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Toxicity and mode of application of essential oil against stored insect pest of maize, (*Sitophilus Zeamais,* Motsch) (Coleoptera: Curculionidae)

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ABSTRACT

The topical, residual action and fumigant test of *Ocimum africanum* (curry), *Curcuma longa* (turmeric) and *Allium sativum* (garlic) oil were investigated against *Sitophilus zeamais* (Moth.) in the laboratory under ambient temperature of 28±2°C and 65±5% relative humidity. Ten microlitres of each essential oil was applied topically on to the dorsal surface of the maize weevils in a Petri-dish with the aid of a micro-syringe. In the second experiment, filter paper was drenched with 60 microlitres of oil from each of the plant materials and air-dried for 5 minutes. The fumigant test was carried out using 500 ml plastic containers (10 cm x 10 cm x 15 cm) with cover containing 2 ml of each essential oil tied in a muslin cloth bag (7 cm x 10 cm) and suspended halfway into the plastic. Ten adult maize weevils were placed in each Petri-dish. Each treatment including control was replicated four times in Completely Randomised Design. Mortality counts were taken and recorded after every 20 minutes for 120 minutes exposure of post treatment. Results of weevil mortality in topical and fumigant test were curry oil (87%; 93%) and turmeric oil (87%; 93%) recorded high weevil mortality within 2 h after exposure period. However garlic oil (60%) had the least mortality from same application and exposure period, which by extension could be employed in an integrated pest management program.

Keywords: *Sitophilus zeamais*; topical; residual; fumigant; essential oil; exposure period

INTRODUCTION

Maize is the third most important cereal crop and USA is the leading world producer of maize, which produces about 40% of the world's total production (Udoh *et al*., 2005). Increasing and improving maize production and utilization have been suggested as one major strategy for alleviating the specter of hunger and malnutrition that appears to be perpetually hanging over many African countries. Maize can be processed for oil, starch, alcohol and adhesives, explosives, paints, ceramics, shoe polish, dyes, rubber substitutes, corn, popcorn, flour corn and sweet corn (Tandy *et al.,* 2013), and it is also a rich source of starch (60 – 80%), protein (8 – 12%), fat (3 – 5%), vitamin (70 – 80%), mineral (1 – 2%), fibers (3.1%), water (13.5%) (FAO, 1992). It contains more carotene, a precursor of Vitamin A, and is therefore nutritionally more valuable (Yayock *et al.,* 1988).

Sitopholus zeamais is a cosmopolitan as well as a major pest of maize which posed a major biotic constraint to utilization of maize in the tropics and subtropics (Akob and Ewete, 2007) and temperate regions of the world (Stoll, 1986). The insect is a field-to-store pest and attacks both standing crops and stored cereal products, (NRI, 1996), such as, wheat, rice, sorghum, oats, barley, rye, buckwheat, peas, dry yam products, groundnut millet, dry cassava and cocoyam in Nigeria (NRI, 1996). Maize weevil does not only damage the grain but also depreciate the weight and quality of

stored grains (Rayhan, 2014). Enobakhare and Law-Ogbomo, (2002) reported that damaged maize grains have reduced weight, poor marketability and low viability. Weight losses in maize up to 80% in storage due to *S. zeamais* infestation have been estimated for many areas (De-Lima, 1987). Consequently, *S. zeamais* damages to stored grains and grain products are around 5 – 10% in the temperate zone and 20 – 30% in the tropical zone (Rahman and Talukder, 2006; Rajendran and Sriranjini, 2008). Such damage may reach up to 40% in countries where modern storage technologies have not been introduced and climate conditions are favorable (Shaaya *et al.,* 1997). Insect damages include direct consumption of kernels, detritus of exuviae, webbing, and cadavers thereby makes the grain unfit for human consumption thus reduce quality and quantity (Shazia *et al*., 2006). Insect infestation manipulate the storage environment resulted in development of hotspots which are congenial for the proliferation of storage fungi and other harmful micro flora (Rajashekar *et al.,* 2012). Problems such as residual effects, pest resurgence, prevalent environmental and ecological hazards, insect pest resistance and economy of farmers associated with currently used synthetic pesticides informed the recent focus on acceptable use of botanicals (Zettler and Cuperus 1990; Elhag, 2000) which are environmental friendly, biodegradable, economic and cost effective.

Effective pest control is no longer a matter of heavy application of pesticides, partly because of rising cost of petroleum-derived products but largely because excessive use of pesticide promotes faster evolution of resistance form of pests, destruction of natural enemies, ability to turn formerly innocuous species into pests and contamination of food (Manohar *et al*., 2017). There is, thus, an urgent need for control agents, which are less toxic to man and more readily degradable. Among which is the use of botanical pesticides with low mammalian toxicity, and can effectively prevent and/or suppress insect pest especially in storage (Golob and Webley, 1980). Turmeric contains pungent, odoriferous oils and oleoresins; the rhizomes have been reported to possess many kinds of biological activities (Ghawana *et al.*, 2011). Insecticidal properties of turmeric have been well documented in the literatures. The insect repellant components in turmeric are turmerones and arturmerone (Tripathi *et al.*, 2002).

Garlic is potent in the control of insects and as efficiently as DDT (Douiri *et al.,* 2013). It has also repellent properties and repeatedly effective against wide range of insects at different stages in their life cycle (eggs, larvae, adult) This includes ants, moths, beetles, termites, tick, etc (Douiri *et al.,* 2013). Therefore, there is the need for methodically use and selective control in aphid infested field since it kills the natural enemies of aphids (Douiri *et al.,* 2013). The objective was to determine the toxicity and mode of application of the plant material (botanicals) used in the control of *Sitophilus Zeamais.*

MATERIALS AND METHODS

Site of Study

The research was conducted at the Crop Protection Laboratory, Department of Crop and Soil Science, Faculty of Agriculture, University of Port Harcourt. The experiment was carried out under ambient temperature at $27 \pm 3^{O}C$ and relative humidity $65 \pm 5\%$, using thermometer and hygrometer.

Source of Maize Seed

Maize (DT.SYNII-W) used was collected from the International Institute of Tropical Agriculture (IITA), Ibadan, Oyo State, Nigeria.

Sources of plant materials

All three (Turmeric, Garlic and Curry leaf) plant materials were obtained from the National Root and Crop Research Institute (NRCRI), Umudike, Abia State.

Identification and sexing of insect
Examination of *S. zeamais* was done with a light microscope of high resolution to correctly identify adult maize weevil that was used for culture. Female was differentiated from male by morphological features (Haine, 1991).

Insect Culture
Infested maize seeds were sieved and adult maize weevil obtained was introduced into the disinfested maize seeds and left for seven days to mate and lay eggs for the F_1progeny to emerge in a 10 litres containers covered with muslin cloth. Only newly emerged adult of *S. zeamais* was used for the experiment.

Seed treatment/Sterilization
Maize seeds were kept in a deep freezer (-4^{O}C) for seven days, and conditioned in the cooled incubator for another seven days to standardize the seeds moisture content.

Extraction of oil from plant materials.
Extraction of oil was carried out at the Chemistry Laboratory, Chemistry Department, Rivers State University Port Harcourt. The extraction was done using soxhlet apparatus/extractor. Solvent used for both turmeric and curry leaf was ethanol, while Hexane was used for garlic. Each plant material (Turmeric, Garlic and Curry leaf) was weighed separately and made available for use. The gram of each plant material was one-third of 1ml of solvent used: $Turmeric - 69.71gram \times 3 = 209.13ml\ of\ ethanol\ used$; $Garlic - 121.385gram \times 3 = 364.155ml\ of\ haxane\ used$ and $Curry\ leaf - 58.52gram \times 3 = 175.56ml\ of\ ethanol\ used$

The gram of each plant material was put into a thine-boll. The solvent was measured into a round bottom flask. And the extraction was done under the following temperature viz turmeric was about 30℃; garlic was about 18^{0}C and for curry leaf it was about 29℃. The three extractions were not time dependent and the extracted oil of each plant material was kept in room temperature to evaporate.

TOXICITY ASSAY
The laboratory tests were conducted using newly emerged maize weevils. The weevils were collected from the cultured lots. Treatments were replicated four times and the number of weevils used per replicate was ten.

Topical application test -Ten weevils were picked from the collection tray into a Petri-dish for each treatment. Ten microlitres of each botanical was applied directly on to the dorsal surface of the weevils in each Petri-dish with the aid of a micro-syringe. Each treatment was replicated four times in Completely Randomised Design. The Petri-dishes were perforated on the top to provide aeration and to prevent either exit of the test materials or entrance of other insects. Mortality counts were taken and recorded after every 20 minutes for 2 hour (which was the maximum time period taken to achieve the highest mortality). A weevil was regarded as dead if it showed no signs of movement when touched lightly with a soft camel hair brush or when it is lying flat on its dorsal part.

Residual contact test - This test was carried out with Petri-dishes fitted with filter papers. The filter paper was drenched with 60 microlitres of the botanicals and allowed to be air-dried for 5 minutes. Ten weevils were placed on the botanical impregnated filter papers with the aid of a soft camel hair brush. Control experiments in which the insects were exposed to similar filter papers treated with distilled water only were set up with each replicate. Each treatment including control was replicated four times in Completely Randomised Design. The Petri-dishes were perforated on the top to provide aeration for the test insects. Mortality counts were taken and recorded after every 20 minutes for 2 hour using the same attributes of identifying a dead insect as earlier described.

Fumigant test - The test was carried out using 500 mls plastic containers (10 cm x 10 cm x 15 cm) with cover and a muslin cloth bag measuring 7 cm x 10 cm. Ten newly emerged weevils were placed in each bag and the mouth tied with an extended rope with which it was lowered half way into the plastic container containing 2 mls of each botanical. Each treatment including control was replicated four times in Completely Randomised Design. The numbers of weevils knocked down or killed during trials were recorded and expressed as percentage mortality. Also, the data were further subjected to the analysis of variance. Means were separated using LSD to test the levels of significance.

Data Collection

Adult weevil mortality was taken for 7days and corrected using Abbott's (1925) formula.

$$Pt = \frac{(P_0 - P_c)}{100 - P_c} \times 100$$

Mortality counts were taken and recorded after.

Statistical Analysis

All data were subjected to Analysis of Variance (ANOVA) using GENSTAT 5.32 version.

Significant means were separated using LSD test at 5% probability level

RESULTS

Table 1 showed that high weevil mortality (87%) each was caused by curry and turmeric oil in topical application after exposure while garlic oil caused least weevil mortality (60%) at the same exposure period. Though curry oil was more immediate in knock down effect on the introduced insects. Therefore, the topical application brings quick contact of botanical oil with the body of weevils. High percent weevil mortality was varied among curry oil (67-87%), turmeric oil (20-60%) and garlic oil (0-47%), which was recorded across durations (40, 60, 120 and 140 minutes). The potent insecticidal activity of curry was quite stable while turmeric oil and garlic oil were gradual and slow against *S. zeamais* respectively. Oil obtained from curry and turmeric achieved highest adult weevil mortality (87%) in 2 h and garlic performed above average (67%). In this study, significantly high weevil mortality was caused by curry and turmeric oil in topical application. It was observed that there is intersection between the turmeric and curry oil as a protectant as shown in the graph (Fig 1).

Superior performance of curry oil was recorded against the adult weevil from the onset of exposure having recorded higher adult weevil mortality (87%), followed by turmeric oil (67%) and the least recorded by garlic oil (47%) (Table 2). However, the turmeric oil caused higher mortality of adult maize weevil as protectant compared to control. Varying high percent weevil mortality due to curry oil, turmeric oil and garlic oil was recorded across durations (40, 60, 120 and 140 minutes). The potent insecticidal activity of the essential oils was gradual across exposure periods in residual test action against *S. zeamais* respectively and no adult weevil mortality recorded after 20 minutes of exposure by garlic oil. Though curry oil and turmeric oil achieved the highest adult weevil mortality and garlic recorded above average mortality against *S. zeamais* at 2 h after exposure. Fig 2 showed the intersection between curry and turmeric oil at the onset of the exposure for the first 20 minutes.

Table 3 shows the fumigant action of the curry, turmeric and garlic oil on percent maize weevil mortality. Curry and turmeric oil evoked significantly high weevil mortality (93%)(93%) in *S. zeamais* at 2 h after exposure, while the least percent weevil mortality (60%) was recorded by garlic. A various percent of weevil mortality caused by curry oil (33-93%), turmeric oil (20-93%) and garlic oil (0-47%) was recorded across duration period (40, 60, 120 and 140

minutes). Curry oil and turmeric oil achieved the highest adult weevil mortality (87%) of *S. zeamais* in 2 h after exposure, while garlic oil only recorded weevil mortality (60%) above average. There was intersection between the turmeric and curry oil as shown by the graph (Fig 3).

Table 1: Effect of exposure time and topical application of botanicals on adult weevil mortality

Plan Oil	20 mins	40 mins	60 mins	120 mins	140 mins	2 hours
Curry	0	67[a]	67[a]	67[a]	73[a]	87[a]
Garlic	0	0c	13b	33b	47c	60b
Tumeric	0	20b	27b	47b	60b	87[a]
Control	0	0c	0c	0c	0d	0c
LSD ($p<0.05$)	-	*	*	*	*	*

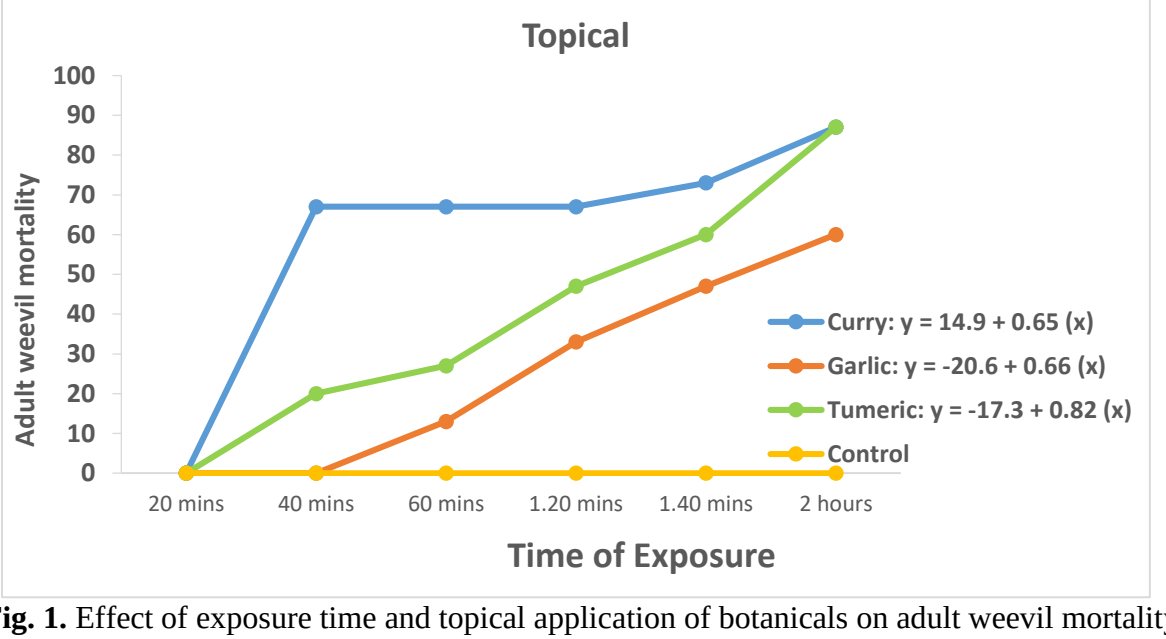

Fig. 1. Effect of exposure time and topical application of botanicals on adult weevil mortality

Table 2: Effect of exposure time and residual application of botanicals on adult weevil mortality

Plan Oil	20 mins	40 mins	60 mins	120 mins	140 mins	2 hours
Curry	0	33a	47a	67a	67a	87a
Garlic	0	0c	13b	33b	40b	47c
Tumeric	0	20b	27b	47b	60a	67b
Control	0	0c	0c	0c	0c	0d
LSD ($p<0.05$)	-	*	*	*	*	*

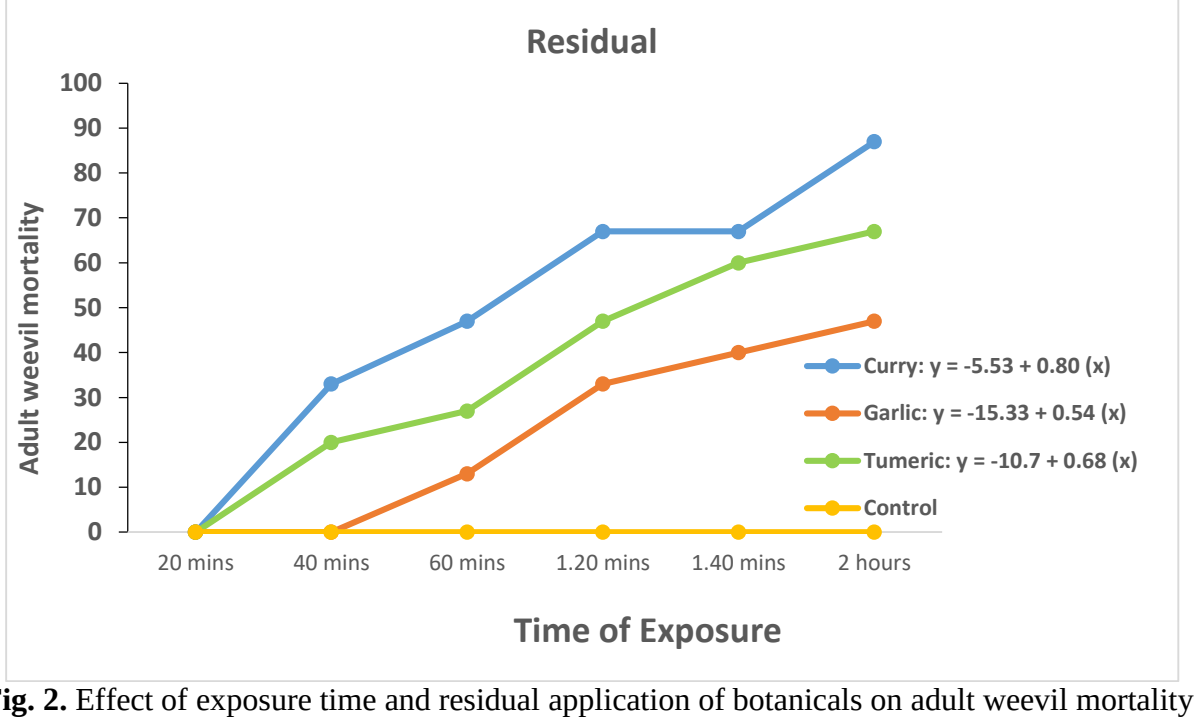

Fig. 2. Effect of exposure time and residual application of botanicals on adult weevil mortality

Table 3: Effect of exposure time and fumigant application of botanicals on adult weevil mortality

Plant Oil	20 mins	40 mins	60 mins	120 mins	140 mins	2 hours
Curry	0	33[a]	47[a]	67[a]	80[a]	93[a]
Garlic	0	0c	13b	33b	47c	60b
Tumeric	0	20b	27b	47b	60b	93[a]
Control	0	0c	0c	0c	0d	0c
LSD ($p<0.05$)	-	*	*	*	*	*

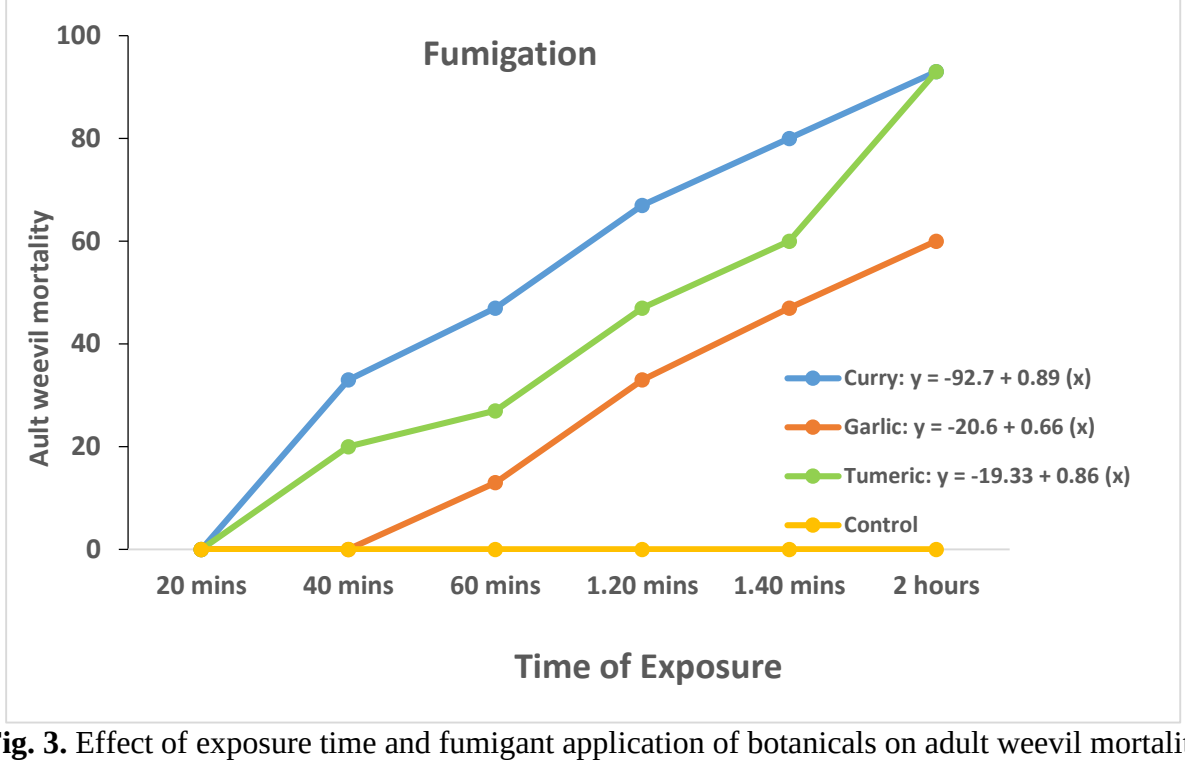

Fig. 3. Effect of exposure time and fumigant application of botanicals on adult weevil mortality

DISCUSSION

Chemical insecticides either used as dust or as fumigants have bad effects on consumers and the environment (Schwab *et al*., 1995), hence residual effects have caused health and ecological hazards, resistance, insect resurgence and deleterious impact on non-target organisms. However, insecticidal plant extracts have broad spectrum action and therefore better option for management of insect pests as these degrade rapidly and do not give the chance to gain genetic resistance in the insect pests (Azad *et al.*, 2013). According to Azeez, (2015) the mode of application of insecticide/botanical through either topical or fumigant action provided

effective control against kola weevil. The topical application brings quick contact of botanical oil with the body of weevils. Since there was direct contact of the essential oil with the body of the insects, due to thin spread of the essential oil on every part of treated adult weevils. The poison was able to penetrate permeable membranes of the test insects. However curry oil was more immediate in knock down effect than turmeric oil on the treated insects. This is in agreement with Barbara *et al*., (2010), who reported in a similar experiment that topical applications of *Hyptis suaveolens* and *H. spicigera* on insects showed that both essential oils had an effective insecticidal activity. The active ingredients of fatty vegetable and rhizomatous plant oils are their fatty acid constituents such as turmerones and arturmerone, oleic, linolenic and arachidonic acids (Don-Pedro, 1990; Tripathi *et al.*, 2002), which has been implicated due to high weevil mortality recorded by curry and turmeric oil. Thus, monoterpenoid contents have also been implicated to confer insecticidal property on some essential oils (Lale, 1987). However, curry oil and turmeric oil achieved adult weevil mortality of *S. zeamais* in 2 h. In a similar finding, Azeez, (2015) reported that ethanolic extracts (*Cymbopogon citratus* and *H. suaveolens*) caused 100% mortality of exposed weevil within 20 minutes whereas the aqueous extracts produced 60% mortality within 120 minutes. Asawalam *et al.,* (2008) reported that the curry oil was found to be moderately repellant to the maize weevil indicating the potential use of the plant in post-harvest control. Therefore, it is a major essential oil crop that possesses a range of biological activities such as insect repellant, nematicidal, anti-bacterial, antifungal and antioxidant activities (Simon *et al.*, 1990, Lee *et al.*, 2005). In similar findings, Kim *et al*. (2003) showed the potent insecticidal activity of extract from cinnamon (*Cinnamomum cassia*) bark and oil, horseradish (*Cocholeria aroracia*) oil, and mustard (*Brassica juncea*) oil against *C. chinensis*, within 1 day after treatment. Okonkwo and Okoye (1996) reported that essential oils of *Dennettia tripetela* and brown pepper (*Piper guineense*) achieved 100% mortality of adults of *C. maculatus* in 24 h. This is to buttress the potency and efficacy of essential oil from the same group of vegetable spices similar to this trial.

Superior performance of curry oil was recorded against the adult weevil from the onset of exposure having recorded higher adult weevil mortality through residual contact action in treated maize seeds. This is in agreement with the findings of Klimankova *et. al., (2008)* that 1, 8-cincole, methyl cinnamate, methyl chavicol and linalool are constituents responsible for the distinct aroma of basil plants. Though, garlic oil is equally effective against adult weevil mortality. The residual method of application was not as effective as topical and fumigant action because it took a long period of time before the effect of toxicity was established on the weevils. The residual mode of action of garlic oil was very slow and may be regarded as slow poison. Garlic is reportedly effective against wide range of insects at different stages in their life cycle (egg, larvae, and adult). However, the turmeric and garlic oil caused mortality of adult maize weevil as protectant compared to control. Insecticidal properties of turmeric have been well documented in the literatures as posited by the report of Ghawana *et al.,* (2011) that turmeric contains pungent, odoriferous oils and oleoresins and many kinds of biological activities. He also reported that powder and oil of turmeric tested significantly caused adult bruchid mortality and exerted toxic effect by disrupting normally respiration activity of the weevil thus results in asphyxiation and subsequent death (Klimankova *et. al., 2008*). Tripathi *et al.*, (2002) reported that turmerone and ar-turmerone are major constituents of *C. longa* rhizome oil and insecticidal constituents of

leaf and root include 82.9% and 16.3% monoterpenes respectively. Consequently, monoterpenes have been well documented to be active as fumigants, repellants or insecticides towards stored grain insects (Obeng-Ofori and Reichmuth, 1997).

From this study, the effectiveness of oil derived from turmeric, garlic and curry was put to test, curry and turmeric oil evoked significantly high weevil mortality in fumigant application, though was not as gradual as recorded in topical application. High fumigant action of the botanicals could be measured by the immediate weevil mortality because it releases fumes which caused knockdown effect. The bio-activity of the tested oil against *S. zeamais* was potent because of its fumes which killed the weevil recorded within a few minutes post treatment. Therefore, curry oil and turmeric oil achieved highest adult weevil mortality of *S. zeamais* in 2 h. In a similar trial, mortality of Kola nut weevils increased with high period of exposure to the botanicals under residual, topical and fumigant action in aqueous extracts. Ethanolic extracts however gave quick knock down effect and achieved high weevil mortality (Azeez, 2015). Curry and turmeric oil sustained the leading consistency in maize weevil control having recorded the significantly adult weevil mortality. This agrees with the findings of Klimankova *et. al., (2008)* that 1, 8-cincole, methyl cinnamate, methyl chavicol and linalool are constituents responsible for the distinct aroma of basil plants. This is also corroborated by Tripathi *et al.*, (2002) who reported that insect repellant components in turmeric are turmerones and arturmerone, which responsible for the toxic effect exhibited by the botanical oil. However, garlic oil is equally effective against the weevil. Garlic possess a lethal property similar and as efficiently as DDT and however effective against wide range of insects at different stages in their life cycle (eggs, larvae, adult) this includes ants, moths, beetles, termites, tick, etc. Paradoxically, it is not recommended for aphid control since it kills the natural enemies of aphids. Other researchers, Nukenine *et al.,* (2011) reported that neem based viz. Neem Azal and NSO (neem seed oil) had sufficient efficacy against *S. Zeamais* to be a component of an integrated management. Another neem product Azadirachtin causes 100% mortality to *Sitophilus oryzae* (Athanassiou *et al.,* 2005). Similarly, Ahmed *et al*. (1999) showed that after three days of insect introduction to beans, 100% of the *C. chinensis* adults were found dead on neem oil-treated beans.

There was intersection between the turmeric and curry oil in both topical and fumigant test action. Consequently, it was observed that the intersection could only take place between curry and turmeric oil at the onset of the exposure for the first 20 mins. The intersection could determine degree of compability between the botanicals and time taken for adult weevil mortality to be achieved. From foregoing, the use of right mode of application e.g. topical or fumigant would save time, prevent waste of pesticide and more economical. Therefore, it is pertinent to employ the best mode of application as a component of control or in development of an integrated pest management program (Ojo and Omoloye, 2016).

CONCLUSION

The most bioactive among the three tested oil was curry while turmeric powder was however promising, while the garlic oil ~~is~~ was equally effective. Mortality of maize weevils increased with high period of exposure to turmeric, curry and garlic powder and oil under the topical, residual contact and fumigant test action

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