MYCOFLORA AND COWPEA BEETLE INFESTATION ON SELECTED COWPEA CULTIVARS AND THE EFFICACY OF SOME PLANT POWDERS AGAINST FUNGAL PATHOGENS

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Abstract

This study was carried out to assess cowpea beetle infestation and identify mycoflora on some selected cowpea grains, and to evaluate the efficacy of three plant powders in the control of fungal pathogens. Seven cowpea varieties; Agonyi, Oloyin, Mala, Milk, Sobo, Drum and Sokoto were obtained from Bodija a local market in Ibadan, Nigeria. Cowpea varieties were stored for six weeks,and cowpea beetle population was monitored weekly. Incidence of fungi were assessed on weekly basis. Fungi were isolated from each of the varieties and identified. Three plant powders; *Moringa oleifera* seeds, *Capsicum annuum* fruit and *Cymbopogon citratus* leaf, each at 0.0 g (control, untreated), 0.5 g, 1.0 g, 1.5 g and 2.0 g were added and thoroughly mixed with 30 g of Oloyin, Sobo, Milk, Agonyi and Drum selected from the earlier seven varieties.. After six weeks of storage, 10 grains of cowpea were selected randomly from the 30 g cowpea grains and surface-sterilized with 10% sodium hypochlorite (NaOCl) and. The grains were incubated for 7 days at a temperature of 28±2°C. Experimental units were arranged in a completely randomized design in three replicates; percentage fungal incidence was determined. Furthermore, treated seeds were planted in 5 kg pots containing sterilised soil and arranged in a randomized complete block design replicated four times. At three weeks after sowing, the effect of these plant powders on the viability of the cowpea varieties was determined. Number of cowpea beetle increased with increase in period of storage from one to six weeks. Beetle population was highest on Sokoto and least on Agonyi across all the weeks. Fungi isolated from cowpea grains were *Aspergillus flavus, Aspergillus niger, Fusarium* sp. *and Mucor* sp. *Aspergillus flavus and A. niger* were the most predominant isolates followed by *Fusarium* sp. *and Mucor* sp. *Aspergillus niger* had the highest incidence in Drum (46%). In the viability test, Oloyin, Agonyi and Drum were the most viable, while Milk and Mala had the lowest viability. Oloyin treated with 1.0 g and 2.0 g of moringa seed powder had the least infection at 4.32 and 3.31, respectively. Among the treatments, pepper fruit powder had the least infection and thus gave the best control against fungal infections.

Keywords: *Aspergillus* spp., *Callosobruchus maculatus,* Cowpea**,** Moringa seed powder, *Capsicum annuum* fruit powder, seed viability

Introduction

Cowpea (*Vigna unquiculata* (L.) Walp.) is an annual herbaceous legume. Cowpea formerly belonged to the family, Leguminosae (Padulosil and Ng, 1997). The crop is a very important food source used widely as grain legume in Asia, Africa, Southern Europe, and Central and Southern America (Chathuni *et al*., 2018). Nigeria is both the world's largest producer and consumer of cowpea (Agbogidi and Egho, 2012; Sheahan, 2012). The world's production is put at 2.2 million tonnes and Nigeria produces about 850,000 tonnes (FAO, 2015). Cowpea seeds are a rich source of amino acid lysine and tryptophan and it is high in calcium and iron (Achuba, 2006; Xiong *et al*., 2016). Apart from its dietary importance (Muoneke *et al.,* 2012), cowpea grain has contributed greatly to increasing incomes of resource-poor farmers (Langyintuo *et al.,* 2005; Baribusta *et al*., 2010, Oluokun, 2005).

However, in spite of its importance and uses, pests and diseases have been great threats to the production of cowpea from the field to the store. The cowpea beetle, *Callosobruchus maculatus* (F.) belonging to the order Coleoptera and family Chrysomelidae, is the main insect pest that causes yield loss in stored cowpea seeds. Also, some storage fungi commonly associated with cowpea are *Aspergillus* sp*., Penicillium* sp. (Gabriel and Puleng, 2013; Ki Deok and Mohamed, 2016;). There is therefore a continuous need to protect the crop against the activities of these pests and diseases (Asiwe, 2005; Hamid *et al*., 2016). During storage, farmers often take little or no consideration of the environmental conditions where they store grains and standard storage procedures are often not followed. These environments often favour the activities of pests and storage fungi which are responsible for the deterioration, especially in weight and quality of grains (Deepak and Prasanta, 2017).

The wide adoption of synthetic pesticides has some health and environmental implications (Agunbiade *et al*., 2014). Many of these synthetic pesticides leave toxic residues on grains and foods for a very long time (Egho, 2009). Due to the hazardous effects posed by these synthetic pesticides on the health and the environment, research on the use of botanicals as an alternative to synthetic pesticides have been on the increase (Mashela and Pofu, 2012; Dipsika *et al.,* 2012, Wafaa *et al.,* 2017). Some plants such as neem (*Azadirachta indica* L.) , lemon grass (*Cymbopogon citratus* Stapf), pepper (*Capsicum* spp.), moringa (*Moringa oleifera*) and many others have insecticidal properties that can inhibit the activities of pests and disease-causing pathogens (Kang *et al.,* 2013; Alabi and Adewole, 2017). Plant materials are easily accessed by farmers, locally and relatively inexpensive compared to the synthetic pesticides and easily biodegradable, hence, more ecofriendly and healthy (Premachandra, 2017). In storage, to maximize the insecticidal and inhibitory effects on pests and pathogens, these plant materials are often processed into powder, extracts and oil (Singh *et al.,* 2010; Ohia and Ana, 2017). The use of these natural products has given promising results and these materials are friendly to both human health and the environment unlike the synthetic pesticides (Cannon *et al.,* 2012; Wafaa *et al.*, 2017).

Therefore the objectives of this study were to assess the population of cowpea weevil and fungal pathogens associated with selected cowpea grains sourced from Bodija market, Ibadan and to determine the effects of three plant powders on the fungal pathogens and viability of the varieties.

Materials and methods

Source of cowpea grains

Seven varieties of cowpea grains; Oloyin, Sobo, Milk, Mala, Agonyi, Drum, and Sokoto, (2 kg each), were randomly sourced from stalls at Bodija market, Ibadan. The grains were placed in clean, moisture-free polythene bags and taken to the Plant Pathology laboratory, Department of Crop Protection and Environmental Biology, University of Ibadan. Grains were kept at ambient temperature and relative humidity for 48 hours in the laboratory.

Source of botanicals

Seeds of *Moringa oleifera* and dried fruits of *Capsicum annuum* were obtained from Ojoo market, Ibadan. The moringa seeds and pepper fruits were air-dried at room temperature, milled using a motorized high speed-grinder (Model no. HS AG 1:120 N11 ID CNC). Powders were stored in separate labelled plastic containers with tight lids. Leaf powder of *Cymbopogon citratus* was obtained from Entomology unit, Department of Crop Protection and Environmental Biology, University of Ibadan. All the materials were used as botanicals at different concentrations to treat the cowpea varieties. Air-dried *Moringa oleifera* seeds and *Capsicum annuum* fruits were ground into fine powder

Cowpea beetle infestation on cowpea varieties

Assessment of *Callosobruchus maculatus* infestation on cowpea varieties was carried out at the Entomology Laboratory, Department of Crop Protection and Environmental Biology, University of Ibadan. Thirty grammes of each of the seven cowpea varieties: Oloyin, Sobo, Milk, Mala, Agonyi, Drum, and Sokoto, were stored in plastic jars (20mL) covered with a fine mesh for aeration and replicated five times. The stored grains were observed weekly for emergence of cowpea beetle for a duration of six weeks.

Isolation and identification of fungi from cowpea grains

After six weeks of storage, ten cowpea grains were randomly selected and surface sterilised with 10% sodium hypochlorite (NaOCl) solution for 1 min and rinsed in three changes of sterile distilled water. The surface sterilised seeds were plated aseptically on layers of filter paper and cotton wool moistened with sterile distilled water in Petri dishes in four replicates (ISTA, 1999). The plates were incubated at 28 ± 2 ºC for 4 days for the growth of the seed-borne fungi. The organisms were sub-cultured to obtain pure cultures. Slides were prepared for identification of the organisms. Percentage incidence of fungi was determined by the formula of Claudius-Cole and Somefun, (2011), and Nnaji and Brook (2016) given below:

$$Fungal\ incidence = \frac{Total\ number\ of\ each\ organism\ in\ a\ variety}{Total\ number\ of\ all\ identified\ organisms\ in\ a\ variety} X\ 100$$

Effect of plant powders on fungal pathogens in cowpea grains

The powders of each of *Moringa oleifera*, *Capsicum annuum* and *Cymbopogon citratus* were weighed (0.5 g, 1.0 g, 1.5 g and 2.0 g) and stored in separate containers and kept aseptically in sterile conical flasks until when needed. Five varieties of cowpea grains were randomly selected from the seven varieties earlier observed to cowpea beetle infestation and fungi infection based on observable high and low incidents of beetle and fungi attacks. These varieties are Oloyin, Sobo, Milk, Agonyi and Drum each at 30 g were weighed into a sterile 90 mm container with different concentrations of each powder and mixed thoroughly. The containers were stored at room temperature for six weeks. Each level was replicated four times and arranged in a completely randomized design.

After six weeks of storage, 10 grains of cowpea were selected randomly from the 30 g cowpea grains coated with different plant powders. The ten grains were surface sterilized with 10% sodium hypochlorite (NaOCl) for 1 min. and rinsed in three changes of sterile distilled water and then, plated aseptically on the moistened three layers of sterile kitchen towel placed in 90 mm containers and incubated for 4 - 7 days (28±2ºC). Percentage fungal incidence was determined.

Effect of plant powders on germination of cowpea grains *in vitro*

;;Ten seeds from each of the cowpea grains coated with plant powders were plated aseptically on layers of filter paper and cotton wool moistened with sterile distilled water in petri dishes in four replicates. Cowpea varieties were observed for percentage germination at the end of 7 days.

Effect of plant powders on germination of cowpea grains in pots

Five varieties, Oloyin, Sobo, Milk, Agonyi and Drum, 30 g each, were transferred into a sterile 90 mm plastic bowl. The three plant powders; *Moringa oleifera* seed, *Capsicum annuum* fruit and *Cymbopogon citratus* were applied as treatments at five various concentration levels of 0.0 g (control), 0.5 g, 1.0 g, 1.5 g 2.0 g, replicated four times. After six weeks of storage in 90 mm plastic bowl, treated seeds were each planted out in 5 Kg pots arranged in a factorial experiment using randomized complete block design with four replications. Number of seeds germinated was recorded three weeks after planting. Percentage seedling viability was calculated by counting only germinated seeds 14 days after planting according to ISTA (1999)

Data Analysis

All data were analysed using descriptive statistics and analysis of variance (ANOVA). Significant means were separated using least significance difference at 0.05 level of significance.

Results

Infestation of *Callosobruchus maculatus* on cowpea varieties

Population of emergent beetles from cowpea varieties increased with increase in duration of storage across all varieties (Figure 1). Sokoto variety had the highest number of emergent beetles among all the varieties from week one (47) to week six (255). Agonyi consistently had the least number of emergent beetles of 2.6 at week one and 26.4 in week 6. Population of emergent beetles in other varieties occurred between those of Sokoto and Agonyi in the order; Sobo> Oloyin> Milk> Mala> Drum.

Percentage infection of freshly obtained cowpea grains

Figure 2 represents the percentage infection of the cowpea grains obtained from Bodija market, Ibadan. Sobo (85%), Milk (85%) and Drum (95%) had the highest fungal infection. High percentage infection was observed in Mala (70%) and Sokoto (65%); but infection was not as high as Sobo, Milk and Drum.Oloyin (45%) and Agonyi (45%) varieties had the lowest fungal infection.

Incidence of fungal pathogens in seven varieties of cowpea after six weeks in storage

Fungi isolated from cowpea grains were *Aspergillus flavus, Aspergillus niger, Fusarium* sp. *and Mucor* sp. *Aspergillus flavus and A. niger* were the most predominant fungi isolates among the seven cowpea varieties followed by *Fusarium* sp. *and Mucor* sp. Drum had the highest incidence of *Aspergillus niger* (46%). Incidence of *A. flavus* was highest in Drum (68%) and Sokoto (60%).Drum had the highest incidence of *Fusarium* sp. (32%), while Agonyi had the lowest incidence of *Fusarium* sp. (6%), Oloyin had the lowest incidence of *Mucor* sp. (10%) among all the varieties (Table 2).

Percentage germination of cowpea varieties in vitro

Figure 3 represents the percentage germination of cowpea varieties *in vitro*. Oloyin percentage germination was the highest (90%), while Drum had the lowest germination percentage of 50%.

Effect of plant powders on fungal infection in cowpea grains

The effect of three plant powders on storage fungi is presented in Tables 3, 4 and 5. In Table 3, Oloyin and Agonyi treated with Moringa seed powder at 1.0 g and 2.0 g had the least infections at 4.32 and 3.31 3.54 and 4.64. Drum treated with 1.0 g and 2.0 g had the highest infection of 9.67 and 9.63, respectively. Table 4 shows the effect of pepper fruit powder in the control of fungal pathogens. Agonyi treated with 1.0 g and 2.0 g had the least percentage infections of 1.70 and 1.74. Drum treated with 1.0 g and 2.0 g had the highest percentage infection of 6.78 and 5.55 respectively. In Table 5, Oloyin treated with 1.0 g and 2.0 g of lemon grass powder had the least infection percentages of 2.00 and 1.25, respectively. Drum not treated (0 g), and Drum treated with 1.0 g both had the highest infection percentages of 14.9 and 10.5 respectively.

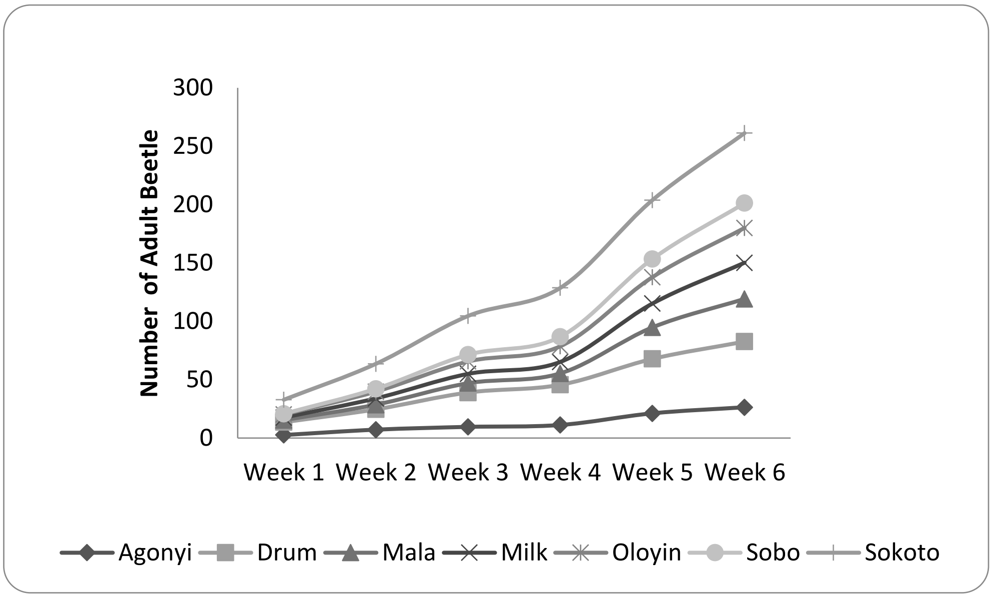

Figure1: Population of *Callosobruchus maculatus* in seven varieties of cowpea over six weeks storage.

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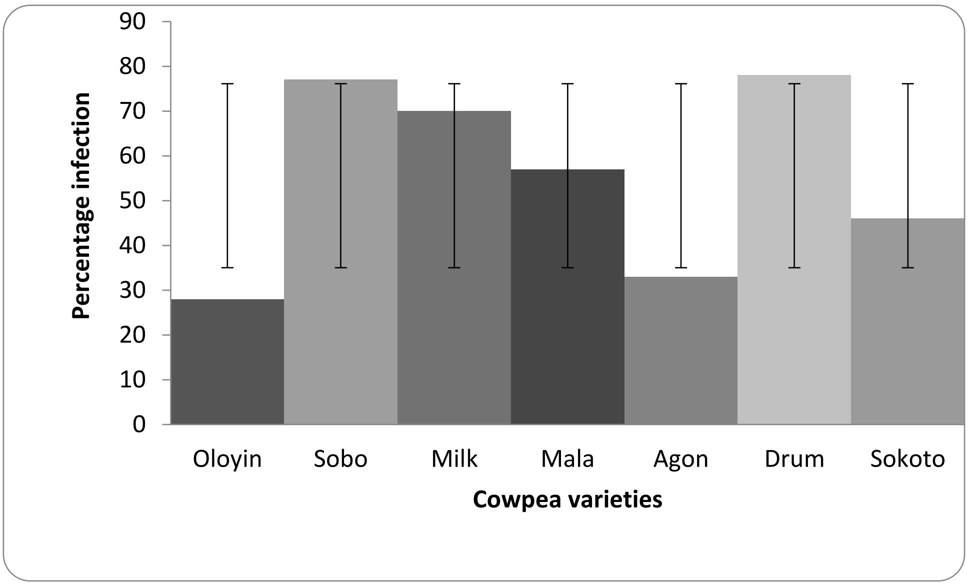

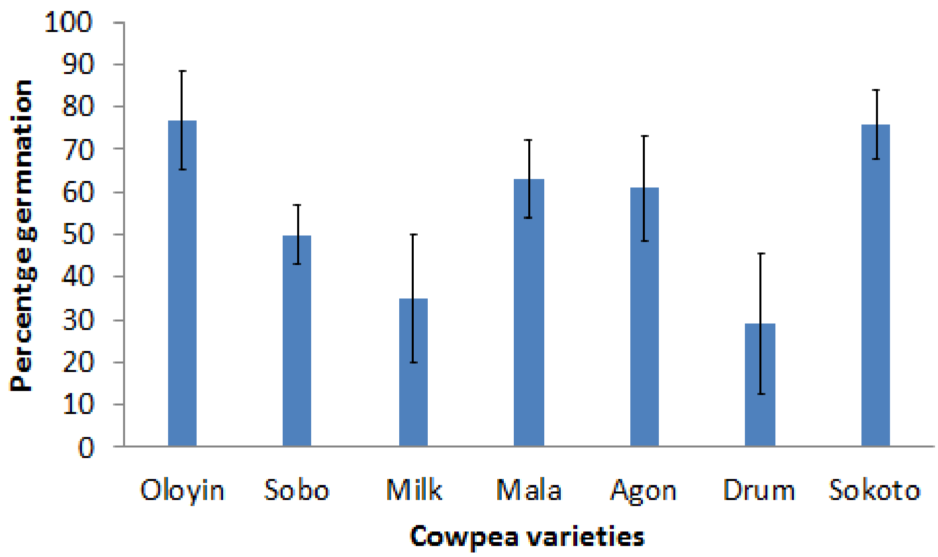

Figure 2: Percentage fungal infection of freshly obtained cowpea varieties

Figure 3: Seed viability test of seven freshly obtained cowpea varieties *in vitro*

Table 2: Incidence of fungal pathogens on seven varieties of cowpea after six weeks in storage in percentages

Percentage incidence (%)

	Oloyin	Sobo	Milk	Mala	Agonyi	Drum	Sokoto
Aspergillus flavus	40	78	45	58	59	68	60
Aspergillus niger	20	28	19	16	10	46	32
Fusarium sp.	14	20	9	11	6	32	26
Mucor sp.	10	32	20	17	11	34	37

Table 3: Effect of *Moringa oleifera* seed powder in the control of fungal pathogens in five cowpea varieties

	Concentration levels	**0 g (control)**	**1.0 g**	**2.0 g**
Cowpea varieties				
Oloyin		5.34a	4.32a	3.31a
Sobo		5.55a	5.24a	5.00a
Milk		5.61a	6.36ab	4.35a
Agonyi		2.62b	3.54a	4.64a
Drum		7.30a	9.67c	9.63b
LSD(0.05)		2.63	2.02	2.58

Values are means of three replicates. Means followed by the same letter in a column are not significantly different ($p \leq 0.05$) using LSD.

Table 4: Effect of *Capsicum annuum* fruit powder in the control of fungal pathogens in five cowpea varieties

	Concentration levels	0 g	1 g	2 g
Cowpea varieties				
Oloyin		4.13a	4.45a	3.35a
Sobo		7.30b	6.60a	2.78a
Milk		6.62b	4.51a	4.32a
Agonyi		4.15a	1.70b	1.74b
Drum		10.00c	6.78a	5.55a
LSD(0.05)		2.15	2.38	1.30

Values are means of three replicates. Means followed by the same letter in a column are not significantly different ($p \leq 0.05$) using LSD.

Table 5: Effects of *Cymbopogon citratus* leaf powder in the control of fungal pathogens in five cowpea varieties

	Concentration levels	0 g	1 g	2 g
Cowpea varieties				
Oloyin		5.56a	2.00a	1.25a
Sobo		7.65a	7.51b	7.00b
Milk		6.67a	4.65a	2.38a
Agonyi		5.42a	4.50a	3.99a
Drum		14.9b	10.5b	9.34b
LSD(0.05)		4.81	3.53	3.24

Values are means of three replicates. Means followed by the same letter in a column are not significantly different ($p \leq 0.05$) using LSD.

Effect of seed treatment with three plant powders on seed germination

Tables 6, 7 and 8 showed the effects of *Cymbopogon citratus* leaf powder, *Moringa oleifera* leaf powder and *Capsicum annuum* fruit powders on the viability of the cowpea varieties. Agonyi (90%) and Drum (85%) treated with C. *citratus* powder were more viable at week 1. At week 2, all varieties were relatively viable. Agonyi (95%), Drum (95%) and Oloyin (95%) were most viable among the varieties at week 3 (Table 6).

Agonyi (90%) and Oloyin (90%) treated with *M*. oleifera seed powder had the highest viability at week 1, while Drum (65%) had the lowest viability. At week 2, Agonyi (95%), Drum (95%) and Milk (95%) had higher viability and had same percentage viability at week 3 (Table 7)

Viability of cowpea varieties treated with *C. annuum* fruit powder is presented in Table 8. Agonyi (95%) and Drum (90%) had the highest viability, while milk (70%) and Sobo (80%) had the least viability at week 1. At week 2, Agonyi (95%) and Drum still had the highest viability percentage, while milk (80%) and Sobo (80%) had the lowest. Agonyi (95%) and Drum (95%) had the highest viability at week 3 (Table 8).

Table 6: Effect of *Cymbopogon citratus* leaf powder on the viability of cowpea varieties at one to three weeks after sowing

Cowpea varieties	Number of weeks		
	1	2	3
Agonyi	90	95	95
Drum	85	90	95
Milk	20	75	80
Oloyin	80	95	95
Sobo	25	70	75

Table 7: **Effect of *Moringa oleifera* seeds powder on the viability of cowpea varieties six weeks after storage**

	Number of weeks 1	2	3
	Percentage viability		
Cowpea Varieties			
Agonyi	90	95	95
Drum	65	95	95
Milk	90	95	95
Oloyin	80	85	85
Sobo	80	85	85

Table 8: **Effect of *Capsicum annuum* fruit powder on the viability of cowpea varieties six weeks after storage**

	Number of weeks 1	2	3
	Percentage germination		
Cowpea Varieties			
Agonyi	95	95	95
Drum	90	95	95
Milk	70	80	85
Oloyin	85	85	85
Sobo	80	80	85

Discussion

Fungal pathogens and insect pests pose a major biotic stress on cowpea at all stages of production leading to great losses from the field to the store. Farmers and researchers are evolving means to curtail the menace caused by these fungal pathogens and insect pests. Among these control strategies is the use of plant materials with fungicidal and insecticidal properties. The population of the cowpea beetle observed showed that, Sokoto had the highest infestation of *Callosobruchus maculatus* population at the end of the sixth week of storage, while Agonyi had the lowest mean infestation. The difference in population of beetles among the varieties suggests that each variety has inherent variation in genetic factors such as differences in physical and biochemical properties that limits or support beetle infestation (Musa and Adeboye, 2017).This agrees with the report of Alabi *et al.* (2003) that cowpea cultivars have different processes by which they withstand insect activities. Several studies have implicated seed physical characteristics (texture, colour, toughness and size) to be responsible for resistance in some cowpea varieties to cowpea beetle (Castro *et al*., 2013; Adebayo and Ogunleke, 2016).

Four species of fungi were isolated from the selected cowpea varieties. The fungi isolated from these varieties were *Aspergillus flavus*, *A. niger, Fusarium oxysporum* and *Mucor* sp. *Aspergillus flavus* and *A. niger* were the predominant fungal species isolated from the cowpea varieties. This study confirms the report of Abd El-Rahim *et al.* (2014) who reported that *Aspergillus flavus* and *A. niger* were the prevalent fungi isolates in cowpea grains. *Aspergillus flavus* and *A. niger* produce aflatoxin in grains which are toxic to man and animals when consumed (Klich, 2007). Milk and Mala varieties were more susceptible to the storage fungi as they showed highest percentages of infection. This suggests that, their high susceptibility to fungal infections could be responsible to their low viability, while Oloyin, Drum and

Agonyi which were far less susceptible to fungal infections showed better viability. This finding is in consonance with Claudius-Cole and Somefun (2011) who found that fungal infection decreased viability in *Vigna subterranean.*
Callosobruchus maculatus is found to predispose cowpea grains in storage to certain fungal infection (Nnaji and Brooks, 2016). Heavily *Callosobruchus maculatus* infested cowpea grains (Sokoto and Mala varieties) after six weeks of storage had more fungal infection rates. This agrees with the finding of Gabriel and Puleng (2013) that, mould infection on cowpea was much higher in heavily beetle-infested seeds than wholesome grains.this could as result of increase in beelte population which led to increase in higher respiration leading to increase in relative humidity.

Milk, Agonyi, and Oloyin cowpea varieties treated with moringa seed powder had higher germination compared to the same varieties treated with lemon grass. This suggested that Moringa seed powder inhibited the growth of some of the storage fungi that would have impaired the viability of the seeds. This agrees with Abo El-Dahab *et al.* (2016) who reported that, moringa extract at 25% concentration enhanced germination and inhibited the growth of seed borne pathogen of sorghum seed. The viability of untreated cowpea has been found in previous research to be adversely affected by seed-borne pathogens (Singh *et al.,* 2002; Islam, 2014). Debnath *et al.* (2012) reported that fungi infection in seeds affect their viability. They observed that seeds that had the highest prevalence of seed-borne pathogens (*Aspergillus flavus*, *Aspergillus niger* and *Fusarium* sp.) and planted without any treatment had the highest germination failure to as high as 29.50%. In this experiment however, the rate of germination failure in cowpea treated seeds was not as high as that reported by Debnath *et al.* (2012). This observation suggests most likely that, treating seeds with the three plant materials before storage can largely reduce germination failure compared to when seeds are not treated.

Untreated Drum and Sokoto had the highest *Callosobruchus maculatus* infestation and were most susceptible to storage fungal infections. It is concluded that, these two varieties should not be stored for a very long period of time without treatment.
Seed treatment especially with *Capsicum* annuum fruit powder and *Moringa oleifera* seed powder could be adopted by farmers because there was no observable incidence of disease infections on treated cowpea.

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