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## EFFECTS OF STORAGE CONTAINER AND STORAGE CONDITION ON SEED HEALTH QUALITY OF COMMON BEAN VARIETY *ENNEPA* IN SOUTHERN GHANA

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### ABSTRACT

*Common bean (Phaseolus vulgaris L.) has been introduced in Ghana for cultivation; however, yields on farmers' fields are low due to poor seed health quality resulting in poor plant stand. This study determined the effect of storage containers (polythene bag vs plastic container) and storage environment (ambient vs cold storage temperature) on the seed health quality of common bean seeds stored for eight months. The fungal species and loads on the seeds were determined using the blotter method at 0, 2, 4, 6, and 8 months after storage. Aspergillus spp., Penicillium spp., and Rhizopus spp. were the major fungi recorded during storage. Seeds stored in plastic containers under an ambient environment showed a significantly lower incidence of Aspergillus flavus than the other treatments. Seeds stored in polythene bags under an ambient environment significantly lowered the incidence of A. niger. Seeds stored in polythene bags recorded a significantly higher incidence of Penicillium spp. after two months in storage irrespective of the storage condition. The lowest incidence of Rhizopus spp. was observed in seeds stored in plastic containers in an ambient environment. There was no observed insect infestation in storage over the eight-month duration. Seeds of the common bean variety Ennepa can safely be stored in a polythene bag under an ambient environment (25.43-29.66°C).*

**Keywords:** Plastic container, polythene bag, ambient environment, cold environment, Aspergillus flavus, Penicillium spp.

## INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is an important grain legume cultivated globally for its nutrition, food security and sustainable land use purposes (Singh *et al.*, 2022). Common bean is a new addition to the grain legume crops cultivated in Ghana. Currently, the first four varieties (Adoye, Ennepa, Nsroma and Semanhyia) officially released by CSIR-Crops Research Institute through the Pan Africa Bean Research Alliance (PABRA), are being promoted for adoption by farmers. However, farmers yields are still low, 0.8 t/ha compared to the potential yield of 2 t/ha (Yeboah *et al.*, 2021). Low seed quality could be one of the contributory factors to the low yield. Seed quality deterioration during storage has been a major problem in the humid climatic conditions (Asiedu *et al.*, 2021). In Ghana, seed producers generally stored their seeds under high temperature and high relative humidity conditions for at least 3-7 months before sale and subsequently planting and sowing. Under such unfavorable storage conditions of high relative humidity of 80 to 97% and minimum of 38 to 72% depending on the season and ecology, and mean daily minimum and maximum temperatures of 22 °C and 35 °C, respectively (Asiedu *et al.*, 2000), carry-over stocks which form about 70% of the stored seeds lose their vigor and viability (Golob *et al.*, 2006).

Storage condition significantly contributes to seed health quality deterioration. Higher temperature, relative humidity (RH) and seed moisture content (MC) predispose seeds to attack by fungi and insects (Mutai, 2018; Pessoa *et al.*, 2016; Mbofung *et al.*, 2012). Seeds maintain lower seed-borne fungi when stored at lower RH (<60%) and temperature (25 °C) (Khan *et al.*, 2018). Seeds stored in equilibrium with a RH of less than 68% inhibit microbial growth (Mutai, 2018). Incidence of seed-borne pathogen is significantly influenced by storage environment compared

to storage containers and period of storage. Storage of seeds in an un controlled condition results in significantly higher *Aspergillus* spp. (Owolade *et al.*, 2011). Storage fungi are responsible for significant deterioration and reduction in storage potential of common bean seeds. On an average 2-3% of the total stored seed is damaged by the activities of storage fungi (Khalequzzaman *et al.*, 2012).

Seed-borne pathogen associated with common bean seeds can be found externally and/or internally and may cause seed and root rots, stem cankers, wilting, necrosis, seedling blight and/or death of infected bean plants. These result in low germination, vigour, germination and field establishment as it destroys the embryo, and ultimately results in low yield (Pradhan *et al.*, 2017; Marcenaro and Valkonen, 2016). Common bean seed fungi infection levels vary depending on location of production, growing conditions, cultivar and storage environments. Estimated yield losses attributed to the incidence of common bean root rot disease ranged between 3.8-76% leading to fewer seeds per plant under commercial production conditions (Naseri and Mousavi, 2015). Fungi have different roles in deteriorating the quality of seeds due to wide range of nutrient requirements and capacity to tolerance for varying environments (Sinha, *et al.*, 1999). *Penicillium* spp. and *Aspergillus* spp. have been found to be the main storage fungi in common bean. They invade the seeds during and after maturation, causing damage when conditions are conducive for their growth (Francisco, and Usberti, 2008).

The rate of development of storage fungi during the storage period is detrimental to the viability and vigour of bean seeds. Incidence of *Fusarium oxysporum* in French bean seeds increased from initial 12.00 to 26.69%, 60 days after storage (Khalequzzaman *et al.*, 2012). Seed rot in French bean can be influenced by storage containers and storage period. Isolated fungus from bean seeds (19-69%)

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followed by *R. solani* (4.2-75%), *M. phaseolina* (4.2-48%) and *F. oxysporum* (2.1-40%) (Nasari and Mousavi, 2015).

The objective of this study was, therefore, to determine the effect of different storage containers and storage condition on the seed health quality of common bean var *Ennepa* in storage.

## **MATERIALS AND METHODS**

### **Seed Source and Seed Multiplication**

Seeds of common bean variety *Ennepa* (white seeded) were sourced from CSIR-Crops Research Institute. Seeds harvested from seed multiplication field established in May, 2019 were used. Manual threshing was done and the seeds were cleaned, sorted and dried by putting harvested pods in sack and carefully beaten with sticks to prevent mechanical injuries, 11% moisture content for the commencement of the storage experiment.

### **Experimental Design and Trial Management**

The experimental design was 2x2 factorial in completely randomized design replicated three times. The first factor was storage containers at two levels, thus plastic containers and polythene bags. The second factor was storage conditions at two levels, thus ambient and cold. One set of each seed lot (50 kg) was kept in moisture-proof polythene bags, (10 cm x 10 cm and 0.2 mm thick) and heat-sealed. The other half (50 kg) of the seeds were packed in plastic containers (0.75 mm thick, 8.4 cm in diameter and 17.5 cm in height). One set of four treatment combinations were stored for 8 months in a warehouse constructed for ambient seed storage at a temperature range of 27-32 °C. Another set of the same four treatment combinations were also stored for the same 8 months in a cold room at a temperature range of 15 + 2

°C. Seed samples were taken at 0, 2, 4, 6, and 8 months in storage for seed health quality analyses. Data on storage room temperature and relative humidity were also taken during the 8 months storage period using HTC 160 Thermo-hygrometer data logger (Dongguan Xintai Instrument Co., Ltd, China).

### **Determination of Seed Fungal Species and Load**

The fungal kinds and loads were determined as a measure of the health status of the seeds using the blotter test after the seeds were stored for 0, 2, 4, 6, and 8 months. One hundred and fifty seeds per treatment were taken and plated on moist blotter paper in Petri dishes. The plated Petri dishes were incubated at 26 °C for 7 days under an alternating cycle of 12 hours of near ultraviolet light and 12 hours of darkness. The seeds were then examined under a low power compound microscope to identify and record the fungal pathogens present and their percentage incidence calculated using the formula by Nandi *et al.* (2017) as follows:

$$\text{Percent Fungal Incidence} = \frac{\text{No. of infected seeds}}{\text{Total no. of seeds plated}} \times 100$$

For insect infestation, seeds with exit holes which was indicative of insect infestation were counted and recorded. The holed seeds were submersed in water for 24 hours to soften and then cut to analyze the internal structures. The number of seeds with insect eggs, larvae, pupae or adults was recorded and expressed in percentages for each treatment (Santos *et al.*, 2016).

Data analysis was performed using Statistix 9.1 statistical software package. Tukey's Honestly Significant Difference (HSD) test was used to separate treatment means at  $P < 0.05$ .

## RESULTS

### Temperature and Relative Humidity Conditions of the Ambient and Cold Room Environments

The highest temperature under the ambient condition was 33.4 °C recorded in November, 2019 while the lowest was 25.4 °C recorded in July, 2020 (Table 1). The highest and lowest relative humidity recorded were 95.45% and

52.17% in October, 2019 and February, 2020, respectively. Under the cold storage condition, the lowest temperature was observed in May, 2020 (15.9 °C) and the highest in January, 2020 (16.81 °C). The highest RH under the cold condition was 70.30% in October, 2019 whilst the lowest was 68.54% in January, 2020.

**Table 1. Temperature and relative humidity recorded during the storage period**

Month	Monthly Average Temperature and Relative Humidity			
	Ambient Condition		Cold Condition	
	Temperature (°C)	Relative Humidity (%)	Temperature (°C)	Relative Humidity (%)
Oct. 2019	26.35	95.45	16.57	70.30
Nov. 2019	33.40	68.53	16.17	68.79
Dec. 2019	28.51	73.92	16.33	68.70
Jan. 2020	29.66	80.44	16.81	68.54
Feb. 2020	29.24	52.17	15.79	69.74
Mar. 2020	29.45	72.59	15.63	69.46
Apr. 2020	26.72	66.35	15.58	69.39
May. 2020	29.06	73.70	15.58	69.18
Jun. 2020	26.72	77.69	15.57	69.54
Jul. 2020	25.43	85.80	15.58	69.47

### Effect of Storage Container and Storage Condition on Percent Incidence of *Aspergillus flavus* of Common Bean Var. *Ennepa* in Storage

Among the storage containers, there were no significant ( $P > 0.05$ ) treatment effects on percent incidence of *Aspergillus flavus* at the initial and four months in storage (Figure 1a). At two and eight months in storage, seeds kept in the plastic containers significantly recorded higher percent incidence of *Aspergillus flavus* than those observed in the polythene bags. However, at six months in storage significantly higher percent incidence was recorded in

seeds kept in the polythene bags than in the plastic containers.

Among the storage conditions, there were no significant ( $P > 0.05$ ) differences on the percent incidence of *Aspergillus flavus* at the initial and six months in storage (Figure 1b). However, significantly ( $P < 0.05$ ) higher percent incidence were recorded in seeds stored at the ambient condition than those stored at the cold condition at two and eight months in storage.

### **Interactive Effect of Storage Container x Storage Condition on Percent Incidence of *Aspergillus Flavus* of Common Bean Var *Ennepa* in Storage**

There were significant ( $P>0.05$ ) storage containers x storage condition interaction on the *Aspergillus flavus* incidence at four months in storage. Seeds kept in the plastic containers and stored under the ambient condition showed significantly ( $P<0.05$ ) lower percent incidence of *Aspergillus flavus* than the other treatment interactions. The highest percent incidence was observed in seeds kept in the polythene bags and stored under the ambient condition (Figure 2a). Among the storage containers, seeds packaged in plastic containers recorded significantly higher percent incidence of *Aspergillus flavus* than those packaged in the polythene bags (Figure 2b).

### **Effect of Storage Container and Storage Condition on Percent Incidence of *Aspergillus Niger* (A. *Niger*) on Common Bean Var. *Ennepa* in Storage**

Among the storage containers, there were no significant ( $P>0.05$ ) difference at the initial, two and four months in storage (Figure 3a). At six and eight months in storage, however, seeds that were kept in the plastic containers recorded significantly ( $P<0.01$ ) higher percent incidence of *Aspergillus niger* than those recorded in seeds kept in the polythene bags.

Among the storage conditions, there were no significant differences at the initial, two and four months in storage (Figure 3b). At six months in storage, seeds stored in the ambient condition recorded the lowest percent incidence of *Aspergillus niger*, significantly ( $P<0.01$ ) lower than those stored under the cold condition.

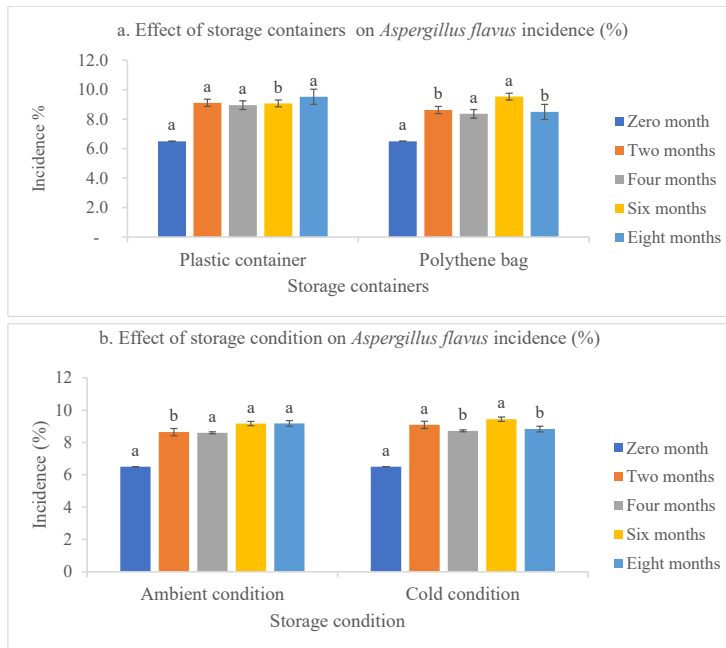
### **Interactive Effect of Storage Container x Storage Condition on Percent Incidence of *Aspergillus Niger* (A. *Niger*) of Common Bean Var *Ennepa* in Storage**

There were no significant differences among the treatment interactions for the percent incidence of *Aspergillus niger* at two, four and eight months in storage. However, significant differences were recorded at six months in storage (Figure 4). Seeds kept in the polythene bags and stored under the ambient condition significantly ( $P<0.01$ ) lowered the percent incidence of *Aspergillus niger* than the other interactions. The highest incidence occurred in seeds kept in the plastic containers and stored under the cold condition (Figure 4).

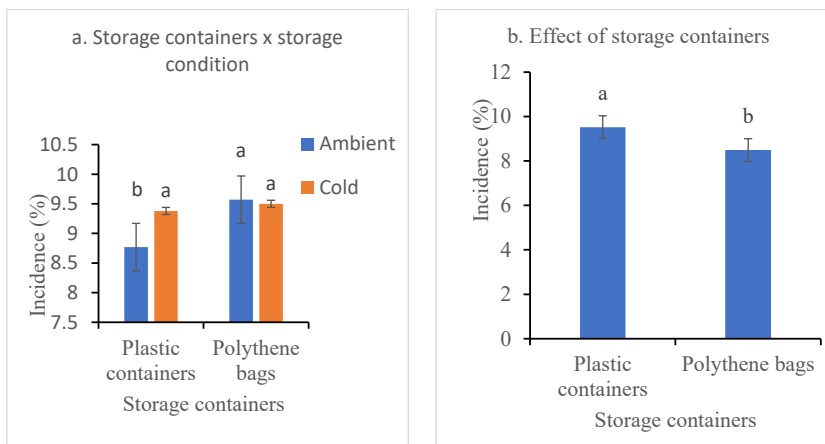
### **Effect of Storage Container and Storage Condition on Percent Incidence of *Penicillium* spp. on Common Bean Var *Ennepa* in Storage**

Among the storage containers, there were no significant differences recorded except at eight months in storage (Figure 5a). Seeds kept in the polythene bags recorded significantly higher percent incidence of *Penicillium* spp. than those observed in seeds kept in the plastic containers.

For the storage conditions, there were no significant differences in the percent incidence of *Penicillium* spp. at the initial, four and six months in storage. However, significant differences occurred at two and eight months in storage (Figure 5b). Seeds stored under the ambient condition had significantly ( $P<0.001$ ) higher percent incidence of *Penicillium* spp. than those stored under the cold condition at two months in storage. At eight months in storage, however, seeds stored under the cold condition recorded the highest percent incidence of *Penicillium* spp., significantly higher than those stored under the ambient condition.

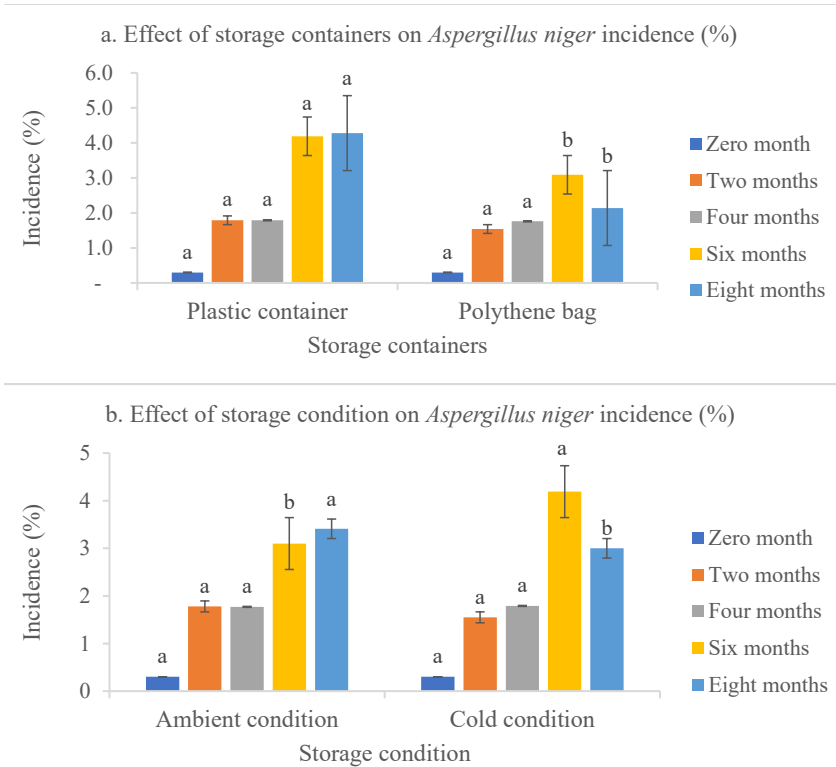


**Figure 1. Effect of storage container (a) and storage conditions (b) on percent incidence of *Aspergillus flavus* on common bean seeds in storage. Letters and vertical bars indicate significance at HSD (0.05).**

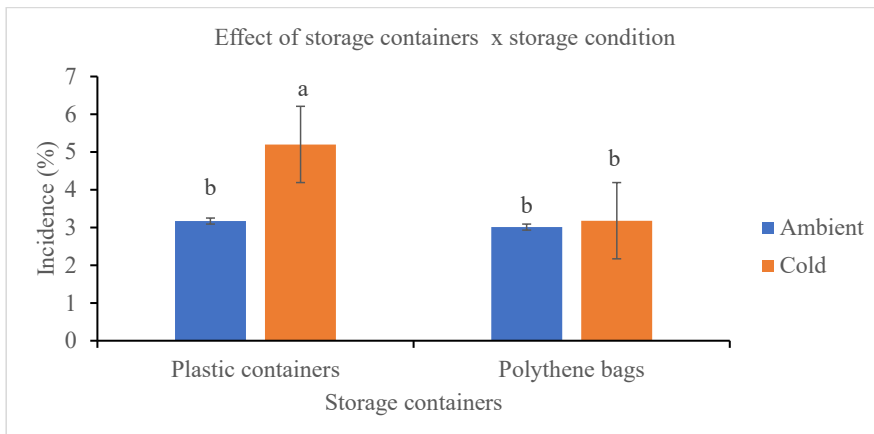


**Figure 2. Interactive effect of (a) storage containers x storage condition, and (b) effect of storage containers on incidence of *Aspergillus flavus* on common bean seeds at six months in storage. Letters and vertical bars indicate significance at HSD (0.05).**

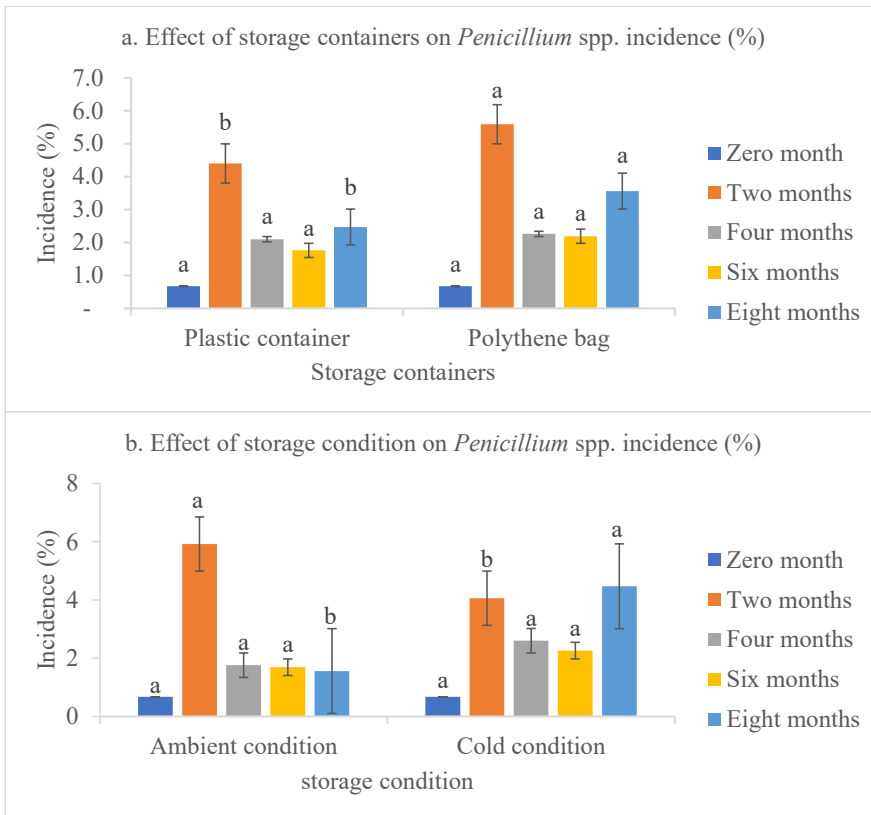
**Effects of storage container and storage condition on seed health**



**Figure 3. Effects of (a) storage containers, and (b) storage condition on the percent incidence of *Aspergillus niger* on common bean seeds in storage. Letters and vertical bars indicate significance at HSD (0.05).**



**Figure 4. Interactive effect of storage containers x storage conditions on incidence of *Aspergillus niger* on common bean seeds in storage. Letters and vertical bars indicate significance at HSD (0.05).**



**Figure 5. Effects of (a) storage containers and (b) storage condition on the percent incidence of *Penicillium* spp. on common bean seeds in storage. Letters and vertical bars indicate significance at HSD (0.05).**

**Effect of Storage Container and Storage Condition on Percent Incidence of *Rhizopus* spp. of Common Bean Variety *Ennepa* in Storage**

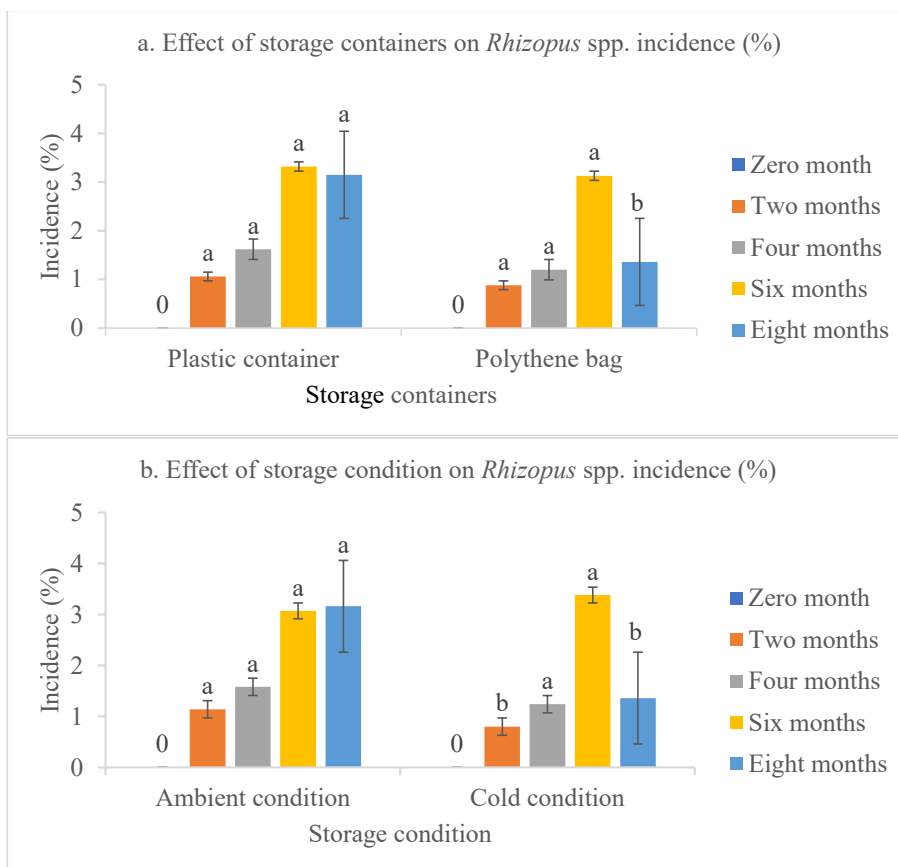
Among the storage containers, no percent incidence of *Rhizopus* spp. was observed at the initial, two, four and six months in storage (Figure 6a). However, at eight months in storage, seeds kept in the plastic containers recorded significantly ( $P<0.001$ ) higher

percent incidence of *Rhizopus* spp., than those recorded in seeds kept in the polythene bags.

Among the storage conditions, no percent incidence of *Rhizopus* spp., was observed at the initial, four and six months in storage. However, at eight months in storage, seeds stored under the ambient storage condition recorded the highest percent incidence of *Rhizopus* spp., significantly ( $P<0.001$ ) higher than those observed in seeds stored under the cold condition (Figure 6b).



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**Figure 6. Effects of (a) storage containers, and (b) storage condition on the percent incidence of *Penicillium* spp. on common bean seeds in storage. Letters and vertical bars indicate significance at HSD (0.05).**

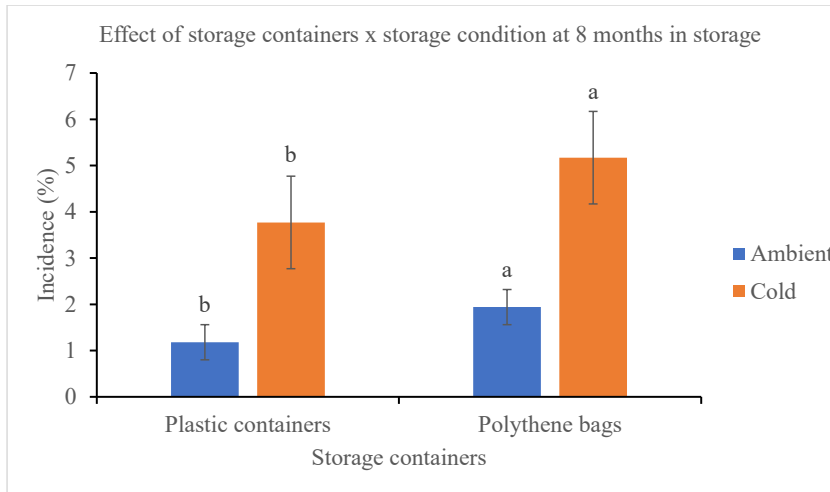
### Interactive Effect of Storage Containers x Storage Condition on Percent Incidence of *Rhizopus* spp. of Common Bean Var *Ennepa* in Storage

At six months in storage, there were significant ( $P < 0.01$ ) storage containers x storage condition treatment interactions on percent incidence of *Rhizopus* spp. (Figure 7). The lowest percent incidence of *Rhizopus* spp. was observed in seeds kept in the plastic containers and stored

under the ambient condition, which was significantly lower than the percent incidence observed in seeds packed in the polythene bags and stored under the cold condition.

### Effect of Storage Container and Storage Condition on Insect Infestation on Common Bean Var *Ennepa* in Storage

There was no insect infestation observed on the common bean var *Ennepa* in storage over the eight month study period.



**Figure 7. Interactive effect of storage containers x storage conditions on percent incidence of *Rhizopus* spp. on common bean seeds at six months in storage. Letters and vertical bars indicate significance at HSD (0.05).**

## DISCUSSIONS

Percentage incidence of seed borne pathogen is significantly influenced by storage environment compared to packaging material and period of storage. For instance, sorghum seeds stored in an uncontrolled condition resulted in significantly higher incidence of *Aspergillus* spp. (Owolade *et al.*, 2011). In the current study, the use of the polythene bags resulted in a lower percentage incidence of *Aspergillus flavus* at 8 months in storage. This could be attributed to the impervious nature of the polythene bags leading to reduced proliferation of the fungi. Storing the common bean seeds under the ambient condition resulted in 1.06 time lower percentage incidence of *Aspergillus flavus*, than the highest percentage which was observed in seeds stored under the cold condition (Figure 1b). The high level of percentage incidence of *Aspergillus flavus* observed in the cold storage condition could be attributed to the relative humidity above 68% (Table 1). Higher temperature and humidity predisposed seeds to high fungal infection as observed in other studies (Mutai, 2018; Pessoa *et al.*, 2016;

Mbofung *et al.*, 2012). The higher temperature and relative humidity can act independently, in concert or interactively in the proliferation of fungal incidence. In the current study these scenarios were observed. From Table 1, at 6 months after storage, the average temperature of 29.35 °C and 62.38% RH under ambient condition the incidence of *A. flavus* was lower compared to 15.69 °C and 69.60% RH under the cold condition. The lower RH observed under the ambient reduced the incidence.

The use of the plastic containers and storage under the ambient condition, resulted in the lowest percentage incidence of *Aspergillus flavus* at 4 months in storage. At eight months in storage, common bean seeds stored under the ambient condition showed increased percentage incidence of *Aspergillus flavus*, 1.09 times higher than the lowest percentage incidence observed in seeds stored under the cold condition (Figure 1). This result corroborates others who have reported that lower temperature and impervious packaging material reduce the rate of deterioration in common bean seed (Scariot *et al.*, 2017; Sharon *et al.*, 2015). This result is similar to another

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study where higher number of French bean seed rot occurred in pervious polythene bags compared to the plastic containers (Khalequzzaman *et al.*, 2012). In the current study, the plastic container with guage of 0.75 mm confers more imperviousness with better barrier against fungi pathogen compared to the 0.2 mm in the polythene bags.

Seeds packaged in the polythene bags and stored under the ambient condition showed significantly lower percentage incidence of *Aspergillus niger* than the other treatment interactions 6 months in storage (Figure 4). This result differs from others who have reported that lower temperature and impervious packaging material reduce rate of deterioration in common bean seed (Silva *et al.*, 2018; Scariot *et al.*, 2017; Sharon *et al.*, 2015). Storage of seeds in an uncontrolled condition, results in significantly higher *Aspergillus* spp. (Owolade *et al.*, 2011). However, the result from the present study shows that for short term storage, the polythene bags and the ambient condition can be used for common bean storage with minimal seed deterioration as viability and vigour were maintained (Adjei *et al.* 2022). In similar studies for 90 days, common bean seed quality was maintained when temperature was below 30 °C (Hendges *et al.*, 2017).

The alternating percentage incidences of *Penicillium* spp. at different storage durations can be attributed to the fact that fungi species have been observed at different temperatures, relative humidity and storage periods. This is due to their different roles in causing deterioration of the quality of seeds which is favoured by these factors (Sinha *et al.*, 1999). At 6 months in storage, the highest percentage incidence of *Penicillium* spp. was observed in seeds packed in the polythene bags and stored under the cold storage condition. Relative humidity above 68% create conducive environment for fungi growth, as explained above the relative humidity in

the cold condition was higher than 68%. In the present study, the relative humidity, storage duration and pervious packaging material, may have caused the increase in the percentage incidence of *Penicillium* spp. in the cold storage condition and this is similar to the finding by Mutai (2018).

Compared to the polythene bags, the plastic containers have better imperviousness (O dian, 2004); therefore seeds stored in the plastic containers were expected to show lower percentage incidence of *Rhizopus* spp. This was not the case in the present study as the seeds in the polythene bags had lower *Rhizopus* spp. levels. However, this result compares well with that of Nahar *et al.*, (2009) who reported lower percentage fungal increase in bean packaged in the polythene bags compared to tin, at 69 days in storage. In another study, seeds packed in the polythene bags maintained seed quality better than pervious materials such as jute sack and cloth (Silva *et al.*, 2019; Patel *et al.*, 2018; Sena *et al.*, 2016).

Although no seed treatment chemical was used, no insect infestation was observed during the 8 month storage period. The result indicates that either the factors studied were able to prevent insects' infestation within the period or common bean insects were not prevalent in the locations where the study was conducted. Other studies have observed insect infestations with increased storage period and moisture content (Tibagonzeka *et al.*, 2018; Pessoa *et al.*, 2016). The present results could also be attributed to the fact that the crop is relatively new in Ghana and therefore low or no hot spots for storage insects exist. It is therefore, recommended that this study is repeated when the crop has been cultivated for a long time in Ghana, to ascertain which of the two factors mentioned above contributed to the no insects infestation.

Seeds of the common bean variety Ennepa can safely be stored in the polythene bag and

stored under ambient condition. This will not only preserve the seed quality but will also reduce cost associated with the operation and maintenance of cold storage condition.

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