Research Article

Comparative Analysis of Impact of OBD Organic and NPK Inorganic Fertilizers on Viability and Fungal Incidence in Maize (Zea mays) Seeds

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ABSTRACT

An experiment was conducted at the experimental site of the Institute of Agricultural Research and Training, Moor Plantation, Ibadan, Nigeria to examine the effect of different combination levels of OBD organic based and 300 kg/ ha NPK fertilizers on viability and fungal incidence of harvested maize (Zea mays) seeds. Six treatment combinations of OBD and NPK fertilizers were applied as a split plot in a randomized complete block design in three replicates. The treatment levels were OBD-plus organic fertilizer at 5t/ha (OBD5), 10t/ha (OBD10) and 15t/ha (OBD15), and in combination with NPK 15-15-15 mineral fertilizer applied at 300 kg/ha: (OBD5NPK, OBD10NPK and OBD15NPK). Fertilizer NPK applied at 300 kg/ha, served as control treatment. Twelve weeks after planting, the seeds were harvested, shelled, dried and stored for 4 weeks at room temperature. They were cultured weekly using the blotter method and scored for viability and fungal incidence; while the data collected were subjected to ANOVA using the GLM procedure of SAS. Isolated fungi include: Aspergillus flavus, A. niger, Penicillium notatum, Mucor species and Fusarium verticillioides. Occurrences of F. verticillioides and A. flavus were significantly higher than other fungi in that order ($p \le 0.01$, R²=0.66). F values for viability, isolated fungi, treatment, model, week of storage, interactions between treatment and fungi, and between week of storage and fungi were all highly significant (p>0.001). Generally, fungal incidence in seeds treated with OBD only was significantly higher than in control (NPK only). However, F. *verticillioides* and A. *niger* in seeds treated with OBD only were significantly lower than in control ($p \le 0.05$). Fungal incidence in seeds treated with OBD only was significantly lower than that in seeds treated with OBD and NPK ($p \le 0.01$, $R^2=0.66$). Viability ($p \le 0.01$, $R^2=0.62$) and fungal incidence in the seeds ($p \le 0.01$, $R^2=0.66$) increased significantly with increase in week of storage. In storage, viabilities of seeds treated with OBD only were significantly higher than those treated with OBD and NPK ($p \le 0.01$). Thus, separate application of OBD fertilizer is strongly associated with lower fungal incidence in maize seeds and higher seed viability. Higher application of OBD fertilizer may be associated with increased viability and lower incidences of certain fungi in maize seeds. Appropriate use of OBD fertilizer only in the field by farmers might sustain viability and appreciably reduce fungal incidence in maize seeds during long storage.

Keywords: Fungal incidence; Viability; Maize seeds; OBD; 300 kg/ha NPK; Organic and inorganic fertilizers.

INTRODUCTION

Maize (Zea mays) is extensively cultivated all over the world [1-4] it is the third most important cereal in the world and the most important in sub-Saharan Africa, providing food and income to over 300 million resource-poor smallholders [5]. In Nigeria, it is the most important staple cereal after sorghum and millet with the widest geographical spread in terms of production and utilization [6,7]. However, the seed yield and quality have been severally reported to be affected by many pathogens [8,9]. Some of the

pathogens are known to produce different mycotoxins in maize, examples of which include aflatoxins, fumonisins, zearalenone, and ochratoxin [10-15]. Fink-Gremmels [16] reported that almost 25% of grains harvested worldwide have mycotoxin contamination.

The use of fertilizers, both inorganic and organic, in improving yield of crops cannot be over emphasized [17-21]. However, the use of inorganic fertilizer has been reported not to be helpful under intensive agriculture because it is often associated with reduced crop yield, soil acidity and nutrient imbalance [22-24]. Soil

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Received: April 08, 2019; Accepted: April 22, 2019; Published: April 30, 2019

Citation: Sobowale AA, Aduramigba-Modupe AO, Aduramigba-Modupe VO (2019) Comparative analysis of impact of OBD organic and NPK inorganic fertilizers on viability and fungal incidence in maize (*Zea mays*) seeds. Plant Pathol Microbiol 10:480. doi: 10.35248/2157-7471.10.480

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degradation, brought about by loss of organic matter accompanying continuous cropping has also been reported to become aggravated when inorganic fertilizers are applied repeatedly. Excessive nitrogen fertilizer applications is also reported to cause pest problems by increasing the birth rate, longevity and overall fitness of certain agricultural pests [25,26].

Organic fertilizers on the other hand are reported to aid abundance of soil microorganisms by providing organic matter and micronutrients. They have also been reported to improve biodiversity and long term productivity of soil [27-32]. Some studies however submitted that combined application of organic manures and mineral fertilizers gave superior effects in terms of balanced plant nutrition and improved susceptibility to diseases. Fertilizer application has also been reported to affect severity of disease caused by fungal pathogens in plants [33].

The experiment was thus conducted to examine the separate and interactive effects of inorganic fertilizer (300 kg/ha NPK) and different levels of organic based fertilizer (OBD) on incidence of fungi in maize seeds after harvest (during storage). The probable impact of the same fertilizer treatments on viability of the same maize seeds in storage was also examined.

METHODS

Planting and experimental protocols

The experiment was conducted at the experimental site of the Institute of Agricultural Research and Training, Moor Plantation, Ibadan, Nigeria. The experiment was laid out as a split plot in a randomized complete block design in three replicates. The six treatment levels were OBD-plus organic fertilizer application at 5t/ha (OBD5), 10t/ha (OBD10) and 15t/ha (OBD15), and in combination with NPK 15-15-15 mineral fertilizer applied at 300 kg/ha: (OBD5NPK, OBD10NPK and OBD15NPK). The NPK only, applied at 300 kg/ha, was used as control treatment. OBDplus is a commercial organic fertilizer from Gateway Organic Fertilizer Company, Abeokuta, Ogun State, Nigeria. The plot size was 3.0m × 4.0m (12.0 m²) and maize variety SUWAN 1 was planted at the spacing of 75 cm × 25 cm, and thinned to one plant per stand at 2 weeks after planting (WAP). The OBD organic fertilizer treatments were applied two weeks before planting, while the NPK fertilizer was applied 2 WAP. Manual weedings were done regularly to prevent competition for space and nutrients.

Storage after harvest

The cobs were harvested at 12 WAP, shelled and dried to 15% moisture content [34,35]. The dried seeds were later transferred into sterile sample bags and stored for one to four weeks at room temperature after labeling appropriately.

Viability and fungal incidence in the stored maize seeds

One week after storage, the blotter method [36,37] was used to isolate fungi from the seeds; the seeds were surface sterilized in 1% Sodium hypochlorite (NaOCl) for 30 seconds and were rinsed in five changes of sterile distilled water and later dried between layers of Whatman Filter paper. Five seeds were then transferred (equidistant) into a sterile Petri plate containing a sterile Whatman filter paper on top of a thin film of absorbent cotton wool moistened with 5 ml sterile distilled water. The experiment was done in triplicates. All plates were labeled appropriately before incubation at room temperature for 5 days. The experiment was repeated after 2, 3 and 4 weeks of storage.

Data collection and analysis

The Petri plates were observed daily (24 hour interval) and scored for viability and fungal incidence. The fungi were identified using their cultural characteristics. The treatment means were analyzed using Analysis of Variance (ANOVA) in the Generalized Linear Model of SAS (Version 9.3).

RESULTS

The isolated fungi from the stored maize seeds include Aspergillus flavus, A. niger, Rhizopus nigricans, Penicillium notatum, Mucor species, Fusarium verticillioides and another Fusarium species. Table 1 shows gives the ANOVA result for fungal incidence after different treatment combinations of OBD and 300 kg/ha NPK. F values for isolated fungi, treatment, model, week of storage, interactions between treatment and fungi, and also between week of storage and fungi were all highly significant (P>0.001). Table 2 shows the comparisons among the fertilizer treatments with regards to fungal incidence. Maize seeds that received all treatment combinations except OBD15NPK and OBD10NPK had their fungal incidence significantly higher than control ($p \le 0.01$, $R^2=0.66$). The seeds treated with OBD15 only had their fungal incidence significantly lower (p \leq 0.01, R²=0.66) than those of others; while those that received OBD15NPK had their fungal incidence significantly higher than those treated with OBD5, OBD10 and OBD5NPK ($p \le 0.01$, $R^2 = 0.66$). Table 3 gives the mean comparisons of incidences of 3 of the isolated fungi in maize seeds after different treatment combinations of OBD and NPK. Generally, the incidences of F. verticillioides and A. niger in seeds treated with different levels of OBD only were significantly lower than those in control ($p \le 0.05$). Their incidences were also significantly lower in seeds treated with OBD15NPK (F. verticillioides) and OBD5NPK (A. niger) compared to control. There was however no significant difference in the incidence of A. flavus in maize seeds treated with different levels of OBD only compared to control ($p \ge 0.05$).

Table 4 compares the significant occurrence of any two particular fungi in maize seeds after different treatment combinations of OBD and NPK. The occurrences of the two *Fusarium* species (including *F. verticillioides*) in the treated seeds were significantly higher than others ($p \le 0.01$, $R^2=0.66$). The occurrence of *A. flavus*

Table 1: ANOVA table for fungal incidence in stored maize seeds after different treatment combinations of OBD and 300 kg/ha NPK fertilizers.

Source	DF	Mean Square	F-Value	P > F
Model	80	13319.63	32.15	0.0001*
Week of storage	3	6658.46	16.07	0.0001*
Fungus	7	102551.9	247.52	0.0001*
Treatment	6	2206.91	5.33	0.0001*
Plot	2	110.66	0.27	0.7657
Treatment [*] Fungus	25	2584.08	6.24	0.0001*
Week [*] Fungus	21	8443.38	20.38	0.0001*
Plot*Fungus	14	200.55	0.48	0.9425
Rep	2	45.52	0.11	0.896
Error	1323	414.32		
Total	1483			

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 Table 2: Significant comparisons of fungal incidence among different fertilizer treatments.

Treatment Comparisons	Difference between means
OBD15 – Control (NPK only)	12.56*
OBD10 – Control (NPK only)	6.78^{*}
OBD5 – Control (NPK only)	6.22*
OBD15NPK – Control (NPK only)	-0.18
OBD10NPK - Control (NPK only)	3.65
OBD5NPK – Control (NPK only)	6.17*
OBD15 - OBD10	- 5.78*
OBD15 - OBD5	- 6.33*
OBD15 - OBD15NPK	-12.73*
OBD15 - OBD10NPK	-8.91*
OBD15 – OBD5NPK	- 6.39*
OBD10 - OBD5	-0.56
OBD10 - OBD15NPK	- 6.95*
OBD10 - OBD10NPK	-3.13
OBD10 - OBD5NPK	-0.61
OBD5 – OBD15NPK	-6.4
OBD5 - OBD10NPK	-2.58
OBD5 – OBD5NPK	-0.06
OBD15NPK - OBD5NPK	6.34*
\mathbb{R}^2	0.66
**Comparisons significant at p ≤ 0.01	

Table 3: Incidences of three fungi in maize seeds after different treatment combinations of OBD and 300 kg/ha NPK.

Variables	F. verticillioides	A. flavus	A. niger
Treatment	Means occurrence in the treated maize see		
OBD15	57.78 ^b	18.89°	2.78°
OBD10	63.33 ^b	15.56 ^{cd}	3.33°
OBD5	63.33 ^b	10.56 ^d	3.33°
OBD15NPK	61.11 ^b	26.67b	18.33ª
OBD10NPK	74.44ª	36.67ª	8.89 ^{bc}
OBD5NPK	82.22ª	33.89 ^{ab}	2.78°
Control (NPK only)	77.78ª	11.67 ^{cd}	11.11 ^b
LSD _{0.05}	10.88	7.73	6.32
R ²	0.63	0.77	0.42

Means with different letters are significantly different from each other

was significantly higher than others except the two Fusarium species ($p \leq 0.01$). The occurrence of A. niger was also significantly higher than that of Mucor sp. ($p \le 0.01$) and P. notatum ($p \le 0.05$). Table 5 shows the ANOVA result for viability of stored maize seeds after treatment with OBD and NPK. F values for model, week of storage, treatment, as well as interaction between treatment and week of storage were all highly significant. Table 6 compares the fungal incidence and viability of the treated seeds at different storage periods. The incidence of fungi in the treated seeds differed significantly among weeks of storage, the highest being observed at the 4th week after storage ($p \le 0.01$, R²=0.66). Viability of the treated maize seeds increased significantly with increase in week of storage ($p \le 0.01$, R²=0.62). Table 7 gives the significant comparisons of viabilities of the stored maize seeds after receiving different treatment combinations of OBD and 300 kg/ha NPK. The viabilities of maize seeds that received all the treatment

combinations except OBD5 were not significantly different from control (p ≥ 0.05). The viabilities of seeds treated with OBD15 only were significantly higher than those of seeds that received other treatment combinations except OBD 10 (p ≤ 0.01). The viabilities of seeds treated with OBD10 only were also significantly higher than those of seeds treated with OBD5 only (p ≤ 0.01). The viabilities of seeds treated with OBD 5 only were as well significantly higher than those of seeds treated with OBD 5 only Were as well significantly higher than those of seeds treated with OBD 5 only Were as well significantly higher than those of seeds treated with OBD 5 only Were as well significantly higher than those of seeds treated with OBD 10 NPK (p ≤ 0.05).

Table 4: Significant comparisons of fungi isolated from stored maize seeds after treatments with different levels of OBD and 300 kg/ha NPK.

Fungal Comparisons	Difference between means
F. verticillioides – Fusarium sp.	45.95**
F. verticillioides – A. flavus	47.14**
F. verticillioides – A. niger	60.87**
F. verticillioides – R. nigricans	67.46**
F. verticillioides – Mucor sp.	68.02**
F. verticillioides – P. notatum	68.57**
Fusarium sp. – A. flavus	1.19
Fusarium sp. – A. niger	14.92**
Fusarium sp. – R. nigricans	21.52**
Fusarium sp. –Mucor sp	22.06**
Fusarium sp. – P. notatum	22.62**
A. flavus – A. niger	13.73**
A. flavus – R. nigricans	20.32**
A. flavus – Mucor sp.	20.87**
A. flavus – P. notatum	21.43**
A. niger – Mucor sp.	7.14**
A. niger – P. notatum	7.70 [*]
"Comparisons significant at p ≤ 0 p ≤ 0.05	0.01, *: Comparisons significant a

Table 5: ANOVA table for viability maize seeds after different treatment combinations of OBD and 300 kg/ha NPK fertilizers.

Source	DF	Mean Square	F-Value	P≻F
Model	49	2138.36	6.82	0.0001*
Week of storage	3	18369.25	58.61	0.0001*
Treatment	6	1189.4	3.79	0.0013*
Treatment*Week of storage	18	1090.91	3.48	0.0001*
Rep	2	849.31	2.71	0.069
Error	202	313.44		
Total	300			
*Highly significant				

Table 6: Viability and fungal incidence in the treated maize seeds at different week of storage.

Week	Means of fungal incidence	Means of viability
1	13.28a	63.49a
2	18.12b	82.86b
3	27.64c	99.68c
4	31.78d	99.05c
LSD _{0.01}	3.96	8.2
R ²	0.66	0.62

Means with different letters are significantly different each other

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 Table 7: Significant comparisons of viability of stored maize seeds after different treatment combinations of OBD and 300 kg/ha NPK.

Treatment Comparisons	Difference between means
OBD15 – Control (NPK only)	5.69
OBD10 – Control (NPK only)	- 0.87
OBD5 - Control (NPK only)	- 12.22**
OBD15NPK - Control (NPK only)	- 7.22
OBD10NPK - Control (NPK only)	- 3.33
OBD5NPK – Control (NPK only)	- 4.44
OBD15 - OBD10	6.56
OBD15 - OBD5	17.91**
OBD15 - OBD15NPK	12.91**
OBD15 - OBD10NPK	9.02*
OBD15 – OBD5NPK	10.14*
OBD10 - OBD5	11.35**
OBD5 - OBD10NPK	8.89*
R ²	0.62

**Comparisons significant at $p \le 0.01$

DISCUSSION

Most of the isolated fungi from the stored maize seeds have been reported to be pre and postharvest fungi of the crop. Aspergillus, Fusarium and Penicillium are documented examples of common field and storage fungi of maize [8,38-40] concluded that Aspergillus flavus and Fusarium verticillioides are capable of colonizing maize kernels and contaminating them with mycotoxins. These fungi are reported to produce their toxins mostly in stored seeds especially grains. They are documented to be among storage fungi that can infect grains after harvest, grow on them during storage and cause mycotoxin contamination [41]. Aspergillus flavus and Fusarium verticillioides are famous for the production of aflatoxin and fumonisins respectively in several crops especially grains [42-45] also reported that Aspergillus section Flavi and several Fusarium species are the main mycotoxigenic fungi found in maize.

The significant F value (p>0.0001) for the model fitted for fungal incidence in the treated maize seeds showed appropriateness of the fitted model (i.e. goodness of fit). This means the level of occurrence of fungi in the maize seeds depends on certain other variables. The significant F value (p>0.0001) for treatment shows the important role played by the fertilizer applications in determining occurrence levels of fungi in the seeds. It shows that the effects of fertilizer treatments (OBD and/or 300 kg/ha NPK) on fungal incidence differed significantly from one treatment level to the other. It may thus be submitted that two different treatment levels will not always have the same impact on fungal incidence in the maize seeds. The significant F value (p>0.0001) for week of storage also showed the important impact of storage duration on occurrence levels of fungi in the treated maize seeds. This means the incidence of fungi in the treated seeds differed significantly from one week of storage to the other. Significant F value (p>0.0001) for isolated fungi also shows the significant differences in occurrences of the fungi in the treated maize seeds. It shows that the incidences of the isolated fungi in the treated maize seeds differed significantly among themselves. The significant F value (p>0.0001) for interaction between treatment and fungi shows that the two variables combined to significantly affect incidence of fungi in the treated seeds. This means the impact of any particular treatment level (OBD and/or 300 kg/ha NPK)

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on incidence of any particular fungus in the maize seeds differed significantly from impact of the same treatment on incidence of another particular fungus in the same maize seeds. It means one treatment level of fertilizer will have significantly different effect on occurrences of different fungi in the maize seeds. This explains that the results obtained in Tables 2 and 3 are complimentary rather than contradictory. The highly significant F value (p>0.0001) for interaction between fungus and week of storage shows that any two different fungi will always differ significantly in their occurrences in maize seeds during storage. It may thus be suggested that the same storage duration would have considerably different impact on incidences of different fungi in maize seeds.

The significantly lower fungal incidence in seeds treated with OBD15 only compared to others even at $p \leq 0.01$, strongly suggests the effectiveness of this treatment level in checking fungal incidence in maize grains compared to other treatment levels. Application of 15t/ha OBD only may then be submitted to impact on fungal incidence in maize seeds much more significantly than other treatment combinations. Its preference over other treatment levels in checking fungal incidence in maize seeds might be very important to consider. The lower occurrence of fungi in seeds treated with 10t/ha OBD only compared to those treated with 5t/ha OBD only, though not significant, is however suggestive. It might be stated that the higher the level of OBD applied, the lower the fungal incidence in the maize grains. There could be said to be a strong association between increased applications of OBD fertilizer and lower fungal incidence in maize seeds. Veresoglou et al. [33] had also reported that application of fertilizer reduces severity of plant diseases caused by fungal pathogens. Organic fertilizers have been reported to increase the abundance of soil microorganisms by providing organic matter and micronutrient for the organisms thereby improving biodiversity and long term productivity of the soil [19,27,46].

The significantly lower fungal occurrences in seeds treated with OBD10 and OBD5 compared to those treated with OBD15 NPK could be indicative of the existence of a pattern. It could be suggesting that application of any of the levels of OBD only would better check fungal incidence in maize seeds than any treatment combination of OBD and 300 kg/ha NPK. This fact is made more evident by the generally lower fungal incidence in seeds treated with OBD only compared those treated with combinations of OBD and NPK. However, the significantly higher incidences of fungi in seeds treated with different levels of OBD compared to control may as well be suggesting the important role also played by NPK in checking fungal incidence in maize seeds. It could then be generally said that applications of OBD fertilizer only or NPK only will likely impact much more appreciably on fungal incidence in maize seeds than combination of both in treatment.

The results obtained in Table 3 however strongly suggest that application of OBD fertilizer only will reduce incidences of certain individual fungi (such as *F. verticillioides* and *A. niger*) much more significantly than NPK only will do. It also shows that for certain individual fungi (like *F. verticillioides* and *A. niger*), combinations of OBD and NPK will impact more significantly on their incidences in maize seeds than application of NPK alone; whereas such may not be the case for certain other fungi (like *A. flavus*) in the same maize grains. The results from Table 3, which examined the effect of separate treatment level on incidence of individual fungus could thus be said to be a necessary compliment for that obtained in Table 2, which pooled the effect of separate treatment levels on

incidences of all isolated fungi. The results in both tables together establish the credibility of the highly significant F value (p>0.0001) for interaction between treatment and fungus in Table 1. The occurrences of *Fusarium* spp. and A. *flavus* in the treated maize seeds, which was highly significant, compared to other isolated fungi, underscore the close relationship of these fungi with maize grains. *F. verticillioides* and A. *flavus* has been severally reported to be close 'companions' of maize [39,40,44,45].

The highly significant F value (p>0.0001) for model for viabilities of the treated maize seeds shows its goodness of fit. The highly significant F value (p>0.0001) for weeks of storage strongly suggests that storage duration played a critical role in determining viabilities of the treated maize seeds. This means viabilities of the treated seeds in storage would certainly be significantly different from one storage regime to the other. The highly significant F value (p>0.0013) for treatment also underscores the important role played by the type of fertilizer treatment in affecting viabilities of maize seeds after harvest. It suggests that maize seeds that received different treatment levels of fertilizer in the field would most likely have significantly different viabilities in storage. However, the highly significant F value (p>0.0001) for interaction between treatment and weeks of storage shows that these two factors combined significantly influenced viabilities of the treated maize seeds in storage. It strongly suggests that seeds which received a particular fertilizer treatment would have significantly different viabilities in different weeks of storage. Sobowale et al. [47] also recorded a similar observation in the course of their work.

The significant difference in viability and incidence of fungi among weeks of storage shows that increased storage duration will always give significant increase in viabilities of the maize seeds as well as incidence of fungi in them. The results obtained here ascertained the credibility of the highly significant F values (p>0.0001) obtained for week of storage in the ANOVA Tables. It means there is a strong likelihood of appreciable increase in viabilities of maize seeds with long storage duration. However, if this is considered to be an advantage of long storage, it is important to note that long storage duration will not only improve viabilities of the seeds, but will also likely result in appreciable increase in their fungal incidence. Farmers who use seeds stored for long period for planting may thus be appropriately advised to be wary of this. Mycotoxin contamination as a result of fungal incidence in seeds remains a great concern till date [48].

Marin et al. [49] argued that if seeds are poorly managed in storage, it can cause increase in mycotoxins content produced by these fungi in the seeds. They submitted that *Fusarium* species, which are predominantly, field fungi can produce fumonisins at postharvest when storage conditions are poorly managed. Kossou and Aho [50] reported that fungi could cause about 50–80% of damage on farmers' maize during the storage period if conditions are favourable for their development. Though long storage of maize grains may be desirable, imperative and inevitable, appropriate measures may have to be taken, before and/or during storage to check the concomitant significant increase in fungal incidence during long storage. Beckingham [51] reported different ways by which incidence of pathogens in maize can be checked from the field before storage.

It can be generally said that either OBD or NPK fertilizer applications will most likely have similar impact on viabilities of the maize grains in storage. This was given by the non-significant difference in the viabilities of seeds that received different

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treatment levels of OBD compared with the control. When viability becomes a critical factor, farmers could then be advised to employ any of these two types of fertilizers (OBD or NPK) but not their combinations. This is shown by the significantly increased viabilities of seeds treated with different levels of OBD only over those that received combinations of OBD and NPK. The results here also suggest there is a strong likelihood of significant increase in viabilities of the maize seeds (during storage) with increased level of OBD fertilizer. A linear relationship (R^2 =0.62) can thus be said to exist between applications of OBD fertilizer in the field and viabilities of maize seeds in storage.

CONCLUSION

It can thus be concluded that application of OBD or NPK fertilizers only would give better viabilities and reduction in fungal incidences in maize seeds than combinations of both. Application of OBD fertilizer only would cause significantly better reduction in incidences of certain fungi in maize seeds than NPK application. Increased application of OBD fertilizer might also be associated with increased viability and lower incidences of certain fungi in maize seeds. Thus a strong inverse relationship exists between OBD fertilizer application and incidence of fungi in maize seeds. Longer storage duration is as well directly proportional to increased viability and fungal incidence in maize seeds. Farmers therefore need to pay more attention to the appropriate pre- and postharvest management of the crop.

ACKNOWLEDGMENTS

The authors are grateful for the support of the technical and field staff members of Land and Water Resources Management Programme, IAR&T, Moor Plantation, Ibadan. The contributions of Olasunkanmi Yetunde A., Odumboni Oluwasola M., Oyetunji Latifat B., Achea-Obuobi Anna B., Oghonoghor Ada J., and Ololade in the data collection are also appreciated.

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