Original article – Thematic Issue

The effectiveness of locally produced protein bait improved with papain in capturing fruit flies of mango

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Summary

Introduction – **Fruit flies adversely affect the fruit industry in Nigeria, especially small holders who cannot afford the cost of imported insect control inputs. Consequently, local alternatives are being developed to benefit these farmers.** *Materials and methods* – **Locally made protein bait from autolysed brewery yeast waste (BYW) to which different concentrations of crude papain were separately added in traps was tested. The treatments included: X_1 = 188.5 mL BYW + 11.5 mL crude papain mixed in 1,300 mL of water; X_2 = 191 mL BYW + 9.0 mL crude papain in 1,300 mL of water; X_3 = 200 mL BYW in 1,300 mL of water. One pellet of Torula yeast in 200 mL of water (T_0) served as the check. A 7-ha orchard planted to 'Tommy Atkin' mango variety was partitioned into three blocks (replicates). The four different baits at 300 mL trap^{-1} were allotted randomly to mango trees 50 m apart in each block. The baits were replaced every 7 days after removal of trapped insects.** *Results and discussion* – **Weekly observations showed that captured fruit flies ranged from mean of 1.7–7.6 flies trap^{-1} day^{-1} out of which 95% were females. The species collected included *Bactrocera dorsalis*, *Ceratitis cosyra*, *C. silvestri* and *C. dittissima* with a dominance of *B. dorsalis* (over 90%). There was no significant ($p > 0.05$) difference between the number of fruit flies attracted by T_0 compared to X_1 at the end of the trial. However, the number captured by T_0 and X_1 were significantly ($P < 0.05$) higher than X_2 and X_3.** *Conclusion* – **The result showed that BYW enhanced with crude papain can be as effective as Torula® in attracting different fruit fly species.**

Keywords
Bactrocera dorsalis, brewery yeast waste, *Ceratitis cosyra*, papain, protein bait

Significance of this study

What is already known on this subject?

- Protein baits have been reported to be produced, either through hydrolysis or autolysis of brewery waste (BW), for capturing various species of fruit flies. Various home-made autolysates from various input sources and with various degrees of attractiveness are reported from other parts of the world. In Africa, locally produced protein bait from brewery waste by autolysis, similar to our present study, was tested on *Bactrocera dorsalis* only in Kenya. Another report in which a different source of papain was included to test the attractiveness in capturing *Bactrocera* (= *Zogodacus*) *cucurbitae* came from Mauritius.

What are the new findings?

- The simple method for the production of protein bait from brewery waste which implies the use of any locally available pots with simple method by farmers.
- The method of crude papain extraction which can be easily performed by any farmer and added to the BW.
- The volume of added papain to increase attractiveness of the bait to *B. dorsalis* in the present work is different from that of any similar work reported.

What is the expected impact on horticulture?

- This study was aimed at developing, for resource-poor farmers, an effective trapping method for detection, monitoring and control of different fruit fly species; especially *B. dorsalis* and *Ceratitis cosyra* that constitute serious problems to the production and exportation of mango in West Africa. The application of the technique as part of IPM practice by small farmers will have impact by restoring their confidence in mango production and thus encourage them to increase their production. They could also apply the technique in the production of other fruit crops.

Introduction

Fruit production is faced with serious fruit fly problems in Nigeria (Umeh *et al.*, 2008a; Umeh and Ibekwe, 2012), thus affecting both domestic and export trades of fruits such as mango and citrus. Fruit flies cause fruit damage with consequent drops of premature or mature fruits. Observations have shown that fruit fly damage occurs at all times when fruits are set in most parts of Nigeria (Umeh and Garcia, 2008). The majority of mango producers are unable to solve the perennial problem of fruit fly damage. As a result, large proportions of annual yields are lost. Most farmers often use inappropriate control measures or apply wrong doses of pesticides on the fruits, thereby endangering human health and creating environmental hazards. Selective insecticides and appropriate protein sources are used as baits in traps or as cover sprays to reduce fruit fly populations below the economic threshold (Umeh and Garcia, 2008; Umeh *et al.*, 2020; Peck and McQuate, 2000).

Ideally, protein hydrolysates and/or para-pheromones (synthetic lures) are used in baits to attract fruit flies away

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from fruits and kill them, the so-called “attract and kill” (Umeh and Onukwu, 2011; Umeh *et al*., 2020; Manrakhan, 2016). Many fruit fly species require both sugar and protein during their adult life for survival and reproduction. The attraction of fruit flies to protein sources has been exploited in developing protein-based attractants used for fruit fly monitoring and control (Epsky *et al*., 2014). Presently, there are two main types of protein-based attractants commercially available for fruit fly detection, monitoring and control: (i) Liquid protein hydrolysates, and (ii) Synthetic lures containing volatiles found in protein hydrolysate lures. The adoption and utilization of liquid protein from brewery yeast waste for fruit fly control gained recognition as a result of the possibility of reducing production cost, especially when produced through the process of autolysis. Since the majority of Nigerian mango producers are smallholders, the production of low cost liquid protein baits from brewery yeast waste that are readily available and sustainable will be useful in reducing fruit fly populations in commercial orchards. The effectiveness of protein bait in the control of fruit flies has been reported by various authors (Umeh and Garcia, 2008; Ross, 1993; Gopaul and Price, 1999; Piñero *et al*., 2009). The attractiveness of brewery yeast waste for detection and suppression of fruit flies has been found to be enhanced by lacing with other materials such as ammonium acetate, papaine, *etc*. (Sookar *et al*., 2002; Piñero *et al*., 2017). The present study is aimed at developing a baiting system that can be used to detect, monitor and suppress fruit fly populations, particularly female fruit flies by enhancing its attractiveness with crude papain obtained from pawpaw (*Carica papaya*). The ultimate goal is to use the enhanced bait in combination with other mass trapping techniques such as Male Annihilation Technique (MAT) to effectively reduce fruit fly population below economic thresholds in mango orchards.

Materials and methods

Plant host and experimental sites

Sites used for the trials were chosen from major mango producing areas in Nasarawa state in the Guinea Savanna agro-ecological zones of the country (08°50.288’N; 07°58.753’E). A 7-ha orchard planted with ‘Tommy Atkin’ mango variety (one of the predominant mango varieties in the area) was used for the trials. The orchard was large enough to contain all the treatments in three replicates with a minimum of 50 m between replicates.

Baiting materials

The baiting materials used include locally made protein bait from brewery yeast waste and conventional Tephri trap. The test material meant to enhance the attractiveness of the protein bait to fruit flies was crude papain extracted from papaya fruits. The extraction was done between 6:30 and 9:00 am by scarifying green papaya fruits on trees and collecting the white sieve in beakers. The collected crude papain was conditioned to dryness by oven drying at 45 °C in the laboratory at NIHORT for several hours. This was a modification of the methods of Sookar *et al*. (2002). The required dilutions were made and mixed to locally prepared brewery yeast waste (BYW) to boost its attracting properties to fruit flies.

Preparation of protein bait from brewery waste

Bulked brewery yeast waste of Star™ and Gulder™ lager beer which contained *Saccharomyces cervisae* was used at a pH of 4.5–6.0 and solid content of 15–20%. The brewery waste was poured into a 6-L aluminum pot immersed in a 10-L open vessel containing water. The vessel was heated and left to boil for 14–15 h. By the process of autolysis (a modification of the method of Gopaul and Price (Gopaul and Price, 1999; Lloyd and Drew, 1997), Alcohol was eliminated, thus resulting in an amber colored product attractive to fruit flies. The product had about 7.5% protein content (determined by Kjeldahl method).

Field layout and evaluation of baits

The trials were conducted during mango season, starting from the juvenile fruit stage to few weeks after harvest (April to July) to coincide with the period when fruit fly attack on mango is usually noticed. The layout was a randomized complete block design with three replications. The three blocks of the orchard were divided into plots corresponding to the number of treatments to be tested. Treatments were allotted randomly to mango trees in each block. There were four treatments. Each (in a trap) was allotted to a tree per plot while maintaining a minimum of 50 m between the earmarked trees.

Autolysed brewery yeast waste (BYW) to which three different concentrations of crude papain were separately added and kept for 24 h at a temperature of 75 °C were used as baits in Tephri traps. The crude papain solution was obtained by diluting 100 g of the powder to 200 mL of water. These treatments were compared to a check of hydrolyzed commercial protein bait (Torula®). The treatments were as follows:

X_1 = 200 mL (188 mL of BYW + 11.5 mL crude papain solution) mixed with 1,300 mL of water;

X_2 = 200 mL (191 mL of BYW + 9.0 mL of crude papain solution) mixed with 1,300 mL of water;

X_3 = 200 mL (BYW + 0 mL of crude papain solution) mixed in 1,300 mL of water;

T_0 = Torula yeast (1 pellet in 200 mL of water).

One Torula yeast pellet (hydrolyzed protein bait) was dissolved in 200 mL of lukewarm water (50 °C). The solutions were separately distributed to the Tephri traps at a volume of 300 mL trap^{-1} and set up according to the treatments above. The traps were hung on mango at 1.5–2 m from the floor. Trap catches were emptied at 7-day intervals, sorted out and recorded according to species and sex. The baits were replaced after each removal of trapped insects and the position of the traps changed in a clockwise direction in each block to eliminate bias due to tree position on the number of captured fruit flies.

Twenty mango fruits were randomly sampled per tree in the trees having traps (on 12th June, the major harvest date). The collected fruits were separately stored in polythene bags according to treatment and taken to the laboratory at NIHORT for incubation until the emergence of adult fruit flies. Each fruit from the treatments was separately incubated in a plastic container with a layer of fine soil and a perforated lid. These fruits were used for damage assessment. Number of emerging adult fruit flies were recorded according to treatment and the identification of species done using identification keys by White and Elson-Harris (1992). The numbers of fruit fly attacked fruits were determined from the fruit culture for each treatment.

Data analysis

Weekly data recorded on fruit fly numbers collected from traps baited with the various concentrations of enhanced BYW and Torula yeast were plotted into a graph to show the population dynamics during the period of the study. The data were later calculated as number of fruit flies per trap per day and subjected to analysis of variance (ANOVA) using SAS statistical package. The analyses were carried out for data collated from April to May and April to July to know the periods that the cumulative effects of the treatments will show significant differences in the number of attracted fruit flies. The numbers of attacked fruits in each treatment was calculated as percentages. The number of adult fruit flies that emerged from the fruits sampled from the various treatments and control were also subjected to analysis of variance. Means of significantly different tests were separated using Duncan's Multiple Range Test (DMRT). All tests were judged significant at $P = 0.05$.

Results and discussion

Observations on the population dynamics (Figure 1) of fruit flies showed that Torula® yeast (T_o) and the enhanced BYW (X_1) attracted more fruit flies throughout the period of study (mango season). Population of fruit flies increased drastically from late May to June before harvest probably due to availability of maturing fruit. Throughout the population studies, the attractiveness of BYW mixed with 11.5 mL of papain solution was superior to all other locally made BYW judging from the number of trapped fruit flies (Figure 1). The locally made BYW mixed with 9.0 mL solution of papain (X_2) showed similar level of attraction to fruit flies as with BYW where no papain was added. A peak population was observed at harvest period (June) due to the presence of a high proportion of mature and ripening mango fruits. Similar observations were reported by other authors on the cultural practice of some citrus farmers whereby harvesting was late thus allowing more than 50% of the fruits to ripen (Umeh *et al.*, 2004). These mature or ripe fruits were more attractive to fruit flies (Umeh *et al.*, 2008b; Ortiz *et al.*, 1987). The population dynamics therefore suggest that fruit fly control by the use of protein bait, either BYW enhanced with papain or the commercial Torula yeast, must commence at least a month prior to fruit maturity rather than during fruit maturity or ripening stages when more preferred fruits are available for attack (Umeh and Garcia, 2008; Lloyd and Drew, 1997).

Statistical analyses of trap captures were made at two stages to show the cumulative effect of the various treatments on population at the initial fruit stage and at maturity (harvest stage). The results showed that there were no significant ($p > 0.05$; df = 17) differences between the number of fruit flies obtained from the brewery yeast waste (BYW) mixed with papain solution and the check Torula® yeast after 2 months of observations (April–May) (Figure 2); although higher numbers of fruit flies trap^{-1} day^{-1} were captured in Torula and X_1 traps compared to others. However, statistical analyses at the end of the study covering 4 months April–July) indicated significant difference ($p < 0.05$; df = 35) between BYW mixed with 11.5 mL of papain (X_1) and the other concentrations, except with Torula yeast which was not significantly different from X_1 (Figure 3). Thus, the descending order of fruit fly numbers captured in the various baits were as follows: Torula yeast (T_o) = BYW + 11.5 mL papain (X_1) > BYW + 9.0 mL of papain (X_2) > BYW + 0 mL of papain (X_3). Observations in the present study corroborate those of Gaupal *et al.* (2000) that observed higher attractiveness with increase in papain concentration in Mauritius. They observed that fruit fly catches on mango were greater when protein autolysate was prepared with higher concentrations of papain (2.0 or 4.0 g L^{-1}). In similar experiments in Kenya, conducted in mango orchards in February 2000, locally produced autolysate bait formulations out-performed commercial hydrolysate in attracting *Ceratitis cosyra*.

In field evaluation trials, the total number of oriental fruit fly, *Bactrocera dorsalis*, captured in Kenya in waste brewer's yeast was superior to the standard NuLure, although Torula yeast gave the highest catch (Ekesi and Tanga, 2016). In Tonga, weekly treatment of chilli with protein bait developed from waste brewer's yeast reduced fruit damage due to *B. facialis* from 90 to 7% compared to the untreated control plot which had damage increase of 27 to 100% (Heimoana *et al.*,

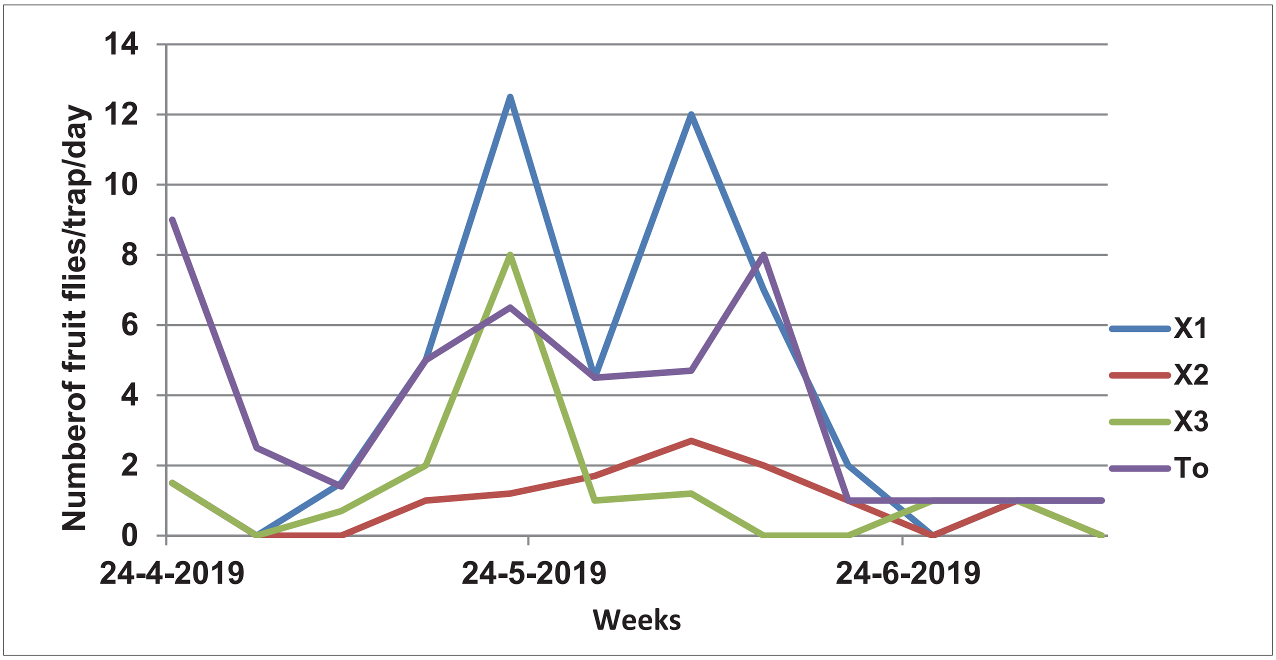

FIGURE 1. Population dynamics of fruit flies in an orchard subjected to baits with brewery waste enhanced with different concentrations of papaine compared with commercial protein bait Torula. X_1 = BW + 11.5 mL papaine; X_2 = BW + 0 mL papaine; X_3 = BW + 9.0 mL papaine; T_0 = Torula yeast.

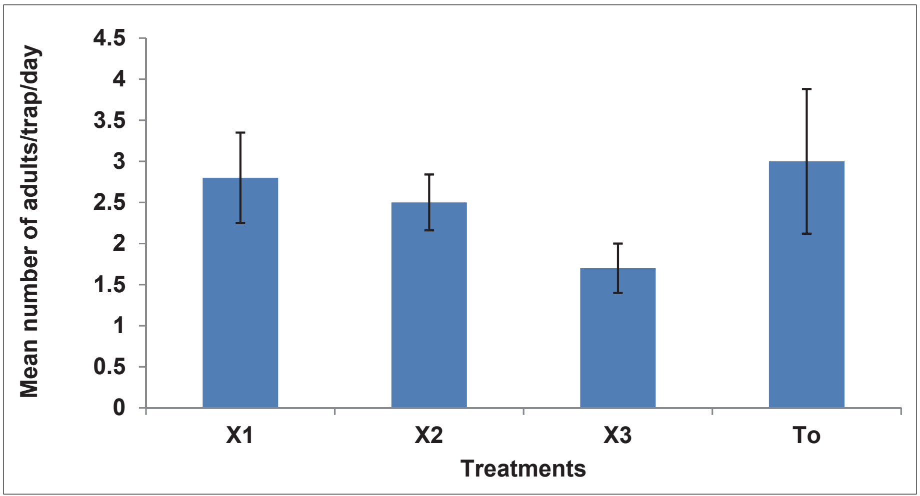

FIGURE 2. Population of fruit flies captured in different protein bait traps in an orchard in Nasarawa state between April and May. X_1 = BW + 11.5 mL papaine; X_2 = BW + 0 mL papaine; X_3 = BW + 9.0 mL papaine; T_o = Torula yeast.

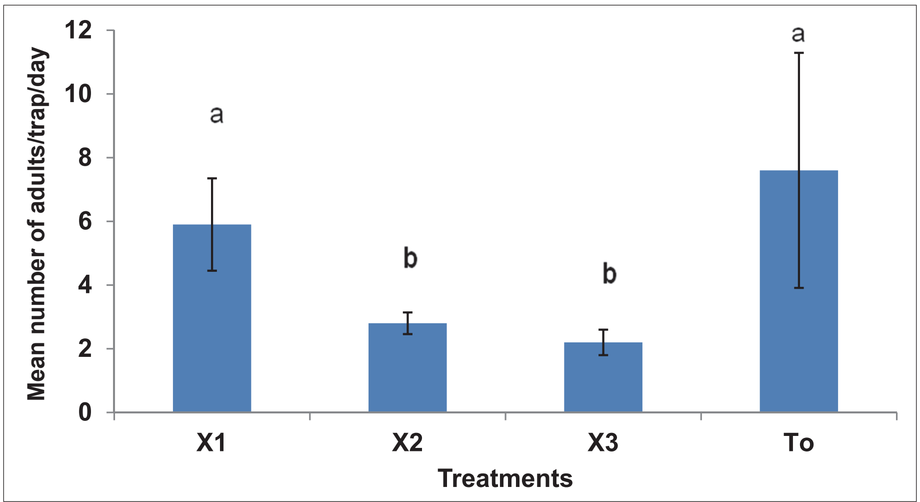

FIGURE 3. Population of fruit flies captured in different protein bait traps in an orchard in Nasarawa state between April and July. X_1 = BW + 11.5 mL papaine; X_2 = BW + 0 mL papaine; X_3 = BW + 9.0 mL papaine; T_o = Torula yeast.

TABLE 1. Number of fruit flies emerging from mango fruits collected from trees with different protein baits in an orchard in Nasarawa state, Nigeria.

Treatment	Mean no. of fruits treatment^{-1}	Mean weight of fruits (kg)	Mean no. of *B. dorsalis* fruit^{-1}	% of infested fruits
X_1	20	8.8	4.2 b	10
X_2	20	9.8	3.6 b	15
X_3	20	9.2	10.2 a	20
T_o	20	8.6	4.0 b	10

Means in the same column followed by the same letter are not significantly (p >0.05) different by DMRT.
X_1 = BW + 11.5 mL papain; X_2 = BW + 9.0 mL papain; X_3 = BW + 0 mL papain; T_0 = Torula yeast.

1997). Similar observation was made when BYW was used as spot spray on citrus compared to untreated stands (Umeh and Garcia, 2008). The protein bait sprays significantly reduced the damage of sweet orange by lowering the infestation level of *Bactrocera invadens* Drew Tsurata & White (= *B. dorsalis*), and consequently reducing the number of dropped fruits (Umeh and Garcia, 2008; Umeh and Onukwu, 2011). The captured fruit flies in the present study ranged from 1.7–7.6 flies trap^{-1} day^{-1} out of which >95% were females. The species collected were limited to *B. dorsalis*, *C. cosyra*, *C. silvestri* and *C. dittissima* with a dominance of *B. dorsalis* (over 90%). The ability of the enhanced bait to attract different species of fruit flies on mango is advantageous despite the negligible number of these species. The dominance of *B. dorsalis* in the traps may be due to the seeming displacement of other fruit fly species by this invasive species as was observed by Ekesi *et al.* (2009) between *B. invadens* (= *B. dorsalis*) and *C. cosyra* on mango.

The mean numbers of fruit flies that emerged from fruits harvested from the trees baited with different concentrations of the BYW enhanced with papain solution or those with Torula yeast were significantly ($p<0.05$) low compared to those harvested from trees baited with BYW devoid of papain (Table 1). We opined that the mean number of fruit flies that emerged from fruits harvested within trees with Torula yeast or papain enhanced BYW were significantly lower because some of the fruit flies that could have infested the fruits were trapped compared to those in the trees with non-enhanced BYW. However, there was no significant ($p>0.05$) difference between the mean numbers of fruit flies that emerged from fruits in the trees baited with Torula yeast and those baited with BYW enhanced with papain solution. Although higher percentages of fruits were attacked in the trees baited with BYW that are not mixed with papain solution, these were not significantly different from the percentage of fruits attacked in trees with the papain-enhanced baits or Torula (Table 1). This might be as a result of the fruit flies attacking more of the fruits than getting attracted to the bait without papain; while the BYW with papain attracted most of the fruit flies that could have attacked the fruits, thus resulting in the lower percentage of attacked fruits observed in trees with papain-enhanced baits. In field suppression trials, mango fruit infestation by *B. dorsalis* in treatments receiving protein bait in combination with other management methods incurred 6.7–20.0% fruit damage compared to 65.6% fruit damage in the untreated control (Ekesi and Tanga, 2016). This implies that, apart from using locally made baits enhanced with papain for fruit fly detection in orchards, they could be incorporated into IPM packages to suppress fruit fly populations.

Conclusions

The locally made brewery yeast waste (BYW) enhanced with the higher concentration of crude papain, 11.5 mL was effective in attracting different species of fruit flies, especially the invasive *Bactrocera dorsalis.* It proved efficacious in trapping the fruit flies infesting mango when compared with commercial Torula. This locally produced protein bait will be useful for small-scale farmers who may not be able to acquire the imported protein hydrolysate baits such as Torula yeast when needed due to their cost and the lengthy procedures involved in their importation. Further studies will involve the extraction of pure of papain and its validation trials for recommendation to farmers.

Acknowledgments

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