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Research Article

Occurrence of Pathogens on Grains of Cowpea (*Vigna unguiculata* Walpers) and Maize (*Zea mays* L) Infested by *Callosobruchus maculatus* (F.) and *Sitophilus zeamais* Mots

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Abstract

Experiments to determine occurrence of pathogens on grains of cowpea (*Vigna unguiculata*) and maize (*Zea mays*) infested by *Callosobruchus maculatus* (F) and *Sitophilus zeamais* (Mots) was conducted at the Department of Crop, Soil and Pest Management, Federal University of Technology Akure (FUTA). The cowpea varieties (Oloyin, Sokoto white and Drum) and the maize varieties (SWAN 1 SR, White and yellow maize) were used for the experiment. Two hundred grammes of cowpea and maize grains were infested with 0-48 hrs old adults of *Callosobruchus maculatus* and *Sitophilus zeamais* respectively. After 14 days of oviposition, the insects were sieved out and the setup left on the laboratory bench for the emergence of fresh adults. After the emergence of the first generation of insects, ten infested grains were selected and plated to isolate the associated pathogens. Similarly, ten grains were selected after the emergence of the 2nd generation of insects for the isolation of associated pathogens. The results from the study showed that the infested and uninfested grains of cowpea and maize contained several pathogens associated with the grains. The microbial load count of Bacterial increased from 27 Cfu/g to 80 Cfu/g while Fungi increased from 1 Cfu/g to 22 Cfu/g. and yeast increased from 122 Cfu/g to 200Cfu/g with period of infestation. It was also observed that moisture content of the infested cowpea grains increased from 6.32% before infestation to 19.84% after infestation while that of maize grains increased from 7.02 before infestation to 11.95% after infestation which consequently enhanced the proliferation of pathogens on the grains. The pathogens isolated and identified include several species of bacteria (*Bacillus cereus, Agrobacterium spp* e.t.c), fungi (*Fusarium equiseti, F. graminearum* e.t.c) and yeasts. Based on the results from the study, it was concluded that pathogens occurred in both the infested and uninfested grains of cowpea and maize. Moisture content increased with infestation which consequently increased the microbial loads on the grains. Therefore, appropriate strategies must be used for the protection of the grains against infestation from the insects.

Keywords: Pathogen; *Vigna unguiculate; Zea mays; Callosobruchus maculatus; Sitophilus zeamais*

Introduction

The maize weevil, *Sitophilus zeamays* Motschulsky (*Coleoptera: Curculionidae*), is one of the most destructive stored product pests of grains, cereals, and other processed and unprocessed stored products in sub-Saharan Africa [23]. *S. zeamays* causes qualitative and quantitative damage to stored products, with grain weight loss ranging between 20 to 90% for untreated stored maize [22], and the severity of damage depends on factors which include storage structures and physical and chemical properties of the produce. Heavy infestation of adults and larvae of maize weevil on stored maize grains which cause postharvest losses have become increasingly important constraints to storage and food security in the tropics [19].

Cowpea (*Vigna unguiculata* Walpers) is an important legume in many developing countries [1], grown mainly for its grains [11]. It is one of the cheapest sources of plant protein in the diet of people that cannot afford animal protein such as meat and fish [18,24,30]. Cowpea is favoured by farmers because of its ability to

maintain soil fertility [8], income [29,31], its use as animal fodder and comparably high yields in harsh environment where other food legumes do not thrive [28]. Cowpea feeds millions of people in developing worlds with an annual world-wide production estimated around 4.5 metric tons on 12-14 million ha [10]. However, cowpea production is faced with a wide range of biotic constraints like virus (Cowpea Aphid-Borne Mosaic Virus, CABMV), bacteria (*Xanthomonas campestris* pv *vignicola*), fungi (*Choanephora spp*), insects (*Aphis carccivora, Megalurothrips sjostedti, Callosobruchus maculatus* [4] and parasitic plants (*Striga gesnenoides* (Bilid) and *Alectra vogeli (Alectra*). In storage, *Callosobruchus maculatus*, also called cowpea beetle, cowpea weevil or bruchid, is regarded as the most important and common pest of cowpea both in Africa and Asia [15]. This weevil has caused losses both in quality and quantity of the stored seeds [3]. Estimates of storage losses are highly variable ranging widely from 4 - 90% [14,32] due to perforations by this weevil, thus reducing the degree of usefulness and making the seeds unfit either for planting or human consumption [5,14]. Sub-Saharan Africa has been known to provide a favourable environment for the pest. The common control methods for the control of this pest are the use of chemical insecticides, biological control, and botanical insecticides [26] among others. Several attempts to preserve the seeds mainly through chemical means apart from being expensive sometimes result in the poisoning of cowpea and environmental toxicity [24]. Therefore when infestation occurs, the conditions of the seeds changed and this influenced proliferation of pathogens [2].

The objective of this study is to determine the occurrence of pathogens on grains of cowpea and maize infested by *Callosobruchus maculatus* and *Sitophilus zeamais*.

Materials and Methods

Experiment location

The experiment was conducted at the Pest Management Laboratory of the Department of Crop, Soil and Pest Management, the Federal University of Technology Akure (5^0 14″ E and $7^0$30 N″), under ambient laboratory conditions of temperature and relative humidity of 28±3°C and 70±5% respectively.

Collection of materials

The clean maize and cowpea grains used for the experiment were obtained from the Ondo State Agricultural Development Project, Akure (ADP). The following cowpea varieties were used for the experiment; Oloyin, (Sokoto white) and Drum while SWAN 1 SR and Akure Local (White and yellow maize) varieties. The clean seeds showed no visible presence of adult insects or their exit hole were disinfested by storing them in a deep freezer for two weeks and then acclimatized in the open laboratory for 4 hours before use to prevent mouldiness [25].

Culturing of insects

The adults of *S. zeamays* and *C. maculatus* used for this experiment were derived from a colony originating from infested maize and cowpea seeds obtained from Erekesan Market, Akure, Ondo State, Nigeria. The emerged adults of *S. zeamays* were sub-cultured on maize grains while the emerged adults of *C. maculatus* were sub-cultured on cowpea grains in the laboratory. The culture was maintained in 2 L white plastic containers with a tight lid cover in the laboratory.

Experimental procedure on infestation of cowpea and maize

Two hundred grammes of cowpea and maize grains each were measured and infested with 0-48 hours old adults of *C. maculatus* and *Sitophilus zeamais* old. After 14 days of oviposition, the insects were sieved out and the setups and left on the laboratory bench for the emergence of fresh adults.

Isolation and identification of pathogens from the infested grains of cowpea and maize

After the emergence of the first generation of insects, ten infested grains were selected and plated to isolate the associated pathogens. Similarly, ten seeds were selected after the emergence of the 2nd generation of insects for the isolation of associated pathogens. Nutrient agar (NA) and Potato Dextrose Agar (PDA) were the culture media of choice used for this investigation. They were prepared according to manufacturer specification, sterilized by autoclaving at 121°C for 21 minutes before pour-plated.

Microbial enumeration and identification of test samples

Microbial enumeration was done using standard plate count method. About 10 grammes each of sample was aseptically transferred into a separate beaker containing 90ml of sterile distil water and allowed to soak for about 30 minutes. It was macerated with a sterile glass rod and mixed thoroughly on a mechanical orbital shaker for ten minutes to dislodge the microbial propagules in water. One (1ml) aliquot of the sample was pipette in to a test tube and serially diluted in another sets of test tubes containing 9ml of sterile distill water to dilution factor 10^{-6}. It was plated on Nutrient agar (NA) and potato Dextrose agar (PDA) respectively. Nutrient agar (NA) plates were incubated at 37°C for 18 – 24hrs while PDA plates were incubated at 25°C for 48 – 72hrs. At the end of the incu-

bation period, number of viable microbial colonies were examined and counted with the aid of colony counter. After counting, their population was determined using the following formula

$$C = \frac{n}{vxd}$$

Where

C: Microbial colony population

x is a multiplication sign

n: Average number of colony counted from each plate

v: Volume of the aliquot of sample transferred to the plate for culturing

d: Dilution factor 10^{-6}

Subculture was carried out to obtain pure cultures. Pure isolates were obtained by successful streaking and sub culturing on freshly prepared plate and various distinct colonies were streaked and identified. Identification of pathogens was carried out by cultural and biochemical methods according to the manual for the identification of medical Bacteria by [9]. Biochemical tests such as Gram's Reaction test, Catalase test, Motility test, Spore staining test, Fermentation of sugar test, Indole test, Methyl Red test, Coagulase test etc. were carried out for the identification according to Bergey's manual for the identification of medical bacteria [9].

Identification of fungal isolates and yeast isolates

Pure isolates of fungi obtained were identified by staining with Lactophenol cotton blue stain and observed microscopically. A wet mount of fungal isolate was prepared by placing a drop of lactophenol blue in the center of a clean slide. Mounting needle was sterilized in the Bunsen flame, allowed to cool and used to transfer a little of fungal mycelia into the drop of the stain solution on the slide. The hyphae were carefully teased with the needle. Then the preparations were covered with cover slips avoiding air bubbles and then examined under the low power objectives of the microscope (X10 –X40 objectives). Cultural characteristics of the fungal isolate, the shape and colour of the hyphae, microscopic and macroscopic observation such as the shape and appearance of the individual spores, conidiophores, phialides etc were employed in the identification and characterization of different species of fungal isolate.

Moisture content of the sample was determined by oven drying methods [7]. Procedures of determining moisture content;A clean and labelled moisture can was weighed after it has been air-dried (W1) Test sample was added into the moisture can and weighed (W2) The moisture can containing the test sample was transferred into thermo-setting oven at about 105 °C for about 24 hours or to a constant weight. The moisture can was removed and allowed to cool in a desiccator for about 1 hour. Then the oven dried moisture can containing the test sample was weighed (W3). The moisture content of the test sample was calculated. The loss in weight after drying in the oven is the moisture content of the test sample.

The percentage moisture content was calculated using the following formula

$$\% \text{ moisture content} = \frac{\text{loss in weight}}{\text{weight of test samples after drying}} \text{ X } 100$$

$$\% \text{ moisture content} = \frac{w2-w3}{w3-w1} \text{ X } 100$$

Where W1= Weight of dried moisture can or crucible dish

W2: Weight of can + wet test samples

W3: Weight of can + oven dried test samples.

Data collection

Data were collected on the moisture content of the test samples, colony of bacteria, fungal and yeast pathogens and their microbial load count were calculated.

Results

Table 1 showed the microbial load/population for the microorganisms isolated from the infested and uninfested cowpea seeds. After calculating the microbial loads of the infested and uninfested cowpea seeds, the results shows that the microbial loads of the infested cowpea seeds were more than the uninfested ones. The microbial load of bacteria, fungi and yeast for the infested cowpea (Oloyin) were 80, 22 and 200 respectively while the microbial load of the uninfested cowpea (Oloyin) are 58, 2 and 188 respectively. The moisture content before infestation was 5.86% it increased to 19.84% after infestation by *C. maculatus.*

Microorganisms were isolated from both infested and uninfested cowpea (Oloyin, Drum and Sokoto white). The microbial load of bacterial, fungi and yeast of the infested Drum cowpea are 52, 18 and 180 respectively while the uninfested are 27, 1 and 122. The microbial load of the infested Sokoto (cowpea) with a value of bacterial, mould and yeast at 55, 48 and 205 showed they are more than the uninfested ones with 27, 26 and 83 respectively.

	Cowpea Varieties after First Generation				
Varieties	**Bacterial (x^{10-4})**	**Fungi/Mould Cfu/g (x^{10-4})**	**Yeast (x^{10-4})**	**Moisture Content. (%)**	**Microorganisms Isolated/Identified**
Oloyin Infested	80	22	200	19.84	*Staphylococcus aureus, Soratia rubidae, Xanthomonas spp, Flavobacterum spp, Neurospora spp, Toralopsis spp, Rhizopus stolonifer, Mucor mucedo, Collectotrichum spp, Kluveromycis spp, A. fumigatus, A. flavus, A. niger.*
Uninfested	58	2	188	5.86	*Staphylococcus aureus, Bacillus subtilis, Proteus mirabilis, Erwinia spp, Mucor mucedo, Rhyzopus stolonifer, Collectotrichum lindimuthanum.*
Drum Infested	52	18	180	10.34	*Staphylococcus saprophyticus, Bacillus cereus, Agrobacterium spp, Xanthomanas phaseoli, Pseudomonas spp, Helminthosporum toxicum, Cercospora spp, Mucor mucedo, Penicillum spp.*
Uninfested	27	1	122	6.32	*Staphylococcus aureus, Micrococcus spp, Streptococcus spp, Bacillus cereus, Aspergillus fumigatus, Mucor mucedo, Rhyzopus spp. Collectotrichum gleosporoides.*
Sokoto Infested	55	48	205	20.00	*Candida spp, Corynebacterium spp. Staphylococcus epidemidis, A. niger, A. fumigatus, Penicillum spp, Collectotrichum spp, Geotrichum spp, Fusarium spp, Curuularia spp.*
Uninfested	27	26	83	7.72	*Serratin spp, Staphylococcus epidemidis, Staphylococcus aurens, Sacharomyces spp, Camdida spp, Corynebacterium spp, Lactobacillus spp, Collectotrichun lindimathianum, A.nigen, Rodotorula spp, A. nigericum, Flavobacterium spp. Bacillus pumilus.*

Table 1: Microbial Loads and Population of Pathogens on Infested and Uninfested.

The results in table 2 indicated that showed the microbial load for the microorganisms that were isolated from infested and uninfested maize varieties (White and Yellow). The microbial load for the infested maize varieties (White and Yellow) was more than the uninfested ones. The microbial load of bacterial fungi and yeast for the infested white maize were 58, 68 and 36 while the uninfested were 28, 48 and 13. The value showed an increase in the number of microorganisms as the seeds was infested by *Callosobruchus maculatus.* The infested and uninfested yellow maize have a microbial load of 62, 60 and 86 and 38, 44 and 48 respectively. The microorganisms present include *Helminthosporium spp, S. aureus, A. niger* and *Lactobacillus spp*. among others.

In the table 3, the microbial population for the organisms isolated from cowpea varieties (Oloyin, Drum and Sokoto white) before and after infestation was revealed. The microbial load showed an increase in the microorganisms present in the cowpea varieties after infestation. The microbial load of the Oloyin cowpea after infestation is 165, 98 and 240 for the bacterial, fungi and yeast respectively. The microbial load for the uninfested Oloyin cowpea was 58, 2 and 188 for bacteria, fungi and yeast respectively. The microorganisms isolated include *Bacillus subtilis*, *Servatin rubidae*, *Rhizopus stolonifer* among others. The moisture content of the Oloyin cowpea increased from 5.86% uninfested to 26.27% infested. The microbial load for uninfested drum cowpea and sokoto cowpea increased after infestation. The microbial load for Drum cowpea was 27, 1 and 122 for the bacterial, fungi and yeast before infestation. It increased to 124, 68 and 240 after infestation. The microbial load for the Sokoto white cowpea also increased from 27, 26 and 83 for the bacterial, fungi and yeast before infestation to 101, 140 and 320 after infestation. The microorganisms isolated include *Chrohebacteria spp*, *Micrococcus spp*, *Camidida spp,* and *Lactobacillus spp* among others. The moisture content also increased from 7.72% in uninfested seeds to 28.35% after infestation by *C. maculatus.*

	Varieties after First Generation				
Varieties	**Bacterial (x^{10-4})**	**Fungi/ Mould Cfu/g (x^{10-4})**	**Yeast (x^{10-4})**	**Moisture Content. (%)**	**Microorganisms Isolated/Identified**
White Maize Infested	58	68	36	11.95	*Xanthomonas phaseolu, Pseudomonas glycinea, Bacteroides, Clavibacter spp, Staphylococcus saprophyticus, Streptomyces spp, Geotrichum spp. Aspergillus niger, A. funmigatus, A. flavus.*
Uninfested	28	48	13	7.74	*Protus mirabilis, Lactobacillus spp, Eriwimus spp, Micrococcus spp, Bacteroides, Lactobacillus spp, Candida spp, S. saprophyticus, A. fumigates, A. nigericum, A. flavus, Sacharomyces cerevesine.*
Yellow Maize Infested	62	60	86	13.28	*Helminthosporium spp, Penicillium spp, Pseudomonas aureginosa, Corynebacterium spp, Streptococcus spp. Penicillium griseoflavum, Helminthosporum mydis, A. flavus, A. fumigatus, A. niger, Penicularium spp. Lactobacillus spp, Cercospora spp.*
Uninfested	38	44	48	7.02	*S. aureus, flavobactein spp, Chromobuctin spp, Micrococcus spp, Lactobacillus spp, Candida spp, Mycoplasma spp, Mucor mucedo, Rhyzopus stolonifer, Bacillus cereus, Aspergillus niger.*

Table 2: Microbial Loads and Population of Pathogens on Infested and Uninfested Maize.

	Cowpea Varieties after Second Generation				
Varieties	**Bacterial (Cfu/g) (x^{10-4})**	**Fungi/Mould (Cfu/g) (x^{10-4})**	**Yeast (Cfu/g) (x^{10-4})**	**Moisture Content (%)**	**Microorganisms Isolated/Identified**
Oloyin Infested	165	98	240	26.27	*Bacillus subtilis, Staphylococcus aureus, Flavobacterium spp, Aerococas spp, Xanthomonas spp, Servatin rubidae, Collectotrichum indimutinaun, Apergillus niger, Fubarium oxyporuin, A. flavus, Veruniculum spp, Geotricun gendidum, pericilu spp.*
Uninfested	58	2	188	5.86	*Staphylococcus aureus, Bacillus subtilis, Proteus mirabilis, Erwinia spp, Mucor mucedo, Rhyzopus stolonifer, Collectotrichum lindimuthanum.*
Drum Infested	124	68	240	14.94	*Bacillus punilus, Staphylococus epidemidis, Serratin marcescers, Cercospora spp, Lactobacillus spp, A. niger, Penicillu griscoflavum, Proteus mirabilis, Corynebacteria spp, Fusarium spp, Pediococas spp.*
Uninfested	27	1	122	6. 32	*Staphylococcus aureus, Micrococcus spp, Streptococcus spp, Bacillus cerens, Aspergillus fumigatus, Mucor mucedo, Rhyzopus spp. Collectotrichum gleosporoides.*
Sokoto Infested	101	140	320	28.35	*Pseudomonas glycinea, Chrohebacteria spp, Pseudomonas syringine, Peincillum rotatum, Cephalosporin spp, Micrococus spp, Collectotrichum spp, Rhyzoctomin spp, A. funmigatus, A. flavins, Mucor spp.*
Uninfested	27	26	83	7.72	*Serratin spp, Staphylococcus epidemidis, Staphylococcus aurens, Sacharomyces spp, Camdida spp, Corynebacterium spp, Lactobacillus spp, Collectotrichun lindimathianum, A. nigen, Rodotorula spp, A. nigericum, Flavobacterium spp. Bacillus pumilus.*

Table 3: Microbial Loads and Population of Pathogens on Infested and Uninfested.

The results of microbial load and microorganisms isolated from infested and uninfested varieties of maize (White and Yellow) were presented in table 4. The microbial load of the White maize before infestation was 28, 48 and 13 for bacterial, fungi and yeast while the microbial load was 135, 152 and 76 for bacterial, fungi and yeast after infestation. The moisture content was 7.74% before infestation and 16.95% after infestation. The microbial load for the yellow maize was 38, 44 and 48 before infestation and increased to 115, 120 and 125 after infestation. The moisture content increased from 7.02% to 18.30% before and after infestation. The microorganisms present include *Pseudomonas spp*, *B. cereus*, *Mucor mucedo* among other microorganisms.

	Varieties after Second Generation				
Varieties	**Bacterial (x^{10-4})**	**Fungi/Mould Cfu/g (x^{10-4})**	**Yeast (x^{10-4})**	**Moisture Content. (%)**	**Microorganisms Isolated/Identified**
White Maize Infested	135	152	76	16.95	*Agrobacterium spp, Cumphylobacter spp, Xanthomonas spp., Acinetobacter spp, Collectotrichum lindimuthium, A. fumigatus, A. flavins, Serratin spp., Curvalarin spp, Pithium spp, Neurospora sporogenes, Mucor spp, Sporotrichun spp, Glocosporium spp*
Uninfested	28	48	13	7.74	*Protus mirabilis, Lactobacillus spp, Eriwimus spp, Micrococcus spp, Bacteroides, Lactobacillus spp, Candida spp, S. saprophyticus, A. fumigates, A. nigericum, A. flavus, Sacharomyces cerevesine*
Yellow Maize Infested	115	120	125	18.30	*Helminthosporim toxicin, Cercuspora spp, Curvulavin spp, Penicillin spp, Pseudomonas spp, Erwina spp, B. cereus, S. saprophyticus, A. niger, A. flavus, A. fumigatus, Botrytis cineria, Geotrichun albidum, Mucor spp, Saccharomyces spp, Debaromyces spp.*
Uninfested	38	44	48	7.02	*S. aureus, flavobactein spp, Chromobuctin spp, Micrococcus spp, Lactobacillus spp, Candida spp, Mycoplasma spp, Mucor mucedo, Rhyzopus stolonifer, Bacillus cereus, Aspergillus niger.*

Table 4: Microbial Loads and Population of Pathogens on Infested and Uninfested Maize.

Discussion

The study established that cowpea seeds either infested or not contained pathogens. Pathogens were isolated from infested and Uninfested Oloyin and Drum cowpea after the emergence of the first and second fillia generations. This study conforms to a study earlier conducted where it was stated that cowpea production is faced with a wide range of biotic constraints like virus (Cowpea Aphid-Borne Mosaic Virus, CABMV), bacteria (*Xanthomonas campestris* pv *vignicola*), fungi (*Choanephora spp*). The study also established the fact that fungi isolated from cowpea include *Fusarium spp*. [16] had reported that fungi on cowpea seeds include *Fusarium equiseti, F. graminearum, F. semitectum, F. chlamydosporum, F. sambucium and F. subglutinans.* [17] isolated *Fusarium verticillioides (Gibberella fujikuroi*) from cowpea seeds.

The study revealed that the uninfested grains have been previously infected by pathogens present in the field before storage. A pathogenic analysis done on the uninfested grains of cowpea revealed that *Staphylococcus aureus, Micrococcus spp, Streptococcus spp, Bacillus cereus, Aspergillus fumigatus, Mucor mucedo, Rhyzopus spp. Colletotrichum gleosporoides* were present on the cowpea seeds. This conforms to a study conducted by [13] that *Ascochyta sp, Colletotrichum lindemuthianum, Rhizoctonia solani, F. oxysporum, F. solani, Macrophomina phaseolina, Septoria vignae* and *Corticium rolfsii* were the pathogens isolated from seed samples collected from the northern parts of Nigeria. Furthermore [21] isolated *Rhizoctonia solani, Aspergillus flavus, Cladosporium sp., Aspergillus niger, Penicillium sp., Fusarium oxysporum, F.solani, F.semitectum, Trichoderma viride, Curvularia lunata, Mucor sp.,* and *Verticillium sp*. from the seed samples collected from Maharashtra, India. [6] isolated *Alternaria longissima, A. flavus, A. fumigatus, A. niger, Botryodiplodia theobromae, Colletotrichum sp., Mucor himelis, M. phaseolina, R. oryzae* using agar and blotter methods. In many of the studies conducted by several researchers, some of the common pathogens were found to be associated with cowpea grainss irrespective of the geographical location or part of the world.

It was observed that as moisture increases, the microbial load of the maize and cowpea grains increases. This was indicated by a correlation between moisture contents and pathogen proliferation on the infested grains. Furthermore, a study conducted by [27] on the effect of moisture contents and temperature on storage molds, found that the higher the initial moisture contents the greater the infection of maize kernels. According to [20], the growth and development of storage fungi in grain are governed by three main factors, crop (nutrients), physical (temperature, moisture) and biotic (insects, interference competition) factors. The inclusion of moisture among the factors indicates the significance of moisture to the growth of pathogens. It was further observed that the moisture content of the infested grains increased compared to the uninfested grains. This agreed with a study earlier conducted by [12] which found that both warmth and high moisture contents can result in rapid deterioration of the grains and promote the growth of microorganisms (e.g. fungi and bacteria) and insects in the grains. In a similar study [2], found out that microbial load count of seeds increased with insect infestation. They further opined that the increase might be due to the increased moisture content which favoured the proliferation of the pathogens.

Conclusion and Recommendations

The infestation by the insects caused increase in moisture content of the grains thereby increasing the microbial load on the grains as a result of proliferations of pathogens. It was also concluded that uninfested grains from field bear certain pathogens which might be soil or air borne.

It is therefore recommended based on the results from this study that;

1. Grains of maize and cowpea should be properly handled right from harvest to preventing contamination.
2. Appropriate moisture content should be obtained before grains are stored
3. Contaminants that might habour pathogens should be removed from the grain lots.

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