Journal of Life Sciences 13 (2019) 18-24
doi: 10.17265/1934-7391/2019.02.003

Efficient Protein-based Bait Formulation for Attraction and Feeding Response of Fruit Flies (Diptera: Tephritidae) in Myanmar

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Abstract: Protein baitsprays were produced from many different sources for the controlling fruit flies in the integrated pest management program around the world. Here, fruit fly baits were formulated based on cost-effective bait mixtures such as local jaggery, brewers spent grain (BSG), apple cider vinegar, wheat bran powder and local red wine with the fresh enzyme from papaya and pineapple. Fruit fly Baits were prepared by mixing the small amount of local detergent as insecticide. Our results indicated that the best efficient fruit fly bait for area wide integrated pest management (AW-IPM) is the BSG-based bait whereas the second efficient bait is local red wine-based bait. The highest fruit fly per trap per day (FTD) value by BSG-based bait traps was discovered. The highest female captured percentage is BSG-based bait with papaya solution. The field analysis of one week and one month old protein-based bait showed that FTD values decreased slightly while only one month old wheat bran-based bait dropped to the base. The BSG-based baits analysis is effective not only female fruit flies but also cockroaches male in Myanmar's tropical region. Thus our results reveal that the efficient protein-based bait is the fresh BSG-based bait with papaya solution.

Key words: Protein-based bait, brewers spent grain, trapping management, fruit flies, cockroach.

1. Introduction

Fruit fly is the one of the most destructive pest with higher infestation percent in Myanmar's tropical region and the dominant of oriental fruit fly, *B. dorsalis* among the other fruit flies is 57% [1]. The analysis of AW-IPM programme with the combination sterile insecticide technique, male annihilation technique, protein-bait spray technique, field sanitation technique and fruit bagging management show the effectiveness with significally decreasing the infestation of fruit flies in mango orchard, Kenya [2].

BSG extracts can be used as functional foods and dietary supplements as rich source of free amino acids and peptides [3]. The spent hops from the beer byproduct can be used as low-cost, eco-friendly insect pest repellents in the protection of stored foods as an excellent source of EO and chemicals [4]. New value-added products were developed from BSG for animal food supplement and also stimulated the researcher to explore for further experiments [5]. Protein-starved fruit flies, *B. dorsalis* were much more likely to feed on protein compared to protein-fed fruit flies [6].

The formulation of bait containing spinosad which has low toxicity show the higher mortality compared without spinosad baits [7]. By-product of the large industrial breweries containing BSG has no disposal options and it can be used in other option such as agricultural, livestock etc. [8]. Bait spray application control method cannot stand-alone for effective suppression of fruit flies but it is an effective method in integrated pest management (IPM) system [9]. One

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of the cost-effective fruit fly control system include the bait-spray applications using treated yeasts with the different assessments [10].

The research was focused on the efficient formulation of protein-based baits for the fruit fly controlling program in Myanmar's tropical region. 10 different baits were performed to select the best efficient bait and evaluated the assessment of fruit flies in festation. The aim of the research is to produce the best bait to control the target female for the AW-IPM approach. BSG-based bait, jaggery-based bait, local red wine-based bait, apple cider vinegar-based bait and wheat bran-based bait were analyzed with fresh pineapple and papaya solution.

2. Materials and Methods

2.1 Sample Collection and BSG Maintenance

BSG were obtained from local brewery industry at Mandalay, Myanmar. Local red wine, jaggery, apple cider vinegar and wheat bran were purchased from local markets. The samples were brought to laboratory, Biotechnology Research Department, Ministry of Education, Kyauk-se, Myanmar.

The fresh BSG which is high humidity with brewer yeast were incubated 30°C for 7 hours. Drying process to avoid loss of nutrients for long time storage were performed 5 hour in room temperature. BSG were mixed with a spoon to pull out the barley and toss, then stored into fine container for long time. The ten list of protein content percentage in BSG was shown in Table 1.

2.2 Preparation of Ingredients

Basic ingredients (yeast, sucrose, potassium sorbate, ammonium acetate, honey and H_2O) were used for all the baits with different main ingredients such as jaggery, BSG, local red wine, apple cider vinegar (export in China) and wheat bran. Pineapple and papaya fresh solution were used as enzyme reaction for both nutrient and flavor. Basic ingredients of yeast, sucrose, potassium sorbate, ammonium acetate, honey and H_2O were prepared as 6.25%, 12.5%, 6.25%, 6.25%, 6.25% and 56.25% respectively for further mixture with main ingredients (Table 2).

2.3 Bait Incubation

The basic ingredients were mixed homogeneously, and then incubated 35°C for 50 mins. The baits were kept in 4°C for 15 mins. The protein source of BSG solution, Jaggery solution, local red wine, apple cider vinegar and wheat bran solution were prepared in 40°C for 15 mins, mixed together with basic composition. The final ingredients of pineapple and papaya solution were added and then shake at 75°C (120 rpm) for 24 hours. Protein-based bait formulation base on the BSG, local red wine, apple cider vinegar, jaggery and wheat bran powder was shown in Table 3.

2.4 Location of Site Selection

The location was selected based on the occurrence of the economically important fruit fly host plants and geographically important fruit production area. The field site is located at 21°0′0″ N, 96°0′0″ E, Mandalay region, Myanmar. The field is situated at huge number fruits in large production areas of mangoes and guava.

2.5 Field Assessment of Protein-Based Bait

The 10 traps of fresh different protein-based baits were prepared for three replications. Local detergents were added only one drop for each trap before

Table 1 The 10 lists of protein content percentages in Beer Spent Grain (BSG).

No	References	Protein Content (%)
1	Kanauchi et al. 2001	24
2	Santos et al. 2003	31
3	Carvalheiro et al. 2004	24.6
4	Mussatto and Roberto 2006	15.2
5	Celus et al. 2006	26.7
6	Xiros et al. 2008	14.2
7	Jay et al. 2008	15–17
8	Robertson et al. 2010	20-24
9	Waters et al. 2012	22.1
10	Meneses et al. 2013	24.7

Table 2 The same composition of basic ingredients for 10 different protein-based baits.

Ingredients	Bait 1	Bait 2	Bait 3	Bait 4	Bait 5	Bait 6	Bait 7	Bait 8	Bait 9	Bait 10
Yeast (g)	5	5	5	5	5	5	5	5	5	5
Sucrose (g)	10	10	10	10	10	10	10	10	10	10
Ammonium acetate (g)	5	5	5	5	5	5	5	5	5	5
Potassium Sorbate (g)	5	5	5	5	5	5	5	5	5	5
Honey (ml)	5	5	5	5	5	5	5	5	5	5
H2O (ml)	45	45	45	45	45	45	45	45	45	45

Table 3 Protein-based bait formulations based on the BSG, local red wine, apple cider vinegar, jaggery and wheat bran powder.

Ingredient	Bait 1	Bait 2	Bait 3	Bait 4	Bait 5	Bait 6	Bait 7	Bait 8	Bait 9	Bait 10
BSG solution	33.3 %	33.3 %	-	-	-	-	-	-	-	-
Local Red wine	-	-	33.3 %	33.3 %	-	-	-	-	-	-
Apple Cider Vinegar	-	-	-	-	33.3 %	33.3 %	-	-	-	-
Jaggery solution	-	-	-	-	-	-	33.3 %	33.3 %	-	-
wheat Bran Powder	-	-	-	-	-	-	-	-	33.3 %	33.3 %
Basic Ingredients	33.3 %	33.3 %	33.3 %	33.3 %	33.3 %	33.3 %	33.3 %	33.3 %	33.3 %	33.3 %
Pineapple fruit solution	33.3 %	-	33.3 %	-	33.3 %	-	33.3 %	-	33.3 %	-
Papaya fruit solution	-	33.3 %	-	33.3 %	-	33.3 %	-	33.3 %	-	33.3 %

trapping in the field. In trapping management, each trap was hanged on about 72-inch-high branch of host plants with 20 meter far away each other including basic ingredient trap as control. Fly per traps per days (FTD) values were evaluated.

After one week and one month storage at room temperature (around 30°C, the old protein-based baits were also analyzed according to the above field assessment of fresh baits. FTD values of both one week and one month old protein-based bait were evaluated and compared.

2.6 Female Captured Percentage of Protein-based Bait

The captured fruit flies and cockroaches were brought to the entomology laboratory at Biotechnology Research Department, Kyauk-se, Myanmar. The fruit flies were identified using stereo-microscope with binocular lens by observing the morphological characteristics. The capture percentages of female fruit flies and cockroaches from all (fresh, one week old and one month old) protein-based baits were also evaluated.

3. Results

3.1 The Efficiency of the Fresh Protein-based Bait on B. Dorsalis

Field assessment of 10 different fresh protein-based bait (BSG-based bait, jaggery-based bait, local red wine-based bait, apple cider vinegar-based bait and wheat bran-based bait) at August, 2019 were shown in Fig. 1. FTD value of BSG-based bait with papaya solution (38.3±1.5) is the highest among the different protein-based bait at Mandalay region of Myanmar.

3.2 FTD Values of the B. Dorsalis with One Week and One Month Old Protein-based Bait

The results of field evaluation analysis on the one week and one month old protein-based bait were discovered that the captured percentage of one month old wheat bran-based bait decrease significantly while decrease slightly with one week old baits (Fig. 2). FTD values of one week and one month old protein-based bait decrease than the fresh bait.

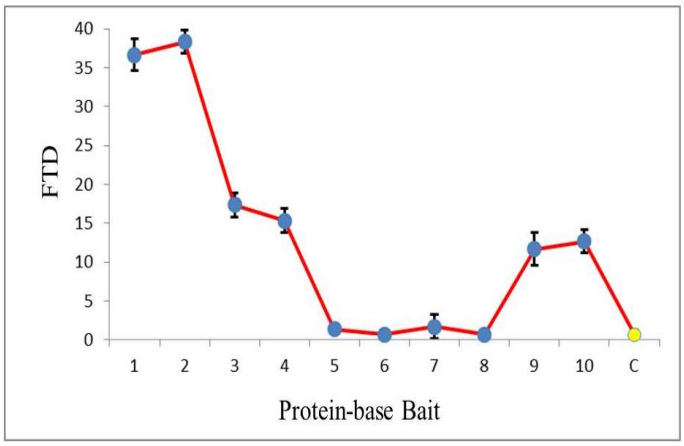
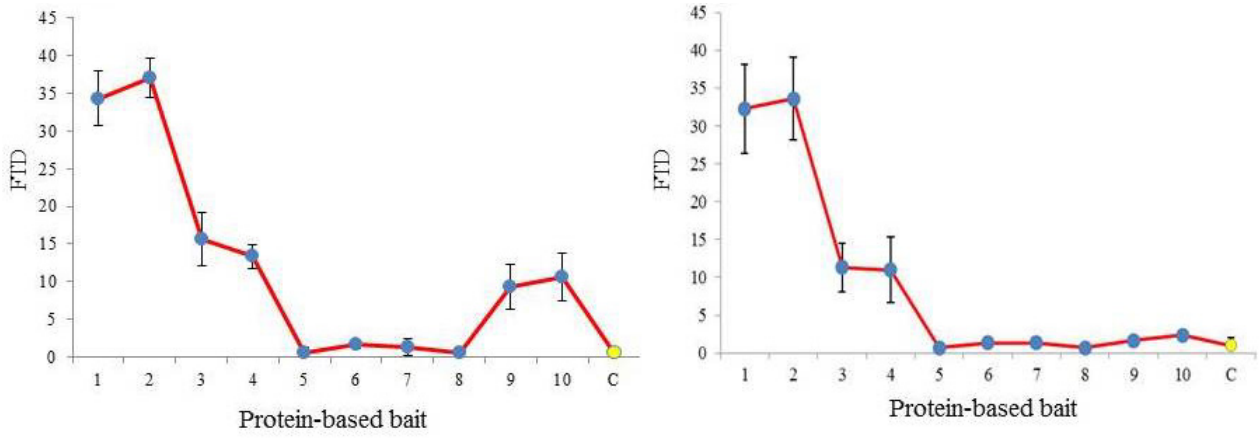


Fig. 1 FTD values of the oriental fruit fly, *B. dorsalis*by the application of 10 different fresh protein-based bait with BSG-based bait (1,2) , local red wine-based bait (3,4), jaggery-based bait (5,6), apple cider vinegar-based bait (7,8) and wheat bran-based bait (9,10) in Myanmar.



(a) (b)

Fig. 2 FTD values of the oriental fruit fly, *B. dorsalis*by the application of 10 different protein-based baits (A) one week old protein-based bait (B) one month old protein-based bait with BSG, local red wine, jaggery, apple cider vinegar and rice bran with pineapple and papaya solution in Myanmar.

3.4 Female Captured Percentage of B. Dorsalis

The *B. dorsalis* female captured percentage of all baits (fresh, one week old and one month old) showed that BSG-based bait is the highest among the different protein-based baits. BSG-based bait with papaya enzyme is the highest female captured percentage as 89%. All the protein-based bait female captured percentages at August in Mandalay, Myanmar were shown in Fig. 3.

3.5 Female Captured Percentage of Cockroach

The cockroaches female captured percentage of all baits (fresh, one week old and one month old) showed that the lower percentage of on BSG-based baits and local red wine-based bait while other 50% together (Fig. 4).

4. Discussion

The field assessment of protein-based bait such as pinnacle and Thailand bait found that female *B. dorsalis* were captured than male on bitter gourd plot in Thailand [11]. In this study, the overall protein-based bait captured results indicated that the female fruit flies need more protein to mature ovaries and oviposition process as an evidence of higher female

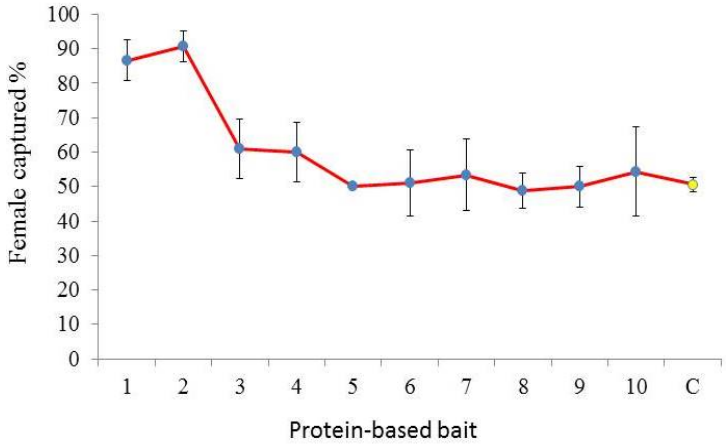


Fig. 3 The captured percentage of female oriental fruit flies, *B. dorsalis* in 10 different protein-based baits with BSG-based bait (1,2) , local red wine-based bait (3,4), jaggery-based bait (5,6), apple cider vinegar-based bait (7,8) and wheat bran-based bait (9,10) in Myanmar.

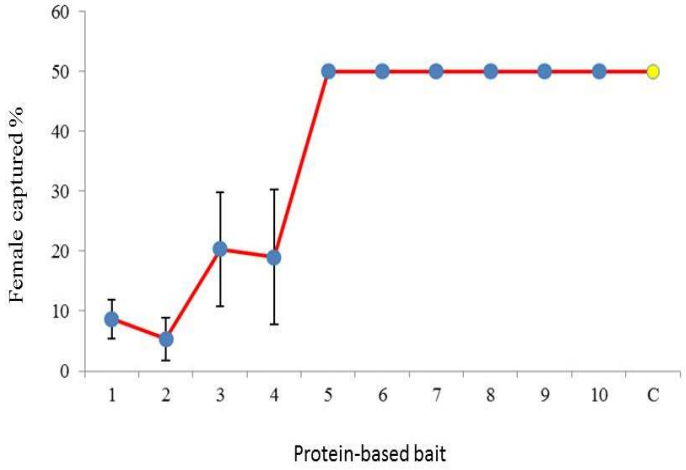


Fig. 4 The captured percentage of the cockroach females in 10 different protein-based bait with BSG-based bait (1,2) , local red wine-based bait (3,4), jaggery-based bait (5,6), apple cider vinegar-based bait (7,8) and wheat bran-based bait (9,10) in Myanmar.

captured percentage. In fruit fly monitoring and suppression programme, the commercial products of protein-based bait from beer yeast waste were used effectively ammonium acetate for the attractiveness of *C. capitata* [12]. Here, ammonium acetate were used as a basic ingredient of protein-based bait especially with main ingredient BSG and also we found that it is very good addictive ingredient for attractiveness of *B. dorsalis*.

In the cucurbit agro-ecosystem, *B. cucurbitae*were controlled as sustainable pest management by the protein hydrolysate as an effective attractant of protein-based bait [13]. We used even general yeast as an addictive of bait is a good attractant of protein-based bait for suppression of *B. dorsalis*in AW-IPM. Unripe papaya can produce 80-90% papain enzyme activities and the efficacies of enzyme activities were analyzed by TLC, spectrophotometer before and after the treated with papain enzyme [14]. The results of fresh unripe papaya solution and ripen pineapple solution for efficiency of enzyme activities indicated that the possibility of best efficient protein-based bait.

The population dynamic of *B. dorsalis* attracted from methyl eugenol (ME) which is the best pheromone attractant in August, 2016-2017-2018 is

around 70 FTD values in each year [1]. We showed that FTD value in August, 2019 is 38.3±1.5 only even protein-based bait attractant which is not ME. *Drosophila suzukii Matsumura* is attracted to fermentation volatiles as protein-rich food which is made by apple cider vinegar because of baits volatiles in search of oviposition substrate [15]. We found that apple cider vinegar-based bait can't attract like BSG because this exported apple cider vinegar from china contain less protein source. In the insecticide-bait sprays programme, the mixture of yeast extract and sucrose is a good attractant for *Anastrepha oblique* but only sucrose is not effective in the experiment [16]. Here, we used the mixture of yeast, sucrose, honey, potassium sorbate, ammonium acetate and H_2O as a basic ingredient. In mangoes production zone of tropical region, Mexico, the protein-based bait controlling method with less insecticides is the effective method under rainy condition of ground spraying technique [17]. We used very less insecticides even trapping section of protein-based bait in tropical region, Mynanmar.

Cockroach males ate more proteins than females and copulated more frequency. The male that were allowed to copulate more times died sooner than less times males [18]. Cockroach males prefer a high carbohydrate diet for protein content suggesting that makes more attractive to females for their reproductive fitness [19]. Here, cockroach male captured percent is higher than female by the BSG-based bait and local red wine-based bait. German cockroach males specifically select a more protein-biased diet as the requirements nutritional values for mating competitiveness [20]. Myanmar cockroach males were also choosing more protein-based diet than fruit flies.

5. Conclusion

Our results reveal that the formulation of BSG-based bait for fruit fly controlling system is the best efficient bait among the above ten different protein-based bait. Female fruit flies need more protein to mature ovaries and oviposition process. We found that male cockroaches need more protein for reproduction process while they need more protein for frequent copulation, more energy, defending territories and mating success because we captured more cockroach males although our research was targeted especially on fruit flies. On the other hand, our baits were proved that they have nutritional values because of the captured of more cockroach males not only more oriental fruit flies females.

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