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Research Article

Comparative Pesticidal Activities of Essential Oils Extracted from Indigenous Plants Against *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae)

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Abstract

Background and Objectives: *Tribolium castaneum* is a major pest of stored grains that developed resistance against synthetic insecticides. This study was aimed to compare and evaluate the pesticidal activities of essential oils of *Piper nigrum*, *Syzygium aromaticum* and *Monodora myristica* against 4th instars of *T. castaneum*. **Materials and Methods:** Extracted essential oils of these 3 botanicals were tested for repellency and larvicidal activities against 4th instar larvae of *T. castaneum* at different doses for specific experimental periods. The 4th instars were subjected to repellency bioassay of 5, 10, 15 and 20 μL ML^{-1} test solutions at intervals of 1 h for 4 h in 3 replicates. While the larvicidal activities were assayed using 5, 10, 15 and 20 μL mL^{-1} test solutions for 24, 48, 72 and 96 h. **Results:** The 3 essential oils exhibited repellency degree in order of *P. nigrum*>*S. aromaticum*>*M. myristica*, respectively at 20 μL mL^{-1} within 4 h exposure time. *Piper nigrum* has the highest and significant toxicity ($p<0.05$) at 20 μL mL^{-1} for 96 h. The *P. nigrum* exhibited the lowest lethal concentration (20.4 μL mL^{-1}) to attain 50% mortality of 4th instar larvae of *T. castaneum* within 24 h of treatment exposure time as compared to *S. aromaticum* and *M. myristica*, respectively. **Conclusion:** *Piper nigrum* exhibited highest repellent and larvicidal activities against 4th instars of *T. castaneum* compared to *S. aromaticum* and *M. myristica*. Based on this study, *P. nigrum* essential oil is recommended for indigenous adoption as an aspect of integrated pest management strategies to protect local stored grains in south west Nigeria.

Key words: Indigenous insecticidal botanicals, essential oils, *Monodora myristica*, *Piper nigrum*, *Syzygium aromaticum*, larvicidal activities

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Insects are the most successful animals on earth due to their adaptive and arthropodal characteristics. So, due to these characteristics insects are competing successfully with human population for scarce food and other ecological sources. *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) is a major pest of stored grains such as; cereals, flour, nuts, pasta, peas, biscuits and spices that showed resistance against synthetic insecticides. The feeding activities and fecal materials caused economics damage and great losses[1-3]. The feeding and reproductive activities of adult *T. castaneum* reduces the quantity and quality of invaded grains in food shortage, thereby it is affecting human population negatively in terms of human sustainability[4]. This warranted needs to reduce *T. castaneum* population to mitigate its excessive activities of which chemical insecticides were effectively used[5]. However, the excessive use of synthetic insecticides led to negative and costly consequences on non-target organisms and the environment, including insecticide resistance developed by *T. castaneum* (Pest)[5]. These challenges were not peculiar only to *T. castaneum* but also exhibited by other identified insects of pest status. This increases the quest for alternative natural botanical insecticides to mitigate the activities of *T. castaneum*. Natural insecticidal botanicals were considered due to their eco-friendly properties despite the rich bioactive chemical components against insect pests excluding non-target organisms[4,6]. Essential oils of some natural botanicals are tested and found to be effective against insect pests of agricultural crops and store produce. The essential oils are used either as fumigants, natural insecticides, repellents, antifeedants, anti-ovipositors or larvicides against pest of post-harvest produce[6]. Excessive uses of chemical insecticides by indigenous crop farmers and stored produce handlers of different regions in different countries including Nigeria, have created instability in the ecosystem[4,7]. This has caused serious ecological challenges and health hazard effects for human population resulting to economic instability due loss of human resources[8]. Substantial economic losses were recorded by local farmers and marketers of stored produce in south western Nigeria. Hence, the aim of this study was to evaluate the pesticidal activities of essential oils of the indigenous plants against *T. castaneum* to identify the most potent insecticidal and repellent botanicals against the insect pest.

MATERIALS AND METHODS

Collection of plant essential oils: The essential plant oils of *S. aromaticum* (Clove oil), *P. nigrum* (Black pepper) and *M. myristica* (Calabash nutmeg) were provided by Post-graduate Research Laboratory of Department of Chemical Sciences, Faculty of Basic and Applied Sciences, Osun State University, Osogbo, Osun state, Nigeria.

Culture of test organisms: At the initial stage of the study, there was challenge in culturing *T. castaneum* in the Laboratory which was resolved by expertise advice. The adults (males and females) of *T. castaneum* were collected from infected stored grains of rice purchased from a local market at Oke-Baale, Osogbo, Osun state, Nigeria. *Tribolium castaneum* with substantial rice grains were taken to the laboratory and cultured with the same rice medium renewed as at when necessary within duration of the study. The culture was maintained in plastic container (6.0 L) at laboratory temperature of 31.5±2.3°C and 72.5±2.5% RH. First filial generation of the culture were sorted out from which 4th instar larvae were sieved and separated for the bioassays. The comparative study was between September, 2016 and August, 2017.

Repellent activities: Repellency bioassay was carried out in glass petri dish. Test solutions of each essential plant oils were prepared by dissolving 5, 10, 15 and 20 µL in 1 mL of acetone each. Whatman No. 1 filter paper (8 cm diameter) was cut into two equal halves and each essential plant oil solution was applied to each half of filter paper uniformly as possible with the aid of micropipette. While the other halves of each filter papers were treated with acetone only as appropriate. The essential plant oil and acetone treated filter papers were air-dried for 5 min to evaporate the solvent completely. Both treated and untreated halves were joined carefully with cellophane tapes and placed at the bottom inside the glass petri dishes. Ten larvae of 4th instars of *T. castaneum* were released at the center of each filter paper disc and then sealed tightly. Three replicates were set for each concentration of each essential plant oils. The set-up was kept in dark aerated laboratory cupboard at 31.5±2.3°C and 72.5±2.5% RH. Observation of the number of larvae on both treated and untreated halves was recorded after every 1 h for 4 h from commencement of experimental set-up to assess their repellency activities[8].

Percentage repellency values:

$$PR = \frac{NC - NT}{NC + NT} \times 100\%$$

where, PR is Percentage Repellence, NC is number of larvae on control portion and NT is number of larvae on treated portion. The mean percentage repellency value was calculated and assigned to repellency classes from 0 to V: Class 0 (PR<0.1%), Class I (PR = 0.1-20%), Class II (PR = 20.1-40%), Class III (40.1-60%), Class IV (PR = 60.1-80%) and Class V (PR = 80.1-100%), respectively[8].

Larvicidal activities: Replicates of 3 g of half broken rice were coated with concentrations of 5, 10, 15 and 20 μL mL^{-1}, respectively in glass petri dishes. For evaluation of larvicidal activities, newly molted 4th instar larvae of first filial generation of *T. castaneum* were exposed to the different concentration of 5, 10, 15 and 20 μL mL^{-1} of each essential oils of *P. nigrum*, *S. aromaticum* and *M. myristica*, respectively and covered tightly. Acetone only was used for control set-up. Mortality was recorded every 24 h for 4 days and the percentage of mortality was calculated by using Abbott method[9].

Statistical analysis:

$$\text{Abbott's percentage corrected mortality} = \frac{\text{Mortality in treated (\%)} - \text{Mortality in control (\%)}}{100 - \text{Mortality in control (\%)}} \times 100$$

Data were analyzed with one-way ANOVA and the lethal concentration (LC values), 95% confidence intervals and Chi-square were calculated by using Probit analysis with Microsoft Excel 2016 version.

RESULTS

Degree of repellency: Generally, the essential oils of *P. nigrum*, *S. aromaticum* and *M. myristica* exhibited repellent activities against 4th instar larvae of *T. castaneum* in order of *P. nigrum* >*S. aromaticum*> *M. myristica*, respectively at 20 μL mL^{-1} within 4 h time of exposure. However, the essential oil of *P. nigrum* exhibited remarkable and significant repellent effect (95.3±1.2%)(V) against 4th instars of *T. castaneum* after 4 h exposure compared to *S. aromaticum* (93±1.3%)(V) and *M. myristica* (86.7±1.2%)(V) (Table 1).

Lethal concentration of essential oils of each indigenous plant to achieve 50% mortality: From probit analysis, 45.70 μL mL^{-1} of essential oil of *S. aromaticum* will achieve 50% mortality of 4th instar larvae of *T. castaneum* at 24 h treatment exposure time compared to 2.40 μL mL^{-1} at 96 h. It showed that as the experimental duration increases, the lethal concentration of essential oil of *S. aromaticum* required to achieve 50% mortality deceases (Table 2).

For essential oil of *M. myristica*, 38.9 μL mL^{-1} will achieve 50% mortality of 4th instar larvae of *T. castaneum* at 24 h treatment exposure time compared to 1.74 μL mL^{-1} at 96 h. This also showed that as the experimental duration increases, the lethal concentrations of essential oil of *M. myristica* required to achieve 50% mortality decreases (Table 3).

Fifty percent mortality of 4th instar larvae of *T. castaneum* would be achieved with 20.42 μL mL^{-1} of essential oil of *P. nigrum* compared to 11.38 μL mL^{-1} at 96 h treatment exposure. As the experimental duration increases, the lethal concentrations of essential oil of *P. nigrum* required to achieve 50% mortality decreases as well (Table 4). From Table 2-4, *P. nigrum* has the lowest lethal concentration (20.4 μL mL^{-1}) to achieve 50% mortality of 4th instar larvae of *T. castaneum* within 24 h of treatment exposure time compared to *S. aromaticum* and *M. myristica,* respectively.

Larvicidal activities of the 3 indigenous plants: Figure 1a showed that mortality percentage of 4th instar larvae of *T. castaneum* increases from 33-76.7% as the concentration of essential oil of *P. nigrum* increases from 5-20 μL mL^{-1} at 96 h duration. For essential oil of *S. aromaticum*, the mortality percentage of 4th instar larvae of *T. castaneum* increases from 57-70% as the concentration increases from 5-20 μL mL^{-1} at 96 h exposure time (Fig. 1b). Also, *M. myristica* exhibited the

Table 1: The percentage Means±SD (%) of repellent activity of *P. nigrum*, *S. aromaticum* and *M. myristica* against 4th instar larvae of *T. castaneum*

		Duration of exposure (Means±SD (%) (PR)			
Plant names	Concentration (μL mL^{-1})	1 h	2 h	3 h	4 h
S. aromaticum	5	54.7±1.4 (III)	56.7±1.5 (III)	63.3±0.6 (IV)	66.7±0.6 (IV)
	10	63.3±1.2 (IV)	65.3±1.2 (IV)	66.7±1.5 (IV)	73.3±1.2 (IV)
	15	66.7±1.2 (IV)	70.0±1.0 (IV)	71.0±1.7 (IV)	80.0±1.0 (IV)
	20	83.3±1.5 (V)	90.0±1.7 (V)	91.0±1.5 (V)	93.0±1.3 (V)
M. myristica	5	43.3±2.1 (III)	50.0±1.7 (III)	53.2±0.6 (III)	63.4±0.6 (IV)
	10	53.3±1.5 (III)	63.3±1.1 (IV)	66.7±0.6 (IV)	73.3±0.6 (IV)
	15	66.7±0.6 (IV)	73.1±1.2 (IV)	76.3±0.6 (IV)	80.7±1.0 (V)
	20	73.3±0.6 (IV)	75.5±1.6 (IV)	78.7±0.6 (IV)	86.7±1.2 (V)
P. nigrum	5	52.3±1.2 (III)	53.3±0.6 (III)	56.7±0.6 (III)	60.0±0.6 (III)
	10	66.7±0.6 (IV)	68.4±0.2 (IV)	70.0±0.6 (IV)	75.0±0.8 (IV)
	15	70.0±0.6 (IV)	76.7±0.7 (IV)	80.0±0.0 (IV)	84.0±1.0 (V)
	20	82.5±1.5 (V)	86.2±0.6 (V)	88.7±1.5 (V)	95.3±1.2 (V)

Repellency classes: Class III (PR = 40.1-60.0%), Class IV (PR = 60.1-80%), Class V (PR = 80.1-100%), h: Hours, PR: Percentage repellence

Table 2: LC_{50} values and related information on the larvicidal toxicity test of *S. aromaticum*

Duration (h)	LC_{50} with 95% confident limits (µL mL^{-1})	Intercept	Slope	X^2 (df = 3)	p-value
24	45.70	3.3705	0.9837	31.9734	0.0010
48	36.31	3.9700	0.6585	16.2440	0.0038
72	6.03	4.7891	0.2714	47.7265	0.0004
96	2.40	4.7813	0.5759	76.2249	3.2205

Table 3: LC_{50} values and related information on the larvicidal toxicity test of *M. myristica*

Duration (h)	LC_{50} with 95% confident limits (µL mL^{-1})	Intercept	Slope	X^2 (df = 3)	p-value
24	38.90	2.9283	1.3833	28.4963	0.0012
48	30.90	3.9430	0.7083	19.8996	0.0025
72	10.00	4.7329	0.2678	1085.042	8.4907
96	1.74	5.0604	0.2463	63.5844	0.0003

Table 4: LC_{50} values and related information on the larvicidal toxicity test of *P. nigrum*

Duration (h)	LC_{50} with 95% confident limits (µL mL^{-1})	Intercept	Slope	X^2 (df = 3)	p-value
24	20.42	2.9041	1.5502	7.5486	0.0171
48	16.60	2.8008	1.7832	6.0823	0.0260
72	15.03	3.0124	1.7075	8.7686	0.0128
96	11.38	3.1383	1.7952	4.2345	0.0515

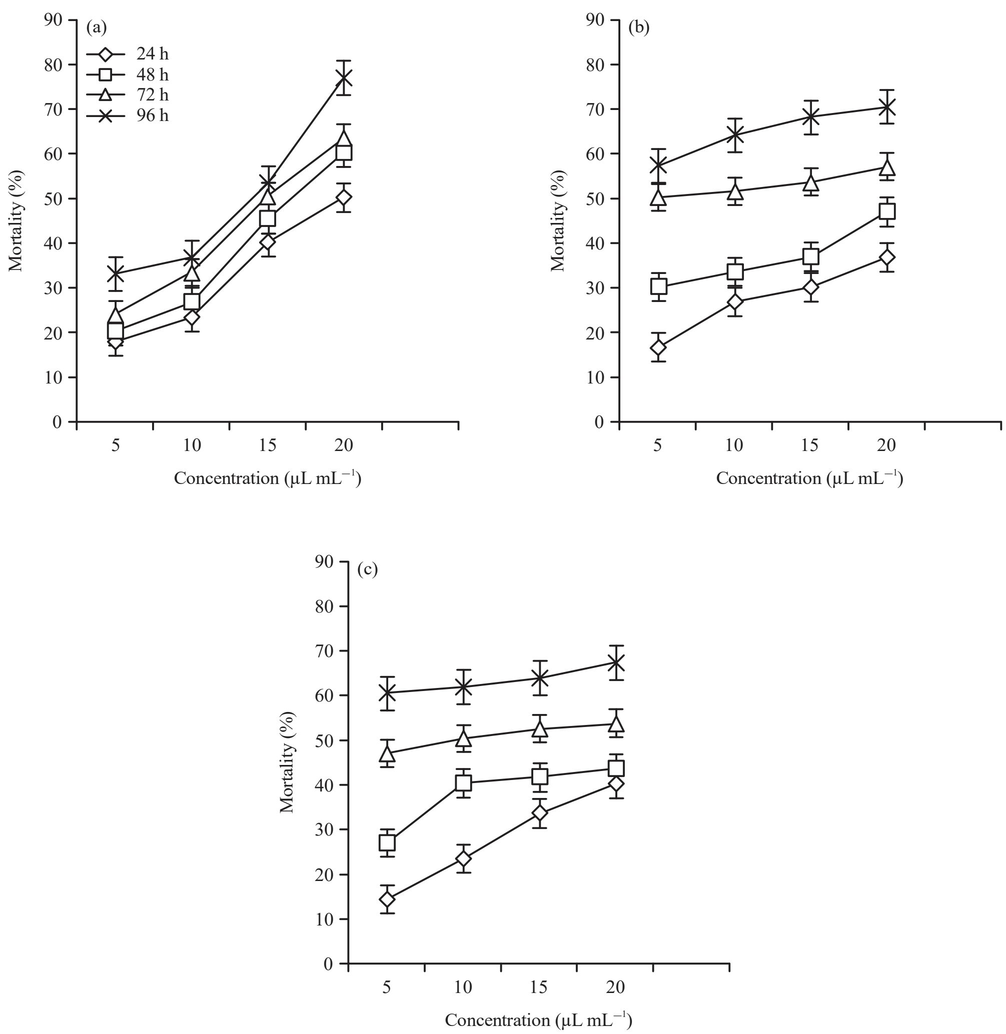

Fig. 1(a-c): Comparative larvicidal activities of essential oils of the 3 identified indigenous plants (a) *Piper nigrum*, (b) *Syzygium aromaticum* and (c) *Monodora myristica* against 4th instars of *T. castaneum* after 24, 48, 72 and 96 h exposures same phenomenon of which the mortality percentage of 4th instar larvae of *T. castaneum* increases from 60-66.7% as the concentration increases from 5-20 μL mL^{-1} at 96 h (Fig. 1c). Generally, the essential oils of all the 3 indigenous plants exhibited the same phenomenon, but the essential oil of *P. nigrum* showed the highest larvicidal activities comparatively.

DISCUSSION

In different ecozones, indigenous eco-friendly plants were sourced to have potentials insecticidal compounds which gained tremendous attention in the quest to replace synthetic insecticides harmful to non-target organisms and the environment. Series of studies were reported globally on the insecticidal activities of essential oils and extracts of natural plants against insect pests of stored products including *T. castaneum*[5,10]. The larvicidal test of the essential oils of the 3 indigenous plants extracts showed that *P. nigrum* (Black pepper) exhibited significant larvicidal activities at higher concentrations than the other two plants extracts at varied treatment durations. Results revealed that the essential oil of *P. nigrum* exhibited the highest and statistically significant toxicity ($p<0.05$) at 20 μL mL^{-1} for 96 h. The essential oil of *P. nigrum* was reported to be highly repellent and toxic against the larval stages of *T. castaneum*[11] as confirmed by this study. It was also observed that *Piper nigrum* exhibited the highest repellent and larvicidal activities against 4th instars of *T. castaneum* compared to *S. aromaticum* and *M. myristica* while rough lemon (*Citrus jambhiri*) was reported to show best result as insecticidal botanical and natural repellant[12] although different from the botanicals considered in this study. Also, *Allium sativum* (garlic) and *Zingiber officinale* (ginger) were also identified as effective insecticidal botanicals against *T. castaneum*[13]. *Ocimum sanctum* (Tulsi oil) was noted to have more repelling property than other oils tested against *T. castaneum*[14]. Significant repellent and larvicidal activities of tested citriodora, citronella, clove, gaultheria and lemon grass oils against *T. castaneum* were observed[8], but citriodora oil showed more significance than the other four oils because it recorded maximum repellent activity (95.24%) and larval mortality (81.86%) against the larvae of *T. castaneum* at 20 μL mL^{-1}. Hence, citriodora oil showed bio-pesticides potential in the management of *T. castaneum* pest[8]. The essential oil of *Mentha arvensis* L. strongly repels *T. castaneum* and *S. oryzae* even at low concentration, but its repellency was stronger towards *S. oryzae*[15]. The garlic extract of *Allium sativum* L. (Amaryllidaceae) had strong repellent activity towards *T. castaneum*[16]. From literatures, several studies have identified potential insecticidal botanicals against the insect pests of stored products, but this study compared the essential oils of *S. aromaticum* (Clove oil), *P. nigrum* (Black pepper) and *M. myristica* (Calabash nutmeg) and identified *P. nigrum* as the most potent insecticidal botanical comparatively.

The chemical constituents of the local *M. myristica* and *P. nigrum* were identified to be trans-13-octadecenoic acid (25.18%) and linalool (21.73%), respectively[17]. The chemical constituents of *P. nigrum* probably inhibits the acetylcholinesterase of the 4th instar larvae of *T. castaneum* resulting to significant larval mortality during the study. Eugenol [2-Methoxy-4-(2-propenyl) phenol] (80.5%) and Eugenyl acetate (4-Allyl-2-methoxyphenyl acetate) (5.01%) were identified as the major constituents of essential oil of *S. aromatium* (Nigeria sourced)[18]. Eugenol also has anti-cholinesterase potential property[19] which may be responsible for the appreciable mortality of the 4th instar larvae of *T. castaneum* as observed in this study. *Corymbia citriodora* (Citriodora oil) was ranked as the most effective insecticidal and repellant botanicals than *S. aromaticum* against the larvae of *T. castaneum*[8]. Hence, *S. aromaticum* is a potential insecticidal botanical which can also be exploited locally, although ranked second in this study. According to Upadhyay and Jaiswal[11] adults of *T. castaneum* were repelled significantly by *P. nigrum* at 0.2% concentration (v/v) and above. While the LC_{50} values for 4th instar larvae and adults of *T. castaneum* were 14.022 and 15.262 μL mL^{-1}, respectively which indicated that *P. nigrum* is one of the promising and eco-friendly potential insecticidal botanicals to protect stored grains against *T. castaneum* as confirmed by this study. The essential oils from leaves and barks of some botanicals may be explored as potential natural insecticides for stored-products insect pests based on their high repellency and insecticidal activities[20]. This comparative study clearly established that the *P. nigrum* essential oil is a promising natural insecticide, which can be exploited to effectively reduce indirectly the red flour beetles' population in stored grains.

CONCLUSION

Piper nigrum had the highest significant toxicity against 4th instars of *T. castaneum.* The degree of repellency and larvicidal activities of the essential oils of the 3 indigenous plants were in the order of *P. nigrum*> *S. aromaticum*> *M. Myristica* at 20 μL mL^{-1} for 4 h (repellency) and 96 h (Larvicidal activities) exposure treatment, respectively. From this study *P. nigrum* appeared to be more promising than the other two indigenous plants which had the potentials to replace chemical pesticides.

SIGNIFICANCE STATEMENT

This study observed that the *P. nigrum* can be beneficial as bio-pesticide in the management of insect stored-product pests such as *T. castaneum*. This study will help researchers to uncover the critical areas of insect pest control using comparatively potent natural and indigenous botanicals that many researchers were not able to explore. A new theory on bio-pesticides may be arrived.

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