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The Efficacy of Three Plant Powders as an Entomocide against *Sitophilus Oryzae* (Linnaeus) Infesting Rice Grains in Nigeria

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Abstract: *The efficacy of powders of Eugenia aromatica, Piper guineense and Zingibar officinales as an entomocide against Sitophilus oryzae infesting rice grains was investigated at ambient temperature (30±2^{o}C) and relative humidity (74±4%). Weevils were exposed to different doses (0.10-2.00g/10g of rice grains) of each plant material and mortality was assessed at 24, 48, 72 and 96 hours post-treatment respectively. The result showed that weevil mortality increased with increasing doses of plant materials and exposure time. However, E. aromatica was the only plant material that elicited complete weevil mortality (100%) after 72 h at 2.0g/10g of rice while Z. officinales evoked less than 40% weevil mortality irrespective of the exposure time. LT_{50} values showed that weevil exposed to E. aromatica powder required the lowest time (1.63-1.06 h) while their counterpart exposed to P. guineense (3.98-1.07 h) and Z. officinales (15.18-9.75 h) showed the median and highest Lethal time (LT_{50}) respectively. Based on LT_{50} values, the order of toxicity of plant materials to S. oryzae from highest to lowest is: E. aromatica > P. guineense > Z. officinales. This study shows that powders of E. aromatica and P. guineense could serve as an alternative wherewithal to synthetic insecticides in controlling S. oryzae infesting rice.*

Keywords: *Entomocide, Eugenia aromatica, Piper guineense, Zingibar officinales, mortality, exposure time.*

1. Introduction

Rice (*Oryza sativa* L.) is one of the world's most important cereal crops providing food for more than one third of the world's population (Younan *et al*., 2011). It is considered as a food for common man both in urban and rural populations due to its ability to grow in a wide range of environment and its richness in essential nutrients (Sasaki and Burr, 2000). Although most of the world's rice is grown and consumed in Asia; production in African countries is increasing with a production of 26.4 million tones reported in year 2012 (FAO, 2013). Nigeria, being the largest producer, consumer and importer of rice in West-Africa with a local production of 3.5 million metric tonnes in year 2012 is making concerted effort to meet her local rice demand (Nelson, 2014). Government is boosting local production through various measures being put in place to reduce rice importation and encouraging local production. Thus, past years in Nigeria has witnessed increasing amounts of rice grains being stored to meet the increasing population's demand for marketing and use as a seed. This has raised rice production in Nigeria from 3.5 million metric tonnes in year 2012 to 5.5 million metric tonnes in year 2013 (Nelson, 2014).

However, due to lack of good and efficient storage facilities in Nigeria, most rice grains are still being stored traditionally in granaries either in the threshed or unthreshed form which pre-disposes them to insect pest attack (Odeyemi *et al*., 2010). As a result, post-harvest losses to storage insect pests such as *Sitophilus oryzae* (L.) (Coleloptera: Curculionidae) remained a serious menace militating against its production in the country. A survey on the prevalence of major pests of rice in diverse ecological areas of Nigeria showed, in general, that the extent of mutilation caused by *S. oryzae* was high (Soto *et al*., 1976). Losses of rice grains ranging from 10 to 20% of overall production due to this insect pest have also been reported (Rajendran and Muralidharan, 2001; Philips and Throne, 2010). Both the larval and adult stages devour the kernel, causing weight losses.

The use of synthetic insecticides has gained prominence as a means of controlling this insect pest in recent decades. Nevertheless, its continuous usage as preservatives is being discouraged due to various adverse environmental, biological and economic consequences associated with its usage (Park *et al*., 2003). This has necessitated the need for an alternative and effective preservative during storage. The use of botanical base insecticides is one of several methods being giving attention. Plant materials have played a vital role in farmer's quest towards dealing with insect pest of their farm produce. Hitherto, several plant materials have been investigated for their insecticidal activities against stored product insect pests. Notable among them are dry flower buds of *Eugenia aromatica* (Baill) (Clove), the seeds of *Piper guineense* (Schum and Thonn) (African black peeper) and the corms of *Zingibar officinales* (Roscoe) (Ginger) (Franz *et al*., 2011; Ukeh, 2009; Ukeh *et al*., 2012). Each of these plant parts is edible and usually use as spices by Nigerians in their diet (Iwu, 1993).

Crude powders of plant materials are believed to contain different phytochemicals working synergistically with many farmers and crop merchants in Nigeria using some of these plants in controlling pest on their produce (Jenson *et al*., 2006; Akinkurolere, 2007). Likewise, plant materials that are safe for human consumption and have the ability to retain their insecticidal potency overtime should be recommended to farmers. Thus, this research work investigated the entomotoxic effect of powders of *E. aromatica*, *P. guineense* and *Z. officinales* against *S. oryzae* infesting rice grains with the aim of recommending the most potent of the plant materials to Nigerian farmers and crop merchants.

2. MATERIALS AND METHODS

2.1 Insect Culture

The rice weevil, *Sitophilus oryzae* L. used for this study was obtained from a culture in the Storage Research Laboratory of Biology Department, Federal University of Technology, Akure, Ondo state, Nigeria. Clean polished rice was disinfested in the freezer at -18^{o}C for two weeks and thereafter allowed to equilibrate on the laboratory shelf for three days. 200g of disinfested rice were introduced into 1.5Litres plastic containers and moths from starter culture were later reared on the disinfested rice. The containers were covered with perforated lid and muslin cloth to prevent the escape of insects and allow air into them. The plastic containers used for insect rearing were kept inside cages at ambient temperature (30 ±2^{o}C) and relative humidity (74±4%) in the insect rearing cage till the new generation of adults emerged.

2.2 Preparation of Plant Materials

Dry flower buds of *Eugenia aromatica*, seeds of *Piper guineense* and fresh corms of *Zingibar officinales* belonging to the families Myrtaceae, Piperaceae and Zingiberaceae respectively were obtained from Oba market in Ondo, Ondo state, Nigeria. Fresh corm of *Z. officinales* were peeled, rinsed in clean water, cut into smaller pieces and dried under sunlight. The dried materials of each plant materials were allowed to cool at room temperature and separately ground into powder with the aid of NAKAI NJ-1731 electric blender. Powders of each plant material were later sieved with a mesh size of 1mm^2 before being separately stored in plastic containers with airtight lids for subsequent use to avoid the plant powders from absorbing moisture and prevent loss of active ingredients in these plants.

2.3 Contact Toxicity

Each plant material was tested separately at ‘0’ (control), 0.1g, 0.2g, 0.5g, 1.0g and 2.0g/10g of rice grain in separate plastic Petri dishes and each treatment had 3 replicates. 10g of clean, undamaged and disinfested rice grains were weighed with the aid of Mettler PB 3002 weighing balance into disposable Petri dishes. Different experimental doses of plant powders were weighed and introduced into the dishes with the aid of spatula. The dishes were shaken properly for thorough mixing to ensure uniform coating of the powders on the rice grains. Twenty unsexed adult 7-14 days old *S. oryzae* were then introduced into each Petri dish containing rice grains and plant materials. The dishes were covered immediately to prevent any escape of the insects or escape of the plant aroma. Mortality counts were taken at 24, 48, 72, and 96 hours post-treatment. This procedure was carried out separately for all the 3 plant materials at all the experimental doses. The control replicates were without plant materials. The test insects were certified dead if they do not respond when probed slightly with office pin.

2.4 Statistical Analysis

Abbott (1925) formula was used to correct all data on adult mortality counts using control mortality. The data were later subjected to analysis of variance (ANOVA) at $p<0.05$. Data on adult mortality were also subjected to probit analysis (oyeniyi) to determine the lethal time required by each plant powder to kill 50% (LT_{50}) of *S. oryzae* (Finney, 1971). All analyses were carried out using SPSS 17.0 software package.

3. RESULTS

3.1. Effect of Powders of Three Botanicals on Weevil Mortality

Tables 1-3 show the percentage mortality of *S. oryzae* treated with *E. aromatica* (Tab.1), *P. guineense* (Tab. 2) and *Z. officinales* (Tab. 3) respectively. The result showed that, generally, weevil mortality increased with increase in the doses of each plant material and exposure time. However, there was no significant effect of doses of *E. aromatica* ($F_{5, 12}$ = 1.94; p =0.161) and *Z. officinales* ($F_{5, 12}$ = 1.56; p =0.244) on the mortality of *S. oryzae* after 24 h post-treatment. For weevils exposed to *P. guineense* at 24 h, mortality at the highest experimental dose (2.0g) significantly differ (p = 0.002) from that of control and other doses (0.10-2.00g/10g of rice). Likewise, there was significant effect (p<0.0001) of doses of *E. aromatica* and *P. guineense* on the weevil mortality after 48, 72 and 96 h post-treatment respectively.

Table1. *Percentage mortality of S. oryzae treated with E. aromatica flower buds*

Doses (g/10g)	Exposure time (Hours)			
	24	48	72	96
0.00	$0.00±0.00^{a}$	$0.00±0.00^{a}$	$0.00±0.00^{a}$	$0.00±0.00^{a}$
0.10	$23.30±9.30^{b}$	$53.30±10.15^{b}$	$90.00±0.00^{b}$	$96.70±1.65^{b}$
0.20	$30.00±8.80^{b}$	$70.00±5.00^{c}$	$93.30±1.65^{b}$	$96.70±1.65^{b}$
0.50	$33.30±3.35^{bc}$	$76.70±0.33^{c}$	$96.70±1.65^{b}$	$100.00±0.00^{b}$
1.0	$36.70±1.65^{bc}$	$90.00±0.00^{d}$	$96.70±1.65^{b}$	$100.00±0.00^{b}$
2.0	$43.30±1.65^{c}$	$96.70±1.65^{d}$	$100.00±0.00^{b}$	$100.00±0.00^{b}$

Each value is the mean ± S.E of three replicates. Means followed by the same letter (s) in the same column are not significantly different (p>0.05) using Turkey's multiple range test.

However, Table 1 showed that no significance difference (p>0.05) existed among the tested doses of *E. aromatica* after 72 and 96 h of application. *E. aromatica* was also the only plant material that elicited complete weevil mortality (100%) after 72 h at 2.0g/10g of rice (Tab. 1). It was however observed in Table 2 that the mortality of *S. oryzae* exposed at a dose of 0.10g of *P. guineense* after 48, 72 and 96 h post-treatment was significantly lower (p<0.05) than those exposed at higher experimental doses. Complete weevil mortality (100%) was also observed after 96 h at 0.10 and 0.20g of *P. guineense* powder. In Table 3, there was no significant difference (p>0.05) among the tested doses after 48, 72, and 96 h respectively. The effect of doses of *Z. officinales* on the mortality of *S. oryzae* after 48 h ($F_{5, 12}$ = 6.64; p = 0.003), 72 h ($F_{5, 12}$ = 4.80; p = 0.012) and 96 h ($F_{5, 12}$ = 11.04; p < 0.0001) post-treatment was however significant. Less than 40% mortality was also observed in weevils exposed to *Z. officnales* irrespective of the exposure time.

Table 2.*Percentage mortality of S. oryzae treated with seed of P. guineense*

Doses (g/10g)	Exposure time (Hours)			
	24	48	72	96
0.00	$0.00±0.00^{a}$	$0.00±0.00^{a}$	$0.00±0.00^{a}$	$0.00±0.00^{a}$
0.10	$0.00±0.00^{a}$	$3.30±1.65^{a}$	$23.30±3.35^{b}$	$50.00±2.90^{b}$
0.20	$13.30±6.65^{b}$	$40.00±5.00^{b}$	$90.00±2.90^{c}$	$96.70±1.65^{c}$
0.50	$26.70±4.40^{bc}$	$76.70±9.30^{c}$	$96.70±1.65^{c}$	$96.70±1.65^{c}$
1.0	$26.70±3.35^{bc}$	$96.70±1.65^{d}$	$96.70±1.65^{c}$	$100.00±0.00^{c}$
2.0	$42.00±5.00^{d}$	$96.70±1.65^{d}$	$96.70±1.65^{c}$	$100.00±0.00^{c}$

Each value is the mean ± S.E of three replicates. Means followed by the same letter (s) in the same column are not significantly different (p>0.05) using Turkey's multiple range test.

Table3. *Percentage mortality of S. oryzae treated with corms of Z. officinales*

Doses (g/10g)	Exposure time (Hours)			
	24	48	72	96
0.00	$0.00±0.00^{a}$	$0.00±0.00^{a}$	$0.00±0.00^{a}$	$0.00±0.00^{a}$
0.10	$13.30±4.40^{b}$	$13.30±4.40^{b}$	$23.30±3.35^{b}$	$30.00±2.90^{b}$
0.20	$13.30±3.35^{b}$	$16.70±1.65^{bc}$	$26.70±4.40^{b}$	$30.00±2.90^{b}$
0.50	$15.30±1.65^{bc}$	$20.00±0.00^{bc}$	$26.70±1.65^{b}$	$33.30±1.65^{b}$
1.0	$16.70±1.65^{bc}$	$23.30±1.65^{c}$	$30.00±1.65^{bc}$	$34.10±1.65^{b}$
2.0	$20.00±2.90^{bc}$	$33.30±1.65^{d}$	$33.30±0.00^{bc}$	$36.70±1.65^{b}$

Each value is the mean ± S.E of three replicates. Means followed by the same letter (s) in the same column are not significantly different (p>0.05) using Turkey's multiple range test.

3.2. Impact of Exposure Time on the Susceptibility of S. Oryzae to Three Botanicals

The lethal time (LT_{50}) needed by each plant material to achieve 50% mortality in *S. oryzae* is presented in Table 4. LT_{50} values decreased as the doses of each plant material increased. However, weevil exposed at each of the doses of *E. aromatica* powder showed the lowest LT_{50} values ranging from 1.63 to 1.06 h. This is closely followed by those exposed to *P. guineense* (3.98-1.07h) while those that were exposed to *Z. officinales* showed the highest LT_{50} values (15.18-9.75h). There was no significant difference (p>0.05) between the LT_{50} values observed in *E. aromatica* and *P. guineense* at a dose of 0.20g to 2.0g due to overlapping fiducial limits. However, LT_{50} values of weevils exposed at each of the doses of *Z. officinales* significantly differ (p<0.05) from those observed at each of the doses of *E. aromatica* and *P. guineense* as there was no overlapping of fiducial limits. In summary, the order of toxicity of plant materials to *S. oryzae* from highest to lowest based on LT_{50} values was: *E. aromatica* > *P. guineense* > *Z. officinales*.

Table4. *Lethal time (LT_{50}) of different doses of three plant materials on S. oryzae*

Doses (g/10g of rice)	LT_{50} (Hours) of plant materials		
	E. aromatica	*P. guineense*	*Z. officinales*
0.10	1.63 (0.63-2.63)	3.98 (3.70-4.26)	15.18 (7.12-23.24)
0.20	1.38 (1.24-1.52)	1.89 (0.08-3.70)	13.02 (6.52-19.52)
0.50	1.28 (1.15-1.41)	1.36 (1.23-1.49)	12.20 (6.14-18.26)
1.0	1.16 (1.04-1.28)	1.22 (1.03-1.41)	11.86 (5.86-17.86)
2.0	1.06 (0.97-1.15)	1.07 (0.88-1.26)	9.78 (4.94-14.62)

Values in parenthesis represents 95% fiducial limits

4. Discussion

The use of plant materials as an entomocide is a common and age-long practice in Nigeria and other African countries. Consequently, plant parts of several floras have been investigated for their insecticidal activities against stored product pests and this has helped in reducing losses associated with most of these pests. In this study, the entomotoxic effect of powders of three plant materials was investigated. The result obtained indicated that powder of *E. aromatica* and *P. guineense* were effective in the control of *S. oryzae* and their effectiveness was dependent on doses and exposure periods. However, powder of *Z. officinales* was less effective in controlling *S. oryzae*.

E. aromatica powder was the only plant material that elicited complete weevil mortality at the highest experimental dose (2g) after 72 hours while *P. guineense* was also observed to be effective against *S. oryzae* after 72 hours post treatment causing more than 90% mortality from 0.20g to 2.0g/10g of rice. Similar observation has been reported by Lajide et al, (1998), Adedire and Lajide (2001) as well as Ofuya and Dawodu (2001). This high toxicity of powders of both *E. aromatica* and *P. guineense* could be linked to phytochemical constituent of each plant material. Piperine and chavicine are two bioactive compounds that have been established to be insecticidal in *P. guineense* while eugenol caryophylline and oleanolic were reported for their insecticidal activity in *E. aromatica* (Pungitore et al, 2005; Idoko and Adesina, 2012; Bhowmik *et al*., 2012; Oyeniyi *et al*., 2015). The mode of action of bioactive compounds in *P. guineense* has been reported by Olaiya *et al*. (1987) to be by contact toxicity while that of *E. aromatica* is by blockage of spiracles leading to insect suffocation and death (Sokker *et al*., 2012). However, high mortality may not be due to contact toxicity of these phytochemicals due to thick exoskeleton of *S. oryzae* which is expected to have conferred on them some level of resistance to these powders. The high weevil mortality could therefore be possibly linked to stomach poison by these bioactive compounds. Adult weevils might have picked up high dosage of both plant materials especially during normal oviposition and feeding where adults bore holes into the rice grains to lay eggs before cementing it with gelatinous materials.

The high pungent smell of powder of *E. aromatica* and *P. guineense* could also be responsible for high mortality observed in weevils exposed to them when compared to their counterpart exposed to *Z.*

officinales. The toxic effect of any insecticide depends on the point of entry of the toxins (Franz *et al*., 2011). One of such points is through inhalation which occurs through the spiracles of the weevils. High pungent smell of bioactive compounds in *E. aromatica* and *P. guineense* could have caused serious injury to the cells controlling opening and closing of the spiracles. This could have blocked the spiracles leading to suffocation and death of weevils (Sokker *et al*., 2012). This might be responsible for complete mortality observed in weevils exposed at higher dosage which is expected to contain more of these compounds.

Powder of *Z. officinales* was the least effective of these botanicals. Although, the insecticidal effect of *Z. officinales* on *C. maculatus* and *E. vapidella* was very high (Echendu, 1991; Ashamo and Akinneye, 2004); its effect on *S. oryzae* as observed in this study was very low with less than 40% mortality after 96 hours post-treatment. Thus, it can be inferred that *Z. officinales* has a very low insecticidal effect on weevils while it shows some high level of efficacy against bruchids and moths. The ability of weevils to tolerate *Z. officinales* could be linked to the nature of exoskeleton in weevils when compared to bruchids and moths. Thicker exoskeleton might have afforded them the ability to tolerate the active compounds in *Z. officinales* when compared to bruchids and moths. *Z. officinales* has been reported to contain geranial, α-zingiberene, α-farnesene and neral as the main bioactive compounds (Franz *et al*., 2011). Gerenial is acyclic monoterpene aldehydes, usually called citral and it has been identified to possess high insecticidal activity but with lesser concentration (less than 41%) in *Z. officinales* (Franz *et al*., 2011). This lower amount of citral in *Z. officinales* coupled with thicker exoskeleton in *S. oryzae* might have afforded them innate ability to withstand this plant material when compared to bruchids and moths. Likewise, LT_{50} values revealed that *E. aromatica* powder possess the highest entomotoxic effect against weevils followed by *P. guineense* while the least entomotoxic effect was observed in weevils exposed to *Z. officinales*.

5. Conclusion

Various result obtained in this study showed that powders of *E. aromatica* flower buds and *P. guineense* seeds could therefore be recommended to Nigerian rice farmers for controlling *S. oryzae* during storage. Also, since powders of these two botanicals are less laborious to prepare and can be easily separated from the grains after application without affecting the physical appearance of the grain; Nigeria rice farmers should be heartened to use this simple and efficient method of control. Also, government and non-government organizations should also support researches geared towards ensuring commercial extraction and formulation of active ingredients in these two botanicals for the usage of Nigerian farmers. This will help the country to achieve the much desired self-sufficiency in rice production

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