



BIOEFFICACY OF THREE PLANT PRODUCTS AS POST-HARVEST GRAIN PROTECTANTS AGAINST *Sitophilus oryzae* Linnaeus (COLEOPTERA: CURCULIONIDAE) ON STORED WHEAT (*Triticum aestivum*)

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ABSTRACT

The bioactivities of three plant material were carried out on the rice weevil, *Sitophilus oryzae* L. reared on wheat grains (*Triticum aestivum*). Twenty grams of wheat grains were used for each treatment and concentrations used were 5%, 10%, 20% (w/w) for powder and 0.2ml, 0.5ml, 1ml (v/w) used for the oils. Mortality of *S. oryzae* depended on the plant powders, oil concentration and exposure time. The oils were more effective than the powder. 100% mortality was observed after 72 and 96 hours at 20%(w/w) concentration of *A. indica* and *A. occidentale* powders respectively, while 64% mortality was observed on treatment with *Moringa oleifera* powder at same concentration. Adult emergence from treated grains decreased with increase in concentration of plant products. No insects emerged from grains treated with *A. indica* oils. The inhibition rates for treatment with oils of *A. indica*, *A. occidentale*, *Moringa oleifera* and control were 100, 95.7, 81.2 and 0% respectively. No significant difference occurred in germination of grains treated with the three plant products and control after 12 weeks in storage. *A. indica* oil was the most efficacious of the three plant products and can be used as biopesticides against *S. oryzae*

KEY WORDS: germinability, *Azadirachta Indica*, *Anarcadium occidentale*, *Moringa oleifera*.

INTRODUCTION

Wheat (*Triticum* spp) is the major source of protein in human foods, having higher protein content than maize or rice, the other major cereal grains. In terms of total production tonnages used for food, it is currently second to rice as the main human food crop (Ileke and Bulus 2012). Wheat is infested by various insect pests between harvest and storage. The most economically important insect pests of stored wheat are the granary weevils, *Sitophilus granaries*; maize weevils, *Sitophilus zeamais*; rice weevils, *Sitophilus oryzae*; lesser grain borer and *Rhizopertha dominica* (Ileke and Bulus 2012). The rice weevil feeds on rice, wheat, barley and on other raw or processed cereals such as pasta. The larva feeds within the kernel and consumes the endosperm. The adult leaves a large, ragged exit hole in the kernel and feeds on damaged kernels. The rice weevil adult gathers and reproduces in stored grains. There is a steady increase in the use of medicinal plant products as a cheaper and ecologically safer means of protecting stored products against infestation by insects. The objectives of this research work were to evaluate the efficacy of powders and oil extracts of *Azadirachta indica* seeds, *Anarcadium occidentale* nuts and *Moringa oleifera* seeds against *Sitophilus oryzae* and on seed germination after three months of storage.

MATERIALS AND METHODS

Insect culture

Adults of *Sitophilus oryzae* were obtained from infested wheat grains at the Biology Research Laboratory, Federal University of Technology, Akure, Ondo State. Clean wholesome wheat grains were used for this experiment. The grains were first sorted out and disinfested by putting them in a deep freezer at -4°C for 7 days. The grains were then removed, air-dried for 1 hour to prevent mouldiness and poured into two plastic jars. Seventy pairs of *Sitophilus oryzae* were introduced into each of the jars. The containers were covered with muslin cloth and held in place with the aid of rubber band. The jars were kept aside at ambient temperature 26±3°C and 65±5% relative humidity.

Plant materials

The plant materials used were *Azadirachta indica* seeds, *Anarcadium occidentale* nuts and *Moringa oleifera* seeds. *A.indica* seeds were acquired from neem trees at the front of Crown Estate Garden, Lekki Epe Axis, Lagos. The nuts of *A.occidentale* were sourced from Ajah Market, Lagos State. *M. oleifera* seeds were sourced from IITA, Ibadan, Oyo State. The plant parts (Table 1) used were sun dried for 2weeks. They were made into powdery form with a blender (HR2021: PHILIPS MODEL, CHINA). The plant powders were then divided into two different portions. A part was used for dust treatment and other portion was subjected to oil extraction process. The powders were packed in plastic containers with tight lids and stored in a refrigerator prior to use.

TABLE 1: Plants evaluated for insecticidal activities against *S. oryzae*

Scientific name	Family	Parts used	Common name
<i>Azadirachta indica</i>	Meliaceae	Seed	Neem
<i>Anacardium occidentale</i>	Anacardiaceae	Nut	Cashew
<i>Moringa oleifera</i>	Moringaceae	Seed	Drumstick

Oil extraction

Ethanol extracts of *A. indica*, *A.occidentale seeds* and *M.oleifera* nuts were carried out using Soxhlet apparatus method. The extraction was carried out between 40–60°C. Excess ethanol was evaporated using a rotary evaporator at 30 to 40°C with rotary speed of 3 to 6 rpm for 8 hours. The resulting extract was air dried in order to remove traces of the solvent

Toxicity of plant powders

Portions of 1g, 2g and 4g of each plant powders corresponding to 5%, 10%, 20% w/w concentrations were weighed and each added to 20g of clean, undamaged and uninfested wheat grains in 250ml plastic containers. The grains in the controls contained no plant powders. The containers with their contents were gently shaken to ensure thorough admixture of the wheat grains and treatment powders. The experiments were set up in a Completely Randomized Design (CRD) and each treatment was replicated four times. Twenty adult *S. oryzae* (2 to 3 days old) were introduced into the treated and control vials. Weevil mortality was assessed every 24 hours for four days. The insects were confirmed dead when there was no response to probing with sharp pin at the abdomen (Adedire *et al.*, 2011). Thereafter, all insects, both dead and alive were removed from each container.

Toxicity of plants extracts

The toxic effect of plants oils on adult *S.oryzae* was carried out using 250ml plastic containers containing 20g of wheat grain with concentration of 0.2, 0.5 and 1.0 % v/w plant oils. The containers were shaken for 5-10 min to ensure uniform mixing and coating. The containers were left open for 30 min so as to allow traces of solvent to evaporate off. Twenty adult insects of *S. oryzae* (2 to 3 days old) were introduced into the treated and control. Weevil mortality was assessed every 24 hours for four days. The insects were confirmed dead when there was no response to probing with sharp pin at the abdomen (Adedire *et al.*, 2011).

Analysis for both powder and oil treatments

At the end of the 4th day, all insects, both dead and alive were removed from each container. The experiment was kept aside for another 42 days for the emergence of the first filial (F₁) generation. The number of adults that emerged from each replicate was counted and recorded. The percentage reduction in adult emergence of F₁ progeny or inhibition rate (IR) was calculated according to the method described by Tapondju *et al.* (2002).

$$\% IR = \frac{Cn - Tn}{Cn} \times \frac{100}{1}$$

Where Cn is the number of emerged insects in the control and Tn is the number of emerged insects in the treated containers.

The % loss in weight was determined and recorded using the method described by Odeyemi and Daramola (2000).

$$\% \text{ Weight loss} = \frac{\text{Initial weight} - \text{final weight}}{\text{Initial weight}} \times 100$$

Percentage germination

10 grains were randomly selected from each treatment after 12 weeks and planted on moistened cotton wool in small plastics. Germination count was taken on the 5th day according to the normal planting methods. Percentage germination was calculated thus:

$$\% \text{ Germination} = \frac{\text{Number of grains that germinated}}{\text{Total number of grains planted}} \times \frac{100}{1}$$

Statistical analysis

Data were subjected to analysis of variance and where significant differences existed, treatment means were separated using the New Duncan’s Multiple Range Test.

RESULTS

Effects of powders and oils of *A. indica*, *A. occidentale* and *M. Oleifera* on *S. oryzae*

Toxicity to Adult Insects

The percentage mortality of *S. oryzae* depended on the plant products, concentration of formulations and exposure time. For all the plant products used, percentage mortality increased as concentration used increase. In wheat grains treated with *A. indica* powder, mortality for all treatment was significantly higher than the control at P<0.05 (Table 2). Mortality rates of 28.75% and 95% were observed at 5% (w/w) concentration after 24 and 96 hours respectively. On the other hand, 66.25% and 100% mortality were observed within 24 and 72 hours respectively in grains treated with 20.0% (w/w) concentration. No mortality was observed in the control (Table 2). Higher mortality values of 43.75 and 100% were observed in the *A. indica* oil treatment after 24 and 48 hours at 0.2 and 1.0ml (v/w) concentration. (Table 3).

TABLE 2: Mortality of adult *S. oryzae* in wheat seeds treated with *A. indica* powder

Conc. (%w/w)	Mean % Mortality ± S. E. with duration of exposure (hours)			
	24	48	72	96
Control	0.00±0.00 ^d	0.00±0.00 ^c	0.00±0.00 ^c	0.00±0.00 ^c
5.0	28.75±7.47 ^c	58.75±5.91 ^b	81.25±8.26 ^b	95.00±2.89 ^b
10.0	51.25±3.15 ^b	68.75±6.88 ^b	93.75±3.75 ^{ab}	100.00±0.00 ^a
20.0	66.25±3.75 ^a	96.25±2.39 ^a	100.00±0.00 ^a	100.00±0.00 ^a

Each value is a mean ± standard error of four replicates. Means followed by the same superscript along the column are not significantly different (P>0.05) using New Duncan Multiple Range Test.

TABLE 3: Mortality of adult *S. oryzae* in wheat seeds treated with *A. indica* oil

Conc. (ml)	Mean % Mortality \pm S. E. with duration of exposure (hours)			
	24	48	72	96
Control	0.00 \pm 0.00 ^c	0.00 \pm 0.00 ^c	0.00 \pm 0.00 ^c	0.00 \pm 0.00 ^b
0.2	43.75 \pm 4.27 ^b	85.00 \pm 3.54 ^b	93.75 \pm 1.25 ^b	98.75 \pm 1.25 ^a
0.5	61.25 \pm 6.25 ^a	95.00 \pm 2.04 ^a	100.00 \pm 0.00 ^a	100.00 \pm 0.00 ^a
1.0	71.25 \pm 7.47 ^a	100.00 \pm 0.00 ^a	100.00 \pm 0.00 ^a	100.00 \pm 0.00 ^a

Each value is a mean \pm standard error of four replicates. Means followed by the same superscript along the column are not significantly different ($P>0.05$) using New Duncan Multiple Range Test.

TABLE 4: Mortality of adult *S. oryzae* in wheat seeds treated with *A. occidentale* powder

Conc. (%w/w)	Mean % Mortality \pm S. E. with duration of exposure (hours)			
	24	48	72	96
Control	0.00 \pm 0.00 ^a	0.00 \pm 0.00 ^d	0.00 \pm 0.00 ^c	0.00 \pm 0.00 ^c
5.0	7.50 \pm 2.50 ^a	22.50 \pm 5.95 ^c	78.75 \pm 4.27 ^b	90.00 \pm 5.40 ^b
10.0	7.50 \pm 3.23 ^a	75.00 \pm 5.40 ^b	83.75 \pm 3.15 ^b	100.00 \pm 0.00 ^a
20.0	21.25 \pm 14.63 ^b	90.00 \pm 3.54 ^a	95.00 \pm 2.04 ^a	100.00 \pm 0.00 ^a

Each value is a mean \pm standard error of four replicates. Means followed by the same superscript along the column are not significantly different ($P>0.05$) using New Duncan Multiple Range Test.

TABLE 5: Mortality of adult *S. oryzae* in wheat seeds treated with *A. occidentale* oil

Conc. (ml)	Mean % Mortality \pm S. E. with duration of exposure (hours)			
	24	48	72	96
Control	0.00 \pm 0.00 ^d	0.00 \pm 0.00 ^c	0.00 \pm 0.00 ^d	0.00 \pm 0.00 ^c
0.2	35.00 \pm 4.56 ^c	35.00 \pm 5.54 ^b	80.00 \pm 3.54 ^c	96.25 \pm 1.25 ^b
0.5	58.75 \pm 5.54 ^b	58.75 \pm 3.75 ^a	92.50 \pm 2.50 ^b	100.00 \pm 0.00 ^a
1.0	72.50 \pm 4.33 ^a	72.50 \pm 5.15 ^a	100.00 \pm 0.00 ^a	100.00 \pm 0.00 ^a

Each value is a mean \pm standard error of four replicates. Means followed by the same superscript along the column are not significantly different ($P>0.05$) using New Duncan Multiple Range Test.

TABLE 6: Mortality of adult *S. oryzae* in wheat seeds treated with *Moringa oleifera* powder

Conc. (%w/w)	Mean % Mortality \pm S. E. with duration of exposure (hours)			
	24	48	72	96
Control	0.00 \pm 0.00 ^c	0.00 \pm 0.00 ^b	0.00 \pm 0.00 ^d	0.00 \pm 0.00 ^d
5.0	6.25 \pm 3.75 ^{bc}	20.00 \pm 2.89 ^a	27.50 \pm 3.23 ^c	40.00 \pm 3.54 ^b
10.0	15.00 \pm 5.40 ^{ab}	22.50 \pm 5.95 ^a	43.75 \pm 3.75 ^b	53.75 \pm 4.27 ^b
20.0	18.75 \pm 1.25 ^a	31.25 \pm 4.73 ^a	57.50 \pm 3.23 ^a	63.75 \pm 3.15 ^a

Each value is a mean \pm standard error of four replicates. Means followed by the same superscript along the column are not significantly different ($P>0.05$) using New Duncan Multiple Range Test.

TABLE 7: Mortality of adult *S. oryzae* in wheat seeds treated with *Moringa oleifera* oil

Conc. (ml)	Mean % Mortality \pm S. E. with duration of exposure (hours)			
	24	48	72	96
Control	0.00 \pm 0.00 ^c	0.00 \pm 0.00 ^c	0.00 \pm 0.00 ^b	0.00 \pm 0.00 ^c
0.2	7.50 \pm 3.23 ^{bc}	22.50 \pm 4.33 ^b	48.75 \pm 4.27 ^a	58.75 \pm 5.91 ^b
0.5	10.00 \pm 3.54 ^b	30.00 \pm 2.04 ^b	57.50 \pm 3.23 ^a	63.75 \pm 2.39 ^b
1.0	23.75 \pm 2.10 ^a	43.75 \pm 6.25 ^a	56.25 \pm 4.27 ^a	75.00 \pm 2.04 ^a

Each value is a mean \pm standard error of four replicates. Means followed by the same superscript along the column are not significantly different ($P>0.05$) using New Duncan Multiple Range Test.

In grains treated with the powders of *A. occidentale*, mortality also increased with increase in treatments. By the 96 hrs mortality in 5% w/w of *A. occidentale* powders was significantly lower (90%) than 10%w/w treatment (100%) which was not significantly lower than 20% w/w treatment (100%) (Table 4). On the other hand, mortality of *S. oryzae* on grains treated with 0.2mls oils of *A. occidentale* was significantly lower (96%) than that of 1.0ml (100%) after 96Hrs of treatment.(Table 5). In grains treated with powder and oil of *M. oleifera*, mortality followed almost the same trend. 100% mortality of *S. oryzae* was not achieved in any of the *M. oleifera*

treatment. Treatment with *M.oleifera* was however also significantly lower ($P<0.05$) at the various concentrations used than the control. Highest mortality of 63.8% was observed after 96 hrs at 20% w/w concentration of the powder treatment while 75% mortality was observed after 96 hours post treatment in the grains treated with oil of *M. oleifera*.(Tables 6 and 7).

Adult Emergence, % Reduction in F1 progeny and % Weight Loss in Powder Treatments

The number of *S. oryzae* adults (F1) that emerged from all powder treated grains was significantly lower than in the

control for all the plant products used. Moreover, the number of adults that emerged from treated grains decreased with increase in concentration of plant products (Table 8). The percentage inhibition rate at all concentrations for every plant product increased significantly when compared to the control. The highest inhibition rate was observed in grains treated with *A. indica* (99.5%) followed by *A. occidentale* (96.5%) and *M. oleifera* (77.2%) powders (Table 8). The weight loss in grains treated with *A. indica* and *A. occidentale* powders were significantly lower than in the control ($P < 0.05$), while, no significant weight loss was observed in grains treated with *M. oleifera* when compared with the control. (Table 8).

Adult Emergence, % Reduction in F1 progeny and % Weight Loss in Oil Treatments

The number of adults that emerged from the oil treated grains was significantly lower than the adults in control. No insects emerged from grains with 1.0ml of *A. Indica* as compared to 2 and 7 insects from grains treated with 1.0ml of *A.occidentale* and *M. oleifera* respectively. The percentage progeny reduction was also significantly lower ($P < 0.05$) in all treated grains compared to the control. The inhibition rate increased with increase in concentration of the plant products with 100% inhibition from 1.0ml for *A. Indica* treatments and 96 and 81% inhibition for *A.occidentale* and *M. Oleifera*. The weight loss observed in samples of grains treated with the oils of *A. indica* and *A. occidentale* were significantly lower than that of control at ($P < 0.05$), while, no significant difference ($P > 0.05$) was observed in weight of grains treated with *M. oleifera* and the control. (Table 9) No weight loss was observed in grains treated with *A.indica*.

TABLE 8: Number of adult emergence, reduction in progeny development of adult *S. oryzae* and weight loss in wheat grains treated with plant powders

Plant powders	Conc. (%w/w)	Mean number of adult emergence	%Inhibition Rate (IR)	% Weight loss
<i>A. indica</i>	5.0	7.25 ^a	93.97±2.05 ^b	4.50±0.24 ^b
	10.0	0.75 ^a	96.24±1.03 ^a	1.13±0.66 ^b
	20.0	0.25 ^a	99.50±0.50 ^a	0.50±0.00 ^b
	Control	21.75 ^b	0.00±0.00 ^c	37.50±4.27 ^a
<i>A. occidentale</i>	5.0	10.25 ^b	88.59±0.00 ^a	6.00±1.06 ^b
	10.0	4.75 ^a	92.50±6.37 ^a	5.38±2.38 ^b
	20.0	0.75 ^a	99.50±7.50 ^a	1.00±1.00 ^b
	Control	21.75 ^b	0.00±0.00 ^c	37.50±4.27 ^a
<i>M. oleifera</i>	5.0	20.25 ^{ab}	67.58±19.46 ^a	27.50±4.33 ^a
	10.0	17.00 ^a	88.96±3.07 ^a	23.75±6.25 ^a
	20.0	16.25 ^a	77.20±12.42 ^a	25.00±5.00 ^a
	Control	21.75 ^b	0.00±0.00 ^c	37.50±4.27 ^a

Each value is a mean ± standard error of four replicates. Means followed by the same superscript along the column (within a plant powder) are not significantly different ($P > 0.05$) using New Duncan Multiple Range Test.

TABLE 9: Number of adult emergence, reduction in progeny development of adult *S. oryzae* and weight loss in wheat grains treated with plant oil extracts

Plant powders	Conc. (ml)	Mean number of adult emergence	% Inhibition Rate (IR)	% Weight loss
<i>A. indica</i>	0.2	1.00	93.33±2.62 ^a	3.75±3.54 ^b
	0.5	0.75	97.01±1.82 ^a	1.50±1.38 ^b
	1.0	0.00	100.00±0.00 ^a	0.00±0.00 ^b
	control	21.75	0.00±0.00 ^b	37.50±7.50 ^a
<i>A.occidentale</i>	0.2	6.75	90.83±2.15 ^a	4.88±1.13 ^b
	0.5	2.25	94.11±0.89 ^a	2.38.50±0.50 ^b
	1.0	1.75	95.65±2.57 ^a	1.75±1.26 ^b
	Control	21.75	0.00 ±0.00 ^b	37.50±7.50 ^a
<i>M. oleifera</i>	0.2	9.50	70.69±4.24 ^a	29.00±5.77 ^a
	0.5	7.38	78.64 ±9.57 ^a	23.75±6.25 ^a
	1.0	6.50	81.15±13.74 ^a	20.25±6.57 ^a
	Control	21.75	0.00±0.00 ^b	37.50±7.50 ^a

Each value is a mean ± standard error of four replicates. Means followed by the same superscript along the column (within a plant oil extract) are not significantly different ($P > 0.05$) using New Duncan Multiple Range Test

Germination

The percentage germination of wheat at 12 weeks after treatment with *A. indica*, *A. occidentale* and *M. oleifera* powders and oil are as shown in Figures 1 and 2

respectively. At the end of the germination period, high germination was observed across all treated seeds as well as the control.

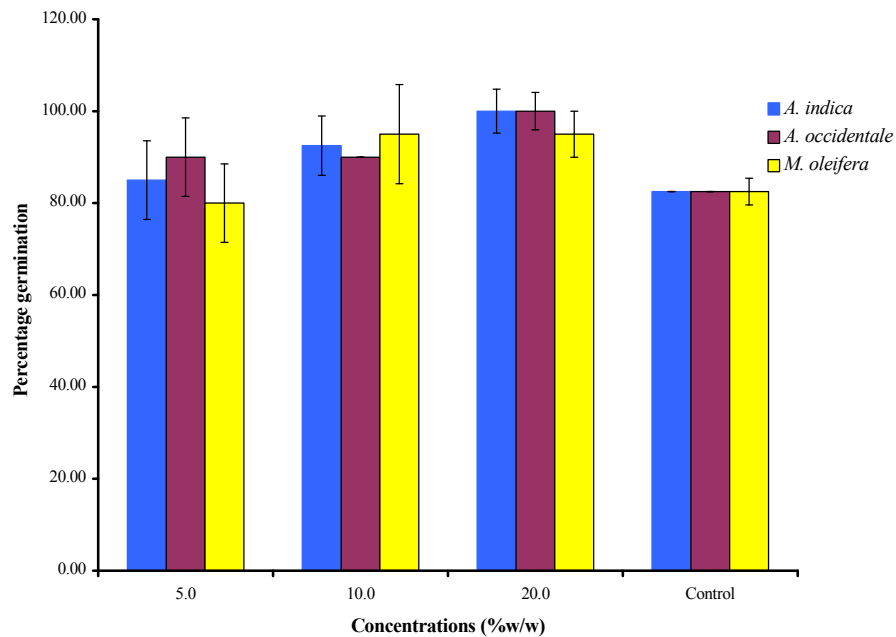


FIGURE: 1 Percentage germination of seeds after treatment with plant powder (with standard error bars displayed)

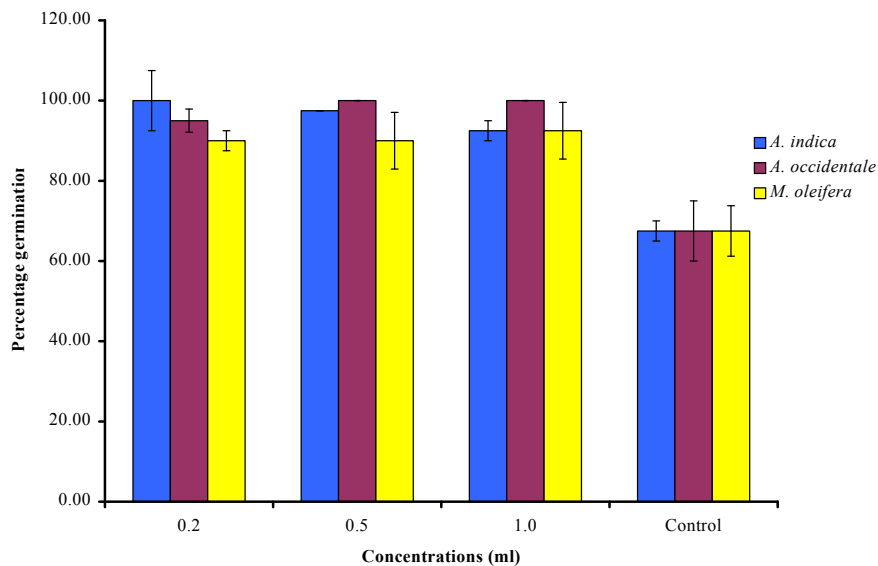


FIGURE 2: Percentage germination of seeds after treatment with plant oil extracts (with standard error bars displayed)

DISCUSSION

The results obtained in this study showed that powders and oils of *A. indica*, *A. occidentale* and *M. oleifera* seeds tested were toxic to the rice weevil, *S. oryzae*. The development of *S. oryzae* was suppressed in all treated wheat grains when compared to control. *A. indica* (Neem) seed powder and extract caused 100% mortality at highest concentrations in adult *S. oryzae* in stored wheat grains. *A. indica* has been found to be effective against the maize weevil, *S. zeamais* and cowpea bruchid, *C. maculatus* (Jackai and Oyediran 1991, Onu and Baba 2003, Ileke and Oni 2011). This has been attributed to the presence of triterpenoids, which include azadirachtin, salanin and meliantriol in *A. indica* (Mbailao et al., 2006; Ileke and Oni 2011). *A. occidentale* (Cashew) also caused 100% mortality in *S. oryzae* at 96 hours post treatment, in stored

wheat grains. This is in agreement with Oparaeke and Bunmi (2006) and Adedire et al (2011). The mode of action could be as a result of the powder and oil coating the treated wheat seeds which prevented contact between the grains and the weevils resulting into starvation. The oil also blocked the spiracles which might have led to suffocation of the insects (Adedire et al. 2011, Ileke and Olotuah 2012). The insecticidal activity of *A. occidentale* oil could be linked to the presence of anacardic acid and cardinal (Rehm and Espig 1991). *Moringa oleifera* at highest concentration also reduced adult emergence of *S. Oryzae*. Mbailo et al. (2006) and Ileke and Oni (2011) reported that high concentration of *M. oleifera* powder significantly reduced the longevity of adult *Callosobruchus maculatus* on cowpea seeds and *S. zeamais* adults on wheat grains respectively. Most insects

breathe through the trachea which usually leads to the opening of the spiracle. These spiracles might have been blocked by the powders and extracts thereby leading to suffocation. The significant reduction in the emergence and lower weight loss could be as a result of higher mortality of adult *S. oryzae*. The higher adult emergence of *S. oryzae* observed in wheat grain treated with *M. oleifera* at different rates may be as a result of concentration used or its inability to protect the grains for a long period. However, the bioactive constituents of the plant materials may be more available in the extract at higher concentrations, which may be responsible for the higher mortality of adult insect within a short exposure time (Ashamo 2007). The plants *A. indica* seeds and *A. occidentale* nuts are of medicinal values, biodegradable, readily available and pose no danger to man and other mammals. They can therefore be used as biopesticides against *S. oryzae*.

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