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Extract of *Pimenta Racemosa* as Attractant for *Bactrocera Dorsalis* in Mango Orchards

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Abstract

The status of *Bactrocera dorsalis* as a destructive pest of fruits including mango continues to be a challenge among farmers at Ghana. Although chemical insecticides have been employed to manage its incidence and damage, there still exist gaps that need be addressed including concerns on toxic residues on fruits and the possibility of resistance evolvement by this pest to insecticides. The alternate management for the fruit fly should therefore be environmentally friendly and with minimal side effects. The objective was to compare the attractiveness of homemade lures of aqueous leaf extract of *Pimenta racemosa* and a commercial attractant containing methyl eugenol. The research involved the use of leaf extracts of *Pimenta racemosa* to trap *Bactrocera dorsalis* was conducted in five mango orchards in two agro-ecological zones in Ghana during the major mango fruiting season of 2017. Three experimental orchards were each sectioned into five blocks of 20 trees each. Four trees in each block formed the sampling trees making 20 sampling trees per orchard received the lures as treatments. The lures were dispensed in homemade traps made of PET containers. A total of 174,388 individual arthropods were captured of which 171,412 were identified as *B. dorsalis* and 2,976 identified as non-target arthropods. There was a significant difference between the performance of the commercial lure and the leaf extracts ($P < 0.05$) which was expected. The ability of the Pimenta extracts in the homemade traps to capture some fruit flies is an indication of its potential as a low-cost option to complement the more expensive commercial for small farm holdings.

Keywords: fruit fly, mango, *pimenta* extract, parapheromone, *Bactrocera dorsalis*

1. Introduction

Mangoes *Mangifera indica* (Anacardiaceae) are produced in the tropics and sub-tropics and consumed all over the world in its fresh, dried, or frozen state as well as in confectionary and beverages (Evans et al., 2017). Over 80 countries cultivate mangoes in a planting area of more than 2.7 million hectares (Jahurul et al., 2015). Mango is one of the major fruit crops grown in Ghana for the domestic and export markets and has a high potential to provide revenue and food security. The taste and nutritional quality are key factors contributing to the worldwide interest in mangoes (Evans et al., 2017). It is high in vitamins and nutrients and has a rich source of vitamins A and C. The rise in global_demand for mango has contributed to the increased production in West African countries (Ræbild et al., 2011) where the climate is ideal. The global mango market is expected to grow by 5% by 2030 and will represent approximately 28 billion US Dollars in trade earnings (FreshFruit, 2022).

The major constraints to production of mangoes include diseases, pests and challenges with high perishability and postharvest losses (Ekesi & Billah, 2007; Oppong et al., 2019; Billah & Honger, 2022). Approximately 260 pest species (Carrillo et al., 2017) including stone weevils, mealybugs, or fruit flies, Diptera: Tephritidae (White & Elson-Harris, 1992; Lux et al., 2003b; Drew et al., 2005; Okorley et al., 2014; Amevoin et al., 2021; Billah & Honger, 2022) have been recorded from seedlings to mature trees at production, harvest, and postharvest stages. In West Africa, mango fruits are infested by fruit flies during the fruit development and fruit ripening stages (Amevoin et al., 2021; Billah & Honger, 2022). The oriental fruit fly *Bactrocera dorsalis* (Hendel) (Diptera:

Tephritidae) is known for its extreme polyphagy, with over 209 recorded hosts in 51 plant families (Clarke et al., 2005). Infestation can lead to 25-30% up to 100% yield loss to the farmer in a season Fruit fly infested fruits lose quality leading to economic loss to farmers. Female fruit flies lay eggs under the skin of the fruit, which hatch into larvae that feed on the flesh and leads to decaying in the crop (Chang et al., 2016). Infested fruits quickly rot and become inedible or drop on the ground. There is also the indirect loss associated with quarantine restrictions imposed by mango importing destinations (Ekesi & Billah, 2007; Qin et al., 2015; Early et al., 2016; Biondi et al., 2018; Karsten et al., 2018).

There is a common concern that the adverse effects of indiscriminate use of chemical insecticides for the control of fruit pests is not sustainable (Sharma et al., 2014; Khan et al., 2018). The high demand for blemish-free vis-a-vis residue-free produce by consumers has led to the crusade which requires for the sustainable management of fruit flies. In effect the management of fruit flies currently, has an increasing focus on replacing chemical pesticides with alternative pest control methods (Benelli et al., 2014; Billah & Wilson, 2016; Billah et al., 2016; Hossain et al., 2020) including natural products (Jaffar & Lu, 2022). Several stakeholders in the mango value chain have explored the possibility of non-chemical alternatives for the management of fruit flies, especially amongst small-holder farmers at Africa. In contrast, the plant extracts show great promise due to their local availability (Aluja & Rull, 2009), perceived safety on humans, low mammalian toxicity, and friendliness to the environment (Tadeo et al., 2017).

Mating disruption using synthetic pheromones for example has been widely adopted as a proactive way to protect crops by preventing insects from reproducing (Sonenshine, 2017). The highest land area under mating disruption as a control strategy in insects has been reported for the spongy moth, formerly- gypsy moth, (*Lymantria dispar* (Lepidoptera: Erebidae), codling moth (*Cydia pomonella* (Lepidoptera: Tortricidae) and the European grapevine moth *(Lobesia botrana*: (Lepidoptera: Tortricidae*)* (Witzgall et al., 2010). The use of baits and lures in the management of fruit flies has also gained popularity due to its reduced risk, biorational attributes, environmentally acceptable and may even be used by organic growers (Leblanc et al., 2009). Techniques such as bait application technology (BAT), male annihilation technology (MAT), Specialized Pheromone and Lure Application Technology (SPLAT) and combination of several control measures have been exploited using cheap and locally derived lures (Tan et al., 2014; Candia et al., 2019). For example, in Brazil, livestock manure was evaluated in the control of fruit flies as an alternative to hydrolyzed protein which was difficult to obtain by small-holder farmers with success (Filgueiras et al., 2016). Vargas & Prokopy (2006) reported the relative attractiveness of protein baits in combination with toxicants such as spinosad and malathion to control fruit flies.

A combination of methyl eugenol and Spinosad was also reported by Vargas et al., (2009) which acted as a convenient ready-to-use MAT formulation. Some of these technologies, however, are still beyond the reach of many small-holder farmers in Africa including Ghana due to costs and may limit their use. Furthermore, farming landscapes in Ghana are located in rural settings with high illiteracy rates and its consequent misunderstanding of the technical profiles of synthetic insecticides. The high cost of commercial pheromones is also an obstacle to the implementation of safer control managements (Walgenbach, 2018).

The need to bioprospect for local and community available ingredients to support small-holder farmers in their quest to develop attractants for pest traps could scale down the cost and improve accessibility of products that could be used to sustainably manage fruit flies. For example, Kimbokota et al., (2013) indicated that extracts of a local plant, *Gynandropsis gynandra* L. (Capparaceae) was attractive to fruit flies in Tanzania. Also in Ghana, Akotsen-Mensah et al., (2013) have indicated that there was promise in the attractiveness of the aqueous leaf extract of *Pimenta dioica* (L.) Merrill (Myrtaceae) applied as baits to attract and kill fruit flies. These observations give credence to the possibility of using local natural products in the development of attractants in fruit fly management. In Akotsen-Mensah et al. (2013), the attractiveness of *Pimenta* extract to male *B. dorsalis* was attributed to the presence of methyl eugenol. In other experiments in West Africa, methyl eugenol was the most effective and had the highest fly capture when tested with other baits (Minhibo et al., 2018). *Pimenta racemosa* likewise, has been reported to contain methyl eugenol (Alitonou et al., 2012) and is abundant and cheap in Ghana. Eugenol is the main compound responsible for the biological potential of the *Pimenta* genus (Contreras-Moreno, 2018). This study was therefore conducted to assess the potential of the aqueous leaf extract of *P. racemosa* in attracting field populations of Oriental fruit flies in mango orchards in two major mango-producing districts in the Southern zone of Ghana.

2. Methods

2.1 Source and Preparation of Attractants

Fresh leaves of *P. racemosa* were collected from the Forest and Horticulture Crops Research Centre of the, University of Ghana at Kade (6 ° 05' North: 0 ° 05' West). They were washed with distilled water to remove adhering materials and shade-dried for about 48 h. The dried leaves were macerated by pounding in a wooden mortar with a pestle until a coarse paste was obtained. Fifty grams of the paste was mixed with 1, 1.5 or 2 L of water and allowed to extract for 10 min to obtain the required concentrations of 5%, 3.3% and 2.5% (wt/vol), respectively. The mixture was decanted with a sieve of mesh size 0.1mm, with the filtrate forming the three extracts at the respective concentrations. The fourth bait, Stop Mating Block™, was a compacted fiber-wood block, soaked in a containing a mixture of methyl eugenol and a killing agent, Deltamethrin in a ratio of 4:1.

2.2 Site Selection

The study was conducted in orchards at Shai-Osudoku and Yilo-Krobo districts in the southeastern part of Ghana, with a vegetation comprising shrubs, grasslands, and semi-deciduous rain forests. Four mango orchards from two neighbouring districts (Figure 1) were selected for the experiments. The orchards were located at Adumanya and Ayikuma in the Shai-Osudoku district (5.8829 ° North, 0.0980 ° West) and the Prosper and Boko orchards in the Yilo-Krobo district (6.1050 °North, 0.0140 °West).

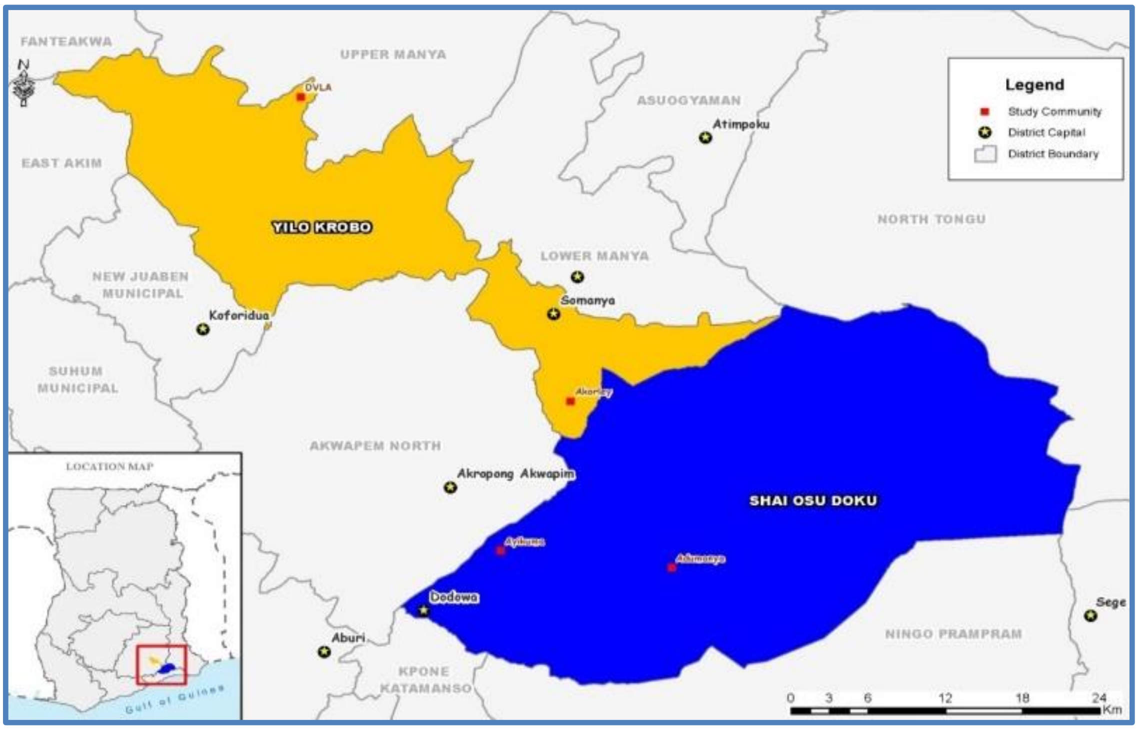

Figure 1. Study locations-Shai-Osudoku and Yilo Krobo districts in Eastern region at Ghana (Geography Department, University of Ghana)

2.3 Experimental Procedures

The planting distances between the mango trees in the selected orchards were 10 x 10 m. The experimental design was a Randomized Complete Block Design (RCBD), with five 20-tree blocks (Figure 2a) in each of the four selected orchards. Four mid-block mango trees were selected to receive experimental treatments (traps), making 20 sets of traps per experimental orchard. A set of traps in a tree thus consisted of four attractants. No single tree received all 4 sets of treatments. The 4 sets of treatments were place on different trees, and at distances of 30 m apart to avoid trap interference.

The homemade traps used to monitor the flies in the orchards consisted of a 500 mL blue-cap PET bottle with two equidistant windows (3.0 x 1.5 m) created on opposite sides on the uppermost part which served as entry holes for the fruit flies. A yellow wide-cap cylindrical PET container which had four round holes of about 2 cm in diameter evenly spaced around its upper half to serve as entrance for the insects was the fourth trap. All the traps had small holes in the lids through which metal wires were attached to the bottle tops to serve as hangers (Figures 2b and 2c) for the traps to be placed on the trees. The traps contained baits specific to the male fruit flies, made from four types of para-pheromones and these formed the four sources of treatment variations. It included:

the commercial Stop Mating Block (SMB™) which was hanged at the tip of the wire inside the yellow wide cap cylindrical PET container trap and three concentrations (5%, 3.3% and 2.5% w/v) of aqueous leaf extract of *P. racemosa*.

2.3.1 Trap Placement and Data Collection

Four trap sets were set in each block (Figure 2a) two weeks after fruiting. Traps were hung at a height of 1.5 - 3 m above ground (depending on the architecture of the canopy and age of the tree). A minimum distance of 40 m was maintained between experimental trees to minimize trap interference (Ekesi & Billah, 2007; N'dépo *et al*. 2013). Trapped insects were protected from predators or scavengers by smearing solid grease on the wire hangers. The leaf extract treatments were replaced each week while the commercial Stop Mating Block™ was replaced after 6 weeks (based on Manufacturer's recommendations).

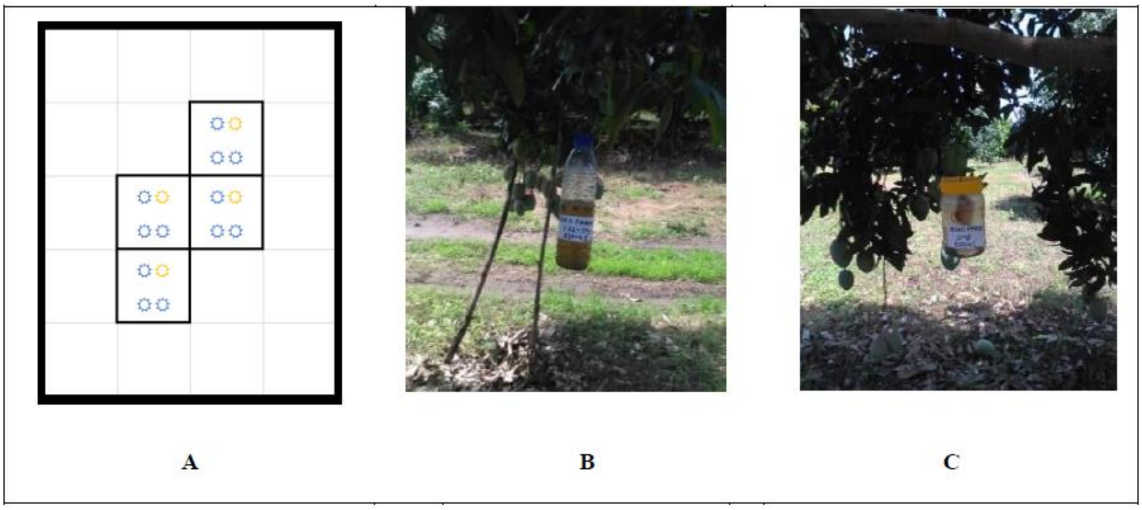

Figure 2. Trap layout in a block and traps containing the baits for the experiment.

A-Four selected trees☐ with set of four traps ☼ per tree within a block ▣comprising 20 mango trees

B-Blue cap PET bottle trap, C-Yellow cap PET container trap

All traps were inspected at weekly intervals. Traps with the leaf extract treatments were sieved to extract drowned insects. Captured insects in the Stop Mating Block™ treatment were collected from the container with forceps. All captured flies were transferred into labelled vials containing 80% alcohol to preserve the insects for identification in the laboratory with the aid of a Digital Lecia EZ4 D stereomicroscope. A taxonomic key by Billah *et al.* (2007) was used in the identification of the captured flies.

2.4 Data Analyses

Catches from different collecting dates were treated as repeated measures. The total trap catches from the four treatments were analyzed with treatment effect being the source of variation (SAS Institute 2014 JMP version 10.0) for each orchard. Data was transformed ($\sqrt{x+0.5}$) where necessary before analyses. Least square means (LSM) estimates for each lure treatment were compared using Tukey's honest significant difference test.

3. Results

A total number of 174,388 (100 %) organisms were captured of which 171,412 (98.3 %) were identified as *B. dorsalis* (Figure 3) and 2,976 (1.7 %) identified as non-target arthropods (Figure 4). The attractant SMB™ had significantly higher trap catches than the leaf extracts ($P < 0.05$) in all the four orchards. Although extracts in traps were replaced at weekly intervals, the trend in trap catch followed a similar trend for all the orchards (Figure 3). Trap catches increased to a peak and then declined. For example, the SMB™ treatment recorded a highest trap catch of 7,903 fruit flies at the Adumanya orchard (Figure 3) at week 4 of the sampling period and declined subsequently.

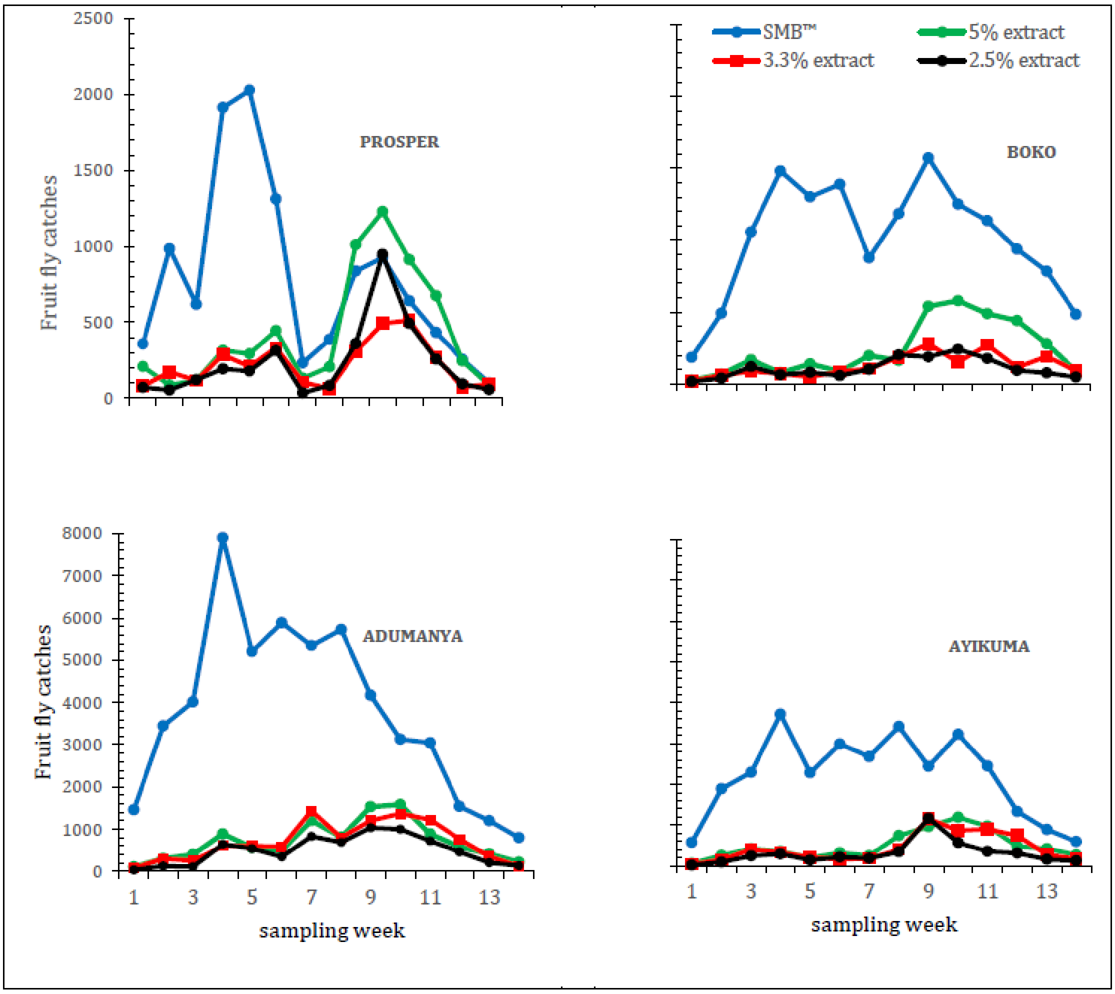

Figure 3. Fruit fly catches at four experimental orchards over a 14-week period

The general performance among the extract concentrations showed no significant differences in terms of trap catches (F = 2.61, df = 2, P = 0.0833). For all the extracts, the number of catches increased between weeks 2 and 10 and declined afterwards. Although the 5% wt/vol extract recorded the highest catches, as for example in week 10 (4,585), it was not significantly different (P = 0.05) from the catches in the other extracts.

Within the orchards, it was only in the Prosper orchard where trap catches from the extract concentrations showed significant differences (F = 6.02, df = 2, P = 0.0254). The 5% wt/vol extract at Prosper orchard recorded the highest mean trap catch per week of 1,190.80 followed by the 2.5% wt/vol extract (648.60) and the lowest was the 3.3% wt/vol extract (619).

A total of 2,976 non-target arthropods from nine orders (Araneae, Blattodea, Coleoptera, Diptera, Hymenoptera, Hemiptera, Lepidoptera, Neuroptera and Orthoptera) were captured in the traps containing the various treatments and were classified as predators, scavengers, or pests (Figure 4). Prosper orchard recorded a total of 640 non-target arthropods, Boko orchard 823, Ayikuma orchard 981 and Adumanya orchard, 532. The Hymenopterans which consisted of ants and wasps were the highest trapped (2,220), followed by other non-target Diptera 282 (flies), Lepidoptera 161 (moths and butterflies), Blattodea 158 (Cockroaches), Araneae 87 (spiders), Coleoptera 60 (beetles and weevils), Orthoptera 5 (grasshoppers), Hemiptera 2 (cotton stainers) and one lacewing, (Neuroptera). Traps in the Ayikuma orchard had the highest number of non-target arthropods (Figure 4).

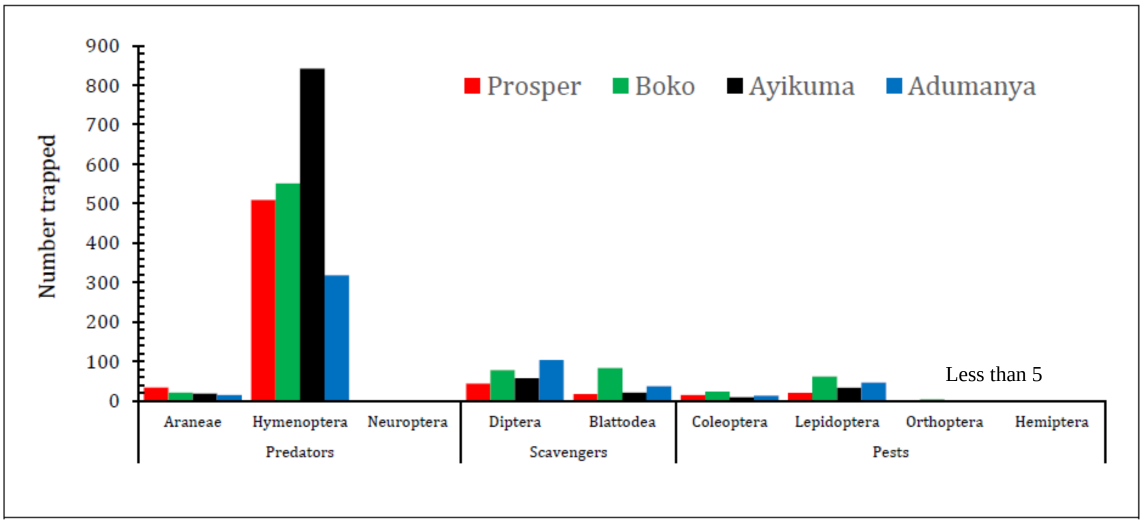

Figure 4. Non-target arthropods captured in traps during the sampling period (14 weeks)

The relative fly density at the orchards as recorded from the treatments (Figure 5) was a function that determined the average number of fruit flies captured per trap per day during the sampling period. The result represents the comparative size of the adult fruit fly population in an orchard in situ. The results obtained were bait dependent with SMB™ at Adumanya recording the highest relative fly density of ca 102 which was significant ($P < 0.05$).

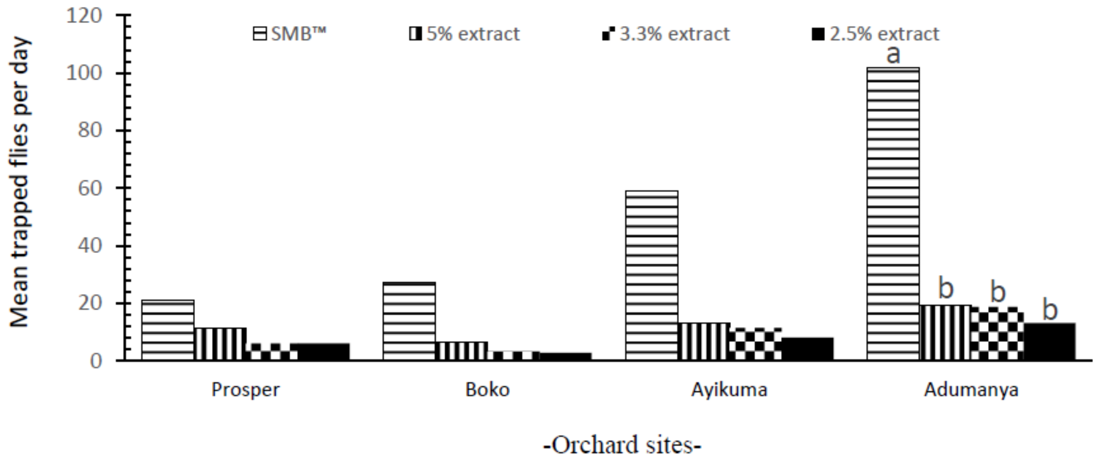

Figure 5. Daily trap density of fruit flies per bait at experimental locations

4. Discussion

Pest monitoring or trapping insects with pheromones is an established and environmentally-sound part of modern-day integrated pest management systems. The pheromone trapping technique adopts the insects' inherent allocrine signalling mechanisms as baits to lure the animal, in this case insects to toxic surfaces and food or interfere with mate-searching efficiency by application of synthetic allelochemicals or its analogues (Verlinden et al., 2014). Mating disruption can be used to disrupt chemical communication in fruit flies. When mating disruption by a lure is successful it is depicted by a drop in trap catches over time (Bakthavatsalam, 2016) which was the case in this experiment during the 14-week sampling period.

Synthetic male lures are well known in the monitoring and mass trapping of fruit flies (Leblanc et al., 2009). Male insects of the genus *Bactrocera* are attracted to either of two well-known kairomones: Cue-Lure [4-(p-acetoxyphenyl)-2-butanone] and methyl eugenol (4-allyl-l, 2-dimethoxybenzene-carboxylate) (Metcalf, 1990). Methyl eugenol is however specific to male flies (Shelly et al., 2012). The two baits studied in this experiment attracted primarily male *B. dorsalis* which confirmed the presence of a specific lure that targets male fruit flies. For the synthetic bait used (Stop Mating Block™), methyl eugenol is explicitly quoted as a constituent active ingredient, however the *Pimenta* used was a crude extract and it is expected to contain methyl eugenol as proven in literature. Pimenta is known to contain at least 48% eugenol (ALrashidi et al., 2022) whereas methyl eugenol is the main active ingredient in the commercial Stop Mating Block™ formulation.

The commercial Stop Mating Block™ outperformed the extracts in attracting more fruit flies which could also be attributed to the added effect of the yellow colour of the trap cap for that bait. Adult fruit flies are known to locate host through visual and olfactory stimuli as reported by Brévault & Quilici (2010). This conforms to the well-established fact that fruit flies react to colours that are comparable to oviposition substrates such as yellow or green mango fruits (Ravikumar & Sviraktamath, 2007). For example, there are reports which indicate that deep yellow-coloured traps attracted greater numbers of *Bactrocera* species (Liu et al., 2018; Bajaj & Singh 2020; Kausar et al., 2022). The fibrous nature of the Stop Mating Block™ contributed to its performance, probably due to its ability to imbibe water and release it by drying up quickly unlike the crude extracts that got diluted from rainwater and reduced its potency. These attributes of the traps were not measured in these experiments. Trap colour and bait retention methods to improve trapping performance has been reported by Broughton & Rahman (2016). Reduction in trap sensitivity over time could be offset by increasing either the frequency of bait replacement or density of traps, although this may have labour implications.

There are also reports indicating that the leaves of *Pimenta* spp. contain volatile compounds and like most phytochemicals may be altered by climatic factors and preparation procedures, for example, environmental factors such as temperature, and UV radiation could modify the composition of phytochemicals (Šamec et al., 2022; Jiménez-Viveros et al., 2023). Specifically, on *Pimenta,* Rao et al. (2012) reported that sunlight variations may alter the composition of volatile oils in *P. dioica* which is in contrast to the commercial Stop Mating Block™ which has the potential of being active for 8 weeks. The potency of the active compounds in crude extracts of *Pimenta* is known to reduce as the extract ages (Akotsen-Mensah et al., 2013). The reduced concentration with age may result in reduced attraction to *B. dorsalis* catches. The extract traps were replaced each week in this experiment to ensure the integrity of the baits.

The recorded fruit fly trappings per day varied for the four experimental locations but it remained high and comparable to other results obtained in Zimbabwe (Musasa et al., 2019) and in Vietnam under farmers practice (Perez-Staples et al., 2019). The high and differential captures of *B. dorsalis* recorded across the orchards indicate the potential ability of the attractants tested to lure fruit flies based on local population densities. The fly catches decreased with time as the season for different mango varieties ended which was an indication of the attraction of different concentrations of kairomones from mango varieties when in season as reported by Mwatawala et al. (2006) and Vayssières et al. (2009). In multi-locational trappings over time in Mozambique for example, Canhanga et al. (2020) reported of differences in *B. dorsalis* catches and population densities peaked over the sampling period to a maximum of 40 flies/trap/day, which was correlated with temperature. The environmental variables were not recorded in our experiments hence the population fluctuation in trap catches was attributable to season duration and host fruit availability in a location (Vayssières et al., 2015; Theron et al., 2017; Grechi et al., 2021). However when fruit fly trap catches are expressed as flies/trap/day, it becomes a standard unit for comparison across different environmental conditions, irrespective of number of traps involved or period of exposure of the traps, and it is internationally used and accepted (IAEA, 2003 & 2009). It is inevitable that non target insects will be captured in traps by accident or due to the attractive scent of decaying fruit flies in traps. The non-target arthropods from nine orders captured were either predators, scavengers or pests which is an impact of beneficial insect diversity. There are concerns as to the non-target impacts of baits on beneficial and endemic insects although most of the previously published records of attraction to methyl eugenol have been attributed to secondary attraction to decaying fruit flies. The recommended strategy to avoid trapping pollinating bees has been to place them in trees after the flowering period in orchards. (Leblanc et al. 2009; Mwatawala, 2015). Some authors including Williams & Whitten (1983), Asquith & Burny (1998) and Sugahara et al. (2013) have speculated that methyl eugenol may either mimic other compounds in flowers or mimic terpenoid pheromones produced by bees.

Other experimental indices that were not compared included the positional height of the traps, However, we placed our traps appropriately within recommendations from others such as Siddiqui et al. (2003) who reported that high number of flies were captured in the traps hung at 10 feet high. Standard heights used in the hanging of traps have always been between 1.5-2.0 m (Billah et al., 2006, 2010; Ekesi & Billah. 2007; Billah & Wilson, 2016; Billah, 2019; Awarikabey et al., 2022).

The use of pheromones as a component of IPM strategies has been reviewed by Witzgall et al. (2010) including the use of mating disruption by Rodriguez-Saona & Stelinski (2009). The high price of commercial products against fruit-flies, and the risk of reinfestation from neighboring orchards, limits options for localized orchard control. This calls for management tactics using low-cost local products and team action among farmers (Bulley, 2012; Banini, 2013; Attafuah, 2017; Billah et al., 2022). Even though, Stop Mating Block™ performed better than the crude extracts, farmers can still achieve economical control or manage *B. dorsalis* with increased number and frequency of the extract traps. This will provide locally sustainable techniques for fruit fly control that could incentivize area-wide cooperation of farmers to use low-cost lures and traps supplemented with commercial lures. With a recommended density of 25 traps per ha to protect a mango orchard Mertilus et al. (2017) cost-effective homemade traps such as the use of *Pimenta* extract can replace commercial traps for mass trapping of fruit flies. These findings have indicated that aqueous leaf extract of *P. racemosa* have the potential of attracting *B. dorsalis* hence can be used in fruit fly management even at lower concentrations (2.5-5% wt/vol). Although this single strategy may not be adequate, the driving force should be integrated strategies to reduce fruit fly damage including adjusting harvest timing, especially by harvesting fruits at the green-mature stage or at the yellow-point onset as reported by Grechi et al. (2021).

5. Conclusions

Insect traps that incorporate attractants are useful tools for either monitoring or directly reducing insect populations over time by mass trapping or mating disruption. Each component of the insect trap has a role to play either as a visual, odour, arrestant, or toxicant and should be considered to increase efficiency. When control programs are accessible, sustainable, and cheap, it improves cooperation among farmers and adoption rate is high, which is necessary for area-wide fruit fly control programs. These results relied on trap colour and odour from a local plant to improve the low-cost options available for the development of pest management strategies based on the use of local bioresources to complement more expensive commercial attractants.

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Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Informed consent

Obtained.

Ethics approval

The Publication Ethics Committee of the Canadian Center of Science and Education.

The journal’s policies adhere to the Core Practices established by the Committee on Publication Ethics (COPE).

Provenance and peer review

Not commissioned; externally double-blind peer reviewed.

Data availability statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Data sharing statement

No additional data are available.

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