Bio-pesticide Treated Jute Bags: Potential Alternative Method of Application of Botanical Insecticides against *Rhyzopertha dominica* (Fabricius) Infesting Stored Wheat

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Abstract Bio-pesticide treated jute bags were evaluated for their insecticidal flurry against *Rhyzopertha dominica* infesting stored wheat. The jute bags were separately treated with 2, 4, 6 and 8ml dosage of *Eugenia aromatic* oil while some bags were deep-soaked in 100ml of the oil. Untreated bags were set as controls. The result obtained showed that the treated bags significantly affect survival of insects as they all achieved above 55% mortality within 72hours of exposure. Moreover, it was observed that jute bag that was deep-soaked in 100ml of *E. aromatic* had the greatest mortality effect on the beetle as it achieved 100% mortality within 48hours of exposure. All the treated bags were found to reduce or prevent the weight loss and damage of the stored wheat after 42 days of storage. The ability of these bags to prevent the weight loss and damage of the stored wheat reduced after six months of storage as only the deep-soaked jute bag was able to prevent seed weight loss and damage. The oil treated jute bags have no effect on the colour, odour and acceptability of the wheat grains stored in them. The result also showed that the treated jute bags did not affect the viability of the stored wheat after six months. Base on the result obtained, the use of *E. aromatic*-treated jute bags may serve as an acceptable method of applying botanical pesticides in protection of stored grains.

Keywords Bio-pesticide; Jute bag; *Eugenia aromatic*; Wheat; *Rhyzopertha dominica*

Introduction

*Rhyzopertha dominica* is an important insect pest of wheat *Triticum* spp. This insect is a field to store pest which its infestation on wheat has led to the reduction in quality, quantity and marketability of this important crop (Ileke, 2011). Use of synthetic chemical insecticides have been the major approach to the control of this important insect pest of wheat but the adverse effects post by these chemical insecticides has led researchers to find a new boulevard of controlling the insect pest of stored products generally. Some of the drawbacks that trimmed down the use of synthetic chemical insecticides include pest resurgence and resistance, lethal effects on non-target organisms, food residues and their effect on both human and environmental health (Garriga and Caballero, 2011; Akinneye and Ogungbite, 2013a; Ashamo et al., 2013). Because of the public awareness of these adverse effects, botanical products have been relied upon as an alternative way of controlling insect pests of stored products as they are believed to be easily biodegradable, ecofriendly and have low mammalian toxicity (Zibaee, 2011). Many botanical powders and extracts have been proved to be effective against stored product insect pest; such botanicals include *Azadiracta indica*, *Anarcardium occidentalis*, *Zanthoxylum zanthoxyloides*, *Aristolochia ringens*, *Piper guineense*, *Eugenia aromatic* among others (Boeke et al., 2001; Akinkurolere, 2007; Idoko and Adeshina, 2012; Akinneye and Ogungbite, 2013b).
Despite the effectiveness of these botanical pesticides, they have not been generally accepted because consumers find the effects posed by these botanical pesticides on foods unacceptable. Such effects include colour change, odour and change in taste of the treated foods (Bagum et al., 2013). Furthermore, some of the well known botanical pesticides have some cons that are thwarting their widespread use. For example nicotine isolated from a number of Nicotiana spp. is insecticidal against wide range of stored product insect pests but with a disagreeable odour and extreme mammalian toxicity (Bagum et al., 2013). Rotenone is also known for its toxic effect on fish as well as some mammals because of its direct contact with the food product it protects (Begum et al., 2013). Therefore an acceptable method of application of these bio-pesticides is required. In USA, pyrethrins and piperonyl butoxide were effective and approved for use as a treatment for insect-resistant packaging on the outer layer of packages or with adhesives (Highland, 1991; Anwar et al., 2005). Neem oil extract was also found to be effective against R. dominica, Sitophilus granarius, Tribolium castaneum and Trogoderma granarium when used to treat jute bag but its efficacy was found to be greatly reduced after 60days of storage (Anwar et al., 2005). Some adult insects of stored products can pass through holes less than 1 mm in diameter and their larvae can enter through even smaller holes (Cline and Highland, 1981). Since the effectiveness of many botanical oils that had been claimed insecticidal on stored product insect pest when used for treatment of packaging material of farm produce have not been fully investigated, this research therefore investigated the efficacy of jute bags treated with oil of E. aromatica, one of the well known bio-pesticides against R. dominica infestation on wheat.

Results

Effect of Jute bag treated with E. aromatica oil on mortality of adult R. dominica.

Table 1 showed the effect of oils of E. aromatica-treated jute bags on mortality of adult R. dominica. At all doses of E. aromatica-treated jute bags, more than 30% beetle mortality was recorded within 24 hours of storage. Jute bags deep-soaked in 100ml of E. aromatica achieved highest percentage of 82.36% insect mortality within 24 hours of storage and its effect was significantly (p<0.05) different from other bags. Also, within 48hours of storage only the jute bags treated deep-soaked in 100ml oil of E. aromatica achieved 100% beetle mortality. Moreover, within 72 hours of storage jute bag treated with 8ml dose of E. aromatica oil achieved complete insect mortality and its effect was significantly (p<0.05) different from others except jute bags deep-soaked in 100 mL of the oil.

Table 1 % mortality R. dominica on wheat seeds stored inside E. aromatica-treated jute bags at different doses

| Doses (ml)       | Mean % mortality ± S.E in hours |
|------------------|---------------------------------|
|                  | 24                              | 48                              | 72                              |
| 2                | 32.00±0.24^b                     | 42.40±0.88^c                     | 60.87±0.58^c                     |
| 4                | 40.20±0.33^b                     | 53.82±0.72^d                     | 80.24±0.84^d                     |
| 6                | 53.87±0.64^c                     | 62.37±0.24^c                     | 87.35±0.28^d                     |
| 8                | 60.00±0.84^d                     | 79.80±0.24^f                     | 100.00±0.00^g                    |
| Deep-soaked      | 82.36±0.88^f                     | 100.00±0.00^g                    | 100.00±0.00^e                    |
| Solvent control  | 4.00±0.24^a                      | 6.00±0.00^b                      | 6.00±0.00^b                      |
| Untreated control| 0.00±0.00^a                      | 0.00±0.00^a                      | 0.00±0.00^a                      |

Note: Each value is the mean ± standard error of seven replicates. Mean followed by the same letters within the same column are not significantly (P > 0.05) different from each other using New Duncan’s Multiple Range Test

Effect of E. aromatica treated jute bags on the adult emergence of R. dominica infesting stored wheat.

The number of adult emergence of R. dominica on stored wheat and percentage inhibition rate of the E. aromatica treated jute bags were presented in Table 2. All the oil treated jute bags were found to significantly reduced or prevent the emergence of the adult beetle. Only the 2 and 4ml E. aromatica treated jute bags was
Table 2 Weight loss and damage caused by *R. dominica* infestation on wheat seeds stored inside *E. aromatica*-treated jute bags at different doses after 42 days of storage

| Doses (ml) | Number of adult emerged | % IR | % weight loss | % damage severity |
|------------|-------------------------|------|---------------|------------------|
| 2          | 3.20±0.32<sup>b</sup>   | 90.34±1.23<sup>b</sup> | 1.62±0.08<sup>b</sup> | 5.11±0.67<sup>b</sup> |
| 4          | 1.4±0.88<sup>a</sup>    | 92.00±0.63<sup>b</sup> | 0.84±0.03<sup>a</sup> | 3.54±0.21<sup>a</sup> |
| 6          | 0.00±0.00<sup>a</sup>   | 100.00±0.00<sup>b</sup> | 0.00±0.00<sup>a</sup> | 0.00±0.00<sup>a</sup> |
| 8          | 0.00±0.00<sup>a</sup>   | 100.00±0.00<sup>b</sup> | 0.00±0.00<sup>a</sup> | 0.00±0.00<sup>a</sup> |
| Deep-soaked| 0.00±0.00<sup>a</sup>   | 100.00±0.00<sup>b</sup> | 0.00±0.00<sup>a</sup> | 0.00±0.00<sup>a</sup> |
| Solvent control | 18.24±0.34<sup>a</sup> | 0.00±0.00<sup>a</sup> | 34.86±0.67<sup>b</sup> | 22.33±0.28<sup>a</sup> |
| Untreated control | 19.65±1.05<sup>a</sup> | 0.00±0.00<sup>a</sup> | 34.92±0.45<sup>ab</sup> | 23.89±0.88<sup>a</sup> |

Note: Each value is the mean ± standard error of seven replicates. Mean followed by the same letters within the same column are not significantly (P > 0.05) different from each other using New Duncan’s Multiple Range Test.

unable to prevent the emergence of adult insect as 3.23 and 1.44 mean number of adult *R. dominica* was observed in them respectively. However, their effect was significantly (p<0.05) different from both the solvent and untreated controls. Also, all the treated jute bags significantly inhibited the emergence of the adult beetle as reflected in Table 2. Only the 6 and 8ml as well as deep-soaked treated jute bags were able to achieve complete inhibition of the adult *R. dominica* but their effect was significantly (p>0.05) different from that of 2 and 4ml dosage of *E. aromatica* treated jute bags.

Weight loss and damage caused by *R. dominica* infestation in wheat stored in jute bag treated with *E. aromatica* oil after 42 days and 6 months of storage.

The effect of *Eugenia aromatica*-treated jute bags on the ability of *R. dominica* to cause seed weight loss and damage at all levels of dosages and their effect was significantly (p<0.05) different from the controls. However, only jute bags that were treated with 6 and 8ml dosage of *E. aromatica* prevented the weight loss of the seeds as well as the damage of the seeds after 42 days of storage. The effect of the bags treated with 4, 6 and 8ml dose of *E. aromatica* was significantly (p<0.05) different from others except bags that were deep-soaked in 100ml of the oil which also prevented the weight loss and damage of the seeds by the insect.

Moreover, only the jute bags that were deep-soaked in 100ml of *E. aromatica* was able to prevent the seed weight loss and damage (Table 3). The bags that were sprinkled with *E. aromatica* at different doses were nevertheless significantly (p<0.05) different from the controls which had 67.23% (solvent control) and 66.86% (untreated control) weight loss; 84.32% (solvent control) and 84.12% (untreated control) seed damage.

Table 3 Weight loss and damage caused by *R. dominica* infestation on wheat seeds stored inside *E. aromatica*-treated jute bags at different doses after 6 months of storage

| Doses (ml) | % weight loss | After 6 months | % damage severity |
|------------|---------------|----------------|------------------|
| 2          | 33.62±0.82<sup>d</sup> | 41.35±2.89<sup>c</sup> |
| 4          | 28.34±0.67<sup>cde</sup> | 37.32±1.54<sup>c</sup> |
| 6          | 25.73±0.53<sup>c</sup> | 27.33±2.09<sup>b</sup> |
| 8          | 16.48±0.63<sup>d</sup> | 23.45±2.45<sup>b</sup> |
| Deep-soaked| 0.00±0.00<sup>a</sup> | 0.00±0.00<sup>a</sup> |
| Solvent control | 67.23±1.88<sup>d</sup> | 84.32±0.88<sup>d</sup> |
| Untreated control | 66.86±2.33<sup>d</sup> | 84.12±1.08<sup>d</sup> |

Note: Each value is the mean ± standard error of seven replicates. Mean followed by the same letters within the same column are not significantly (P > 0.05) different from each other using New Duncan’s Multiple Range Test.
**Effect of E. aromaticus used for treatment of jute bag on the protected grains**

Table 4 showed the effect of the *E. aromaticus* oil treated jute bags used as packaging material on the colour and odour of the stored wheat grains. The result obtained showed that the oil extract treated jute bags have no adverse effect on the colour of the wheat grains after six months of storage regardless of the dosage used. However, it was noted that there was slight change in the odour of the wheat grains stored inside deep-soaked treated jute bags and this also affects the acceptability of the grains.

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| Treatments       | Colour | Odour | Acceptability |
|------------------|--------|-------|---------------|
| 2                | 5      | 5     | 5             |
| 4                | 5      | 5     | 5             |
| 6                | 5      | 5     | 5             |
| 8                | 5      | 5     | 5             |
| Deep-soaked      | 5      | 4     | 3             |
| Solvent control  | 5      | 5     | 5             |
| Untreated control| 5      | 5     | 5             |

Note: Key: 5=very good; 4=good; 3=average; 2=fair; 1=poor

**Effect of treated jute bags on viability of stored wheat grains**

Table 5 presents the effect of bio-pesticide treated jute bags on the viability of stored wheat grains. The effect of treated bags on the viability of treated grains showed that none of the treated bags adversely affect the viability of the wheat grains when compared with the controls after six months of storage. Moreover, only the untreated control and 2ml treated jute bag were able to achieve 100% wheat viability. However, their effect was not significantly (p>0.05) different from other treatments as all of them achieved above 90% seed viability.

| Doses(ml)       | % germination |
|-----------------|--------------|
| 2               | 100.00±0.00  |
| 4               | 94.00±1.08   |
| 6               | 93.24±0.24   |
| 8               | 92.00±0.88   |
| Deep-soaked     | 93.46±0.28   |
| Solvent control | 98.00±0.24   |
| Untreated control| 100.00±0.00 |

Note: Each value is the mean ± standard error of three replicates. Mean followed by the same letters within the same column are not significantly (P > 0.05) different from each other using New Duncan’s Multiple Range Test.

Market share of biopesticides including botanical and microbial pesticides is less than 2.2% of the global pesticides market (Isman, 2000; Kim et al., 2012). This could be due to the adverse effects that are associated with botanical base pesticides because consumers find it difficult to accept any food commodities that have colour change, undesirable odour, change of taste and other effects that are associated with botanical pesticides (Bagum et al., 2013). Therefore, a new method of application of some of these well known active botanical pesticides is required. Moreover, recent government action in the United States, in the form of the Food Quality Protection Act of 1996, will dramatically restrict the use of many conventional insecticides upon which growers have depended for decades (Isman, 2000). Nevertheless, this could serve as an opportunity for botanical based pesticides to grow drastically in the market place if a new method of their application is provided.

The result obtained from this research showed that the bags treated with oil of *E. aromaticus* is highly toxic to adult *R. dominica*. The ability of the bags to cause mortality could be due to the toxic effect of the *E. aromaticus* oil used for the treatment of these bags. Idoko and Adeshina (2012) opined that the oil used for the treatment of these bags has the ability to block the spiracle of the insects thereby causing suffocation. Also, the oils are known to contain phenylpropanoids such as carvacrol, thymol, eugenol and cinnamaldehyde which have been known to have ability to disrupte the

**Discussion**

Researches have been shifted toward plant kingdom for their possibility in replacing synthetic chemical insecticides because they are believed to contain some chemicals that are toxic to insect pests of stored products. However, despite of widespread public concern for the side effects of synthetic pesticides, the
normal respiratory activity of the insects leading to their asphyxiation and subsequent death (Yang et al., 2006). This result did not acquiesce with the findings of Anwar et al. (2005) in which jute bags treated with neem oil was unable to achieve complete mortality of R. dominica, T. castaneum, T. granarium and S. granarius at different concentrations. The ability of the treated jute bag to prevent weight loss and damage of the seeds after 42 days of storage may be due to the ability of the jute bags to retain the oil and its fume for long period. The result obtained showed that the jute bags treated at higher doses prevented the adult emergence of the beetle and as well achieved complete inhibition of the adults. The result obtained on adult emergence could be as a result of high mortality of the beetle which could therefore after mating and egg laying capacity of the insect. This agreed with work of Ashamo et al. (2013) in which oil of Newbouldia laevis was found to prevent the emergence of adult Callosobruchus maculatus. Also, the ability of the bags to reduce or prevent the weight loss and damage of seed stored in them may be due to the ability of the oil used for their treatment to reduce and prevent adult emergence of the beetle as suggested by Idoko and Adeshina (2012). However, result of this work revealed that jute bag treated with E. aromatica could not prevent weight loss and damage of stored seed for long period as there was an increase in the percentage of weight loss and damage of seed stored inside them after six months. This could be that the treated bags were only able to inhibit the egg laid by the beetle from hatching for some period. This also showed that the E. aromatica treated jute bags only disrupted the normal life cycle of the insect. This was in agreement with the work of Murdue-huntz and Nibet (2000) as well as Yang et al. (2006) which suggested that botanical oils are found to disrupt growth and reduced larva survival as well as disruption of life cycle of insects. Moreover, jute bags that were deep-soaked in the oil of E. aromatica showed a level of persistence in their effectiveness as they were able to prevent weight loss and damage of seeds stored in them for six months. The inability of the botanical-treated jute bag to protect the weight loss and damage of stored grains may be due to the fact that botanical oils and powder lost their potency over time as opined by Oruonye and Okrikata (2010). This was in accordance with the result of Anwar et al. (2005) in which neem oil used for the treatment of jute bags was found to reduced greatly in its effectiveness as protectant against four stored product insect pest of wheat. Also the result agreed with the work of Faditan (2013) in which piper guineense oil treated jute bags used for packaging maize grains was found to reduce in its effectiveness over time. After six month of storage, it was found that the wheat grains stored inside treated jute bags have no colour change regardless of the dose used and this may be due to the fact the oil was not applied directly on the wheat grains. This agreed with the findings of Anwar et al. (2005) in which neem oil treated jute bags have no colour, flavour and taste effect on the stored wheat. However, it was observed that there was a little change in the odour of wheat grains stored inside deep-soaked jute bags which in-turn affected its acceptability. The treated jute bags however did not affect the viability of the stored wheat after six months. Regardless of the dose of the oil used for the treatment of the bags, all the stored with achieved high rate of viability. The use of E. aromatica-treated jute bags may serve as an acceptable method of applying botanical pesticides in protection of stored wheat for more than 4-6 months especially when the bags are deep-soaked in the oil. Also, to increase the effectiveness of these treated jute bags, the dose of the oil used for their treatment could be increased as this may have no toxic effect on the consumer (mammals) since it is not in contact with the stored wheat. More so, E. aromatica used for the treatment of the bags is a medicinal plant and does not affect the viability of the stored wheat.

Materials and Methods
Insect culture
The initial culture of R. dominica was obtained from infested wheat grains from the Food Storage Research Laboratory, Federal University of Technology, Akure. The insects were subsequently reared in the laboratory on cleaned wheat seeds at temperature of 28 ± 2°C and relative humidity of 75 ± 5%. The wheat seeds were kept inside plastic containers with the open end covered with muslin cloth to prevent escape of insects and to provide aeration.
Collection of wheat seeds and plant materials

The wheat seeds used for this study were collected from the Agricultural Development Project (ADP), Akure. The seeds were cleaned of foreign matter and disinfested by keeping in freezer at -5°C for 7 days. They were subsequently exposed to air to reduce their moisture before being used for the experiments. The *E. aromatic* seeds used were purchased from local herbal seller in Oba market Akure, Nigeria. The seeds of *E. aromatic* were sundried and pulverized into fine powder using an electrical grinder (model 373) and were kept in air-tight plastic containers for subsequent use.

Preparation of oil extract

Twenty grams of each pulverized plant materials was put in a muslin cloth and transferred into the thimble and extracted with methanol in a soxhlet apparatus. The extraction was carried out for 3-4hr and the extraction was terminated when the solvent in the thimble become clear. Then, the thimble was removed from the unit and the solvent recovered by distilling in the soxhlet extractor. The resulting extracts contain both the solvent and the oil. The solvent was separated from the oil using rotary evaporator, after which the oil was exposed to air so that traces of the volatile solvent evaporates, leaving the oil extract.

Preparation of the storage material

The storage material used was jute bag. The jute bags of 1x1 mm mesh size were cut into sizes (30cm by 30cm), all the sides were sewed and one side was left open through which the samples were filled into the bags. These bags were separately sprinkled with oil of *E. aromatic* using graduated syringes at doses of 2, 4, 6 and 8ml. The bags were air dried for 2 days in the laboratory before use. Another set of bags were deeply soaked in 100ml of *E. aromatic* oil. Untreated bags and bags that were treated with 8ml of solvent (methanol) were set as controls.

Experimental procedures

200 grams of wheat seeds were weighed into each treated jute bag. Twenty 0-24 hours old unsex adults of *R. dominica* were introduced into the treated bags containing wheat. The experiment was set up in a complete randomized design. Each treatment had seven replicates. The bags were tightly sealed by sewing with needle and thread. Mortality was observed at interval of 24, 48 and 72hours. All dead and live insects were removed after 72hours of exposure and the bags were sealed again to determine the number of F1 adult emerged, % adult inhibition rate (%IR), weight loss and level of seed damage after 42 days of storage. The bags were tightly sealed after the observation of the number of emerged adult, seed weight loss and seed damage at 42 days of storage and was left for 6 months after which only the seed weight loss and damage was determined. The formula below was used to calculate the percentage inhibition rate, percentage weight loss and percentage damaged seeds respectively.

\[
\text{%IR} = \frac{C_n - T_n}{C_n} \times 100
\]

(Tapondu et al. 2002)

Where *Cn* is the number of insects that emerged in the control treatment and *Tn* is the number of adult insects that emerged in the treated grains.

\[
\frac{\text{% weight loss}}{= \frac{\text{change in weight} \times 100}{\text{initial weight}}} - 1
\]

\[
\frac{\text{% damage seed}}{= \frac{\text{number of seeds with hole}}{\text{total number of seeds}} \times 100} - 1
\]

The effect of the *E. aromatic* oil used for the treatment of the jute bags on the protected wheat grains were assessed after six months of storage. This was done by subjecting the grains for assessment by ten different panelists. The panelists observed the effect of the oil on the colour and odour of the grains.

Viability bioassay

After six months of storage thirty seed of stored wheat were randomly picked from each treated jute bags and were separately placed on moist whatman filter paper No. 1 inside disposable Petri dishes at the rate of 10 seeds per plate kept in a Gallenkamp incubator. Emerged seedlings were counted at the end of 7 days after planting and % viability was calculated as follow:

\[
\frac{\text{%Viability}}{= \frac{\text{number of seeds germinated}}{\text{total number of seeds planted}} \times 100 - 1}
\]
Statistical analysis
Data obtained was subjected to one-way analysis of variance and where significant differences existed, means were separated by New Duncan’s Multiple Range Test, using SPSS Version 19.

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