Applied Tropical Agriculture
Volume 25, No.2, 10 - 19, 2020

EFFECT OF BINARY MIXTURES OF *Gongronema latifolium* BENTH AND DIATOMACEOUS EARTH AGAINST DAMAGE OF *Callosobruchus maculatus* (F.) ON STORED COWPEA SEED.

Azeez, O. M., Zakka, U. and Onwutuebe, G.

Department of Crop and Soil Science, Faculty of Agriculture, University of Port Harcourt, Choba, Port Harcourt, River State
Email of Corresponding Author: azeezowolabi@yahoo.com

Abstract
Appropriate combinations and varied dosage of Gongronema latifolium (GL) and Diatomaceous earth (DE) powder on cowpea seed damage by Callosobruchus maculatus in storage were assessed under Entomology laboratory condition. Twenty (20 g) Kidney cowpea seeds were treated with the powders at different dosage of 0.25 g, 0.50 g and 1.00 g and binary ratios; 1:1, 1:2, 1:3, 1:4, 5:1, 4:1, 3:1, 2:1, and infested with five pairs of newly emerged C. maculatus separately into netted lid containers and left for 14 days. Each treatment, including the control comprised four replicates in a Completely Randomized Design. Results of the study shows that GL and DE powdered evoked significant (P < 0.05) mortality (30.35%) and (25.41%) after 90 days post treatment. The result also revealed that DE was more effective in achieving mortality of adult bruchids at higher dosage. Diatomaceous earth and Gongronema latifolium treated seeds had 7.33% and 10.00% exit holes; 12.08% and 12.02% seed damage; 3.00% and 2.50% weight loss in all the treatments compared with control (32.30%; 77.50%; 22.50% and 37.00%). The combination GL:DE (1:2) produced most desirable results causing lower exit hole (0.33) and seed damage (3.33%), and least seed weight loss (0.03%). Therefore, effectiveness of GL:DE (2:1), (1:3) combinations may be attributed to synergetic effects exerted on C. maculatus.

Keywords: *Diatomaceous earth, Gongronema latifolium, powders, mortality, combination, synergetism*

Introduction

Cowpea is a grown tropical crop that can endure drought and warm weather under tropical and sub-tropical regions (Tiroesele *et al.,*2014; Mazarin *et al.,*2016). According to Singh *et al*.,(2002) over 5.4 million tons of dried cowpea was produced worldwide with Africa producing 5.2 million. Nigeria is the world's largest producer and consumer of cowpea, which accounts for 61 % production in Africa and 58 % in other part of the world respectively (IITA, 2012).

The legume is consumed as a high-quality plant protein and carbohydrate contents with a relatively low fat and complementary amino acid pattern compared to cereal grains (Jayathilake *et. al*., 2018). It is a major legume and a valuable source of calcium, iron, thiamine and riboflavin (Nielsen *et al*., 1997; Rangel *et al*., 2003). Cowpea is an essential source of proteins, calories, dietary fiber, minerals and vitamins for majority of people, who cannot afford other sources of proteins especially in Africa and other developing countries of the world (Akpapunam and Sefa-Dedeh, 1997; *Gonçalves et al*., 2016). Cowpea has beneficially been used for its anti-cancer, anti-diabetic, anti-hyperlipidemic, anti-hypertensive and anti-inflammatory properties due to the presence of valuable compounds such as soluble and insoluble dietary fibers, polyphenols, proteins and peptides, tannins, flavonoids, anthocyanins and other diverse groups of phytochemicals in the seeds (Mudryj *et al*., 2012; Liyanage *et al*., 2014). Cowpea can be boiled and consumed directly, made into flour, beans puddings or weaning foods for young children and thus ameliorate malnourishment and wastage (Phillips and Dedeh, 2003). The Yoruba in Southwest, Nigeria use cowpea flour to prepare fried cakes (akara) and moin-moin, and a local soup called *gbegiri* (Madodé *et al.* 2011), the bean puddings are often referred to as poor man's meat (Phillips *et al*., 2003; Hamid *et al*., 2016; Carvalho *et al*., 2017). The quality of grains and seeds during storage depends on various factors such as crop or variety, initial seed quality, storage conditions, seed moisture content, insect pests, bacteria and fungi (Amruta *et al.,* 2015).

Callosobruchus maculatus is a major storage insect pest of cowpea seeds (NRI, 1996; Maina *et al*., 2006). It is a field - to - store pest of cowpea in Africa and Asia (Beck and Blumer, 2014) and most destructive because of their comparatively shorter life cycle being a multivoltine insect (Maina and Lale, 2004). In susceptible grain legumes, an initial infestation of 10% could lead to 100% grain loss in storage within 3 to 5 months under ordinary storage conditions (Chhabra *et al*. 1993; Srinivasan, 2014). The insect pests did not only damage the grain but also depreciate the weight and quality of stored grains (Rayhan, 2014). Insect damages include direct consumption of kernels, detritus of exuviate, webbing, and cadavers thereby makes the grain unfit for human consumption and also reduce quality and quantity. Insect infestation manipulates the storage environment which usually resulted in the development of hotspots which are congenial place for the proliferation of storage fungi and other harmful micro flora (Rajashekar *et al.,* 2012). Seed damage results in qualitative and quantitative losses, *consequently* quantitative damage leads to reduction in seed weight as a result of larvae that burrowed while feeding. However, qualitative losses are primarily due to chemical changes in the grains; presence of pathogenic and toxicogenic microorganisms that reduced nutrient value and caused unwanted changes to taste, color, texture, or cosmetic features of food (Buzby and Hyman, 2012). Therefore these activities led to contamination with frass, insect body parts and or reduction in seed viability (Umeozor, 2005; Maina *et al*., 2006). Dead insect pupae, larval cocoons and their integument may contain various carcinogenic compounds such as ethyl, methyl and methoxy quinines which cannot be denatured by baking or boiling (Ileke and Olotuah, 2012). Thus, the damage caused during storage, shipping and transportation, is a very serious problem all over the globe (Upadhyay and Ahmad, 2011).

Conventional insecticides such as pirimiphos methyl, permethrin and Aluminun phosphide have been reported to be effective against *C. maculatus* in the storage. The pest control technology is mostly dependent on synthetic insecticides (Azad *et al*., 2013), having a knockdown effect on targets, and more often the only solution to sudden outbreak of pests. Raupp *et al*., (2014) reported the residual effect of insecticides on insect pests and natural enemies, while inherent high mammalian toxicity and ecological safety are of great concern to both environmentalists and researchers worldwide (Zacharia, 2011). The different problems regarding residual effects, pest resurgence, prevalent environmental and ecological hazards, insect pest resistance and economy of farmers associated with currently used synthetic pesticides have raised recent concerns towards botanicals (Zettler and Cuperus 1990; Elhag, 2000) which are environmental friendly, biodegradable, economic and are equally effective. Plant products, such as aqueous or organic solvent extracts are being used in many countries as protectants of stored products (Fernando and Karunaratne, 2012).

There is need to examine and determine the combinations of two botanicals in different mixing ratio for farmer's use. The objectives of the study were to determine the interaction and synergism effects of suitable binary ratio including its most effective dosage in the management of *C. maculatus* on treated cowpea seed. The combinations of powdered *G. latifolium* and Diatomaceous earth could sustain optimal agricultural production through insect pest management of farm and stored products.

Materials and Methods

Experimental Site - 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 of 27 ± 3°C and relative humidity 65 ± 5 %.

Collection and Preparation of Materials - The leaves of *Gongronema latifolium* were collected from Faculty of Agriculture Experimental farm and identified in the Department of Forestry and Wildlife, Faculty of Agriculture, University of Port Harcourt. The plant leaves were washed in clean water and were later air-dried in room temperature (25°C) and ground into fine powder using Thomas Wiley Machine. The powder was further sieved in 100 µm aperture sieve. *Diatomaceous* earth was obtained from Nigerian Stored Products Research Institute, Port Harcourt. Each plant material was contained in a plastic container and labeled properly for use. Kidney cowpea variety use for the experiment was obtained from Institute of Agricultural Research, Zaria. The cowpea seeds were disinfested using cold storage treatment at 0 – 4°C for seven days. Other materials used were plastic containers, muslin cloth, masking tape and marker pen etc.

Insect Culture - The initial 200 unsexed adult *C. maculatus* were obtained from the culture maintained on Ife Brown cowpea variety in the Entomology laboratory, Department of Crop and Soil Science, University of Port Harcourt, Port Harcourt, Nigeria. Fifty adults were introduced into a 500-ml Kilner jar containing 200 g of clean disinfested Ife Brown cowpea seeds, and 4 jars were prepared in this manner. The Kilner jars were covered with muslin cloth held in place by a screw cap in order to allow for aeration and to prevent the insects from escaping. The set-up was kept under ambient temperature (27±3°C) and relative humidity (65 - 70%). The insects were allowed to mate for seven days and lay eggs in each of the jars after which they were removed to avoid multiple oviposition. The devoured seeds were replaced continuously with the same quantity of freshly disinfested seeds. The newly emerging adult bruchids from the culture were used for the experiment.

Weighing of Cowpea – Twenty (20g) of cowpea was weighed using Mettler weighing balance (Tonedo 223) and introduced into a small plastic container (9 cm x 12 cm) with tightly fitted lids and kept on the work bench in the Entomology laboratory.

Identification of Insect -Examination of bruchids was done with a light microscope of high resolution to correctly identify adult *C. maculatus* that was used for the experiments. Thus, the teneral adult females were easily recognized by their strong markings on the elytra the grain but also depreciate the weight and quality of stored grains (Rayhan, 2014). Insect damages include direct consumption of kernels, detritus of exuviate, webbing, and cadavers thereby makes the grain unfit for human consumption and also reduce quality and quantity. Insect infestation manipulates the storage environment which usually resulted in the development of hotspots which are congenial place for the proliferation of storage fungi and other harmful micro flora (Rajashekar *et al.,* 2012). Seed damage results in qualitative and quantitative losses, *consequently* quantitative damage leads to reduction in seed weight as a result of larvae that burrowed while feeding. However, qualitative losses are primarily due to chemical changes in the grains; presence of pathogenic and toxicogenic microorganisms that reduced nutrient value and caused unwanted changes to taste, color, texture, or cosmetic features of food (Buzby and Hyman, 2012). Therefore these activities led to contamination with frass, insect body parts and or reduction in seed viability (Umeozor, 2005; Maina *et al*., 2006). Dead insect pupae, larval cocoons and their integument may contain various carcinogenic compounds such as ethyl, methyl and methoxy quinines which cannot be denatured by baking or boiling (Ileke and Olotuah, 2012). Thus, the damage caused during storage, shipping and transportation, is a very serious problem all over the globe (Upadhyay and Ahmad, 2011).

Conventional insecticides such as pirimiphos methyl, permethrin and Aluminun phosphide have been reported to be effective against *C. maculatus* in the storage. The pest control technology is mostly dependent on synthetic insecticides (Azad *et al*., 2013), having a knockdown effect on targets, and more often the only solution to sudden outbreak of pests. Raupp *et al*., (2014) reported the residual effect of insecticides on insect pests and natural enemies, while inherent high mammalian toxicity and ecological safety are of great concern to both environmentalists and researchers worldwide (Zacharia, 2011). The different problems regardi $\text{PT} = \frac{P_o - PC}{100-PC} x100$ st resurgence, prevalent environ hazards, insect pest resistance and economy of farmers associated with currently used synthetic pesticides have raised recent concerns towards botanicals (Zettler and Cuperus 1990; Elhag, 2000) which are environmental friendly, biodegradable, economic and are equally effective. Plant products, such as aqueous or organic solvent extracts are being used in many countries as protectants of s (Fernando and Karunaratne, 201 $= \frac{X-Y}{X} \ x\ 100$

There is need to examine and determine the combinations of two botanicals in different mixing ratio for farmer's use. The objectives of the study were to determine the interaction and synergism effects of suitable binary ratio including its most effective dosage in the managem $\frac{B-D}{B} \ x\ 100$ atus on treated cowpea seed. The comb vdered *G. latifolium* and Diatomaceous earth could sustain optimal agricultural production through insect pest management of farm and stored products.

Materials and Methods

Experimental Site - The research was conducted at the C $\text{undamaged seed} = \frac{B-U}{B} \ x\ 100$ nent of Crop and S , University of Port Harcourt. The experiment was carried out under ambient temperature of 27 ± 3°C and relative humidity 65 ± 5 %.

Collection and Preparation of Materials - The leaves of *Gongronema latifolium* were collected from Faculty of Agriculture Experimental farm and identified in the Department of Forestry and Wildlife, Faculty of Agriculture, University of Port Harcourt. The plant leaves were washed in clean water and were later air-dried in room temperature (25°C) and ground into fine powder using Thomas Wiley Machine. The powder was further sieved in 100 µm aperture sieve. *Diatomaceous* earth was obtained from Nigerian Stored Products Research Institute, Port Harcourt. Each plant material was contained in a plastic container and labeled properly for use. Kidney cowpea variety use for the experiment was obtained from Institute of Agricultural Research, Zaria. The cowpea seeds were disinfested using cold storage treatment at 0 – 4°C for seven days. Other materials used were plastic containers, muslin cloth, masking tape and marker pen etc.

Insect Culture - The initial 200 unsexed adult *C. maculatus* were obtained from the culture maintained on Ife Brown cowpea variety in the Entomology laboratory, Department of Crop and Soil Science, University of Port Harcourt, Port Harcourt, Nigeria. Fifty adults were introduced into a 500-ml Kilner jar containing 200 g of clean disinfested Ife Brown cowpea seeds, and 4 jars were prepared in this manner. The Kilner jars were covered with muslin cloth held in place by a screw cap in order to allow for aeration and to prevent the insects from escaping. The set-up was kept under ambient temperature (27±3°C) and relative humidity (65 - 70%). The insects were allowed to mate for seven days and lay eggs in each of the jars after which they were removed to avoid multiple oviposition. The devoured seeds were replaced continuously with the same quantity of freshly disinfested seeds. The newly emerging adult bruchids from the culture were used for the experiment.

Weighing of Cowpea – Twenty (20g) of cowpea was weighed using Mettler weighing balance (Tonedo 223) and introduced into a small plastic container (9 cm x 12 cm) with tightly fitted lids and kept on the work bench in the Entomology laboratory.

Identification of Insect -Examination of bruchids was done with a light microscope of high resolution to correctly identify adult *C. maculatus* that was used for the experiments. Thus, the teneral adult females were easily recognized by their strong markings on the elytra consisting of two large marginal dark patches mid-way the grain but also depreciate the weight and quality of stored grains (Rayhan, 2014). Insect damages include direct consumption of kernels, detritus of exuviate, webbing, and cadavers thereby makes the grain unfit for human consumption and also reduce quality and quantity. Insect infestation manipulates the storage environment which usually resulted in the development of hotspots which are congenial place for the proliferation of storage fungi and other harmful micro flora (Rajashekar *et al.,* 2012). Seed damage results in qualitative and quantitative losses*, consequently* quantitative damage leads to reduction in seed weight as a result of larvae that burrowed while feeding. However, qualitative losses are primarily due to chemical changes in the grains; presence of pathogenic and toxicogenic microorganisms that reduced nutrient value and caused unwanted changes to taste, color, texture, or cosmetic features of food (Buzby and Hyman, 2012). Therefore these activities led to contamination with frass, insect body parts and or reduction in seed viability (Umeozor, 2005; Maina *et al*., 2006). Dead insect pupae, larval cocoons and their integument may contain various carcinogenic compounds such as ethyl, methyl and methoxy quinines which cannot be denatured by baking or boiling (Ileke and Olotuah, 2012). Thus, the damage caused during storage, shipping and transportation, is a very serious problem all over the globe (Upadhyay and Ahmad, 2011).

Conventional insecticides such as pirimiphos methyl, permethrin and Aluminun phosphide have been reported to be effective against *C. maculatus* in the storage. The pest control technology is mostly dependent on synthetic insecticides (Azad *et al*., 2013), having a knockdown effect on targets, and more often the only solution to sudden outbreak of pests. Raupp *et al*., (2014) reported the residual effect of insecticides on insect pests and natural enemies, while inherent high mammalian toxicity and ecological safety are of great concern to both environmentalists and researchers worldwide (Zacharia, 2011). The different problems regarding residual effects, pest resurgence, prevalent environmental and ecological hazards, insect pest resistance and economy of farmers associated with currently used synthetic pesticides have raised recent concerns towards botanicals (Zettler and Cuperus 1990; Elhag, 2000) which are environmental friendly, biodegradable, economic and are equally effective. Plant products, such as aqueous or organic solvent extracts are being used in many countries as protectants of stored products (Fernando and Karunaratne, 2012).

There is need to examine and determine the combinations of two botanicals in different mixing ratio for farmer's use. The objectives of the study were to determine the interaction and synergism effects of suitable binary ratio including its most effective dosage in the management of *C. maculatus* on treated cowpea seed. The combinations of powdered *G. latifolium* and Diatomaceous earth could sustain optimal agricultural production through insect pest management of farm and stored products.

Materials and Methods

Experimental Site - 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 of 27 $\pm$ 3^{0}C and relative humidity 65 $\pm$ 5 %.

Collection and Preparation of Materials - The leaves of *Gongronema latifolium* were collected from Faculty of Agriculture Experimental farm and identified in the Department of Forestry and Wildlife, Faculty of Agriculture, University of Port Harcourt. The plant leaves were washed in clean water and were later air-dried in room temperature (25^{o}C) and ground into fine powder using Thomas Wiley Machine. The powder was further sieved in 100 µm aperture sieve. *Diatomaceous*

Table 1 : Effect of *Gongronema latifolium* and Diatomaceous earth and varied dosage on damage index of *C. maculatus* on stored cowpea seeds

Botanicals	dosage	Adult Mortality%	Exit Hole%	Seed Damage%	Undamaged Seed%	Weight Loss%
Diatomaceous earth (DE)	0.00	0.00^{a}	32.30^{a}	77.50^{a}	22.50^{a}	37.00^{a}
	0.25	36.05^{b}	7.67^{b}	45.05^{b}	54.95^{ab}	12.90^{b}
	0.50	37.48^{b}	9.67^{b}	13.13^{c}	86.87^{c}	3.60^{c}
	1.00	30.83^{c}	7.33^{b}	12.08^{c}	87.92^{c}	3.00^{c}
Gongronema latifolium (GL)	0.00	0.00^{a}	31.05^{a}	87.75^{a}	12.25^{a}	41.67^{a}
	0.25	35.50^{b}	10.30^{bc}	55.58^{b}	44.42^{ab}	8.73^{b}
	0.50	30.35^{c}	11.00^{bc}	16.67^{c}	73.33^{c}	3.85^{c}
	1.00	25.41^{c}	10.00^{bc}	12.02^{c}	87.98^{c}	2.50^{c}

Means are separated by Tukey's Honest Significant Difference Test at 5%. Means followed by the same letter in the same column are not significantly different from one another

Table 2: The Effect of Binary ratio o*f Gongronema latifolium* and Diatomaceous Earth on the Percent Age Adult Mortality and Seed Damage Caused by *Callosobruchus maculatus*

Botanical	Binary Ratio	Percentage Adult Mortality	Percentage Seed Damage
Gongronema latifolium (GL) and Diatomaceous earth (DE)	0:0	0.00^{a}	42.00^{a}
	1:1	26.67^{bc}	11.33^{cd}
	1:2	8.67^{d}	3.33^{d}
	1:3	12.33^{cd}	18.67^{c}
	1:4	17.67^{c}	13.33^{cd}
	1:5	20.33^{bc}	18.67^{c}
	5:1	23.33^{bc}	20.67^{b}
	4:1	36.33^{b}	23.33^{b}
	3:1	30.00^{b}	20.67^{b}
	2:1	18.00^{c}	9.67^{cd}

Means are separated by Tukey's Honest Significant Difference Test at 5%. Means followed by the same letter in the same column are not significantly different from one another

Table 3: The Effect of Binary Ratio of *Gongronema latifolium* and Diatomaceous Earth and Varied Dosage Levels on the Number of Exit Hole and Percent Weight Loss

Botanical	Binary Ratio	Mean of No of Exit Hole	Percentage Weight Loss
Gongronema latifolium (GL) and Diatomaceous earth (DE)	0:0	30.67^{d}	45.67^{d}
	1:1	5.67^{b}	0.10^{b}
	1:2	0.33^{a}	0.03^{a}
	1:3	3.67^{b}	0.07^{a}
	1:4	3.00^{b}	0.07^{a}
	1:5	6.67^{b}	0.13^{b}
	5:1	6.67^{b}	0.20^{ab}
	4:1	23.33^{c}	0.67^{c}
	3:1	14.67^{c}	0.37^{c}
	2:1	5.67^{b}	0.13^{b}

Means are separated by Tukey's honest significant difference test at 5%. Means followed by the same letter in the same column are not significantly different from one another

Table 4 : Binary mixture of *Gongronema latifolium* and Diatomaceous earth on damage index of *Callosobruchus maculatu*s on stored cowpea seeds

Binary ratio	Percentage adult mortality	Percentage seed damage	Mean no of exit hole	Percentage weight loss
0.0	0.00^{c}	61.00^{d}	45.00^{d}	15.00^{d}
1:1	32.00^{b}	30.67^{a}	12.11^{b}	0.29^{b}
1:2	26.89^{b}	5.89^{c}	1.67^{c}	0.04^{a}
1:3	35.67^{b}	12.89^{b}	4.44^{bc}	0.16^{ab}
1:4	33.00^{b}	20.89^{b}	10.67^{b}	0.23^{b}
1:5	46.78^{a}	31.78^{a}	11.00^{b}	0.17^{ab}
5:1	48.67^{a}	27.00^{a}	12.56^{b}	0.24^{b}
4:1	44.33^{a}	36.89^{a}	18.56^{a}	0.39^{c}
3:1	47.44^{a}	34.89^{a}	13.67^{b}	0.41^{c}
2:1	31.44^{b}	22.11^{b}	10.11^{b}	0.31^{c}

Means are separated by Tukey's Honest Significant Difference Test at 5%. Means followed by the same letter in the same column are not significantly different from one another

Discussion

Insect pests did not only damage grains but also depreciate the weight and quality of stored grains (Rayhan, 2014). Consequently, farmers resort to botanicals that have the phyto-toxic/pesticidal effect that could increase seed quality parameters because of problems regarding residual effects, pest resurgence, prevalent environmental and ecological hazards, insect pest resistance and economy of farmers associated with currently used synthetic pesticides (Zettler and Cuperus

1990; Elhag, 2000). Significantly higher bruchid mortality was recorded on cowpea seeds treated with plant powder at higher concentrations while lower percentage adult bruchid mortality was recorded at lowest concentration. Therefore, tested plant powders have shown to have insecticidal properties against *Callosobruchus maculatus*. The mechanism of action of these plant powders includes toxicity, repellency and deterrence effects against storage insect pest (Lale, 1994). The results obtained from this trial showed that *Gongronema latifolium* (GL) and Diatomaceous earth (DE) caused bruchid mortality at different dosage rates. Lale (2001) and Ofuya (2003) have opined that a few plants in the Nigerian flora with confirmed biological efficacies against stored products insects were sufficiently insecticidal to merit scientific formulation. Generally, all the botanicals tested caused significantly higher bruchid mortality compared with the untreated (control). The result from this study has shown that powders made from *Gongronema latifolium* are toxic to obnoxious *C. maculatus*. The lethal effect of *Gongronema latifolium* powder on the bruchid could be as a result of contact toxicity. Diatomaceous earth powder equally caused adult bruchid mortality, reduced progeny emergence, seed damage and weight loss at 0.50 g and 1.00 g dosage level. This is however implicated by Adedire *et al*., (2011) who reported that insects breathe by means of trachea that usually open at the surface of the body through spiracles. The spiracle might have been blocked by the plant powders thereby leading to suffocation of adult bruchids. Diatomite is of value as an insecticide because of its abrasive and physico-absorptive properties, the fine powder adsorbs lipids from the waxy outer layer of the exoskeletons of many species of insects, this layer acts as a barrier that resists the loss of water vapour from the insect's body. Paul *et al*.(2002) reported the damaging effect on the insect outside layer increases the evaporation of water from their bodies so that they dehydrate often fatally. D.E is widely applied for insect control in grain storage as an anti-caking agent as well as an insecticide in order to be effective thus having a mean particle size below about 12µm (Capinera, 2008).

The plant powders also caused reduction in exit holes, weight loss and seeds damage by *C. maculatus* on treated cowpea seeds which led to corresponding high percent undamaged seeds. Cowpea seeds treated with high concentration of *Gongronema latifolium* (GL) and Diatomaceous earth (DE) powder gave significantly lower seed weight loss compared to lower concentrations. Several workers, Okosun and Adedire, (2010); Adedire *et al*., (2011); lleke and Oni, (2011) reported that these powders inhibited locomotion which might have affected mating activities and sexual communication, consequently caused females oviposition deterrence and complete suppression of developmental stages of insects. In a similar finding, these toxic effects have been attributed by various authors to the presence of some chemical compounds of triterpenoids and indole alkaloid group such as alstonine, astonidine and porphine that have been identified from the stem bark of *A. boonei* (Philipson *et al*.,1987;Anonymous,1992;2001).

However, (Azeez & Pitan, 2014; Manohar *et al*., 2017) reported that botanicals proven to be a better option in the control of field and storage pests; thus neither affect the quality of grains or seeds nor destroy the ecosystem or environment. This is also similar to the findings of Shazia *et al*., (2006) who reported that black pepper powder gave significantly better results than the control in suppressing bruchid survival, higher numbers of undamaged seeds and fewer holes per cowpea seed. Previous works have demonstrated the potency of some botanicals to preserve seed quality (Khatum *et al*., 2011; Rana *et al*., 2014); reduced seed damage (Rana *et al*., 2014) and weight loss (Rayhan *et al*., 2014). There was little or no seed damage and percent weight loss in seeds treated with *Gongronema latifolium* and Diatomaceous earth. Botanicals are determined only to target pests, are effective in very small quantities, degrade rapidly and provide pesticide free food and a safe environment for living beings (Joseph *et al*., 2012). Boeke *et al*., (2004) reported that the adult beetles died soon after they came into contact with the powder of *Tephrosia vogelli* and lay few eggs, only very few developed into adults. Musa *et al*., (2009) reported that seed- extract of *H. suaveolens* was significantly more effective in enhancing adult mortality, reducing egg laid and suppressing larval and adult emergence. The different combinations of the botanicals gave significantly reduced number of exit holes compared to the control. The *Gongronema latifolium* (GL) and Diatomaceous earth (DE) powder tested, significantly ($P<0.05$) recorded low seed damage in treated cowpea seeds compared with control. Application of the *Gongronema latifolium* (GL) and Diatomaceous earth (DE) powder on cowpea seeds showing lower number exit holes by *C. maculatus* agrees with Ogunwolu and Idowu (1994) who studies with *Zanthoxylum zanthoxyloides* root bark powder and *Pipper guineense* powder. Also, Dawodu and Ofuya (2000) observed that mixture of the fruit powders of *P. guineense* and *Denettia tripetala* in equal proportions significantly reduced oviposition and adult emergence of *C. maculatus*. The result got from this trial showed that the plant parts applied to cowpea seeds significantly achieved adult bruchid mortality. Combinations of the botanicals at different ratios gave significantly higher adult mortality compared to the control. Emeasor *et al*., (2007) reported in another study that the mixture of *P. guineense* and *Thevetia peruviana* at different percentages caused the highest mortality, least egg counts and significantly suppressed adult emergence. This observation is sustainable because more complex preparations such as combination of substances present in insecticide are likely to become effective to overcome development of resistance by insect pests (Regnault-Roger and Hamraini, 1993). Amruta *et al*., (2015) recorded effective storage insect control and higher seed quality when treated with botanicals and emamectin benzoate. The percentage mortality recorded at combination GL: DE (2:1) was significantly different from other combinations. Combination GL: DE (2:1) and (1:1) recorded significantly lower number of exit hole relative to other combinations. Combinations GL: DE (1:3), (1:1) and (1:4) reduced exit hole when compared with the control. Also, Rayhan *et al*., (2014) reported that the bio-efficacy of neem, mahogani and their mixture were able to prevent seed damage and seed weight loss by rice weevil in storage. Although there may not be differences in the bruchid mortality recorded in the combination compared with single application, the combination is desirable due to reduction in chances of resistance development. Hence, the mixtures of insecticides could also be used because of cost efficiency (AII *et al*., 1977). Different combinations tested had an appreciable reduction in percentage weight loss. The tested plant leaves significantly reduced the development of *C. maculatus* that could lead to the damage of cowpea seed. This was achieved against the developing larva that usually feeds entirely with a single seed, excavating a chamber within the cotyledons as it grew. The varied combinations of *Gongronema latifolium* (GL) and Diatomaceous earth (DE) powder caused the adult bruchid mortality and weight loss of the treated cowpea seeds after 3 months storage. Little or no seed damage and weight loss was recorded with combination GL: DE (1:2). This confirmed similar findings by Azeez and Pitan (2018) who reported that combination of *Hyptis suaveolens*, *Cymbopogon citratus* and *Alstonia boonei* (H:C:A2) produce most desirable results causing higher adult mortality, low offspring emergence, lower seed damage, higher seed viability, and least seed weight loss and therefore the most bio-active mixing ratio against *C. maculatus*. With combination GL: DE (2:1) and (1:1) there was significant reduction in seed damage and weight loss compared to other combinations. This implicated synergistic effect between the two botanicals in this ratio. Therefore, these findings would be readily accepted by the local farmers because peasant farmers in sub-saharan Africa use indigenous plants either singly or in mixtures to protect cowpeas against pest damage during storage (Issa *et al*., 2011; Khatum *et al*., 2011; Ibrahim, 2012; Ignatowicz and Wesolowska, 2015). Therefore, the mixture may give best control of a complex of pests with varying levels of susceptibility to the different components of the mixtures. In the same vein, Azeez and Pitan (2018) observed interaction and synergism effect among the appropriate mixing ratio of *Hyptis suaveolens*, *Cymbopogon citratus* and *Alstonia boonei* against damage of *Callosobruchus maculatus.* Therefore, insects that are resistant to one or more insecticides may be susceptible to a combination of toxicants or synergism may be exhibited by the components (Wolfenbarger and Cantu, 1975).

Conclusion

Higher dosages of *Gongronema latifolium* (GL) and Diatomaceous earth (DE) powders have shown to reduce activity of *C. maculatus* that would have caused high number of exit holes, percentage seed damage, mortality of *C. maculatus* and also reduced weight loss of stored cowpea seeds. However, gave corresponding increased percentage undamaged seeds. Also, the binary ratio of *Gongronema latifolium* (GL) and Diatomaceous earth (DE) powders are equally effective as protectant against infestation of *C. maculatus*. Thus, higher dosages of the insecticidal plant powders have toxic effect on *C. maculatus* that caused infestation to stored cowpea seeds. Consequently, this shows that plant materials tested have the potential of being used as bio-pesticide and could be used as an alternative to conventional synthetic insecticidal for the protection of stored product. Higher dosage of the powder could be more effective since it is expected to contain high active components. However, the adverse effect of chemical fumigants used for stored products protection, in respect of ozone depletion, high mammalian toxicity, insect resistance and health hazard caused a significant contribution to the total loss.

References

Abbott, W., (1925). A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18, 265–267.doi:10.1093/jee/18.2. 265a

Adedire, C. O., Akinkurolere, R. O. and Ajayi, O.O. (2011). Susceptibility of some maize cultivars in

Nigeria to infestation and damage by maize weevil, Sitophilus zeamais (Mostch.) (Coleoptera: Curculionidae). Nigerian Journal of Entomology 28: 55-63.

All JN, Ali M, Hornyak EP and Weaver JB. (1977). Joint action of two pyrethroids with methylparathion, Methomyl and chlorpyrifos on *Heliothis zea* and *Heliothis virescens* in the laboratory and in Cotton and sweet corn. J Econ Entomol. 70 (6): 813-817.

Akpapunam, M. A. and Sefa-Dedeh, S. (1997). Jack bean (*Canavalia ensiformis*): Nutrition related aspects and needed nutrition research. Plant Foods for Human Nutrition, 50(2): 93–99

Amruta, N., Sarika, G., Umesha, Maruthi, J.B. and Basavaraju, G.V. (2015). Effect of botanicals and insecticides seed treatment and containers on seed longevity of black gram under natural ageing conditions. *J. App. Nat. Sci.,* 7 (1): 328 – 334.

Azad, A.K., Sardar, A., Yesmin, N., Rahman, M. and Islam, S. (2013). Eco-friendly pest control in cucumber (*Cucumis sativa* L.) field with botanical pesticides. *Nat. Reso.,* 4(5): 6p.

Azeez, O.M. and Pitan, O.O.R. (2014). Influence of cowpea variety on the potency and deterrent indices of six plant powders against the seed bruchid, *Callosobruchus maculatus* (Fabricius) (Coleoptera: Bruchidae), Archives of Phytopathology and Plant Protection, DOI: 10.1080/032354 08.2014.893637.

production, utilization and nutrient composition. In: Advances in Cowpea Research, B.B. Singh, D.R. Mohan Raj, K.E. Dashiell, L.E.N. Jackai, Editors. I.I.T.A., Ibadan, Nigeria.Sayce Publishing, Devon, UK. 372 pp

Ofuya, T. I. (2003). Beans, insect and man. Inaugural lecture series 35, The Federal University of Technology, Akure, Nigeria. Rajashekar, Y., Bakthavatsalam, N. and Shivanandappa, T. (2012). Botanicals as grain protectants. *Psyche: A J. Ento,* 13 Pp.

Rana, K., Sharma, K.C. and Kanwar, H.S. (2014). Efficacy of aqueous plant extracts on the seed quality of pea (*Pisum sativum* L.) during storage. *Am. Int. J. Res. For., App. Nat. Sci.,* 6(1): 7-11.

Rangel, A.; Domont, G. B.; Pedrosa, C.; Ferreira, S. T. (2003). "Functional properties of purified vicilins from cowpea (Vigna unguiculata) and pea (Pisum sativum) and cowpea protein isolate". *Journal of Agricultural and Food Chemistry.* ***51*** *(19):5792–5797 doi:10.101021/jf0340052.PMID 12952435*

Raupp, M.J., Holmes, J.J., Clifford, S.P., Shrewsbury, P. and Davidson, J.A. (2014). Effects of cover `sprays and residual pesticides on scale insects and natural enemies in urban forests. *J. Arbori.,*27(4):213-214.

Rayhan, M.Z., Das, S., Sarka,R. R., Adhikary, S.K., Tania, S.N., Islam, M.M. and Rabbani, M.G. (2014). Bioefficacy of neem, mahogoni and their mixture to protect seed damage and seed weight loss by rice weevil in storage. *J. Biod. Env. Sci.,* 5(1): 582-589.

Ibrahim, M.Y. (2012). Efficacy of some plant oils against stored-product pest cowpea weevil, *Callosobruchus maculatus* (Coleoptera: Bruchidae) on chickpea seeds. *Per. Gu. Crop Prot.,* 1(1): 4-11. IITA, (2012). Cowpea (*Vigna unguiculata*). http://www.iita.org/ cowpea (accessed 16.08.19

Ileke, K. D. and Olotuah, O. F. (2012). Bioactivity of *Anacardium occidentale* (L) and *Allium sativum* (L) powders and oils extracts against cowpea bruchid, *Callosobruchus maculatus* (Fab.) [Coleoptera: Chrysomelidae]. International Journal of Biology 4(1): 96-103

Ileke, K.D. and Oni, M.O. (2011). Toxicity of some plant powders to maize weevil, *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae) on stored wheat grain (*Triticum aestivum*). African Journal of Agricultural Research, 6(13): 3043 -3048).

Jayathilake, C., Visvanathan, R., Deen, A., Bangamuwage, R., Jayawardana, B. C. Nammic, S. and Liyanagea, R. (2018). Cowpea: An overview on its nutritional facts and health benefits: Nutritional and health properties of cowpea. Journal of the Science of Food and Agriculture.www.ileyonline library.com DOI 10.1002/jsfa.9074

Liyanage, R., Perera, O. S., Wethasinghe, P., Jayawardana, B. C., Vidanaarachchi, J. K. and Sivaganesan, R. (2014). Nutritional properties and antioxidant content of commonly consumed cowpea cultivars in Sri Lanka. Journal of Food Legumes: Indian Journal of Pulses Research, 27: 215–217

Madodé, Y. E., Houssou, P. A. Linnemann, A. R. Hounhouigan, D. J. Nout, M. J. R. and Van Boekel, M. A. J. S. (2011). Preparation, consumption, and nutritional composition of West African cowpea dishes. Ecology of Food and Nutrition, 50(2): 115-136.

Maina, Y.T. and Lale N.E.S. (2004). Efficacy of integrating varietal resistance and neem *Azadirachta indica* A. Juss. seed oil for the management of *Callosobruchus maculatus* infesting bambara groundnut in storage. Nigerian Journal of Entomology, (2004).21:94-103.

Maina, Y.T., Sastawa, B.M and Bidliya, B.S. (2006). Susceptibility of local cowpea (*Vigna unguiculata* L. Walpers) Cultivars to *Callosobruchus maculatus* infestation in storage. *UNISWA Research* Journal of Agriculture, Science and Technology, 9(2): 159-163.

Mazarin A, Nukenine EN, NIUC, and Vincent F.V., (2016). Synergistic effects of wood as and essential oil on fecundity, pupal eclosion and adult mortality of *callosobruches maculatus (*coleoptra: Bruchidae*)* cowpea seed weevil, Am-J. Exp. Agric. 11, 1-12 (doi:10.9734/A-JEA 12016/25306).

Mudryj, A. N., Yu, N., Hartman, T. J., Mitchell, D. C., Lawrence, F. R. and Aukema, H. M. (2012). Pulse consumption in Canadian adults influences nutrient intakes. British Journal of Nutrition,108:27–36

NRI (Natural Resource Institute). (1996). Insect pests of Nigerian crops: Identification, biology, and control. Chantham U.K. NRI.

Ogunwolu, E.O. and Idowu, O. T. (1994). Potential of powdered *Zanthoxylum zanthoxyloides* (Rutaceae) root bark and *Azadirachta indica* (Meliaceae) seed for the control of cowpea seed bruchid, *Callosobruchus maculatus* (F.). Niger. J Afric. Zoo. 108: 521-528.

Okosun, O.O and Adedire, C.O. (2010). Potency to cowpea seed bruchid, Callosobruchus maculatus of African nutmeg seed, Monodora myristica extracted with different solvents. Nigeria J. Entomol., 27: 89- 95

Paul, Allen, Korunic, Mllaughin, Alan Stathers, Tanya July (2002) Standardized for *Diatomaceous* earth (PDF) proceeding of the Eighth, international working conference of stored product pork. U.K. Entomogical Society of Mantoba.

Philips, R.D. and Dedeh, S.S. (2003). Developing

Azeez, O. M. and Pitan, O. O. R (2018). Sole and combined effect of three botanicals against cowpea seedbruchid, *Callosobruchus maculatus* Fabricius. African Journal of Agricultural Research, Vol. 13(7) pp 321-328. DOI: 10.5897/AJAR2017.12737.

Beck, C.W. and Blumer, L.S. (2014). A Handbook on Bean Beetle, *Callosobruchus maculatus*. National Science Foundation,Pp1-14 www.beanbeetle

Buzby, J. C. and Hyman, J. (2012). Total and per capita value of food loss in the United States. Food Policy, 37: 561-570.

Capinera, John L. (2008). *Diatomaceous* earth in Capinera, John L (ed.). Encyclopedia of Entomology (second ed.). Springer. P. 1216 ISBN 978-1-4020-6242-1.

Carvalho, M., Lino-Neto, T., Rosa, E. and Carnide, V. (2017). Cowpea: a legume crop for a challenging environment: Cowpea for a challenging environment. Journal of the Science of Food and Agriculture,97(13): 4273-4284

Chhabra, K. S., Lal, S., Kooner, B. S. and Verma, M. M. (1993). Insect pests of pulses – identification and control Manual. Punjab Agricultural University and Directorate of Pulses Research, India. 88 pp.

Dawodu, E.O. and Ofuya, T.I. (2000). Effect of mixing powders of Piper guineense Schum and Thonn, Dennetia tripetala Baker on oviposition and adult emergence of Callosobruchus maculatus (F.) (Coleoptera: Bruchidae). Applied Tropical Agriculture 5: 156 -160.

Delobel, A and Tan, M. (1993). Lescoleople are these decrees aliment Aires enterprises dandles regions chauda funnel tropical xxxii, ORSTOM editions, Paris, CTA wangeningen, 425 pp

Dobie, P. Hams, P., Hooges, R., J. and Preret, P. F. (1984). Insect as Arachnids of Tropical Stored Products. Their biology and identification. (A training manual). TDRI, U.K., 273 pp

Elhag, E.A. (2000). Deterrent effects of some botanical products on oviposition of the cowpea bruchid *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae). *Int. J. Pest Manag.,* 46(2): 109-113.

Emeasor KC, Emosairue SO, Ogbuji RO. (2007). Preliminary laboratory evaluation of the efficacy of mixed seed powders of *Piper guineense* (Schum and Thonn) and *Thevetia peruviana* (Persoon)Schumagainst *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae). Niger J Entomol.24: 114-118.

Fernando, H.S.D. and Karunaratne, M.M.S.C. (2012). Ethnobotanicals for storage insect pest management: Effect of powdered leaves of *Olax zeylanica* in suppressing infestations of rice weevil *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae). *J. Trop. For. Environ.,* 2(1): 20-25.

Gonçalves, Alexandre; Goufo, Piebiep; Barros, Ana; Domínguez-Perles, Raúl; Trindade, Henrique;Rosa, Eduardo A. S.; Ferreira, Luis; Rodrigues, Miguel (2016). "Cowpea (Vigna unguiculata L. Walp), a renewed multipurpose crop for a more sustainable agrifood system: nutritional advantages and constraints". *Journal of the Science of Food and Agriculture. 96 (9): 2941–2951. doi:10. 1002/jsfa.7644.ISSN 1097-0010PMID 26804459*

Hamid, S., Muzaffar, S., Wani, I. A., Masoodi, F. A. and Bhat, M. M. (2016). Physical and cooking characteristics of two cowpea cultivars grown in temperate Indian climate. Journal of the Saudi Society of Agricultural Sciences, 15: 127-134

Hill, D.S. (1990). Pest of stored product and their control. Behavior Press London 280 pp Ignatowicz, S. and Wesolowska, B. (2015). Potential of common herbs as grain protectants: repellent effect of herb extracts on the granary weevil, *Sitophilus granarius* L. Proceedings of 6th International Working Conference on Stored-products Protection, 2: 790-794.

Issa, U.S., Afun, J.V.K., Mochiah, M.B., Owusu-Akyaw, M. and Braima, H.(2011). Effect of some local botanical materials for the suppression of weevil populations. *Int. J. Plant, Ani. Envir. Sci.,* 1(3): 270-275.

Joseph, B., Sowmya and Sujatha, S. (2012). Insight of botanical based biopesticides against economically important pest. *Int. J. Phar. Life Sci.,* 3(11): 2138-2148.

Khatun. A., Kabir, G., Bhuiyan, M.A.H. and Khanam, D. (2011). Effect of preserved seeds using different botanicals on seed quality of lentil. *Ban. J. Agric. Res.,* 36(3): 381-387.

Lale, N.E.S. (1994). Laboratory assessment of the difference persistence of powders of four spices on cowpea bronchus and maize weevil in an air tight storage facilities, Samna journal of Agriculture Research. 11, 79 – 84

Lale, N.E.S. (2001). Steath thieves in home and food stores, an inaugural lecture series. pp 20 – 21

Manohar Lal, Budhi Ram and Prabhat Tiwari. (2017). Botanicals to Cope Stored Grain Insect Pests: A Review. *Int.J.Curr.Microbiol.App.Sci.* 6(6): 1583-1594. doi: http://doi.org/10.20546/ijcm. 2017.606.186

Musa, A.K., Dike, M.C. and Onu I. (2009). Evaluation of Nitta (*Hyptis suaveolens* Poit.) seed and leaf extracts and seed powder for the control of *Trogoderma granarium* Everts (Coleoptera: Dermestidae) in stored groundnut. Ameri-Eurasi J Agrono. 2(3): 176-179.

Nielsen, S. S.; Ohler, T. A.; Mitchell, C.A., (1997). Cowpea leaves for human consumption:

nutritional and economic value added food products from cowpea. http://wwwispms edu /crsp/Finalreport PDF Downloaded 07/02/2005.

Phillips, R. D., McWatters, K. H., Chinnan, M. S., and Saalia, F. K. (2003). Utilization of cowpeas for human food. Field Crops Research, 82(2-3): 193-213.

Phillipson, J.D., O' Neill, M.J., Wright, C.W., Bray, D.H. and Warhaurst, D.C., (1987). Plants as a Source of Antimalarial and Amoebicidal Compound; Medicinal and Poisonous Plants of the Tropics: Proceedings of symposium 5 – 35 of the 14th International Botanical Congress Berlin. pp 70 – 78.

Regnault-Roger C, Hamraoni A, Holeman M, Theron E, Pinel R. (1993). Insecticidal effect of essential oil from Mediterranean aromatic plants upon *Acanthoscelides obtectus* Say. Coleopteran, bruchid Kidney bean (*Phaseolus vulgaris* L.) J Chem. Ecol. 19: 133-144.

Shazia OWM, Minza M, Rhodes M, Robert NM, Bukheti K, Maulid M, Herman FL, Christine GI, Dastun GM, Loth SM. (2006). Control of cowpea weevil (*Callosobruchus maculatus* F.) in stored cowpea (*Vigna unguiculata* L.) grains using botanicals, Asi. J Pl. Sci. 5(1): 91-97.

Singh, K.K; M.M; Samants, A.K; Kundu, S.S; and Sharma, S.D; (2002). Evaluation of certain feed resources for carbohydrate and protein fractions and in sites digestion characteristics Indian J. Anim. Sci. 72(9): 794-797.

Srinivasan, R. (2014). Insect and mite pests on vegetable legumes: A field guide for identification and management. AVRDC – The World Vegetable Center, Shanhua, Taiwan. *AVRDC Publication*,No. 14-778. 92 pp.

Tiroesele B, Thomas K. and Seketems S; (2014). Control of cowpea weevil, *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) using Natural plant products. Insects 6.77-84 (doi: 10.3390/insects6010077).

Umeozor, O. C. (2005). Effect of the infection of *Callosobruchus maculatus* (Fab.) on the weight *Environ* loss of stored cowpea (*Vigna unguiculata* (L.) Walp). Journal of Applied Sciences and mental Management, 9 (1): 169 – 172

Upadhyay, R.K. and Ahmad, S.(2011). Management strategies for control of stored grain insect pests in farmer stores and public ware houses. *World. J. Agric. Sci.,* 7 (5): 527-549.

Wolfenbarger DA, Cantu E. (1975). Enhanced toxicity of carbaryl when combined with synergists againstlarvae of the bollworm, *Heliothis zea* and the tobacco budworm, *Heliothis virescens*. Flor.Entomol. 58: 103-104.

Zettler, J.L. and Cuperus, G.W. (1990). Pesticide resistance in *Tribolium castaneum* (Coleoptera: Tenebrionidae) and *Rhizopertha dominica* (Coleoptera: Bostrichidae) in wheat. *J. Econ. Entom.,* 83: 1677–1681.

Zacharia, J.T. (2011). Ecological Effects of Pesticides. In: Pesticides in the Modern World - Risks and Benefits, Stoytcheva M (Ed.). InTech Publisher, 560 Pp.