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Possible Association Levels between Fertilizer (300 kg/Ha NPK) Application and Fungal Incidence and Viability of Stored Maize Seeds

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Abstract

The effect of application of 300 kg/ha NPK fertilizer on viability of maize (*Zea mays*) seeds and incidence of fungi in maize seeds was examined. Fertilizer application was done 2 weeks after planting. Control experiment had no fertilizer application. Maize cobs were harvested 11 weeks after fertilizer application and brought into the laboratory. Plating of seeds and incubation was done for five days and repeated for four weeks. Petri plates were later scored for viability and fungal incidence. Data obtained were subjected to ANOVA (Analysis of Variance) using Generalized Linear Model (SAS). Isolated fungi included *F. verticillioides*, *Fusarium* species, *A. flavus*, and *A. niger*. For viability, F values for model (P>0.0004) and week (P>0.0001), were highly significant. Viability of treated maize seeds increased significantly at higher weeks of storage (p=0.01) but not significantly different from control (p=0.05). Incidence of all isolated fungi in treated seeds increased significantly at higher weeks of storage. Incidence of *Fusarium* species (p=0.01), and *A. flavus* (p=0.05) in treated seeds were significantly lower than in control. For all isolated fungi, F values for week (P>0.0001), fungus (P>0.0001), treatment (P>0.0003), interaction between treatment and fungus (P>0.0001), and interaction between week and fungus (P>0.0001) were highly significant. *Fusarium* spp. including *F. verticillioides* and A. flavus (p=0.01) were prevalent in the stored seeds. Farmers need to pay continuous attention to appropriate measures of NPK fertilizer application, for yield improvement and reduction in fungal incidence. They may be advised to use seeds stored under appropriate conditions for appropriate length of time for planting.

Keywords: Viability; Fungal incidence; *F. verticillioides*; *Fusarium* species; *A. flavus*; *A. niger*

Introduction

Maize (*Zea mays*) is a cereal widely grown throughout the world in a range of agro-ecological environments. It is known and called by different names around the world depending on the different locality [1,2]. It plays a vital role in the diet of millions of African people due to its high yield per hectare, wide range of food uses, ease of storage, amidst other characteristics. It is a multipurpose crop used for food, fodder, and grains as well as production of oil, starch and glucose [2]. Maize (*Zea mays*) is an important staple cereal after sorghum and millet with wide geographical spread in terms of production and utilization among the cereals. It is known to be a major source of income to farmers including many resource-poor farmers in many developing countries of the world [3].

There are however several diseases affecting maize being caused by fungi, bacteria, viruses including several insects and pests. The major genera of fungi commonly encountered by maize in tropical regions are *Fusarium*, Aspergillus and *Penicillum* [4-7]. Fungi are important diseases of maize (*Zea mays*) and rank highest as the cause of deterioration and loss of maize [8]. They account for 75% of pathogens found in seeds [9] and they could cause about 50-80% damage on maize during the storage periods if conditions are favourable for their development. However, the quality of maize seeds, amongst other seeds cannot be over- emphasized [10].

Inorganic fertilizer has been reported to exert strong influence on plant growth, development and yield [11]. NPK fertilizer application has actually been documented to significantly increase plant height, stem girth, number of leaves, leaf area, leaf area index, dry matter accumulation apart from the yield of maize [12-14]. However, there has been conflicting reports about the benefits and adverse effect of fertilizer application on crops. Ayoola and Adeniyan [15] reported that the use of inorganic fertilizer sometimes lead to reduced yield and nutrient imbalance amongst other effects. The experiment seeks to examine probable effect of application of 300 kg/ha NPK fertilizer and length of storage on the incidence of fungi in the maize (*Zea mays*) seeds as well as on viability of the maize seeds.

Materials and Methods

Planting in the field

Maize seeds were planted at the Institute of Agricultural Research and Training (IAR&T), Moor Plantation, Ibadan. The land was ploughed and harrowed at minimal tillage level so as not to wear away the top soil. Two seeds were planted per hole at a spacing of 75 cm by 25 cm and the maize seedlings were later thinned to one plant per stand. The treatment (300 kg/hectare of NPK Fertilizer) was applied two weeks after planting. The planting was done in 3 plots and replicated three times. Weeds were controlled using herbicides while insects and rodents were also prevented as good as possible.

Harvesting and shelling of maize seeds

The cobs were harvested randomly after 13 weeks of planting and

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were labeled accordingly before they were taken to the laboratory where husking and shelling were done. The shelled maize seeds were dried in the sun to 15% moisture content [2,9], transferred into sterile sample bags, and labeled accordingly before they were stored at room temperature.

Culturing the maize seeds in the laboratory

The 'blotter method' [16] was used to culture the seeds in the laboratory. Five seeds, that have been surface sterilized by soaking in 2% Sodium Hypochlorite (NaOCl) for 2 minutes and later dried between double layers of Whatman filter paper, after rinsing in 5 exchanges of sterile distilled water, were transferred each into different petri plates in three replicates with the use of sterile forceps. This was done for maize seeds from the three plots. The petri plates were covered, and incubated for 5 days at room temperature. The experiment was done for 4 different weeks after storage.

Data collection and analysis

The petri plates were later observed for the incidence of fungi and viability of maize seeds. The data collected were subjected to analysis of variance (ANOVA) using the General Linear Model option of SAS [17]. This was done to evaluate the effects of the fertilizer application on the incidence of fungi in the maize seeds and also on the viability of the maize seeds after different weeks of storage.

Results

The fungi that were isolated were *F. verticillioides*, *Fusarium* species, *Aspergillus flavus*, *A. niger* and two other unidentified fungi. Table 1 shows the ANOVA table for viability of maize seeds treated with 300 kg/ha NPK in two locations, after weeks of storage. The *F* values for model (P>0.0004) and week (P>0.0001) were both highly significant. However, *F* values for treatment (P>0.4774), location (P>0.4774) and plot (P>0.2219) were not significant. Table 2 shows viability of the treated maize seeds at different weeks of storage. There was no significant difference in the viability of the maize seeds between weeks 1 and 2, as well as between weeks 3 and 4 after storage. However, the viability of the treated maize seeds at weeks 3 and 4 was significantly higher than that at weeks 1 and 2 after storage (p=0.01). The viability of treated maize seeds compared with the control is also shown in table 2. There was no significant difference in the viability of maize seeds that received 300 kg/ha NPK when compared with the control (p=0.05).

Source	DF	Means Square	F. Value	*P>F*
Model	15	804.2593	3.00	0.0004**
Week	3	2269.4444	8.47	0.0001**
Plot	2	408.3333	1.52	0.2219
Location	1	136.1111	0.51	0.4774
Treatment	1	136.1111	0.51	0.4774
Replicate	2	158.3333	0.59	0.5555
Error	128	268.0556		
Total	143			

**=Highly significant

Table 1: ANOVA table for viability of maize seeds treated with 300 kg/ha NPK, in two locations after weeks of storage.

Week	Means	Treatment	Means
1	83.89[a]	300 kg/ha NPK	88.61[a]
2	81.67[a]	Control	90.56[a]
3	97.22[b]	$L.S.D_{0.05}$	5.3993
4	95.56[b]	R^2	0.2601
$L.S.D_{0.01}$	10.09		
R^2	0.2601		

Means with different letters are significantly different from each other

Table 2: Viability of the treated maize seeds compared with control and at different weeks of storage.

Fusarium species		*F. verticillioides*	
Week comparison	Means	Week comparison	Means
4-3	42.78*	3-4	2.22
4-2	73.33*	3-2	16.11*
4-1	73.62*	3-1	18.49*
3-2	30.56*	4-2	13.89*
3-1	30.84*	4-1	16.27*
2-1	0.29	2-1	2.38
t	2.6148	t	2.6148
R^2	0.6548	R^2	0.2576

Significant comparisons are indicated by*

Table 3: Incidence of *Fusarium* species and *F. verticillioides* in maize seeds treated with 300 kg/ha NPK between weeks of storage (p=0.01).

A. flavus		*A. niger*		Other two unidentified fungi	
Week comparison	Means	Week comparison	Means	Week comparison	Means
3-4	18.89*	4-3	5.56	4-1	2.68
3-1	23.71*	4-2	7.78	4-3	4.44
3-2	23.89*	4-1	10.46*	4-2	5.56*
4-1	4.83	3-2	2.22	1-3	1.76
4-2	5.00	3-1	4.91	1-2	2.87
1-2	0.18	2-1	2.68	3-2	1.11
t	2.6148	t	2.6148	t	2.6148
R^2	0.2834	R^2	0.2517	R^2	0.1349

Significant comparisons are indicated by*

Table 4: Incidence of *Aspergillus flavus, A. niger* and other unidentified fungi in maize seeds treated with 300 kg/ha NPK between weeks of storage (p=0.01).

Table 3 shows the incidence of *Fusarium* sp. and *F. verticillioides* in the maize seeds treated with 300 kg/ha NPK at different weeks of storage. The incidence of *Fusarium* sp. was significantly higher at week 4 than at weeks 1, 2 and 3 after storage. The incidence of the fungus was also significantly higher at week 3 than its incidence at weeks 1 and 2 after storage (p=0.01). There was no significant difference in the incidence of *Fusarium* sp. (p=0.01) and *F. verticillioides* (p=0.01) between weeks 1 and 2 after storage. The incidence of *F. verticillioides* in the maize seeds at weeks 4 and 3 was also significantly higher than at weeks 1 and 2 after storage. However, the incidence of the fungus at weeks 3 and 4 after storage was not significantly different from themselves (p=0.01). Table 4 compares the incidence of *A. flavus*, *A. niger* and other unidentified fungi in the maize seeds treated with 300 kg/ha NPK at different weeks of storage. The incidence of *A. flavus* at week 3 after storage was significantly higher than at weeks 1, 2 and 4 after storage (p=0.01). The incidence of *A. niger* in the treated maize seeds was not significantly different among the weeks after storage except the incidence at week 4 which was significantly different from that at week 1 after storage (p=0.01). The incidence of other unidentified fungi in the treated maize seeds was not significantly different among all the weeks of storage except at week 4 which was significantly different from the incidence at week 2 after storage (p=0.01). Table 5 shows the incidence of *Fusarium* sp., *F. verticillioides* and *A. flavus* in the maize seeds treated with 300 kg/ha NPK compared with control. The incidence of *Fusarium* sp. (p=0.01) and *A. flavus* (p=0.05) in the treated maize seeds was significantly lower than that in their respective

Fusarium sp.		*F. verticillioides*		*A. flavus*	
Treatment	Means	Treatment	Means	Treatment	Means
300 kg/ha NPK	26.39[a]	300 kg/ha NPK	80.28[a]	300 kg/ha NPK	10.28[a]
Control	45.92[b]	Control	87.04[a]	Control	16.62[b]
$LSD_{0.01}$	10.89	$LSD_{0.05}$	7.04	LSD0.05	6.18
R^2	0.6548	R^2	0.2576	R^2	0.2834

Means with different letters are significantly different from each other

Table 5: Incidence of some of the isolated fungi from maize seeds treated with 300 kg/ha NPK compared with control.

A. niger		Other two unidentified fungi	
Treatment	Means	Treatment	Means
300 kg/ha NPK	12.50[a]	300 kg/ha NPK	3.89[a]
Control	6.38[a]	Control	1.97[a]
$LSD_{0.01}$	6.35	$L.S.D_{0.05}$	2.66
R^2	0.28	R^2	0.1349

Means with different letters are significantly different from each other

Table 6: Incidence of *A. niger* and other unidentified fungi in maize seeds treated with 300 kg/ha NPK compared with the control.

Source	DF	Mean Square	F Value	Pr>F
Week	3	21514.63	63.38	0.0001**
Fungus	4	155765.83	458.91	0.0001**
Week*Fungus	12	7700.28	22.69	0.0001**
Plot	2	100.56	0.30	0.7437
Location	1	1125.00	3.31	0.0691
Treatment	1	4400.56	12.96	0.0003**
Replicate	2	83.89	0.25	0.7811
Location*Fungus	4	384.72	1.13	0.3396
Treatment*Fungus	4	3776.95	11.13	0.0001**

*=Significant, **=Highly significant

Table 7: ANOVA table for all the fungi isolated from the maize seeds treated with 300 kg/ha NPK after weeks of storage.

controls. However, the incidence of *Fusarium verticillioides* in the treated seeds was not significantly different from that in the control even though the incidence of the fungus in the treated maize seeds was lower than that in the control, (p=0.05).

Table 6 compares the incidence of *A. niger* and other unidentified fungi in maize seeds treated with 300 kg/ha NPK with their controls. There was no significant difference in the incidence of *A. niger* (p=0.01) and other unidentified fungi (p=0.05) in the treated maize seeds compared with their controls. Table 7 shows the ANOVA table for all the fungi isolated from the maize seeds treated with 300 kg/ha NPK weeks after storage. The *F* values for week (P>0.0001), fungus (P>0.0001), treatment (P>0.0003), the interaction between week and fungus (P>0.0001), and the interaction between treatment and fungus (P>0.0001) were all highly significant, while the F values for plot (P>0.7437), location (P>0.0691), and the interaction between location and fungus (P>0.3396) were not significant. Table 8 compares the incidence of all the isolated fungi from the maize seeds treated with 300 kg/ha NPK. The incidence of *Fusarium verticillioides* was significantly higher than the incidence of *Fusarium* sp., *A. flavus*, *A. niger*, and other unidentified fungi. The incidence of *Fusarium* sp. was significantly higher than the incidence of *A. flavus*, *A. niger*, and other unidentified fungi. The incidence of *A. flavus* was also significantly higher than the incidence of *A. niger* and other unidentified fungi. There was no significant difference between the incidence of *A. niger* and other unidentified fungi (p=0.01).

Fungus	Means
Fusarium verticillioides	83.75[a]
Fusarium sp.	35.83[b]
Aspergillus flavus	13.89[c]
Aspergillus niger	7.92[d]
Other two unidentified fungi	4.31[d]
$LSD_{0.01}$	2.6964
R^2	0.25

Means with different letters are significantly different from each other

Table 8: Comparing incidence of all the isolated fungi from the maize seeds treated with 300 kg/ha NPK during storage.

Discussion

The fungi isolated in this experiment agreed with the work of Niaz et al. [2] who isolated different fungi including *Fusarium* and *Aspergillus* species from maize seeds stored at different temperatures and moisture content. Mboya et al. [7] also reported 86-88% of the selected maize stored using sack and roof storage methods to be infected with different fungi including *Fusarium* and *Aspergillus* species and also contaminated with different mycotoxins. The highly significant *F* value (P>0.0004) for model for viability of the maize seeds showed the appropriateness of the model (Table 1), while the significance of the *F* value (P>0.0001) for week means that the viability of the treated maize seeds differed significantly from one week of storage to another week of storage. The non significant *F* value (P>0.4774) for treatment means that the treatment (i.e., application of 300 kg/ha NPK fertilizer) did not significantly affect the viability of the maize seeds after harvest. This fact is also corroborated by the non significant difference in the viability of the treated maize seeds compared to control (p=0.05) in table 2. The non significant *F* value for location (P>0.4774) means that the location where the maize seeds were planted had no significant effect on the viability of the maize seeds after harvest. The significant difference in the viability of maize seeds in the third and fourth weeks after storage (p=0.01) compared to that of the first and second weeks (Table 2) means that viability of the maize seeds increased significantly after third and fourth weeks of storage. This showed that the longer the seeds are stored, the more viable they are likely to become. However, this did not support the work of Niaz et al. [2] who reported significant decrease in germination of maize seeds with increase in storage time due to fungal infection. This may be because of the short period of storage employed in this experiment; perhaps if the seeds were stored for much longer periods, the fungal infection may begin to affect the viability of the seeds.

The highly significant difference in the incidence of *Fusarium* sp. (p=0.01) and *F. verticillioides* (p=0.01) at weeks 4 and 3 after storage (Table 3) compared to weeks 1 and 2 showed that the incidence of these fungi in the maize seeds was significantly higher at weeks 4 and 3 than at weeks 1 and 2 after storage. This means the incidence of these fungi in the maize seeds significantly increased at higher weeks of storage. It thus strongly suggests that longer period of storage could lead to significant increase in incidence of these fungi in the maize seeds. Storage fungi have been reported to be usually present in small quantities before harvest and this small amount of inoculum is known to increase rapidly under favorable storage conditions like high temperatures and moisture [18,19]. Infection by *Fusarium* sp. and its contamination have also been reported to be generally influenced by many factors including environmental conditions such as climate, temperature, humidity amongst others. Appropriate condition of storage can thus be said to be a critical factor in checking significant increase of fungi in maize seeds during storage.

The highly significant difference in the incidence of *A. flavus* (p=0.01) at higher weeks of storage compared to lower weeks (Table 4) showed that the incidence of the fungus in the maize seeds increased significantly at week 3 after storage. *A. flavus* amongst other *Aspergillus* species has been reported to be a common fungus isolated from maize seeds and seeds of several other crops producing aflatoxins in stored grains [1,2,9,20]. The result obtained in table 4 showed that the incidence of *A. flavus* in the maize seeds increased significantly at higher weeks of storage. This suggests that long period of storage could lead to significant increase in incidence of this fungus and indeed of *A. niger* (p=0.01) and other unidentified fungi (p=0.01) in the maize seeds. It suggests that the longer the seeds are stored, the higher is the likelihood of significant increase in incidence of these fungi in the maize seeds. This agreed with the work of Tanaka et al. [21] who reported increase in incidence of *Aspergillus* and *Penicillium* with increase in storage under uncontrolled environmental conditions. However, the incidence of *F. verticillioides* in their experiments decreased sharply with increase in storage period under the same conditions.

The significant difference in the occurrence of *Fusarium* sp. (p=0.01) and *A. flavus* (p=0.05) in the treated maize seeds compared to their controls (Table 5) showed that the fertilizer application significantly reduced the incidence of these fungi in the maize seeds. This strongly suggests that an inverse relationship exists between appropriate measures of NPK application and incidence of these fungi in maize seeds. The fact that this was also observed in the case of *F. verticillioides* (p=0.05) even though the reduction in incidence was not significant still showed the negative effect of the fertilizer application on the incidence of the fungus. However, the non significant difference in the incidence of *A. niger* (p=0.01) and other unidentified fungi (p=0.05) in the treated maize seeds compared to control (Table 6) means that the fertilizer application did not significantly affect the occurrence of these fungi in the maize seeds. It may then be appropriate to submit that while application of this level of NPK fertilizer may impact significantly on the incidence of some fungi in maize seeds, it may not affect the incidence of others.

The highly significant *F* value for week (P>0.0001) in the incidence of all the isolated fungi (Table 7) means that the incidence of these fungi in the treated maize seeds differed significantly from week to week after storage. This is lending credence to results obtained in tables 3 and 4 where incidence of the fungi at higher weeks of storage was significantly higher than that at lower weeks of storage. The highly significant *F* value for fungus (P>0.0001) means that the incidence of all or some of the isolated fungi in the treated maize during storage differed significantly from themselves. This is justifying the results in table 8 where the incidence of all the fungi in the stored maize seeds differed significantly from themselves. The highly significant *F* value for treatment (P>0.0003) means that the fertilizer application significantly affected the incidence of all or some of the isolated fungi in the stored maize seeds. This is also establishing the results obtained in table 5 where the incidence of *A. flavus* and species of *Fusarium* in the maize seeds were significantly affected by the fertilizer application. However, the highly significant *F* value (P>0.0001) for the interaction between treatment and fungus means that the effect of the fertilizer application on the occurrence of any individual isolated fungus in the treated maize seeds differed significantly from fungus to fungus. It means that the effect of the 300 kg/ha NPK on the incidence of any one particular isolated fungus in the treated maize seeds differed significantly from one fungus to the other. This is also corroborating the results obtained in tables 5 and 6 where significant difference in the effect of the fertilizer treatment on all the isolated fungi was observed. This same highly significant interaction between treatment and fungus might as well be one of the reasons for the significant difference among the incidence of all the isolated fungi obtained in table 8. The highly significant *F* value for interaction between week and fungus (P>0.0001) means that the incidence of any individual fungus in the maize seeds differed significantly from week to week after storage. It could thus be explained that the incidence of either *F. verticillioides* or *A. flavus*, or any of the isolated fungi for that matter, in the stored maize seeds differed significantly from one week of storage to the other. Again, this is justifying the significant difference in the occurrence of the fungi in the stored seeds from one week of storage to the other as obtained in tables 3 and 4. However, the non significant *F* values for plot (P>0.7437) and location (P>0.0691) means that the incidence of the isolated fungi in the treated maize seeds did not differ significantly from plot to plot and from location to location. The non significant *F* value (P>0.3396) for the interaction between location and fungus means that the incidence of any individual fungus in the maize seeds did not differ significantly from one location to the other. It means that the location maize seeds are planted does not necessarily determine the occurrence of the fungi in the maize seeds after harvest.

The significant difference in the incidence of all the isolated fungi in the treated maize seeds (Table 8) showed the occurrence of *Fusarium verticillioides* to be significantly higher than the occurrence of all other isolated fungi (p=0.01). The results also showed that the occurrence of *Fusarium* sp. in the maize seeds was significantly higher than the occurrence of others except *F. verticillioides*. *Fusarium verticillioides* has been found to be the most widely spread and most frequent in preharvest and stored maize in Nigeria [22]. Tanaka et al. [21] also reported *F. verticillioides* to be among the fungi with highest incidence in stored maize seeds though in cold conditions. However, in the experiments of Niaz et al. [2], *Aspergillus* species was the highest species of fungi isolated from the stored maize seeds (though at 20% moisture content and at 25°C and 40°C). The significant difference in the incidence of *A. flavus* compared to that of *A. niger* and other unidentified fungi also showed that the occurrence of *A. flavus* in the treated maize seeds was also significantly higher than the occurrence of *A. niger* and other unidentified fungi, the occurrence of both of which was not significantly different from themselves. Species of *Fusarium* and *Aspergillus* are among the fungi that are commonly associated with stored agricultural produce. Amadi and Adeniyi [19] found *Fusarium* sp. and different species of *Aspergillus* including *A. flavus* and *A. niger* to be commonly associated with stored maize grains. Several storage fungi are also known to produce different types of mycotoxins which pose severe danger to both man and animals [23].

Conclusively, it can be said that the location where maize seeds are planted does not determine the viability of maize seeds after harvest as well as the incidence of fungi in them. The mostly isolated fungi from maize seeds could be said to include *Aspergillus flavus*, *A. niger* and *Fusarium* sp., amongst which *Fusarium verticillioides* is prevalent. Application of 300 kg/ha NPK fertilizer does not increase viability of maize seeds after harvest. However, application of same level of NPK fertilizer could reduce the incidence of some fungi, while it may not reduce the incidence of others in the maize seeds. It, therefore, becomes imperative for farmers to pay attention to appropriate measures of NPK fertilizer application, not only for the purpose of yield improvement, but also for the reduction of fungal incidence in maize seeds from the field. Tanaka et al. [21] reported that incidence of some fungi in maize seeds decreased with increase in storage period under uncontrolled atmosphere as compared to cold environment where higher frequency of the fungi was observed. They concluded therefore that storage under uncontrolled environment may reduce incidence of *F. verticillioides* and other important fungi in maize seeds but that this condition may also accelerate seed deterioration. According to them, storage under cold condition on the other hand, may preserve physiological quality of the seeds but may be favorable to fungal incidence. Niaz et al. [2] reported that 8% moisture as well as 4°C and 25°C temperatures to be better for the storage of maize for decreasing the chances of fungal incidence and mycotoxin production. An average local farmer stores his dry grains under room temperature. This experiment showed that the longer the maize seeds are stored under room temperature, the more viable (vigorous) they are likely to become, but the likelihood of increased incidence of fungi in them is higher. Farmers may thus also be advised to pay more attention to appropriate storage conditions that will be appropriate for any desired length of time (storage period). This may not only improve the vigour of the stored seeds, but also address to some extent, the unguarded increase in incidence of fungi in maize seeds during storage.

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