Extraction and Potential Use of Black Pepper (Piper Nigrum) Oil as Attractant for *Bactrocera Dorsalis*

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Abstract *- Mango is considered one of the most valued crops in the Philippines. However, the production of quality mango fruit has been declining due to various environmental factors, inflated cost of production, and severe pest infestations. One of the most damaging pests in the mango industry is Bactrocera dorsalis, commonly known as Oriental fruit fly, which affects the fruit quality and reduces its market value and export potential. Investigation of the effectiveness of black pepper oil as an attractant for mass trapping of B. dorsalis was the aim of this research, since the commercially available lures only target a specific sex of fruit flies. In this research, steam distillation and Soxhlet extraction using two solvents, hexane and ethanol, were used to extract volatiles and essential oils from dried black pepper. The attractancy of fruit flies to the extracted oils was tested using a controlled environment setup. Hexane black pepper oil (BPO) extract obtained the highest number of trapped fruit flies, while BPO from steam distillation showed minimum attractiveness to B. dorsalis. A multi-choice test bioassay showed that the attractiveness of hexane BPO extract was comparable to that of commercially used attractants in the market. These findings suggested a possible use of black pepper oil as an alternative attractant for B. dorsalis control.*

Keywords: mango fruit fly, black pepper, Soxhlet extraction, GC-MS.

I. INTRODUCTION

Mango, Mangifera indica, is considered the national fruit of the Philippines and is one of the largest fruit crops in the country in terms of volume of production, under banana and pineapple (Alcasid et al., 2021; Ventura et al., 2021). While popular in both local and international markets, mango production in the Philippines faces challenges despite its demand in the world market (Ventura et al., 2021). The variety of "Carabao" mango contributed the largest share (77.34%) of total mango production in the first quarter of 2022 (Alcasid et al., 2021), 4.1% lower compared to the production during the same period of 2021. This is attributed to the occurrence of massive infestations and diseases, among other factors.

Pest infestation has been a major problem in mango production in the Philippines. In the first quarter of 2018, the infestation of pests decreased mango production by 9.4%, leaving a total of 10.06 thousand metric tons of loss (Tumang, 2019). Oriental fruit fly, *Bactrocera dorsalis*, is one of the most damaging pests that causes direct and indirect losses to the fruit, affecting its quality and resulting in problems in exports. It is considered a serious threat in terms of losses in quality, a decline in production, and its market value. Cultivators use synthetic pesticides to control pests in different orchards in the country. Others use mass trapping, orchard sanitation, and physical controls, including fruit bagging (The Organic Farmer, 2021). Although the use of pesticides is deemed effective in reducing pests, their use is discouraged as they contaminate the soil and other vegetation and can be toxic to nontargeted organisms such as bees. When used in excess, these pesticides can be hazardous to the environment and leave residue in the fruit (Imam et al., 2022). Insect resistance to pesticides are also associated with the use of pesticides (Aktar et al., 2009; Latif & Abdullah, 2015).

The most used attractant for *B. dorsalis*, methyl eugenol, is proven effective but is known to be gender-specific and is used as a male attractant only (Haq et al, 2018; Fan et al., 2022; Imam et al., 2022). Food-type attractants, such as fermenting sugars, hydrolyzed proteins, and yeast, are used as attractants for both male and female fruit flies, but the lack of potency and their attractiveness to non-targeted organisms are the downsides of this type of attractant (Jayanthi et al., 2012; Sasaki et al., 2019; Imam et al., 2022). Though crude attractants based on fermenting sugars, hydrolyzed proteins, and yeast are readily available in the market, they lack potency and specificity, and their short shelf life (Jayanthi et al., 2012) is the reason cultivators opt for other means. Thus, developing effective, non-gender-specific attractants with long shelf life is needed.

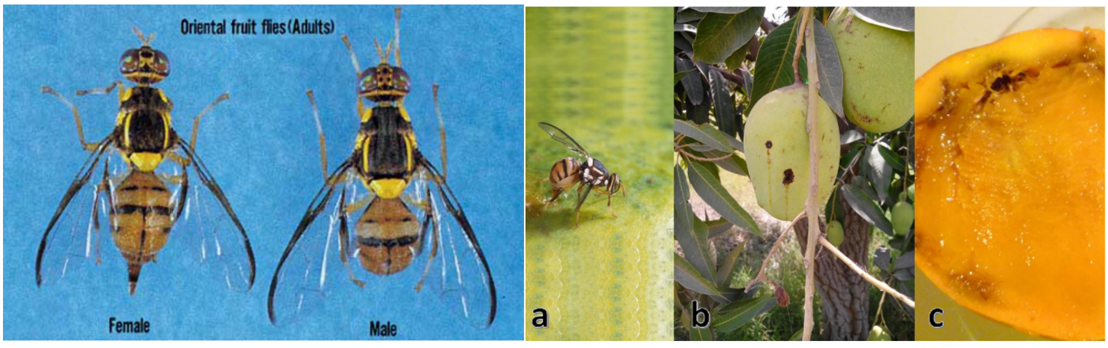
Therefore, a study in the use of plant-based attractants is proposed. This study explored the use of black pepper oil extract as a viable alternative to commercial attractants for *B. dorsalis*. This study used different types of extraction methods, namely, Soxhlet extraction and steam distillation.

II. LITERATURE REVIEW

Oriental fruit fly (*Bactrocera dorsalis*)

Bactrocera dorsalis, commonly known as Oriental fruit fly, is one of the major pests in agricultural fruits and vegetable crops. Its invasion poses a serious biosecurity risk to agricultural trade-based development of economies in Africa (Mutamiswa et al., 2020), is also highly invasive in Asia and certain European countries and has now become the major fruit pest in Africa and Asia owing to its high competitivity (Michael et al., 2021), ability to quickly adjust to a new environment and its high dispersal ability (Zhao et al., 2018). Moreover, *B. dorsalis* has more than 150 hosts of both fruits and vegetables, such as avocados, bananas, citrus, coffee, guavas, mangoes, papayas, passion fruits, pineapples, and tomatoes (Paragas et al., 2020).

The adult fruit fly is usually bigger than a house fly, with a body length of about 8.0 mm and a wing length of about 7.3 mm, which is mostly hyaline. Prominent yellow and dark brown to black markings on the thorax, two horizontal black stripes in the abdomen, and a longitudinal median stripe that runs from the base of the third segment apex are the predominant morphological observations of these flies. Under optimum conditions, the



development from egg to adult fruit flies takes roughly 16 days (Weems et al., 2019). Female *B. dorsalis* puncture their eggs into the host fruits and vegetables (Paragas et al., 2020).

Figure 1. Adult *Bactrocera dorsalis* (left) and Female Fruit Fly Oviposition in Mango fruit (right). a: Female *B. dorsalis* laying eggs on the fruit; b: Punctured mango fruit; c: Rotten mango fruit after infestation. *Source*: Weems et al., 2019; Sidra, 2021

Depending on environmental conditions and temperature, the eggs inside the host will hatch within a day or up to five days in cool conditions (Manrakhan, 2020), and the larvae will live in and feed off the flesh of the host fruits or vegetables through their developing stage (Paragas et al., 2020). When fully grown, the maggots exit the host fruit and pupation of the flies will later happen in the soil until adult flies emerge. Then, sexual maturity will occur after nine days of emergence, then mating ensues, and the cycle continues (Weems et al., 2019).

Essential Oils and Volatiles in Mango

Pandit et al. (2009) investigated the volatile profiles of mango based on cultivar relationships. It was found that a total of 84 volatile compounds were from alcohol, aldehyde, monoterpene hydrocarbons, oxygenated monoterpene, sesquiterpene hydrocarbons, oxygenated sesquiterpene, lactone, ketone, and non-terpene hydrocarbons. Further, β-myrcene, (Z)-ocimene, and δ-3-carene were the major compounds found in the blends of Indian cultivars, while non-Indian cultivars were found to contain δ-3-carene as the major component.

Ramos et al. (2014) reported that terpinolene and δ-3-carene were the major compounds found in the Brazilian mango Espada variety, while α-pinene and β-pinene were found in the Rosa variety. Further, essential oils from the leaves and fruit peels of Nigerian M. indica L. were found to be rich in sesquiterpenes such as δ-3-carene, α-gurjunene, E-caryophyllene, and β-selinene, while the fruit peel oil mostly yielded δ-3-carene and α-pinene (Ana et al., 2014).

According to research, fruit flies rely heavily on odorants in their host-searching behavior. Whilst the fruits and leaves of the host plants have been the most extensively studied organs for fruit fly volatile attractants, the search has been broadened to include non-host plant volatiles. In lieu of this, electrophysiological studies reported the sensitivity of fruit flies to organic compounds such as monoterpenes, sesquiterpenes, pyrazine, carboxylic acids, alcohols, aldehydes, ketones, and esters, the majority of which are volatiles typically found in ripening fruits (Gikonyo et al., 2003; Robacker et al., 2009; Jayanthi et al., 2012; Biasazin et al., 2014).

Black Pepper as a Potential Attractant

Black pepper, Piper nigrum, is one of the oldest and most popular condiments in the world. The fruit of black pepper is not only used in the kitchen but also in the medicinal field as it can be used as a stimulant, rubefacient, and disinfectant when externally applied (Martins et al., 1998). Research revealed that the genus piper contained terpenes—monoterpene hydrocarbons (62.2%), oxygenated monoterpenes (30.7%), sesquiterpene hydrocarbons (26.2%, and oxygenated sesquiterpene (3.2%)—amides, and alkaloids (Gupta et al., 2013). Additionally, limonene, β-caryophyllene, sabinene, β-pinene, α-pinene, δ-3-carene, and myrcene are the major constituents in black pepper oil (Martins et al., 1998; Dosoky et al., 2019). While 1-phyllandrene and caryophyllene are the predominant constituents in the volatile oil of both P. nigrum and P. longum (Bindu Madhavi et al., 2009). Jirovetz et al. (2002) reported that β-caryophyllene, germacrene D, limonene, β-pinene, α-pinene, α-phellandrene, and α-humulene are the major constituents of the three investigated pepper samples, namely P. nigrum (black), P. guineense (black and white). Minor constituents such as δ-carene, β-phellandrene, isoborneol, α-guaniene, sarisan, elemicin, calamine, caryophyllene alcohol, isoelemicin, T-muurolol, cubenol, bulnesol were reported to have a significant role in the odor of pepper.

III. MATERIALS AND METHODS

Samples and Chemicals

The black peppercorns were sourced from a local grocery. Ethanol (95%), hexane (95%), anhydrous sodium sulfate, and methyl eugenol were from a chemical supplier.

Soxhlet Extraction

Black pepper oil was extracted in the Soxhlet apparatus using ethanol and hexane as solvents. The ratio between ground black pepper powder and the solvent was 1:15 (g/mL) in each extraction . Solvents were recovered using a rotary evaporator and were reused for another batch of extraction using the same solvent to minimize waste generation.

Modified Steam Distillation

The modified steam distillation set-up consists of oil bath (heat source), two-neck round bottom flask (contains the solvent), biomass flask (contains the sample), adapter (connects the glassware), 3-way distillation adapter (connects the condenser, biomass flask and thermometer), condenser, thermometer, 105°C distillation adapter with drip tip (distillate passage), separatory funnel for the distillate. For this set-up, 150g of ground black pepper was placed in the biomass flask, and 500mL of distilled water was transferred to the round bottom flask. Distillation was done at 150-160°C for 6 hours. Distillate was collected and dried using anhydrous Na_2SO_4. The distillate was filtered and stored at 4°C.

Fruit Fly

Wild fruit flies were randomly collected from a micro mango farm in Los Baños, Laguna, Philippines. Twenty fruit flies, regardless of maturity, sex, and behavioral movements, were used in each trial for CEB bioassays, while forty fruit flies were used in CEB-MCT. Fruit flies used in this study were submerged in isopropyl alcohol before disposal.

Controlled Environment Bioassay

Controlled environment fruit fly bioassays were built for this study using 5 mL plastic tubes and a small (237 mL) plastic jar. This served as a treatment and control container/trap for the controlled environment bioassays. The dual-choice bioassay test setup was made using a clear box container (41.5 × 26.5 × 15.5 cm) with the lid cut off and replaced with a fine mesh wire to provide ventilation. Two plastic jars with plastic tubes inside, containing the positive control (extract) and negative control (solvent used), were placed in the bioassay container at an equal distance. A total of 20 fruit flies were released and left for 24 hours in each bioassay per test trial.

Another bioassay set-up was made using another clear box container (23×23×25 cm). Four plastic jars were placed inside the bioassay container. Tests were done by releasing 40 *Bactrocera dorsalis* flies that were left for 24 hours to choose between the traps with attractant: three extracts (hexane, ethanol, distillation) and a commercially used attractant (methyl eugenol).

The number of trapped fruit flies, *Bactrocera dorsalis*, in each trap, along with the undecided fruit flies inside the bioassays, was counted and recorded after 24 hours. Each test was replicated three times. Each test in the bioassays used a different set of fruit flies to avoid possible adaptation of the flies due to prolonged and repeated exposures to the extracts (Tait et al., 2018).

Numbers of fruit flies trapped in the treatment (extract) containers and control (solvent) containers from the bioassays were collected after 24 hours in each test. Undecided fruit flies were also counted and recorded per test. Triplicate analyses were done per bioassay. A total of 300 fruit flies were used in this study.

IV. RESULTS AND DISCUSSION

Black Pepper Oil Extraction from Soxhlet and Steam Distillation

Among the three extraction methods used, Soxhlet extraction using hexane as solvent produced the highest percent yield of 12.55%, next was Soxhlet extraction using ethanol as a solvent with 12.52%, and lastly, modified steam distillation with 4.98% yield (Table 1). This may be because the Soxhlet apparatus setup only needs a few grams of sample per extraction, unlike modified steam distillation, which uses quite a lot of samples per distillation setup. The low percent yield from the modified steam distillation was attributed an insufficient amount of water, hence a lower diffusion of oil in the water, resulting in reduced solubility and decreased yield of soluble components.

Table 1. *Results in terms of (%) Yield of Three Extraction Methods*

Extraction method	Solvent used	Total sample used (g)	Extracted oil (mL)	Extracted oil (g)	% Yield
Soxhlet Extraction	Ethanol	114.81	17.3	14.38	12.52
Soxhlet Extraction	Hexane	121.58	19.9	15.26	12.55
Steam Distillation	Water	300	17.1	14.93	4.98

Efficiency of Extracts in Bioassay

The effectiveness of hexane BPO extract from Soxhlet extraction was tested against the solvent used in extraction, which was hexane. Table 2 showed significant differences in trapped flies in the three bioassays performed. Hexane extract had an average of approximately 11 trapped flies, while hexane (solvent used) had an average of 3, and lastly, approximately 6 fruit flies were undecided and stayed outside the arenas after 24 hours.

Table 2. Hexane extract Dual-choice Bioassay Test

Hexane dual-choice bioassay test (2 Traps: 1 extract and 1 negative control)				
	Trial 1 (20 flies)	Trial 2 (20 flies)	Trial 3 (20 flies)	Average
Hexane Extract (+)	12	10	10	10.66 ≈ 11
Hexane (-)	2	4	3	3
Undecided	6	6	7	6.33 ≈ 6
Total	20	20	20	20

Table 3 shows the effectiveness of ethanol BPO extract against the solvent used in the extraction. Significant differences in the three trials were obtained with average count of fruit flies trapped of approximately 9, 4, and 7 for ethanol BPO extract, ethanol (solvent used), and undecided flies.

Table 3. Ethanol extract Dual-choice Bioassay Test

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	Trial 1 (20 flies)	Trial 2 (20 flies)	Trial 3 (20 flies)	Average
Ethanol Extract (+)	9	10	9	9.33 ≈ 9
Ethanol (-)	4	4	3	3.66 ≈ 4
Undecided	7	6	8	7
Total	20	20	20	20

Significant differences were also evident in the water BPO extract and water (solvent used) as shown in Table 4. An average of 6 fruit flies were trapped in the arena containing water BPO extract, while there were no reported flies trapped in the arena with water as an attractant. However, the average count of undecided fruit flies was notably higher than the average count of water BPO extract in comparison to the other two extracts, which denotes that the water extract was not as compelling as the hexane and ethanol BPO extracts. Overall, this proved that the attraction of *B. dorsalis* to the extracts was not owed to the solvents used (control) in the extraction, but to the chemicals and volatiles present in the BPO extracts.

Table 4. Distillation extract Dual-choice Bioassay Test

Ethanol dual-choice bioassay test (2 Traps: 1 extract and 1 negative control)				
	Trial 1 (20 flies)	Trial 2 (20 flies)	Trial 3 (20 flies)	Average
Water Extract (+)	6	4	8	6
Water (-)	0	0	0	0
Undecided	14	16	12	14
Total	20	20	20	20

The responses of *B. dorsalis* to the extracts against the solvents used in extractions were investigated in a bioassay using dual-choice tests. All tests conducted on the data were parametric tests, Student's t-tests. Table 5 shows that all extracts have significant differences from their solvents with p-values of < 0.01, which means that *B. dorsalis* was indeed attracted to the extracted BPOs and not to the solvents used in the extraction processes.

Table 5. Combined Table T-test for the Three Extracts

Extract	Solvent	Mean	Standard Deviation	Mean Difference	Statistic	p-value	Levene's Test
Hexane Extract		10.7	1.15	7.67	8.69	<0.001**	0.609
	Hexane	3	1.00				
Ethanol Extract		9.33	0.577	5.67	12.0	<0.001**	1.000
	Ethanol	3.67	0.577				
Distillation Extract		6.00	2.00	6.00	5.20	<0.001**	0.116
	Water	0.00	0.00				

The high attractancy rate of fruit flies to hexane BPO extract was attributed to the chemical compounds present in the BPO such as β-myrcene, δ-3-carene, α-gurjenene (Pandit et al., 2009; Ramos et al., 2014), terpinolene, β-pinene (Ramos et al., 2014; Li et al., 2019; Malo et al., 2011), caryophyllene (Li et al., 2019), and limonene (Malo et al., 2011) that were also observed in extracts of mango fruit pulp and peels. Consequently, a lower attractancy rate of fruit flies to extracted BPO using ethanol was associated with fewer chemical compounds present in BPO that were also reported in mango fruit such as α-pinene, caryophyllene, 3-carene, and limonene. Moreover, a much lower attractancy rate was observed from steam distillation BPO, possibly due to significantly fewer chemical compounds present, such as β-pinene, caryophyllene, and limonene.

Multi-choice Test Bioassay

The numbers of trapped *B. dorsalis* on different arenas containing the three extracts (hexane BPO, ethanol BPO, and water BPO) and the commercially known attractant, methyl eugenol, inside the multi-choice test bioassay were summarized in Table 6. Hexane extract and methyl eugenol garnered almost the same response from *B. dorsalis*, with an average of 12 and 11 fruit flies trapped, respectively. Likewise, ethanol extract trapped an average of 7 fruit flies, while distillation extract had a lower response compared to the former attractants, with an average of 3 fruit flies trapped. Undecided fruit flies were accounted for in each trial, with an average of 8 untrapped fruit flies.

Table 6. Multi-choice Bioassay Test

Multi-choice bioassay Test (4 Traps: 1 commercial attractant and 3 extracts)				
	Trial 1 (40 flies)	Trial 2 (40 flies)	Trial 3 (40 flies)	Average
Hexane Extract	12	11	12	11.66 ≈ 12
Ethanol Extract	7	6	8	7
Water Extract	3	3	2	2.66 ≈ 3
Methyl Eugenol (+)	10	11	11	10.66 ≈ 11
Undecided	8	9	7	8
Total	40	40	40	40

A one-way ANOVA was performed to investigate the attractive responses of *B. dorsalis* to the three extracts, along with methyl eugenol (ME), in a controlled environment bioassay setup using the multi-choice test. One-way ANOVA revealed a significant difference across the flies attracted to different extracts with an F value of 100.000 and a p-value of less the alpha of 0.05.

A Tukey-Post Hoc test showed that the hexane extract captivated more fruit flies than ethanol extract ($p < .01$) and water extract ($p < .01$), but there was no significant difference between hexane extract and methyl eugenol ($p = 0.369$). This means that hexane extract and methyl eugenol have no significant difference when it comes to the percentage of flies attracted to the traps. This was an indication that hexane extract has chemical constituents correlated to the attraction of fruit flies, since the number of attracted fruit flies to the trap containing hexane extract as an attractant was almost equal to the number of trapped fruit flies in the trap using methyl eugenol.

Compounds in Black Pepper Oil

The black pepper oil from hexane extract using Soxhlet extraction was analyzed using GC-MS. Caryophyllene, D-limonene, δ-3-carene, stearolic acid, and 1-[5-(1,3-Benzodioxol-5-yl) pentanoyl] piperidine were the constituents with the highest percent composition in black pepper oil, with percent compositions of 30.96, 8.49, 8.29, 7.09, and 5.13%, respectively. Likewise, the β-caryophyllene, limonene, β-pinene, α-pinene, δ-3-carene, sabinene, and myrcene, with significant variation in their percentages, were the main components of BPOs frequently reported in the literature (Dosoky et al., 2019). Furthermore, δ-3-carene, which has a sweet and pungent odor (Robbins, 2020), was reported to be the major aroma-contributing component of all varieties of mango (Pino et al., 2005), which was also found in the hexane BPO extract, hence the viable explanation for the attraction of fruit flies to the hexane BPO extract for its mango-like odor.

Conclusion

Among the three extraction methods used, Soxhlet extraction using hexane provided the highest efficiency in terms of the percent yield of oil. Significant differences were observed in the comparison of the extracts against the solvents used in extraction in the double-choice bioassays of all extracts (hexane, ethanol, and water). Black pepper oil from hexane extract performed the greatest among the three extracts when used with the commercial attractant. The number of *B. dorsalis* attracted to the trap with hexane extract as an attractant is notably comparable to the number of trapped fruit flies using the commercial attractant, methyl eugenol, in the multi-choice test bioassay.

Black pepper oil from dried black peppercorns was successfully extracted in Soxhlet extraction using hexane as solvent. Among the three extracts, hexane BPO extract garnered the highest attractancy compared to ethanol and water extracts, with no significant difference from methyl eugenol. Further, characterization of black pepper oil from hexane extract using GC-MS revealed that black pepper oil contains chemical constituents related to the attraction of *B. dorsalis* to mango fruit, hence the possible explanation for the attraction of the fruit flies to the black pepper oil in the controlled environment bioassays. In conclusion, black pepper oil extract can be considered a viable alternative to methyl eugenol as an attractant for mass trapping of *B. dorsalis*.

Various parameters in extraction, such as temperature, time, concentration, solvent, and different extraction techniques, could be further studied and optimized. The researchers recommend hydrodistillation using the Clevenger apparatus. The use of the extracted BPO, especially the hexane extract, in field trapping is also recommended. Lastly, characterization of black pepper oil from Soxhlet extraction using ethanol could also be done to analyze the chemical constituents of black pepper oil extracted from a polar solvent.

Authors' contributions

Louise Raphaelle V. Dela Cruz is the primary author. She was involved in the bioassay, experimentation, collection, verification of the species used, and writing of the manuscript.
Mormie Joseph F. Sarno was responsible for the conceptualization, method development, validation of results, as well as supervision of the research process. Giovani P. Mercado assisted in the technical refinement of the topic, in the experimentation, statistical analysis, and editing of the manuscript. Dexter F. Pajarito, Myrnille Joy Z. Galang, and Viola R. Garduque all contributed to the conceptualization of the topic, editing of the manuscript, and validation of results.

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