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Efficacy of three plant oils against *Sitophilus oryzae* L. (Coleoptera: Curculionidae) on stored polished and local rice

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Abstract

Oil extracts from three tropical plants, *Annona muricata*, *Moringa oleifera* and *Senna alata* commonly used medicinally in treating various ailments were tested in the laboratory under constant conditions at 30±2 °C and 60-65 % relative humidity for their ability to protect local and polished rice grains against *Sitophilus oryzae*, a major pest in storage. Doses at 0.0, 0.5 and 1.0 ml/100 g rice grains were separately applied for assessment of adult mortality in the laboratory for 3 days. Parameters assessed were single effect, combined effects and combined cumulative effects of plant oils on mortality of *S. oryzae* on local and polished rice. The experiment was laid out in a Completely Randomized Design (CRD) with each treatment replicated four times. The results showed that *A. muricata* at 1.0 ml and *M. oleifera* at 0.5 and 1.0 ml each recorded higher ($p<0.01$) adult weevil mortality at 48 and 72 h, respectively. In the polished rice, there were highly significant ($p<0.05$) differences at 24, 48 and 72 h where *M. oleifera* and *S. alata* at 0.5 ml each recorded higher mortalities of adult *S. oryzae*. The combined analysis of local and polished rice showed that the local rice was not significantly different ($p>0.01$) from the polished rice among the plant oil treatments on adult weevil mortality within the period (24, 48 and 72 h). The study also revealed that the treated local rice showed better resistance to weevil attack than the polished brand, and that the experimental plant oils could offer effective protection against *S. oryzae* to reduce dependency on toxic chemicals.

Keywords: Plant oils, *Sitophilus oryzae*, mortality, *Annona muricata*, *Moringa oleifera*, *Senna alata*

Introduction

Rice, *Oryza sativa* L. is an annual monocot belonging to the family Poaceae and a leading widely grown cereal with high global recognition and food value [2, 5]. Rice grain is a major crop cultivated in Africa for food, feed, starch and industrial products [14; 25, 41]. Nutritionally, rice meal nourishes the human body with up to 80 % carbohydrate and has been found to be easy to digest [19]. It is low in fat, cholesterol and has a high nutrient content of proteins, fibre, mineral and vitamins [16, 55]. It has been reported that rice is one of the most important sources of employment and income generation for rural people [13, 36, 54].

Nigeria is the largest producer and consumer of milled rice in Africa with an average production volume of 8 million metric tonnes and consumption volume of more than 7.5 metric tonnes [15, 19, 26, 54]. The country cannot meet her growing domestic demand hence the high demand placed on imported rice that drains her of foreign earnings [40]. This reality is further compounded by the increasing concern about the large scale post-harvest losses [2, 10, 26, 31, 38, 39, 40, 51].

The maximum amount of losses occurs during the storage of the grain due to a lack of adequate infrastructure, insect-resistant varieties and poor sanitation [4, 6, 12, 17, 21, 37, 45]. The rice weevil, *Sitophilus oryzae* L. (Coleoptera: Curculionidae) is most implicated as a field to store pest of rice, causing considerable losses across the sub-Saharan Africa and Nigeria in particular [29, 32, 55]. This weevil can feed directly on intact grain kernels of rice which serve as natural host to the pest [44]. The activities of this primary pest culminate in loss of grain weight, nutrients and seed viability and their activities make grains vulnerable to contamination by mites and fungi [8, 28, 29, 34]. The use of synthetic pesticides has not been fully accepted as to poses substantial risks to human health, non-target organisms and to the environment [1, 16, 20].

The three plant species, namely: *Moringa oleifera*, *Annona muricata* and *Senna atata*, belong to the families Moringaceae, Annonaceae and Leguminosae, respectively. The *M. oleifera* is a fast-growing drought-resistant tree with important phytochemicals such as alkaloids, saponins, tannins, steroids, phenolic acids, etc [22, 50]. The *A. muricata* commonly called soursop is a low-branching and slender evergreen with leaves having offensive smell, alternate, smooth, glossy and dark green on the surfaces [24]. Sesquiterpenes are the major constituents of the soursop leaf essential oils besides other rich deposits of bioactive substances like annonaceous acetogenins and phenolic compounds such as tannins and saponins [24]. The *S. alata* popularly referred to as Christmas candles, is a highly valued plant distributed in the tropical and humid regions. Bioactive compounds found in this plant include kaemferol, luteolin, anthraquinone, glycosides, steroids and volatile oils [48]. Different parts of these three test plants are reported in folk medicine as therapeutic substances for remediation of diverse diseases and infections [4]. The extracts and isolated compounds displayed pronounced pharmacological activities such as antibacterial, antifungal, antimalarial, anthelmintic and insecticidal plant substances [7, 53].

Currently, worldwide interest is centred on bio-rational products such as natural plant derived volatile organic compounds that are biodegradable, environment friendly, cheap and affordable to farmers [11, 27, 43, 44]. This study is aimed at using three plant oils to reduce stored rice grain damage caused by *S. oryzae*.

Materials and Methods

Insect culture

Parent stock of *S. oryzae* was obtained from stock culture maintained in the Teaching and Research Laboratory of the Department of Crop Science, University of Calabar and reared on stored rice in a constant temperature and humidity (CTH) room running at 25°C, 65 % relative humidity on a 12:12 Darkness and Light photoperiod. Freshly emerged (0-24 h) adult *S. oryzae* were used for the experiment.

Collection of rice, plant parts and oil extraction

Rice grains, both local and polished were obtained from Ogoja (Lat. 6.65° N, Long. 8.79° E) and Watt Market (Lat. 4.95° N, Long. 8.32° E) in Calabar. Matured seeds of *M. oleifera* and *A. muricata* were purchased from Marian markets in Calabar while the flowers of *S. alata* were collected from around the University of Calabar. Plant materials were authenticated in the Herbarium of the Department of Crop Science, University of Calabar. The seeds and flowers were separately thoroughly washed, and oven dried for 2 h at 40 °C. The dried samples were weighed and separately pulverized into fine powder using a milling machine (A steel, 11.1 cm diameter sample input, 17.8 cm deep chamber diameter, corona mechanical blender, Landars, Mendellin-Colombia) and sieved with < 2 mm size mesh. The ground powder was packed into separate black polythene

bags.

Oil extracts from the powders were obtained in a Soxhlet apparatus using petroleum ether at 40 or 60 °C boiling point as the solvent. The extraction was cooled in a desiccator after being filtered using Whatmann No. 1 filter paper and filtrate kept in freezer at -5°C before it was put to use.

Weevil mortality bioassay

One hundred grams of the disinfected rice (polished and local) were weighed into transparent plastic containers. Disinfection was done by placing each of the rice variety in a refrigerator maintained at a temperature of 4 °C for 24 h. The oil of each plant was measured using a syringe and applied by direct admixture at three levels of 0.0, 0.5 and 1.0 ml per 100 g of rice grains. The containers were vigorously shaken, except for the controls that received no treatment. Each treatment was replicated four times and laid out in a Completely Randomized Design (CHD).

Ten pairs of freshly emerged (0-24 h) *S. oryzae* adults were placed into each labeled test containers with covers perforated and covered with a muslin cloth to facilitate aeration and confinement of weevils. Observation was made on each treatment for adult insect mortality and recorded in all the containers of local and polished rice at 24, 48 and 72 h. Dead insects were removed and live ones returned to the respective containers each time a count was made. After seven days all live and dead adult weevils were removed and discarded. Data obtained were calculated as percentage mortality using the formula.

% Rice weevil mortality = No. of dead insects / Total No. of insects x 100

Statistical analysis

Analysis of variance (ANQVA) was performed by one-way ANOVA procedures and treatment means separated with SAS-SNK (Student Neumann Keuls) at 0.1% significant level (SAS, 1990).

Results

The effect of *M. oleifera*, *A. muricata* and *S. alata* oils on local rice is presented in Table 1. The results showed that there was no significant difference ($P > 0.05$) between the plant oil treatments and the control, although *A. muricata* oil at 1.0 ml evoked more weevil mortality at 24 and 48 h compared with the rest of the treatments. The *M. oleifera* had the highest mortality at 1.0 ml per 100 g of rice grains at 72 h compared with the rest of the treatments and control. Table 2 shows the effect of the three plant oils on mortality of weevil infesting polished rice. The result revealed that there was a highly significant difference ($p < 0.01$) in *S. oryzae* mortality at 48 and 72 h between plant oil treatments and the untreated.

The combined effect of plant oils on mortality of *S. oryzae* on polished and local rice is presented in Table 3. Within 24, 48 and 72 h, there was no significant difference amongst plant oils at different concentrations in all treated local rice.

Table 1: Effect of plant oils on mortality of *S. oryzae* local rice.

| Treatment | Concentration (ml/100 grice grain) | Mean mortality of <i>S. oryzae</i> (h) | | |
|--------------------|------------------------------------|--|-------------------|-------------------|
| | | 24 | 48 | 72 |
| Control | 0.0 | 00.0 ^a | 0.00 ^a | 0.00 ^a |
| <i>A. muricata</i> | 0.5 | 4.75 ^a | 2.25 ^a | 3.50 ^a |
| <i>A. muricata</i> | 1.0 | 4.75 ^a | 5.25 ^a | 2.50 ^a |
| <i>M. oleifera</i> | 0.5 | 0.50 ^a | 3.00 ^a | 5.25 ^a |
| <i>M. oleifera</i> | 1.0 | 2.50 ^a | 4.00 ^a | 5.75 ^a |
| <i>S. alata</i> | 0.5 | 3.00 ^a | 3.25 ^a | 4.75 ^a |
| <i>S. alata</i> | 1.0 | 2.50 ^a | 3.00 ^a | 4.25 ^a |
| S. E (±) | - | 0.11 | 0.13 | 0.12 |
| | | NS | NS | NS |

Means followed by the same letter are not significant at 1% level of probability.

Table 2: Effect of plant oils on mortality of *S. oryzae* on polished rice.

| Treatment | Concentration (ml/100 g rice grain) | Mean mortality of <i>S. oryzae</i> (h) | | |
|--------------------|-------------------------------------|--|---------------------|--------------------|
| | | 24 | 48 | 72 |
| Control | 0.0 | 00.0 ^a | 0.25 ^a | 0.25 ^a |
| <i>A. muricata</i> | 0.5 | 4.50 ^a | 0.50 ^a | 0.50 ^a |
| <i>A. muricata</i> | 1.0 | 5.00 ^a | 1.00 ^{ab} | 2.00 ^a |
| <i>M. oleifera</i> | 0.5 | 7.25 ^a | 8.25 ^a | 8.75 ^a |
| <i>M. oleifera</i> | 1.0 | 2.00 ^a | 3.00 ^{abc} | 4.50 ^a |
| <i>S. alata</i> | 0.5 | 5.00 ^a | 5.75 ^{bce} | 7.00 ^{cd} |
| <i>S. alata</i> | 1.0 | 4.75 ^a | 5.50 ^{de} | 6.75 ^{cd} |
| S. E (±) | - | 0.13 | 0.09 | 0.08 |
| | | NS | HS(1%) | HS(1%) |

Means followed by the same letter (s) are not significant at 1% level of probability.

Table 3: Combined effects of plant oils on mortality of *S. oryzae* on local and Polished rice

| Treatment | Concentration (ml/100 g rice grain) | Mean mortality (hour) in local rice | | | Mean mortality (hour) in polished rice | | |
|--------------------|-------------------------------------|-------------------------------------|------|------|--|--------------------|--------------------|
| | | 24 | 48 | 72 | 24 | 48 | 72 |
| | | Control | 0.0 | 00.0 | 0.00 | 0.00 | 0.00 ^a |
| <i>A. muricata</i> | 0.5 | 4.75 | 2.25 | 3.50 | 4.50 ^a | 0.50 ^e | 0.50 ^e |
| <i>A. muricata</i> | 1.0 | 4.75 | 5.25 | 2.50 | 5.00 ^a | 1.00 ^e | 2.00 ^e |
| <i>M. oleifera</i> | 0.5 | 0.50 | 3.00 | 5.25 | 7.25 ^a | 8.25 ^a | 8.75 ^a |
| <i>M. oleifera</i> | 1.0 | 2.50 | 4.00 | 5.75 | 2.00 ^a | 3.00 ^{de} | 4.50 ^{de} |
| <i>S. alata</i> | 0.5 | 3.00 | 3.25 | 4.75 | 5.00 ^a | 5.75 ^b | 7.00 ^{bc} |
| <i>S. alata</i> | 1.0 | 2.50 | 3.00 | 4.25 | 4.75 ^a | 5.50 ^{bc} | 6.75 ^{bc} |
| S. E (±) | - | 3.02 | 2.28 | 2.25 | 2.28 | 1.63 | 1.47 |
| | | NS | NS | NS | NS | HS(1%) | HS(1%) |

Means followed by the same letter(s) are not significant at 1% level of probability.

Table 4: Combined cumulative effect of plant oils on mortality of *S. oryzae* on Local and Polished rice

| Type of Rice | Mortality (h) | | |
|--------------|-------------------|------|------|
| | 24 | 48 | 72 |
| Local | 2.57 ^b | 3.07 | 3.82 |
| Polished | 4.11 ^a | 3.46 | 4.22 |
| LSD (P<0.01) | | NS | NS |

Means followed by same alphabets (in 24 h) are not significant at 1% level of probability.

The *M. oleifera* treated polished rice at 0.5 ml per 100 g rice grains resulted in a higher mortality after 72 h compared with the other treatments. However, there were significant differences ($p < 0.01$) amongst the oils at 48 and 72 h in polished rice. Similarly, *M. oleifera* at 0.5 ml in 48 and 72 h evoked higher weevil mortality than *S. alata* at same concentration. The data presented in Table 4 show the combined cumulative effect of plant oils on mortality of *S. oryzae* on both local and polished rice. The results indicate

that in 24 h, there was significant difference ($p < 0.05$) in mortality of weevils infesting both local and polished rice, whereas at 48 and 72 h, there was no significant difference on weevil mortality between the two rice brands.

Discussion

In this study, the marked differences observed on the mortality of *S. oryzae* on treated and untreated polished rice grains (Table 2) indicate that all the plant oil extracts used in the experiment exerted remarkable effects on the mortality of the rice weevils. This result is consistent with the report that plant extracts often consist of complex mixtures of bioactive constituents which may produce toxic effects [3, 42, 44]. It is deduced from the present study that the effectiveness of these oils could be due to their toxicity which caused blocking of the insect's specimen spiracles and as a result hindered their movements in the treated containers. This insecticidal effect may have subsequently hindered the weevils from feeding on the rice coated with plant oils and thereby leading to starvation and death [12]. Reported that the application of oil extract covered the outer layer (testa) of the seeds which served as food poison to *S. oryzae*. The oils may have also disrupted the normal respiratory activity of the insects' leading to asphyxiation and death.

In this study, *A. muricata* oil at 1.0 ml per 100 g of rice evoked higher *S. oryzae* death at 24 and 48 h. This is at variance with the work of [23] who observed that there was a gradual increase in the mortality rate with increasing oil concentration of celery (*Apium graveolens*) and camphor (*Cinnamomum camphora*) at 1.0 ml of the exposure time. It is suggested that the insecticidal properties of *A. muricata* could be attributed to the presence of acetogenins, alkaloids and phenols as major active ingredients in Annonaceae which are insecticides like rotenone. *M. oleifera* and *S. alata* oils in the study also showed remarkable bioactivities with gradual increase in mortality and exposure time, and with their insecticidal potential manifesting greatly from 24 to 72 h. This could suggest that the plant oils are slow acting insecticides and supports the findings of [11] who reported that the mortality of *S. oryzae* was increased with increased time of exposure. The oils of *M. oleifera* and *S. alata* are both extensively used in treating various ailments. It is thought that their inherent active medicinal properties may have played major roles in killing the weevils [33]. ascribed the biological activity of *M. oleifera* to the presence of chavicine and piperine. The pungent smell of the oil has been reported to deter weevil from Seeding on grains [47].

Furthermore, in this study, there was no significant differences amongst the plant oils at different concentrations in treated local rice but *M. oleifera* treated polished rice

resulted in significant differences (Table 3). This finding is however, not consistent with [35] who reported that oils of *Camellia reticulata* and some other plant oils recorded significant mortality against *S. oryzae* and *Tribolium castaneum* [52]. reported that plant oils when used alone were less effective against the beetles than oils combined with either 1, 8 cineole, eugenol or camphor, Mortality significantly decreased with time after application except in treatments combining plant oils and chemicals, which achieved complete control of all beetles exposed after 90 days storage following application.

The observation from this study suggests that the treated local rice is resistant to the *S. oryzae* while the treated polished brand is susceptible to the same weevil attack at 48 and 72 h. It is deduced from this observation that although the polished rice was produced from improved grain post-harvest systems, they did not perform better than the local rice in terms of resistance to *S. oryzae*. Our findings are, however, not consistent with the belief that local rice, products are more susceptible to *S. oryzae* than the polished rice. This further suggests that rice brand resistance to weevils is not entirely dependent on the efficiency of the post-harvest treatment but could also be attributed to origin and other genetic factors.

Finally, these findings also indicate that at 24 h, there were significant differences in mortality of *S. oryzae* in local and polished rice but they were no significant differences at 48 and 72 h (Table 4). This observation shows that the plant oils have varying performing abilities given their genetic compositions. It can also be deduced that the plant oils were more effective on polished rice than the local variety. This finding is in agreement with [30] who reported the activity of plant oils from leaves of four plant species (*Ficus carica*, *Eucalyptus globulus*, *citrus* and *lemon*) on *S. oryzae* and found them slightly effective compared to the control.

Conclusion

Findings of this study showed that all the plant oils used in the experiment are good potential candidates for controlling *S. oryzae* on stored rice grains. Currently, rice varieties with relative resistance to *S. oryzae* and other major insect pests have been identified. These varieties can be planted by farmers to reduce damage caused by the *S. oryzae* and other insect pest infestations both in the field and store, and when infestation is high, they can be treated with any of the oil extracts used in this work, thus reducing reliance on synthetic pesticides. The oils used in this study are harmless to mammals at least at the dosage used and efforts should be intensified toward their production, packaging and application on a large scale, as botanical insecticides and subsequently incorporated into a sustainable pest management programme, for the control of stored product insect pests such as *S. oryzae* on stored rice.

Conflict of interests

The authors have not declared any conflict of interests.

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