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Field evaluation of various food attractants for the fruit fly *Bactrocera* species in pear orchard

M. HAMAYOON KHAN, M. SALMAN, S. J. AHMAD SHAH✉
Principal scientist, Senior scientist, Deputy Chief scientist, Plant Protection Division, Nuclear Institute for Food and Agriculture (NIFA), Peshawar, Pakistan

Abstract

Field studies were carried out to evaluate the attractiveness of different food attractants *viz*. protein hydrolysate 10%, torula yeast 10%, yeast instant 7%, casein 5% and sugar molasses 10% towards the fruit fly, *Bactrocera* species in pear orchard. Different concentrations of food baits (as mentioned above) were prepared in 200 ml of water and transferred to locally designed traps made up of 1.5 liter cold drink plastic bottles. The traps were hung on fruit trees at a height of about 2 m and at appropriate distance from each other. No insecticide was used in the food attractants. Data were regularly recorded at weekly intervals on the total number of *B. zonata* or *B. dorsalis* captured their sex ratio and aggregate population density of both the species. Results showed that yeast instant attracted significantly higher population of *B. zonata* and *B. dorsalis* and hence the highest cumulative population of both species followed by treatment of sugar molasses and protein hydrolysate. Among all the treatments, torula yeast was found to be the least preferred attractant for *B. zonata* and *B. dorsalis*. Sex ratio (%) of *B. zonata* and *B. dorsalis* revealed that all the baits attracted significantly higher number of females than males with highest female percentage in protein hydrolyzate and the lowest in sugar molasses. The results showed that yeast instant, sugar molasses and protein hydrolysate were highly effective and could further be exploited in combination with certain chemicals to enhance their effectiveness and developing a strong fruit fly attractant.

Keywords: Attraction, food baited traps, population, sex ratio, trapping

ارزیابی صحرایی جلب کننده‌های غذایی مختلف مگس‌های میوه *Bactrocera* در باغ گلابی

محمد همایون خان✉، محمد سلمان، سید جواد احمد شاه
استادیار، استادیار، دانشیار، بخش حفاظت گیاهان، موسسه ستادی غذا و کشاورزی، پیشاور، پاکستان

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چکیده

بررسی‌های صحرایی به‌منظور ارزیابی میزان جلب کنندگی پروتئین هیدرولیزات ده درصد، مخمر ترولا ده درصد، مخمر هفت درصد، کازئین پنج درصد و ملاس قند ده درصد روی مگس میوه جنس *Bactrocera* در باغ‌های گلابی انجام شد. جلب‌کننده‌های غذایی فوق به‌همراه ۲۰۰ میلی‌لیتر آب تهیه و به داخل تله‌های طراحی شده، منتقل و در ارتفاع دو متر از سطح زمین در باغ‌های میوه روی درختان مورد نظر نصب شدند. هیچ ترکیب شیمیایی در تله های جلب‌کننده غذایی استفاده نشد. نسبت جنسی، تعداد حشرات شکار شده گونه‌های *B. zonata* و *B.dorsalis* و تعداد کل حشرات شکار شده مربوط به هر دو گونه به‌طور هفتگی ثبت شد. نتایج نشان داد که مخمرهفت درصد، ملاس قند و پروتئین هیدرولیزات به‌ترتیب بیشترین شکار از هر دو گونه *B. zonata* و *B.dorsalis* را داشته‌اند. همچنین در بین تمامی تیمارها، مخمر ترولا ده درصد کمترین میزان جلب کنندگی را نشان داده است. بررسی‌های نسبت جنسی نشان داد که تعداد حشرات ماده شکار شده در تمامی تله‌های جلب‌کننده غذایی به‌جز ملاس قندی نسبت به حشرات نر بیشتر بوده است. همچنین بیشترین شکار حشرات ماده در تله جلب‌کننده غذایی با پروتئین هیدرولیزات ده رصد مشاهده شد. بر اساس نتایج به‌دست آمده تله‌های جلب-کننده غذایی مخمر هفت درصد، ملاس قند و پروتئین هیدرولیزات با توجه به کارایی بالا قابل استفاده در برنامه‌های کنترلی بهره‌برداری هستند.

واژه‌های کلیدی: تله‌گذاری، تله‌های جلب‌کننده غذایی، جلب‌کننده، جمعیت، نسبت جنسی

✉ **Corresponding author: mhkhan170@gmail.com**

Introduction

Fruit flies (Diptera: Tephritidae) are serious pests of horticultural crops across the globe, causing tremendous losses to a wide range of fruits and vegetables (Ilyas *et al.*, 2017; Khan *et al.*, 2021). Fruit flies are regarded as one of the major quarantine pests in international trade of fruits and vegetables (Peck and McQuate, 2004; Khan *et al*., 2021) by creating hindrances in the export and reducing their average per hectare yield (Ahmad *et al.*, 2005; Khan *et al*., 2021). In favorable conditions, production from the entire crop can be wiped out by these pests and the whole agricultural economy of the infested area could be ruined (Mahamod and Mishkhatullah, 2007; Ilyas *et al.*, 2017). The genus *Bactrocera*, which includes about 651 described species, is reported to have been the most economically significant fruit fly genus. About 50 species in this genus are considered as highly destructive and major polyphagous pests of horticultural crops (Ahmad *et al.*, 2005; Vargas *et al.*, 2015). Among these, the *Bactrocera zonata* is a widespread pest causing heavy losses to a wide range of fruits such as guava, mango, peach, pear, plum, apricot, loquat, etc. in Pakistan at farm level with added losses to traders, retailers and exporters (Chauhan *et al.*, 2011; Awad *et al.*, 2014; Salman *et al.*, 2022).

A huge amount of pesticides is being used annually for the control of fruit flies in fruit orchards and vegetable fields which are continuously affecting the biotic and abiotic factors of the environment (Rehman *et al.*, 2009). It is a known fact, that often only 1% of the active ingredients reach the target pests, while 99% of these materials, some of which are highly toxic, deteriorate the eco-system (Khan *et al.*, 2010). Non-selective use of pesticides is responsible for water pollution, air pollution, soil degradation, insect resistance and resurgence, destruction of native flora and fauna. Pesticides are responsible for ozone depletion and contribute to the greenhouse effect (Naeem *et al.*, 2012). Moreover, there is much concern about the export of various fruits from Pakistan to other countries either due to the presence of fruit flies larvae/ eggs or insecticide residues that are applied for its control (Rehman *et al.*, 2009).

It is therefore, highly imperative to develop alternative control strategies that can easily be incorporated in Integrated Pest Management (IPM) system against these quarantine pests. Different eco-friendly techniques available for fruit fly control include MAT (Male Annihilation Technique), BAT (Bait Application Technique), biological control and botanical pesticides. Among these, the MAT is extensively being used by the fruit and vegetable growers (Shelly, 2001). However, the lure used in MAT is highly male specific which attract and kill only male flies and has zero attraction for the female flies. Research is therefore, needed to explore certain protein baits which could either be used alone or in combination with chemicals for the attraction of both sexes of fruit flies.

Application of bait spray in combination with a toxicant for the control of potentially damaging population of fruit flies is reported to have been started in 1908, in South Africa. The concept of a bait spray is to attract the flies to droplets of the mixture, where they feed and die (Chambers *et al.*, 1974). Until 1952, the principal baits used for medfly were sugars, molasses, syrups or other sorts of carbohydrates. However, in the 1950's, enzymatic protein hydrolysates were found to be more attractive to medflies than carbohydrates (Steiner *et al.*, 1961). One particular bait spray which was adopted in the 1960's remains the standard today in many countries. It consists of a mixture of protein bait plus Malathion for fruit fly suppression mainly medfly (Roessler, 1989). Food attractants such as molasses, fermented sugar and yeast's have been used to attract both sexes of fruit flies. Similarly mixtures of different chemicals like putrescine, ammonium acetate, trimethylamine, acetic acids etc. have also been used effectively in traps for the attraction of *Ceratitis capitata* (Wiedemann) females (Ahmad *et al.*, 2005).

Food rich in amino acids may have a dramatic impact on the adolescent of fruit flies (Lemos, *et al*., 1992) with particular reference to ovary development and fertile egg production (Vargas *et al*. 2002). Qasim and Zada, (2016) used Methyl Eugenol, Cue Lure and protein based local product for trapping of *B. dorsalis, B. zonata* and *B. cucurbitae* in the field in modified traps of McPhill. Protein based local product was found as the most efficient bait for capturing of adult flies of all the three species. Field studies of various lure mixtures comprised of ammonia and protein sources mixed with sugar, honey, and vinegar revealed that protein-hydrolysate based lure-mixture, yeast-extract based

lure-mixture and ammonium-acetate based lure-mixture proved to be the promising lure mixtures for attracting *B. cucurbitae* (Iqbal *et al.* 2020). Abro *et al.* (2021) evaluated the effectiveness of different bait sprays such as Nu-lure, Protein hydrolysate and Prima in controlling *B. cucurbitae* in Sindh province of Pakistan. It was observed that Protein hydrolysate had the highest attraction followed by Nu-lure. Contrary to this, Hussain *et al.* (1998) reported significantly higher attraction of *B. dorsalis* to McPhail traps baited with coffee liquid compared to those baited with Nulure or sugar water. Khan *et al.,* (2020) applied various management practices for the suppression of peach fruit fly, *B. zonata* in Quetta Balochistan region of Pakistan and found that the application of protein hydrolyzate in conjunction with sanitation practices was effective in fruit fly population reduction. Similarly, Leblanc *et al.,* (2010) reported that water solution of torula yeast was more effective attractant than Bio Lure for fruit fly, *Bactrocera* when applied in multi traps.

However, there is very little information available for monitoring and controlling *Bactrocera* species using targeted products for both sexes, the present study is therefore, aimed to explore various nutritious substances that could be used for the attraction and trapping of both male and female individuals of Tephritid fruit flies.

Materials and Methods

Studies on the attractiveness of different food baits to the fruit fly, *Bactrocera* species i.e *B. zonata* and *B. dorsalis* were conducted at the experimental farm (pear orchard) of Agricultural Research Institute (ARI) Tarnab, Peshawar Pakistan during 2021. Different concentrations of food attractants/ baits *viz.* protein hydrolyzate, torula yeast, yeast instant, casein and sugar molasses were evaluated in traps for their attraction to the fruit fly *Bactrocera* species.

The experiment consisted of the following treatments (food attractants/ baits) prepared at the given concentrations.

All the above treatments were prepared by mixing them at the mentioned rate in 200 ml of water and put in traps made up of 1.5 liter plastic bottles. Protein hydrolyzate and casein were obtained from the fruit fly rearing laboratory of the Nuclear Institute for Food and Agriculture (NIFA), Peshawar province. Torula yeast and Yeast Instant were obtained from Nuclear Institute of Agriculture, Tandojam Sindh and local market, respectively. The experiment was laid out in a Randomized Complete Block Design (RCBD) comprising of 20 traps (5 treatments replicated four times).

The traps were locally designed and prepared from 1.5 liter plastic bottles by making four small holes (0.5 inch diameter) on the sides about 6 inches above the base for fly entrance. A small wire was fastened to the bottle neck of trap for onward hanging in the tree. The traps were hung at appropriate distance from each other in pear orchard about 1.5-2 m above the ground level. No toxicant was added to the bait solutions in traps. The traps were checked weekly for data collection and recharged if needed. For data collection, the trap containing bait solution with flies was emptied in a sieve (75 size mesh) placed over a small bucket. The captured flies remained on the sieve whereas solution went down to the bucket. The traps were further cleansed with a little water to recover left over flies in the traps. Water was also used to wash flies on the sieve in order to remove coloration and impurities of the bait and to make them clearly visible. Once the flies were clearly differentiable as male, female or species, observations were recorded on the total number of *B. zonata* or *B. dorsalis* captured their sex ratio and total number of flies of both the species (Ravikumar and Viraktamath, 2007).

Table 1. Different concentrations of treatments (food baits)

No.	Treatments	Abbreviation	Concentration
1	**Protein hydrolysate**	PH	10%
2	**Torula yeast**	TY	10%
3	**Yeast instant**	YI	7%
4	**Casein**	CS	5%
5	**Sugar molasses**	SM	10%

Statistical Analysis

Data recorded on the field evaluation of different food attractants for the fruit fly, *Bactrocera* species in pear orchard were subjected to analysis of variance (one-way ANOVA) using statistical analysis software STATISTIX 8.1. Multiple comparisons among the means were made using LSD test ($P < 0.05$).

Results

Results on the attractiveness of different food baits to the fruit fly *Bactrocera* species revealed that YI 7% proved to be the most superior food bait attracting significantly higher number of female *B. zonata* (21.25±2.54/trap/week) followed by treatment of SM 10% (18.33±1.89/trap/week) over an exposure period of four weeks (Table 2). Treatment of PH 10% also attracted substantial number of female *B. zonata* (15.08±2.14/trap/week). TY 10% was found to be the least preferred food bait for *B. zonata* showing attraction of 11.41± 1.58 flies/trap/week. Combined population of male and female flies of *B. zonata* was found to be the highest in traps treated with YI (35.58±3.72/trap/week) followed by treatment of SM where total population recorded was 34.0±3.34/trap/week (Table 2).

Table 2. Mean number of fruit fly, *Bactrocera zonata* attracted to different food baits

Treatments	Male	female	Total *B. zonata*
TY 10%	7.66 ± 0.86 ^{c}	11.41 ± 1.58 ^{d}	19.08 ± 2.67 ^{c}
PH 10%	9.91 ± 1.17 ^{b}	15.08 ± 1.55 ^{c}	25.0 ± 2.14 ^{b}
YI 7%	14.33 ± 1.41 ^{a}	21.25 ± 2.54 a	35.58 ± 3.72 ^{a}
CS 5%	8.33 ± 1.19 ^{bc}	13.41 ± 2.26 ^{cd}	21.75 ± 3.40 ^{bc}
SM 10%	15.66 ± 1.47 ^{a}	18.33 ± 1.89 ^{b}	34.0 ± 3.34 ^{a}
LSD	1.858	2.605	3.777

Means within a column followed by different letters are significantly different at 5% level of significance ($P \leq 0.05$).

In case of *B. dorsalis*, YI again proved to be the most superior attracting the highest number of female flies (15.08±1.47/trap/week) which was non significantly different with 2nd higher population of female flies attracted by treatment of SM 10% (13.41±1.5 /trap/week, Table 3). In the rest of the treatments, population of female *B. zonata* was found non-significantly different among all the treatments.

The overall population of *B. dorsalis* (male + female flies) attracted to different baited traps was found to be highest in traps treated with YI with average population of 25.502.16 /trap/week), followed by traps baited with SM with average population of 25.082.58 /trap/week (Table 3). The cumulative population of both species of fruit flies attracted to different food baited traps also followed the same pattern (Fig. 1). The highest collective population of fruit flies was recorded in traps treated with YI i.e. 61.08±4.16 /trap/week which was found non significantly different with treatment of SM (59.08±5.16 /trap/week). TY proved to be the least favored food bait for both species, attracting substantially less mean cumulative populations (32.66±2.09 /trap/week) of *B. zonata* and *B. dorsalis* (Fig.1).

Table 3. Mean number of fruit fly *Bactrocera dorsalis* attracted to different food baits

Treatments	Male	Female	Total *B. dorsalis*
TY 10%	5.66 ± 1.14 ^{b}	7.91 ± 1.55 ^{b}	13.58 ± 2.41 ^{b}
PH 10%	5.83 ± 0.68 ^{b}	9.16 ± 1.23 ^{b}	15.00 ± 3.44 ^{b}
YI 7%	10.41 ± 0.84 ^{a}	15.08 ± 1.47 ^{a}	25.50 ± 2.16 ^{a}
CS 5%	5.33 ± 0.76 ^{b}	7.75 ± 1.27 ^{b}	13.08 ± 1.96 ^{b}
SM 10%	11.66 ± 1.1 ^{a}	13.41 ± 1.5 ^{a}	25.08 ± 2.58 ^{a}
LSD	1.619	1.828	3.148

Means within a column followed by different letters are significantly different at 5% level of significance ($P \leq 0.05$).

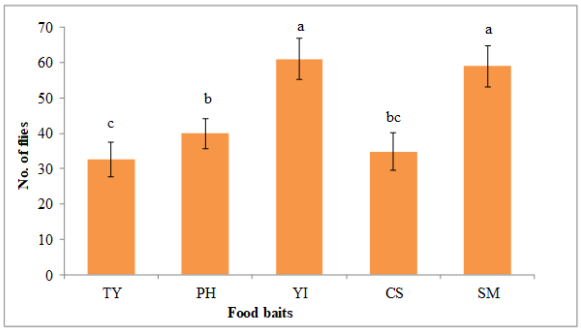

Fig. 1. Overall population of *Bactrocera zonata* and *B. dorsalis* captured in different food baited traps.

Sex Ratio (%) in traps

Percentage of sex ratio of *B. zonata* captured in different food baited traps showed that all the baits attracted higher number of female flies compared to male flies (Fig. 2). The highest percentage of females was recorded from traps baited with PH and CS (60.6%). Then, the lowest female percentage was recorded in SM (53.5%)(Fig. 2). Sex ratio (%) of *B. dorsalis* showed a higher percentage of female flies than male (Fig. 3). The lowest and the highest percentage of female of *B. dorsalis* were respectively, obtained in SM (53.1%) and YI (58.7%).

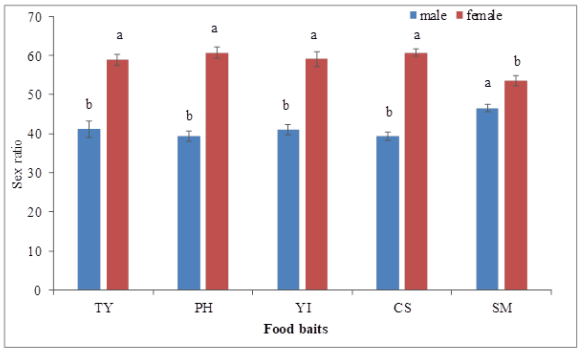

Fig. 2. Sex ratio (percent males and females) of *Bactrocera zonata* attracted to different food baited traps.

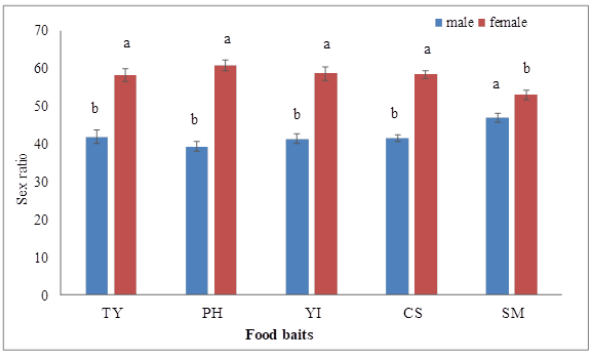

Fig. 3. Sex ratio (percent males and females) of *Bactrocera dorsalis* attracted to different food baited traps.

Weekly population

Highest population of *B. zonata* was captured during the third week of observation after which it was in the decreasing order in the subsequent week and dropped to much lower level in the sixth week i.e. the 3rd week of Jun (Fig. 4). The same trend was also true for *B. dorsalis* (Fig. 5).

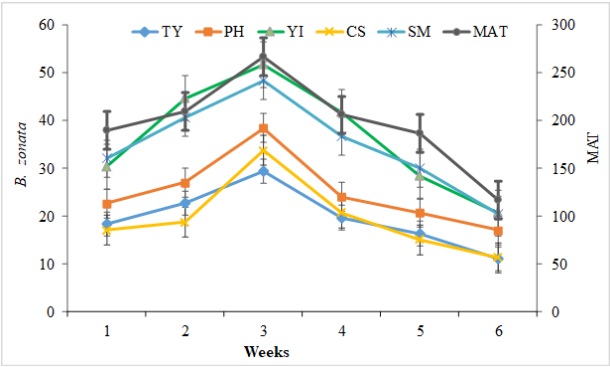

Fig. 4. Population of *Bactrocera zonata* attracted to different food baited traps at weekly interval.

Weekly population monitoring also revealed that overall density of *B. dorsalis* was far lower than that of *B. zonata*. The results revealed a higher population of flies captured in MAT traps compared to those caught in food baited traps.

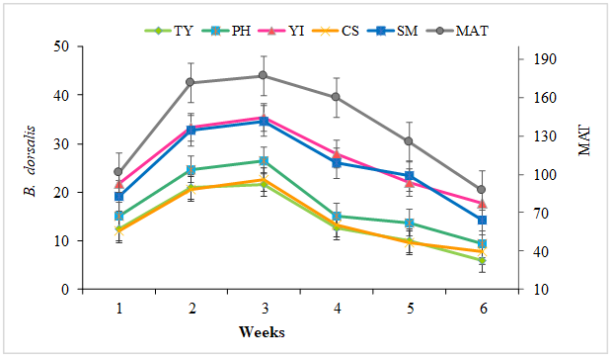

Fig. 5. Population of *Bactrocera dorsalis* attracted to different food baited traps at weekly interval.

Discussion

Among various eco-friendly management studies of fruit flies, the Male Annihilation Technique (MAT) is extensively being used in in horticultural crops. However the lure applied in MAT is highly male specific attracting only male individuals and leaves the female flies. It is also well recognized that female flies are the primary sources flies multiplication in the form of eggs production and hence, there is a dire need of using female attractive baits for fruit fly monitoring and control (Mazor *et al.* 2002). Fruit flies are attracted to different food baits in order to fulfill their nutritional requirements. Food baits such as molasses, fermented sugar, protein hydrolysate, nulure, torula yeast, brewer's yeast etc. have been used in traps for the attraction fruit flies in horticultural crops (Economopoulos and Haniotakis, 1994; Ismail, 2012). Our experimental results with regard to the use of various food baits in locally designed traps for the attraction of both sexes of *B. zonata* and *B. dorsalis* revealed that YI and SM being the most effective treatments in terms of attracting higher population of *B. zonata* and *B. dorsalis* (Both sexes) followed by treatment of protein hydrolyzate. These results demonstrate that food-based lures are able to attract both females and males of Tephritid fruit flies (Epsky *et al.*, 1999; IAEA, 2003). Tsitsipis (1989) reported that sources of proteins,

carbohydrates, amino acids and minerals act as phagostimulants for the fruit flies. Traps baited with YI attracted significant numbers of other species of flies too and got fully packed with these species in a span of a week. This could be due to higher fermentation in the bait that released CO_2 together with acids and alcohols containing gases that are attractive to flies (Morton and Bateman, 1981).

It is important to mention that SM though attracted higher population of flies but the different between male and female flies ratio was insignificant. Moreover SM also attracted a large number of red wasps and some other species of flies which thoroughly filled the solution and increased the replenish frequency. On the other hand, Delgado *et al.* (2022) reported that sugar molasses was not effective in attracting females of *C. capitata* and *A. fraterculus*. Malavasi *et al.* (1990) also reported the ineffectiveness of sugar molasses against *A. fraterculus* and *A. grandis*. However, irrespective of these reports, molasses is still commonly used in baits mixed with a toxicants in southern Brazil (Nava & Botton 2010) and Uruguay (Delgado *et al.* 2022), and the same is also considered very efficient by the citrus growers.

Treatment of protein hydrolyzate was found to be the most effective in attracting higher percentage of female flies of both species with less attraction of other fly species. Proteins are needed by the female flies for full ovarian development and egg production thus, in the field they readily search for protein sources (Vargas and Prokopy, 2006). Hence, being major source of protein, protein hydrolysate is highly attractive to fruit flies (Putruele *et al.*, 1993), especially female flies (Metcalf, 1990). Navarro *et al.* (2008) reported that protein baited traps may attract and trap both male and female flies of medfly, *C. capitata*. Among different protein sources, Potein hydrolysate was found to be the most potent attractant for both sexes of Medfly, *C. capitata* and Mexican fruit fly, *A. ludens* (Epsky *eta al.* 1999). Khan *et al.*, (2020) found that the application of protein hydrolyzate in conjunction with sanitation practices was effective in fruit fly population reduction. Fabre *et al.* (2003) and Duyck *et al*. (2004) reported that Solbait (protein hydrolysate) was the most effective food bait in mass trapping of females of melon fly *B. cucurbitae*. Owing to its attractiveness towards fruit flies, protein-based attractants are extensively being used as an important component of commercial food lures in many Tephritid fruit fly control programmes, (Alyokhin *et al.*, 2000; Epsky *et al.*, 2014).

Although casein and torula yeast are concentrated sources of protein, they were inferior as an attractant, compared to protein hydrolysate. Our results with regard to casein (milk protein) are supported by Hossain *et al.*, (2020) who reported casein additive in the adult diet had no remarkable effect on different biological parameters of the melon fly, *B. cucurbitae*. So the far effect of torula yeast is concerned, unlike to our findings, Leblanc *et al.*, (2010) reported that water solution of torula yeast was more effective attractant than BioLure for fruit fly, *Bactrocera*. Similarly, commercial products, Mazoferm E802 and Torula yeast were found to be the most effective attractants for *B. invadens* capturing significantly higher flies than the standard Nulure (Ekesi *et al*., (2014). On the other hand, Vargas *et al* (2003) suggested that attraction of fruit flies to different baits may vary based on the type of protein used in the baits.

It is also worth mentioning that the baits in traps usually dry up within a few days depending upon the weather conditions especially temperature and air moisture level (Taneja *et al.*, 1986). Fermentation process in the bait decreases with the reduction in moisture level leading to a slow release of volatile chemicals and hence a significant decline in fly attraction (Morton and Bateman, 1981). That is why further studies are needed to further refine these attractants and improve their attractiveness towards fruit flies.

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