. Using chipboard block as dispenser in lure traps demonstrated also a long lasting effect which culminated in increasing refreshing time interval and finally reducing the control costs. However, for a sustainable control program of *B. zonata*, a comprehensive scheme need to be made in which cultural, mechanical and chemical approaches are perceived. Use of resistant mango cultivars and other baited lure traps like protein hydrolysate are the other fruitful approaches which can be involved against *B. zonata* in an integrated pest management. From the horticultural point of view, it is necessary to avoid mango intercropping with susceptible species such as ziziphus. Furthermore, volunteer ziziphus plants in the mango plantation should be also removed from the orchard.

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کارایی تله‌های مختلف متیل اوژینول در کنترل مگس میوه انبه
Bactrocera zonata (Diptera: Tephritidae)

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چکیده: مگس میوه انبه *Bactrocera zonata* (Saunders) (Diptera: Tephritidae) یکی از آفات بسیار مهم درختان میوه و صیفی‌جات می‌باشد که تاکنون موزع‌های مختلفی برای کنترل آن مورد استفاده قرار گرفته است. در این پژوهش کارایی موزع‌های مختلف متیل اوژینول به‌همراه سم مالاتیون روی کنترل مگس میوه انبه طی سال‌های ۱۳۸۵ و ۱۳۸۶ مورد بررسی قرار گرفت. هم‌چنین کارایی این موزع‌ها از نظر شکار مگس میوه در ماه‌های مختلف بررسی شد. نتایج به‌دست آمده نشان داد که تیمارهای مختلف هم از نظر کنترل مگس میوه انبه و هم از نظر شکار آن دارای اختلاف معنی‌داری با یک‌دیگر بودند. به‌طور کلی نتایج نشان داد که کاربرد متیل اوژینول با استفاده از قطعه نئوپان دارای کم‌ترین آلودگی به مگس میوه و بیش‌ترین شکار این آفت در هر دو سال انجام آزمایش می‌باشد. بررسی تغییرات جمعیت آفت نشان داد که این مگس دارای یک اوج جمعیتی در شهریور ماه یعنی زمان رسیدگی فیزیولوژیکی میوه انبه می‌باشد. براساس نتایج به‌دست آمده از این پژوهش می‌توان دریافت که روش غنی‌سازی نئوپان با متیل اوژینول در تله‌ها به‌خاطر کارایی بالاتر و دوام طولانی‌تر بهترین روش در کنترل مگس میوه انبه بوده است.

واژگان کلیدی: *Bactrocera zonata*، متیل اوژینول، مالاتیون، موزع، موزع نئوپان