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Evaluation of Solid Lure Plugs and Insecticide Dispensers on Capturing Dacine Fruit Flies and Non-target Insects

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ABSTRACT

The present study investigated the efficacy of solid male lures *viz.*, cuelure (C-L), methyl eugenol (ME) and tri-med lure (TML) formulated with insecticide for the capture of Tephritid fruit flies in three different green areas of Bangladesh. We also evaluated non-target attraction effects to traps baited with these male lures. Traps were placed at nine locations in each of three experimental fields of Savar, Dhaka, Bangladesh during May-September, 2015. The experimental areas were i. Atomic Energy Research Establishment (AERE) colony, ii. AERE office campus, and iii. Jahangirnagar University (JU) campus, comprising agricultural fields, backyard gardens and mixed plantation. The flies were collected at weekly interval over 18 weeks. Total capture of *Zeugodacus cucurbitae* (Coq.), *Bactrocera dorsalis* (Hendel), *Zeugodacus tau* (Walker), and *Bactrocera zonata* (Saunders) were determined. The number of non-target insects attracted to different lure baited traps was also recorded. The prevalence of Dacine fruit flies was significantly higher at the JU campus comprising 98.41% *B. dorsalis* (538.05±62.28 fly/trap/week (FTW)) captured by ME. The comparatively higher number of *Z. cucurbitae,* and Z. *tau* trapped by C-L from AERE office campus, and JU campus, respectively. No *Bactrocera s*pp. was attracted to the TML. Saprophagous non-targets mostly Diptera, *Drosophilla*, Milichiidae, Hymenoptea (black ants) were abundant in traps baited with C-L and ME. It was revealed that the response to lures was species-specific. Tested solid lures and DDVP strips did not exert any detrimental effects on non-target beneficial insects and were found effective for mass-trapping of Dacine fruit flies.

Keywords: Solid lures, Insecticide strips, Dacine, Tephritid fruit fly, Capture, Non-target insects.

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INTRODUCTION

The Dacini fruit flies within Tephritidae (Diptera: Tephritidae) are mainly florivorous or frugivorous and approximately 10 percent of the 932 recently recognized species are pests of various vegetables and fruits [1, 2]. The genus includes various highly invasive and/or serious polyphagous pest species *viz.*, the melon fly, *Zeugodacus cucurbitae* (Coquillett), the pumpkin fruit fly, *Zeugodacus tau* (Walker), *Bactrocera dorsalis sensustricto* (Hendel), the oriental fruit fly, the peach fruit fly, *Bactrocera zonata* (Saunders) and many others. *Ceratitis* is also a genus of Tephritidae having around 65 species found in tropical and South Africa with many pest species. The Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) which has spread to almost all warm temperate and tropical areas worldwide and captured using food baited traps in Hawaii and also traps baited with different amount of trimedlure (TML) [3, 4]. From an economic perspective, different fruit fly species of these genera: i. inflict direct and extensive damage to fleshy vegetables and fruits, ii. cause quarantine restrictions on infested areas, iii. need commercial fruits to undergo postharvest and protective treatment before export, and iv. provide a breeding reservoir for their introduction into other parts of the world [5]. Recently, the alarming invasion of these insects has been increased due to the increased human travel and global trade worldwide. These insects have been suppressed and even eradicated through the area-wide utilization of male lures. In addition to detection programs, the male lures also have been used to control or suppress through the male annihilation technique (MAT) [6-9]. The most commonly used Tephritids male lures for detection are TML (tert-butyl 4- and 5-chloro-cis- and trans-2-methylcyclohexane-1-carboxylate), raspberry ketone (RK) (4-(p-hydroxyphenyl)-2-butanone), Cue-lure (C-L) (4-(p-acetoxyphenyl)-2-butanone), and Methyl eugenol (ME) (4-allyl-1, 2-dimethoxybenzene-carboxylate). These are powerful male-specific lures. Males of above mentioned *Bactrocera* fruit flies are attracted to either C-L/RK or ME. TML is known to attract numerous male *Ceratitis* species (e.g., *C. capitata* and *C. rosa* Karsch) and is a mixture of eight isomers. ME is a widely distributed natural plant product and is found in >200 plant species in 32 families mainly found in the tropics. C-L has not been isolated as a natural product but it is rapidly hydrolyzed and forms RK, a very effective lure for Z. *cucurbitae*. An investigation recently reported that the CL hydrolysis is negligible and it remains intact in the atmosphere in the time-frame of the compound acting like a fruit fly lure [10]. Moreover, C-L was recently discovered in 2 daciniphilous flowers- *Bulbophyllum hortorum* [11, 12] and *Passsiflora maliformis* L. [13]. However, RK was isolated originally from *Dendrobium superbum* Rchb. F. A novel fluorinated ana-log of raspberry ketone, raspberry ketone trifluoroacetate (RKTA) found to attract significantly more Q-flies, *Bactrocera tryoni* (Froggatt) than cuelure or melolure [14, 15]. However, of the 54 Dacini species (comprised of the 2 main genera *Dacus* F. and *Bactrocera* Macquart) that are agricultural pests, 16 respond to ME and 26 to C-L/RK.

In fruit fly suppression and detection programs, various types of traps were used baited with these male lures (plus a toxicant) usually in liquid form. Some of the common traps used for detection with C-L and ME are bucket, Champ, Jackson, and Steiner traps [16]. A surveillance reported on non-target insects captured in tephritid fruit fly traps in South Korea and also a novel dispensing system for male lures used to detect invasive fruit flies [17, 18]. MAT carriers including molded paper fiber, Min-U-Gel, cotton wicks, and fiberboard blocks are commonly used in different countries. For instance, fiberboard blocks impregnated with ME and different organophosphate insecticides including naled and malathion were utilized to eliminate *B. dorsalis* from Okinawa, and papaya fruit fly, *Bactrocera papaya* Drew and Hancock from Australia. Usually, the liquid lures have been a mixture of ME or C-L and liquid insecticides *viz.*, nailed or malathion, placed on a cotton wick. These involve significant handling to measure and apply the liquids, and also potential health risks due to pesticide exposure [6, 19-21]. However, eventually, there is progressing toward replacement of liquid C-L and ME and insecticides with solid formulations (such as C-L plugs or Scentry ME cones, North Bend, WA, ME wafers, Farma Tech (FT), Boseman, MT) [3, 22, 23] and with solid lure/insecticide (such as DDVP) combinations [9], which proved convenience and safe for workers. Again, it was revealed that traps lacking an insecticide and containing a male lure generally captured fewer *Z. cucurbitae* or *B. dorsalis* males compared to those containing a naled plus lure or a separate DDVP strip [19]. It was also demonstrated that the presentation of a male lure plus spinosad, a low-risk pesticide, did not increase the effectiveness of the trap more than what was observed for traps with no insecticide. There is not any suitable alternative to organophosphate insecticides, and fruit fly surveillance programs continue to use them to retain insects in the traps [4, 17, 24, 25]. 'Given this constraint, it was recommended that pre-packaged DDVP strips, which are safer and easier to handle than lure-naled solutions, can be as effective as these solutions in detecting infestations or monitoring Tephritids populations. The HAWPM (Hawaii Fruit Fly Area-Wide Pest Management Program) (2000-2009) program effectively-researched, developed, and registered novel fruit fly monitoring and control technologies (IPM package, *i.e.*, (i) monitoring, (ii) field sanitation,

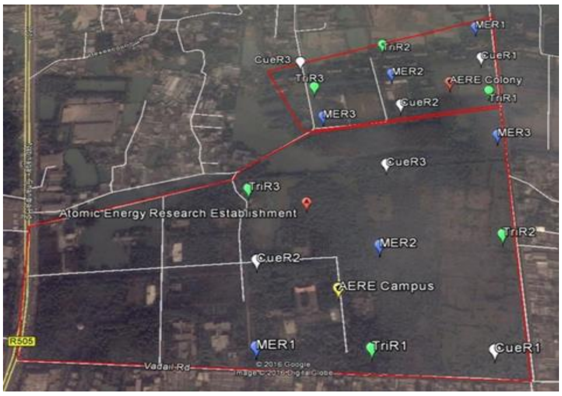
(iii) protein bait sprays, (iv) MAT, (v) augmentative parasitoid releases, and (vi) sterile insect releases) [22]. The HAWPM has set one of the best examples of using traps baited with solid dispensers of male lures in MAT and monitoring of *Bactrocera* fruit flies. However, there has been much concern about the possible non-target effects of such lures on beneficial insects. The use of male lures for fruit fly control may impact non-target insects or risk possible extinction of small endemic populations in large-scale fruit fly eradication programs [26].

In Bangladesh, a new species and 33 new country records for Tephritid fruit flies were reported [27-30]. Four species in particular, *B. zonata*, *B. dorsalis, Z. tau,* and *Z. cucurbitae* - inflict serious damage to fruits and fleshy vegetables production in Bangladesh. Recently, the pheromone traps have gained popularity and become a vital tool for pest monitoring in a wide range of crops in Bangladesh. The design of the pheromone trap [31, 32], its placement, and the ratio of the chemical components are the factors influencing the number of insect capture [33]. The formulation of different lures, use of novel lures [34-41], combination of lures and traps [42, 43] are also considered as critical issues for the capture of pestiferous fruit flies. There was also scanty of literature on the use of solid formulation of male lures and the impact of these lure baited traps on non-target and beneficial insects in Bangladesh. The present study, therefore, has been undertaken to determine the efficiency of three solid single lure plugs (ME, C-L, and TML) in conjunction with insecticidal strips (DDVP) baited traps on the capture of four economically important Dacine fruit flies in Bangladesh. We also evaluated non-target attraction effects to traps baited with these lures.

MATERIALS AND METHODS

Study sites

In the present study the capture of two cucurbit pests *Z. cucurbitae, Z. tau*, and the fruit pests *B. dorsalis* and *B. zonata* were recorded at three green areas of Bangladesh during May-September, 2015. The experimental areas **(Figures 1a and 1b)** were: i. Atomic Energy Research Establishment (AERE) colony, Savar, Dhaka (8.64 ha) 23°57'35.60"N, 90°16'54.02"E, ii. AERE office campus, Savar, Dhaka (112.276 ha), 23°57'14.62"N, 90°16'44.79"E), and iii. Jahangirnagar University (JU) campus (214.62 ha) 23°52'8.85"N, 90°16'1.50"E) with mean monthly rainfall 394.5 mm, (minimum 185mm, maximum 623mm), mean monthly temperature 29.17 °C (minimum 25.7°C, maximum 31.8°C), and mean monthly relative humidity 77% (minimum 71%, maximum 81%). These areas mainly comprised of agricultural fields, backyard gardens, and mixed plantation with a diversity of vegetables and fruit trees planted, including jack fruit (*Artocarpu sheterophyllus* Lam.), guava (*Psidium guajava* L.), mango (*Mangifera indica* L.), and Oranges (*Citrus*), Star fruit (*Carambola* or *Averrhoa*), banana (*Musa*) and also various vegetable hosts including melon (*Cucumis*), pumpkin (*Cucurbita*), Brinjal (*Solanum melongena*), chili peppers (*Capsicum*), *etc.* along with other non-host trees. The three experimental areas reflect typical of existence fruit and vegetable production and are commonly infested with Dacine fruit flies across much of Bangladesh.



a)

b)

Figure 1. a) Traps locations at AERE colony (23°57'35.60"N, 90°16'54.02"E), AERE office campus (23°57'14.62"N, 90°16'44.79"E). b) JU campus (23°52'8.85"N,90°16'1.50"E), (average temperature 30˚C and precipi. 55mm-601mm).

Three different solid single lure plugs: i. C-L ii. ME and iii. TML (Scentry Biologicals, Billings, Montana, USDA-APHIS-PPQ) and DDVP strips (10% dichlorvos (2,2-dichlorovynil dimethyl phosphate) (Vapertape® II, Hercon Environmental, Emingsville, Pennsylvania, USA) were used in traps. Traps baited with three different lures were placed at nine locations in each of the three experimental fields and were hung in tree branches about 1.5m above the ground in shaded areas using a metal hanger. Traps were made of a plastic container (1/2 ltr) with two round holes (10 mm) near the top of the container, to allow fly entry. The flies were collected at a weekly interval over 18 weeks. Traps were emptied once every week and all flies and non-target Arthropods captured were transported to the laboratory of Insect Biotechnology Division (IBD), AERE in plastic bags for counts. Weekly captured flies of the *Zeugodacus/ Bactrocera* spp. were identified to species level and recorded on MS Excel spreadsheet. To compensate for position effects, traps within an area were rotated clockwise after each week. Daily rainfall and temperature data for Dhaka were collected from the Bangladesh Meteorological Department, Agargaon, Dhaka, Bangladesh.

Statistical analysis

Compiled data were subjected to ANOVA (Analysis of Variance) using statistical Software Mini-Tab, USA (version-2017). The treatment means were compared using the Tukey HSD Test at P=0.05 probability level.

RESULTS AND DISCUSSION

Five Dacine fruit flies (Z. *cucurbitae*, *B. dorsalis*, Z. *tau*, *B. zonata*, and *B. nigrofemoralis* (White & Tsuruta)), and one *Dacaus* species (*Dacus longicornis* (Widemann) were captured during the trapping experiment conducted at three green areas of Savar, Dhaka, Bangladesh. The prevalence of Dacine fruit flies was significantly higher at the JU campus comprising 89.56% *B. dorsalis* (mean 1614.1±14.9 fly over 18 weeks)/ (538.05±62.28fly/trap/week (FTW)) captured by ME baited traps. The comparatively higher number of *Z. cucurbitae* (138.0±21.83 FTW) and Z. *tau* (35.11±7.13 FTW) trapped by C-L from AERE office campus **(Figure 3)** and JU campus **(Figure 4)**, respectively. No *Bactrocera* or *Zeugodacus s*pp. was attracted to TML indicated the absence of *Ceratitis* or *Anastrepha* spp. at the experimental fields during the trial.

Z. cucurbitae captured from different campuses differed significantly (df=2, 51; F=6.12; P=0.004). Although the capture of *Z. cucurbitae* in the AERE office campus (138.0±21.83 FTW), and JU campus (122.44±13.53 FTW) did not differ significantly. But with the lowest capture of *Z. cucurbitae* (62.25±11.20 FTW) at the AERE colony differed statistically from the capture of two other campuses. The capture of *Z. tau* (35.11±7.13FTW) was significantly higher (df=2, 51; F=13.64; P=0.000) in JU campus. However, the fly capture was only 7.83±1.86 FTW and 6.30±1.82 FTW, respectively at the AERE office campus and AERE colony, and did not differ significantly. Significantly highest capture of *B. dorsalis* (538.05±62.28 FTW) was recorded on the JU campus (df=2, 51; F=33.32; P=0.000). The capture was 204.69±37.07 FTW, and 64.00±9.20 FTW at AERE office campus and AERE colony, respectively during the experimental time and significantly differed from *B. dorsalis* capture of JU campus. The capture of *B. zonata* was remarkably low from all three experimental sites and did not differ statistically (d=2, 51; F=2.89; P=0.065). The mean capture ranges from 0.77±0.37 to 4.06±1.28 FTW.

On the other hand, the comparative capture of four *Bactrocera* and *Zeugodacus* spp. at AERE campus differed significantly (d=3, 68; F=21.27; P=0.000). The higher number of *B. dorsalis* (204.7±157.2 FTW) captured followed by *Z. cucurbitae* (138.1±92.6 FTW), *Z. tau* (7.83±7.9 FTW), and lowest *B. zonata* (4.06±5.4 FTW). In the AERE colony the capture of four *Bactrocera* and *Zeogodacus* spp. also differed significantly (d=3, 68; F=20.80; P=0.000) **(Figure 1)**. The higher number of *B. dorsalis* (64.00±39.07 FTW) was captured here followed by *Z. cucurbitae* (62.3±47.6 FTW), *Z. tau* (6.31±7.7 FTW), and lowest *B. zonata* (4.0±5.7). Significantly higher capture of *B. dorsalis* (538.05±62.28 FTW) (d=3, 68; F=59.80; P=0.000) was recorded from the JU campus followed by *Z. cucurbitae* (122.4±57.2 FTW), *Z. tau* (35.11±30.28 FTW). The capture of *B. zonata* (0.7±1.5 FTW) was significantly lowest among four spp. Total 132, 1304, and 21 non-target insects were captured in C-L, ME, and TML baited traps from AERE colony, AERE office campus, and JU campus, respectively **(Table 1)**. The non-target insects *viz.*, Drosophilidae, Hymenoptea (black ants), Milichiidae, Muscidae, were abundant in traps baited with ME and C-L and mostly attracted to decaying fruit flies in the trap. Control traps hardly capture non-target insects. Scavengers are the non-target species in the most commonly captured families.

Table 1. The capture of non-target insects in traps baited with ME, C-L and TML along with decaying fruit flies compared with control traps placed at three experimental fields of Savar area during May-September, 2015.

Experimental fields	Order/Family/Genus/Species	Mean (±se) number of non-target insects captured in trap/week			
		Cue-lure (C-L)	Methyl- eugenol (ME)	Trimed-lure (TML)	Control
AERE Colony	Coleoptera (beetles)	-	0.16 ±0.1	-	-
	Drosophilidae	0.3±0.1	0.27±0.6	0.11 ±0.1	-
	Hymenoptera (black ants)	1.66±0.7	1.16 ±2.5	-	0.11±0.1
	Lonchaeidae	-	0.11±0	-	-
	Milichiidae	0.11±0.1	0.96 ±0	0.16±0.2	-
	Muscidae (*Atherigona*)	0.55±1.1	0.33 ±0.5	-	-
	Platystomatidae (*Agadasys hexablepharis*)	0.01±0 .1	-	-	
AERE Office Campus	Arachnids (jumping spiders- Salticidae)	0.33 ±0.2	0.05 ±0.1	-	-
	Braconidae	-	0.1±0.1	-	-
	Coleoptera (beetles)	-	0.01±0.2	-	-
	Drosophilidae	14.0±33.0	21.6±47.2	-	-
	Hymenoptera (Pompilidae, weaver ants- Oecophylla, black ants)	2.94±1.7	7.6±7.8	0.55±0.1	-
	Lepidoptera (moths)				-
	Orthoptera (grasshoppers)	-	0.16±0.5	-	0.2±0.1
	Milichiidae	10.33±14.6	4.2 ±11.0	-	-
	Muscidae (*Atherigona*)	1.4±1.8	1.6±0.9	-	-
	Platystomatidae	1.5±2.0	0.6±1.5	-	-
	(*Agadasys hexablepharis*)		0.01±0.1	-	-
	Sarcophagidae	0.01±0.1	-	-	-
JU campus	Arachnids (jumping spiders-Salticidae)	0.16±0 .1	0.22±0	-	-
	Bugs	0.72±0.4	0.16±0	-	
	Coleoptera (beetles)	0.55±0.1	-	-	-
	Drosophilidae	-	0.60±0.1	-	-
	Hymenoptera (black ants)	3.0±0.7	0.66±0.2	-	-
	Lepidoptera (moths)	0.16 ± 0.5	3.22 ±2.1	-	-
	Milichiidae	5.16±1.6	3.16±0.5	-	-
	Muscidae	0.83±0.8	-	0.11±0.1	0.10±0.1
	Platystomatidae (*Agadasys hexablepharis*)	1.5±2.0	2.94±3.6	-	-
					-

In the present study, the highest number of *B. dorsalis* was captured than *Z. cucurbitae*, *Z. tau,* and *B. zonata* using solid lure plugs of ME, and C-L baited traps at AERE office campus, which has partial similarities with Hossain *et. al*. 2019 [44] findings who reported the presence of polyphagous fruit fly pest dominated mainly by *B. dorsalis* (58.0%), followed by *Z. cucurbitae* (23.6%) and *Z. tau* (13.5%), and non-pest *B. rubigina* (3.6%) using traps baited with solid lure plugs of ME, C-L and zingerone during their two years survey at AERE office campus. Our experiment with solid lure plugs of ME, C-L and TML baited traps over 18 weeks also revealed that the overall Dacine fruit fly capture was higher in JU campus than AERE office campus and AERE colony **(Figures 2-4)**. However, the parahormone lure stick commonly used by fruits and vegetable growers of Bangladesh consists of a small cotton wick/rope impregnated with 2 ml of lure (Safe Agriculture Bangladesh Ltd. (SABL) and a cotton ball was placed inside each trap soaked with 4% sevin-solution (contact poison of ACI. Limited, Bangladesh) to trap and kill the flies. An experiment conducted at a mango orchard in

Chapai Nawabganj revealed that traps baited with a solid single lure plug of ME (Scentry Biologicals, Billings, Montana, USDA APHIS-PPQ) captured a comparatively higher number of *B. dosalis* and *B. zonata* than traps baited with commercially available ME impregnated cotton rope/wick plug (Ispahani Co. Ltd., Bangladesh) (unpublished data).The solid lure plugs of ME also used to study the population fluctuation of male *B. dorsalis* and to reveal the abundance of peach fruit fly, *B. zonata* in mango orchards [45, 46]. Field studies shown that traps baited with solid dispensers of male lures and liquid lures catch an almost similar number of *Bactrocera* males [20, 24]. Although most of these studies [17, 20, 25] support the adoption of an alternative delivery system of lures, still the solid dispensers or lure impregnated cotton wick/rope tested for trapping invariably need to use an insecticide (either nailed or DDVP or any contact poison) together with the male lure.

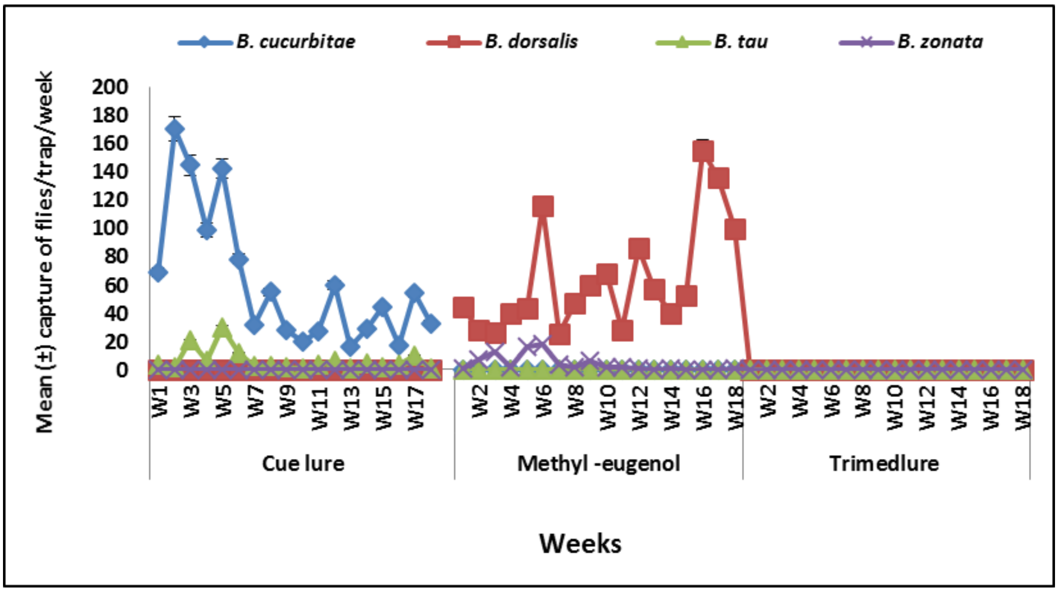


Figure 2. Mean (±se) weekly capture of *B. zonata*, *B. dorsalis*, *Z. tau*, and *Z. cucurbitae* using three solid lure plugs (C-L, ME, and TML) and insecticide strips baited traps at AERE colony, May-September, 2015.

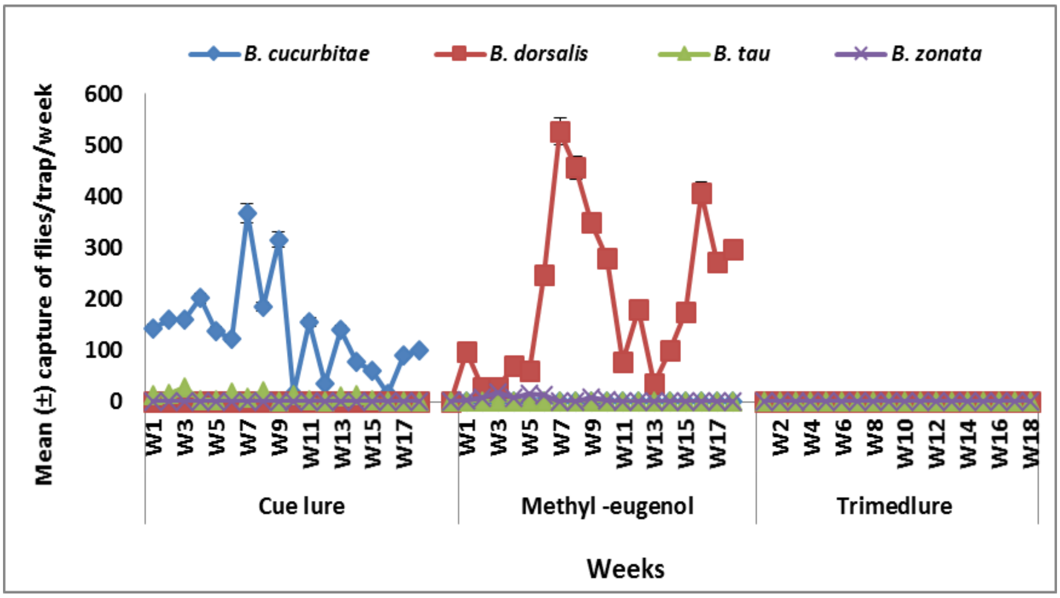


Figure 3. Mean (±se) weekly capture of *B. zonata*, *B. dorsalis*, *Z. tau*, and *Z. cucurbitae* captured per week using three solid lure plugs (C-L, ME, and TML) and insecticide strips baited traps at AERE office campus, May-September, 2015.

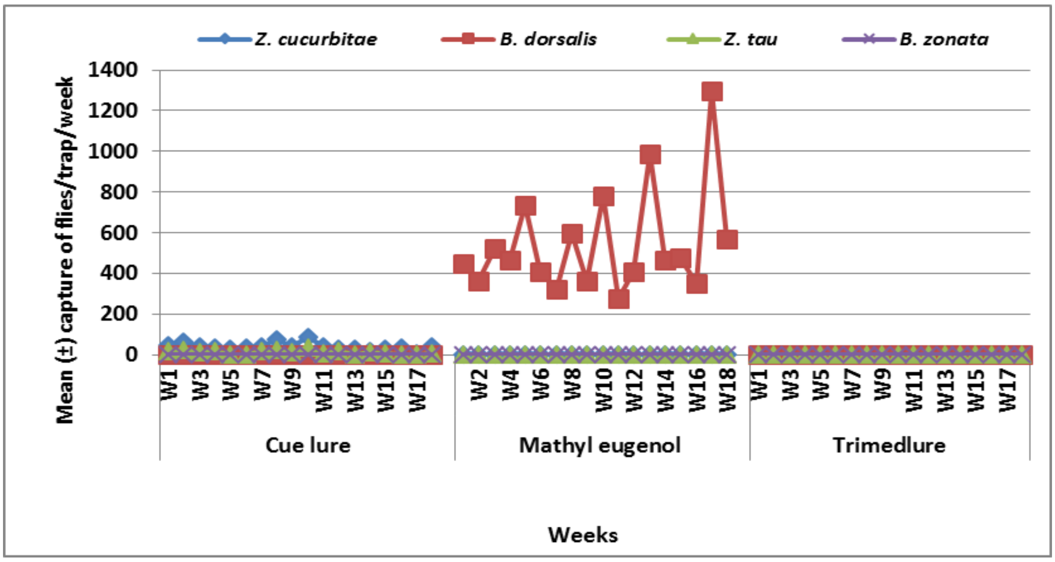


Figure 4. Mean (±se) weekly capture of *B. zonata*, *B. dorsalis*, *Z. tau*, and *Z. cucurbitae* captured per week using three solid lure plugs (C-L, ME and TML) and insecticide strips baited traps at JU campus, May-September, 2015.

The present findings on capture of non-target insects in different lure baited traps have similarities with Leblanc *et al.* 2010a; 2010b [47, 48] reported capture of a broad diversity of non-target insects, dominated by the Drosophilidae, Ceratopogonidae, Cecidomyiidae, Chloropidae, Calliphoridae, Neriidae, Muscidae, Sarcophagidae, and Corylophidae while comparing traps baited with multi lure *i.e.*, three different food attractants for Tephritid fruit flies in Hawaii. The present findings also agree with other reports published on scavenger attraction to food lures [26, 47, 48] and decaying fruit flies in traps baited with male lure [49]. However, the capture rate of non-targeted insects was much lower than the above-mentioned studies due to the use of synthetic lures rather than food lures. The study was conducted over a comparatively short period (18 weeks, May-September, 2015), and, so, the results apply only to the weather conditions and host availability during the period of the particular year *i.e.*, 2015. Results may vary in the autumn and cooler winter months.

CONCLUSION

This work suggested that the effect of the solid lure plugs on Dacine fruit fly species were usually species-specific. Solid lures and DDVP insecticide strips were found convenient in handling and effective for mass-trapping of Dacine fruit flies and did not exert much detrimental effect on non-target beneficial insects. Non-target insects were not only attracted to lures baited traps but also to randomly capture decaying *Bactrocera* species. The negative non-target impact of male lures is likely to be minimal. Further investigation should be focused on the use of novel lure matrix using natural products, as well as the formulation of lures, and find much safer alternative insecticide on the capture of Dacine fruit fly species. These will eventually help to use in detection and MAT in conjunction with protein bait sprays, sanitation, and environmentally friendly technique like the Sterile Insect Technique in Area-Wide Integrated Fruit Fly Management Program (AW-IFFMP) in Bangladesh.

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CONFLICT OF INTEREST: The authors declare that they have no conflict of interests.

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ETHICS STATEMENT: All the procedures performed in this experiment involving human participants were subjected to the ethical standards approved by the local ethics committee of Bangladesh.

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