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Research Article

Radial growth of three Aspergillus species isolated from two Ghanaian maize varieties Abeleehi and Obaatanpa

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ABSTRACT

Three *Aspergillus* species; *A. flavus, A giganteus, A. ochraceus*. (= *A.alutaceus*) isolated from two newly-developed maize (Zea may L.) Abeleehi and Obaatanpa under varying ambient equilibrium relative humidity (ERH's) (55, 60, 65, 70, 75, 80 85, 90, and 95%) representative of the Ghanaian ambient conditions. The humidity was provided by glycerol ; water mixtures at temperature of 28-30°C for 36 days. The vegetative (radial) growth of the three *Aspergillus* species were investigated on five solid media namely; Czapek-Dox, Malt-Extract, Maize-meal (Abeleehi), Maize-meal (Obaatanpa) and Potato Dextrose at varying temperatures of 18, 30, 35, and 40°C to determine their optimum growth conditions. Generally, the optimum growth of *A. flavus* was obtained on all media at between 30°C and 35°C . Growth was depressed at 40°C but not at 18°C except on Czapek-Dox Agar. The best medium for growth of *A. flavus* was Maize Meal Agar prepared from Obaatanpa variety. Radial growth was poorest on Potato Dextrose Agar and Czapek-Dox Agar attaining a radius of 50mm after 7 days at 30°C as compared to 86 mm after 7 days of growth at 30°C on Maize Meal Agar prepared from Obaatanpa variety. *A. giganteus* grew best at 30°C followed by 18°C on all media tested. Growth on Czapek-Dox Agar and Malt Extract Agar lagged behind the rest. A temperature of 40°C was unsuitable as radial growth remained nearly static after 2-3 days *A. alutaceus* (*A. ochraceus*) grew best at 35°C on Czapek-Dox Agar and Malt Extract Agar and on Maize Meal Agar. Radial growth of the fungus was considerably depressed at 40°C for all media tested except on Czapek-Dox Agar which at 40°C the fungus attained a radius of 30 mm in 7 days as compared with 50mm on Czapek-Dox Agar. Interestingly, radial growth of *A. alutaceus* at 35°C was nearly the same as at 40°C on Maize Meal Agar prepared from Abeleehi and Obaatanpa varieties. The radial growth of the *Aspergillus* species was influence by both the medium and the temperature of incubation.

Keywords ; Abeleehi, *Aspergillus alutaceus*, Equilibrium relative humidity, Obaatanpa and Maize-meal agar.

INTRODUCTION

Maize (Zea may. L) or corn undoubtedly is the most important cereal crop in Ghana and forms an important part of the food and feed system and also contributes significantly to income generation for rural households. Being a seasonal crop, especially in sub-Sahara Africa, maize is stored as dry grains and forms an enormous reserve of food. Unfortunately, considerable amount of grains in storage are attacked by a variety of insects, fungi and other bioderioagents. Losses in storage due to insects and fungi is estimated at 25- 30% of the annual harvest[1] and [2]. The role of fungi in quality deterioration of grains is well-documented in developed countries[3,4,5] but there is limited information regarding fungal flora

of maize in West Africa and the role such fungi play.[6] provided an extensive list of fungi associated with maize stored in Nigeria. Later, [7,8] extended the list by eleven fungal species belonging to the genera *Aspergillus* and *Penicillium.*

Two ecological categories of fungi that invade seeds are field fungi and storage fungi. Field fungi are those that invade seeds on the developing plants in the field. They may be saprophytes (e.g. *Alternaria tenuis, Cladosporium herbarum, Epicoccum nigrum*) or in some seed pathogenic fungi (e.g. *Fusarium moniliforme(=F. verticillioides)* and *Verticillium alboatrum*). Storage fungi are those that contaminate stored products. These fungi are xerophilic and the predominant species are *Aspergillus* and *Penicilium* which can survive in seeds as long as 4-8 years.[9] Grain deterioration can be severe under prolonged storage at moderately high humidities. Fungal activities lead to; loss of dry weight, death of the product and hence loss of planting material, development of off-odour, and changes in the texture of the tissues.

Recently, a new storage problem of maize grains has been reported in certain parts of Africa[10] called stackburn. Maize grains stored in woven polypropylene bags in the warehouse heated (up to 40^{O}C or more) caused discolouration of the seed coat and the germ region turning from white to varying shades of brown. The grains are subsequently downgraded and disposed off cheaply. This constitute a great loss to the farmer and the warehousing agents and above all, a great threat to food security in sub-Sahara Africa. The Crops Research Institute of the Council for Scientific and Industrial Research, CSIR of Ghana has through the Grains and Legumes Improvement Programme, developed high lysine content maize grains including Abeleehi and Obaatanpa .There is hardly any information on the mycoflora associated with these grains which have to be stored for prolonged periods as seed grains for the next planting seasons.

This investigation, however, is a followed up work by[11] who reported three *Paecilomyces* species (*P carneus, P. puntoni*, and *P. varioti*) isolated from two newly-developed Ghanaian maize varieties Abeleehi and Obaatanpa showed varied radial growth on five mycological media depending on the media and temperature of incubation. Furthermore, the culture filtrate of the three species severely depressed length of the two maize varieties by 45-90% at the highest concentration applied. The reduction in length of radicle was severer on Abeleehi variety as compared to Obaatanpa,

This paper therefore, aimed at investigating the radial growth of *Aspergillus flavus, A. giganteus* and *A. ochraceus* (= *A. alutaceus*) isolated from Abeleehi and Obaatanpa maize varieties on five different solid media at varying temperatures of 18, 30, 35, and 40^{o}C to determine their optimum growth conditions.

MATERIALS AND METHODS

Materials:

The maize varieties used Abeleehi and Obaatanpa were purchased from Aglow Seed Company, Accra. The fungal species, *Aspergillus flavus*, *A. giganteus*, *A.ochraceus* (= *A. alutaceus*) used in these investigations were isolated from the Abeleehi and Obaatanpa maize varieties.

General Methods:

Maize Sample kept under humidity chamber

Maize sample of Abeleehi and Obaatanpa varieties were kept at 55, 65, 75, 85 and 95% Equilibrium Relative humidity (ERH) provided by glycerol : water mixtures at temperature of 28-31^{o}C for 36 days.

Direct - Plating Method

The maize grains were surface-sterilized by washing in Milton's reagent (1% sodium hypochlorite + 16.5% sodium chloride) for 5 min and then rinsed with three changes of sterile water. Sodium hypochlorite treatment was used with the aim of reducing or removing completely external saprophytes which compete with pathogens. Ten (10) surfaced-sterilized grains were placed on either Sabouraud Dextrose Agar (Oxoid CM 41), Dichloran Glycerol Agar, DG 18 (Oxoid CM 727) in Petri plates without further treatment.

The plates containing Sabouraud's Agar and DG 18 were incubated until fungi grew. There were 25 replicates for each grain variety.

Serial - Dilution Method

A 10g sample of the grains was weighed and transferred aseptically into 100ml 0.1% Peptone in 250ml, Erlenmeyer flasks and then shaken in Gallenkamp Orbital at 140rev./min for 30mins. From this stock suspension, serial dilution was employed up to 1: 10v/v and spores raised either in Sabouraud;s Agar (Oxoid CM 41) or Oxytetracycline Glucose Yeast Extract Agar (Oxoid CM 545). The objective of using two media is to recover a wider range of fungal species from the incubated grains.

The plates were incubated at 28-31^{O}C until fungi grew (7-14) days.

Maintenance of stock Cultures

Stock cultures of *A, flavus, A. giganteus, A, ochraceus* (= *A, alutaceus*), were maintained on

slopes of Potato Dextroe Agar, slants in MaCartney tubes and sub-cultured every two weeks.

Preparation of Media:

Potato Dextrose Agar

Two hundred grams (200g) of Irish potato was peeled, weighed and cut into slices. The cut slices were boiled in 500ml of water to become soft thereafter strained through cheese cloth and the slurry made up to the 1litre mark. Twenty grams (20g) of glucose and fifteen grams (15g) of agar were weighed separately and added into the solution. After heating on hot-plate for a few minutes to homogenize, the medium was sterilized in an autoclave at 121^{O}C for 20mins.

Maize Meal Agar Prepared from either Abeleehi or Obaatanpa

Similarly, 200g maize weighed and blended and 500ml distilled water added. This was heated for a few minutes. The suspension was filtered through Buchner funnel to obtain a near clear solution. Twenty grams (20g) of glucose and fifteen grams (15g) agar were added and made up to 1litre mark with sterile distilled water. The medium was sterilized in an autoclave at 121^{O}C for 20mins.

Method of Inoculation:

Two diameters at right angles to each other were drawn at the bottom of the Petri plates (9cm) with grease pencil after the agar medium had set. Each plate was held in inverted position, the lid was removed and the plate inoculated at the intersection of the two diameters with conidia on 2mm Agar disks at the tip of a flamed - sterilized inoculation pin. The lid was placed back and the plates incubated in the inverted position. This method of inoculation completely obviated the usually sprinkling of powdery spores of *Penicillium* and *Aspergillus* species on plate inoculated in the upright position. In the case of *Paecilomyces* and *Fusarium* species, the agar disks bearing the inoculation was placed directly at the centre of the plate. The plates were inoculated in triplicate for each species and were inocubated at 18^{o}, 30^{o}, 35^{o},and 40^{o}C respectively.

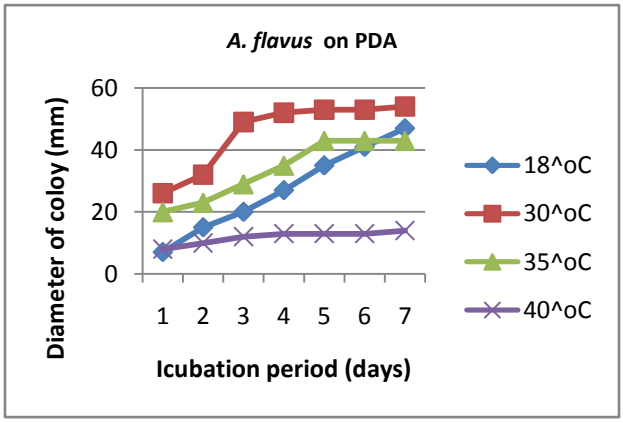
Seed Viability Test:

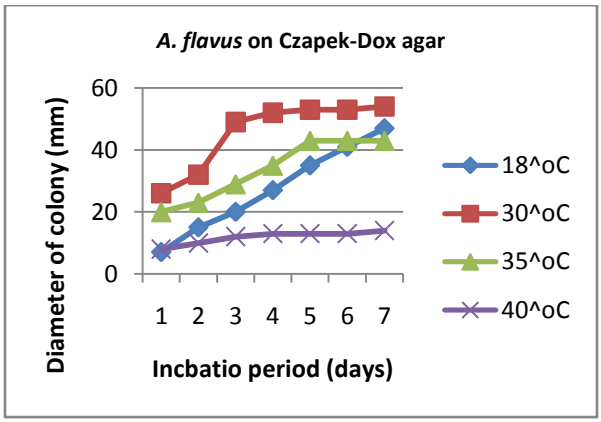
Maize seeds completely free from fungal attacks were used in the viability test. Fifty seeds each of Abeleehi and Obaatanpa varieties were cut longitudinally to expose the germ region and then placed in sterile Petri dishes containing Tetrazolium Chloride solution. There were five replicates for each maize variety. The plates were incubated in total darkness for at least three hours. Thereafter the number of seeds showing characteristic pinkish colour in the germ region were counted and the percentage viability calculated.

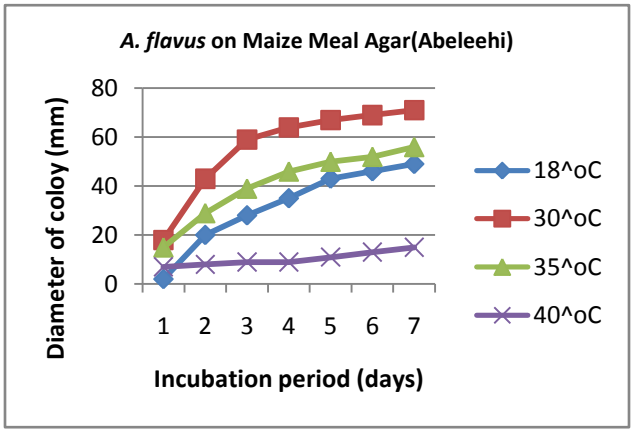
***In vitro* studies on the effect of Fungal Metabolites on Germination and Radicle Elongation**

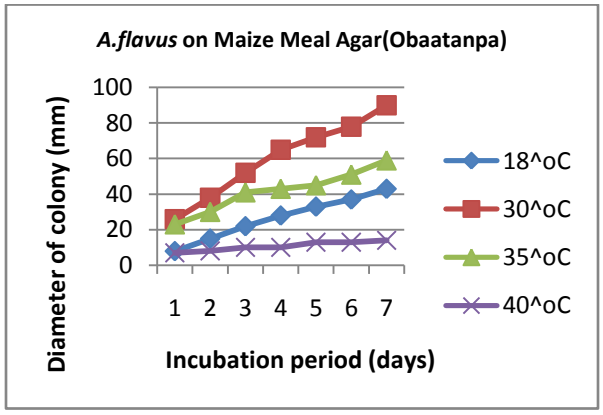
Liquid static culture filtrate of the local isolates of *Aspergillus flavus*, *A. giganteus*, *A. ochraceus* (=*A. alutaceus*) were obtained by raising the listed fungi (aliquot of 1.2 - 1.8 x 10^{5} spores/ml per flask) in either 30ml of Potato Dextrose Broth (PDB), Maize Meal Broth (MMB) prepared from both Abeleehi and Obaatanpa varieties. The mycelium was harvested after 2, 4 and 8 days at 28-31^{o}C. Vegetative growth was assessed by the convectional dry weight method and the cultural filtrates stored separately in 500ml Erlenmeyer flasks covered with black bags for immediate use.

The pH of the filtrate were taken using TOA pH meter HM - 60s (TOA Company Japan). The culture filtrate were used either undiluted or diluted (1:1 ,1:2, 1:5 and 1:10v/v). Ten (10) grains of either Abeleehi or Obaatanpa varieties were placed on sterile filter paper in 9.0cm Petri dishes moistened with 10ml distilled water (control) or with 10ml of culture filtrate of the listed fungi. There were 250 grains for each dilution level of the culture filtrates and the period of growth, that is 2, 4, and 8 days of the respective fungi. Percentage germination was calculated after 5 days incubation at 28- 31^{o}C and the length of radicles noted. The length of the radicles (hypocotyl) are given as ratio (%) to those of the control seedlings in distilled water [12]









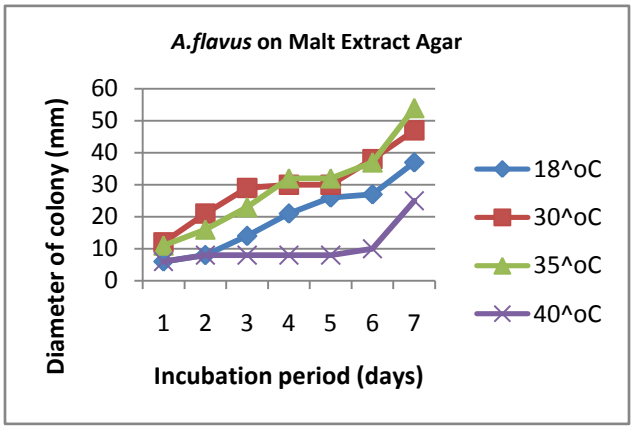


Fig 1
Radial growth of *Aspergillus flavus* on five different mycological media (Indicated).

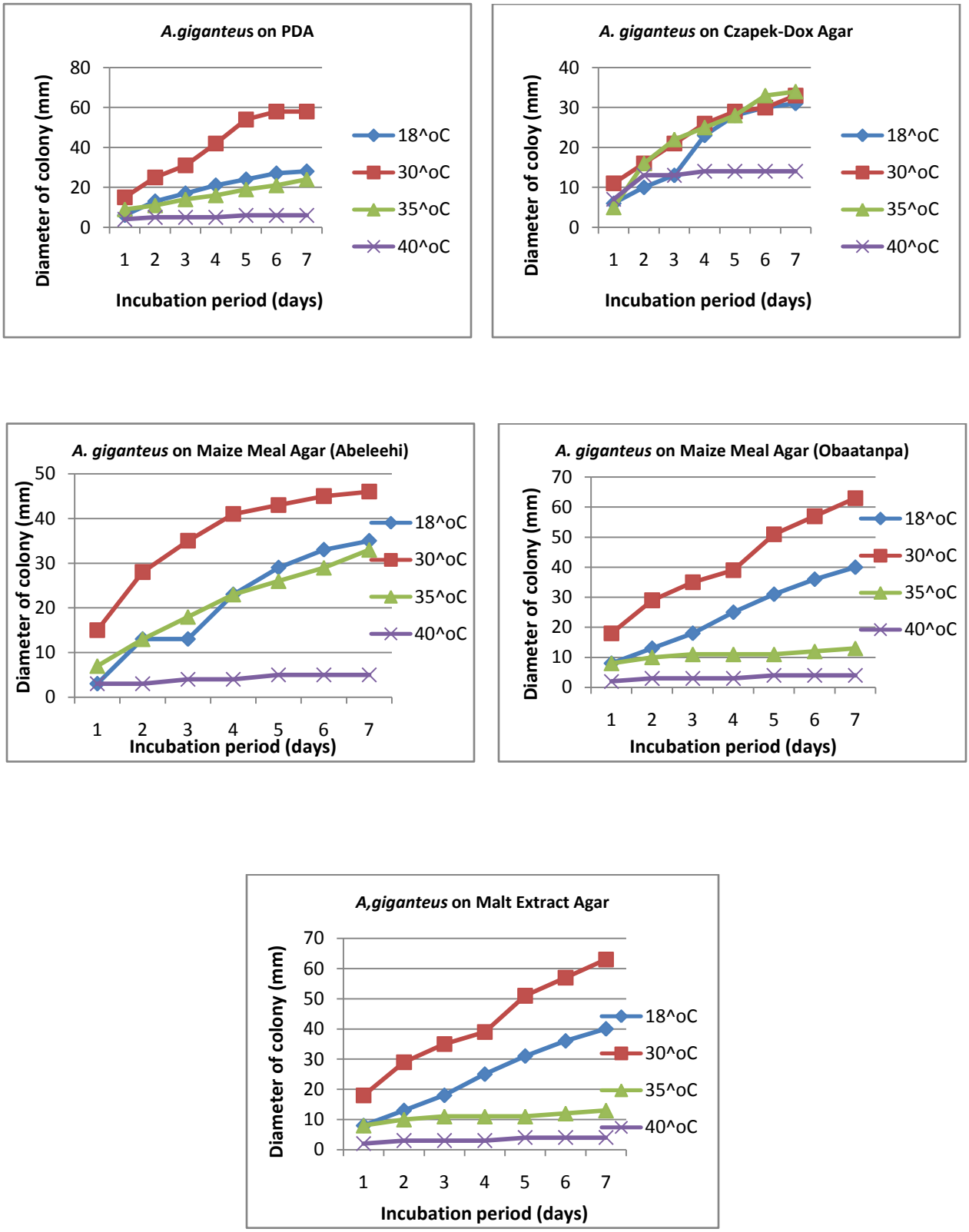


Fig.2
Radial growth of *Aspergillus giganteus* on five different mycological media (Indicated).

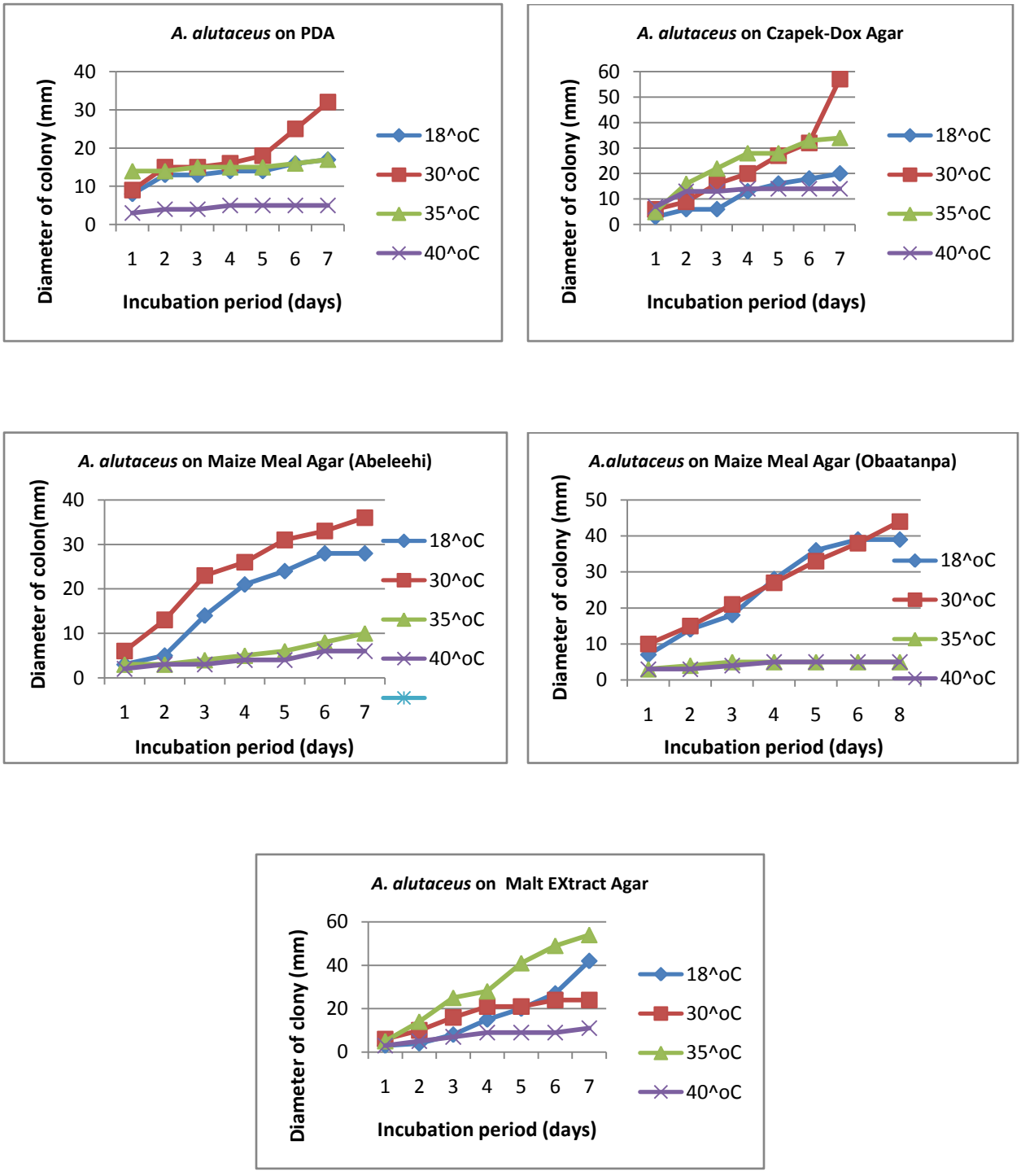


Fig.3

Radial growth of *Aspergillus ochraceus* (= *A alutaceus*) on five different mycological media indicated.

Table 1

Culture filtrate of *Aspergillus ochraceus* (= *A. alutaceus*) growing on Maize meal broth (Obaatanpa) used as germination medium for maize varieties - Abeleehi and Obaatanpa).

Age	Dilution ratio	Radicle length of abeleehi as % of control	Dilution ratio	Radicle length of obaatanpa as % of control
2 - DAY OLD	Undiluted	40	Undiluted	57
	1 : 1	50	1 : 1	62
	1 : 2	55	1 : 2	76
	1 : 5	60	1: 5	86
	1 : 10	80	1 : 10	95
4 - DAY OLD	Undiluted	20	Undiluted	28
	1 : 1	33	1 : 1	43
	1 : 2	48	1 : 2	54
	1: 5	75	1: 5	69
	1 : 10	82	1 : 10	70
8 - DAY OLD	Undiluted	32	Undiluted	26
	1 : 1	44	1 : 1	51
	1 : 2	63	1 : 2	67
	1: 5	84	1: 5	82
	1 : 10	88	1 : 10	93

Table 2

Culture filtrate of *Aspergillus ochraceus* (= *A. alutaceus*) growing on Potato Dextrose broth (Obaatanpa) used as germination medium for maize varieties - Abeleehi and Obaatanpa).

Age	Dilution ratio	Radicle length of abeleehi as % of control	Dilution ratio	Radicle length of obaatanpa as % of control
2 - DAY OLD	Undiluted	36	Undiluted	44
	1 : 1	45	1 : 1	56
	1 : 2	47	1 : 2	78
	1 : 5	82	1: 5	83
	1 : 10	91	1 : 10	89
4 - DAY OLD	Undiluted	24	Undiluted	25
	1 : 1	29	1 : 1	29
	1 : 2	35	1 : 2	43
	1: 5	39	1: 5	55
	1 : 10	57	1 : 10	61
8 - DAY OLD	Undiluted	48	Undiluted	52
	1 : 1	54	1 : 1	87
	1 : 2	72	1 : 2	90
	1: 5	80	1: 5	95
	1 : 10	94	1 : 10	97

Table 3

Culture filtrate of *Aspergillus ochraceus* (= *A. alutaceus*) growing on Maize meal broth (Abeleehi) used as germination medium for maize varieties - (Abeleehi and Obaatanpa).

Age	Dilution ratio	Radicle length of abeleehi as % of control	Dilution ratio	Radicle length of obaatanpa as % of control
2 - DAY OLD	Undiluted	38	Undiluted	70
	1 : 1	50	1 : 1	76
	1 : 2	54	1 : 2	81
	1 : 5	65	1: 5	90
	1 : 10	77	1 : 10	95
4 - DAY OLD	Undiluted	20	Undiluted	6
	1 : 1	22	1 : 1	16
	1 : 2	35	1 : 2	28
	1: 5	41	1: 5	30
	1 : 10	74	1 : 10	80
8 - DAY OLD	Undiluted	21	Undiluted	18
	1 : 1	31	1 : 1	36
	1 : 2	72	1 : 2	55
	1: 5	81	1: 5	73
	1 : 10	97	1 : 10	83

RESULTS AND DISCUSSION

In Ghana, the major cereal grains produced are maize, millet, sorghum and rice. Various seeds are produced in large quantities and are commercially very important. The major ones are legumes especially groundnuts, cowpea, bambara groundnuts, pigeon pea, winged bean among others and to a lesser extent other seeds like *Citrullus* species. Of these, maize is widely cultivated and utilized as food, livestock feed, and as a raw material for industrialized products. About 40 million bags of maize is consumed annually in Ghana and the total maize production is done by about 70% small holder farmers. Being a seasonal crop, particularly in sub-Sahara Africa maize is dried, bagged and stored in warehouses to serve as enormous food reserve in the lean seasons.

Two ecological categories of fungi that invade seeds are field fungi and storage fungi. Field fungi invade seeds on the developing plants in the field they may be saprophytes such as *Alternaria tenuis*, *Cladosporium herbarum*, *Epicoccum nigrum* or in some cases pathogenic fungi such as *Fusarium moniliforme* and *Verticillium alboatrum*. Storage

fungi are those that contaminate stored products (grains). Most of them are able to grow without free water. Most of the storage microbiota are species of *Aspergillus* and *Penicillium.* Some fungi; *Aspergillus*, *Penicillium* can survive in seeds as long as 4- 8 years [9]. Metabolites of these fungi may have beneficial or adverse effects on plant growth including suppression of seed germination, malformation and retardation of growth of seedlings.[13,14] root growth promoters[12]. Seed-borne mycoflora is one of the major components reducing seed quality, protein and carbohydrate contents, reducing germination capacity, seedling damage, resulting in the reduction of crop yield [15,16,17]. Fungi mostly involved are members of the *Aspergillus*, *Fusarium*, *Penicillium*, *Alternaria* among others. One cannot but agree with,[18,19] who reported that high degree of mould contamination in stored grains and animal feeds is a measure of their quality assurance.

A previous work by[20] on the effect of fungi on germination of six maize cultivars revealed that the effect on germination on the maize seeds cultivars significantly differ from cultivar to cultivar with the highest germination failure recorded in a locality in which maximum prevalence of various fungi were recorded. A finding which agreed with the one reported by [21].

Pathogenicity test carried out with two most frequently isolated fungi, *F. moniliforme (=F. verticillioides)* and *A. niger* on maize revealed that the two toxigenic moulds were highly effective by producing mycotoxin that retarded maize seeds germination and seedling growth [22.] In a related work [23] reported the occurrence of *F. moniliforme (=F. verticillioides)* in pre harvest maize ear which was dominant in the infected ear kernel.[24] stated that *F. moniliforme (=F.verticillioides)* produces toxic

metabolites such as *fumosin* which is responsible for many cancers in the digestive systems of human animals.

The increase attempt by man to cultivate new varieties of crops more suited to his climate has necessitated breeding programmes that require crossing of local varieties with imported grain varieties which are not indigenous to Africa. The attendant problem is the production of new varieties whose versatility in terms of drought tolerance, yield and susceptibility to local indigenous diseases have not been thoroughly investigated prior to the introduction of the crop to the farmers. About 12 *Aspergillus* species including *A, flavus, A.ochraceous* (= *A. alutaceus*) and *A, giganteus* were isolated from two recently- developed high lysine content maize grains in Ghana, Abeleehi and Obaatanpa. The radial growth of the *Aspergillus* species were investigated on five solid mycological media namely; Czapek-Dox, Malt-Extract, Maize-Meal (Abeleehi), Maize-Meal (Obaatanpa) and Potato-Dextrose at varying temperatures of 18,30 35 and 40^{o}C to ascertain the effect of temperature and culture media on their vegetative growth. The mycological media as well as temperature of incubation influenced radial growth of *Aspergillus* species (*A. flavus, A.giganteus* and *A. alutaceus* (= *A. ochraceus*) tested.

Generally, the optimum growth of *A. flavus* was obtained on all media at between 30^{o}C and 35^{o}C (fig.1). Growth was depressed at 40^{o}C but not at 18^{o}C (fig.1) except on Czapek-Dox Agar. The best medium for growth of *A. flavus* was Maize Meal Agar prepared from Obaatanpa variety. Radial growth was poorest on Potato Dextrose Agar and Czapek-Dox Agar attaining a radius of 50mm after 7 days at 30^{o}C as compared to 86 mm after 7 days of growth at 30^{o}C on Maize Meal Agar prepared from Obaatanpa variety. (fig.1). *A. giganteus* grew best at 30^{o}C followed by 18^{o}C on all media tested. Growth on Czapek-Dox Agar and Malt Extract Agar lagged behind the rest. A temperature of 40^{o}C was unsuitable as radial growth remained nearly static after 2-3 days (fig.2).

A. alutaceus (*A. ochraceus*) grew best at 35^{o}C on Czapek-Dox Agar and Malt Extract Agar and on Maize Meal Agar. Radial growth of the fungus was considerably depressed at 40^{o}C for all media tested except on Czapek-Dox Agar which at 40^{o}C the fungus attained a radius of 30 mm in 7 days as compared with 50mm on Czapek-Dox Agar (fig.3). Interestingly, radial growth of *A. alutaceus* at 35^{o}C was nearly the same as at 40^{o}C on Maize Meal Agar prepared from Abeleehi and Obaatanpa varieties. The Optimum growth for this fungus therefore was between 30 and 35^{o}C. Interestingly, two-day old culture filtrate of *A. ochraceus* (=*A. alutaceus*) isolated from Abeleehi depressed seed germination by 50 to 70% of both Abeleehi and Obaatanpa maize varieties. The same culture filtrate adversely affected radicle elongation of germinating maize grains of Abeleehi and Obaatanpa by 60-90% (Tables 1,2, and 3).

CONCLUSION

This paper contains data which reveals that the mycological media as well as temperature of incubation influenced radial growth of all the *Aspergillus* species [*A. flavus*, *A.giganteus*, and *A. alutaceus* (= *A. ochraceus*) tested]. The optimal growth of *A. flavus* was obtained between 30^{o}C and 35^{o}C. Growth however, was depressed at 40^{o}C but not at 18^{o}C.The best medium for the growth of *A. flavus* was Maize Meal agar prepared from Obaatanpa variety. Radial growth of *A. flavus* was poorest on Potato Dextrose agar and Czapek-Dox agar. *A. giganteus* grew best at 30^{o}C followed by 18^{o}C on all media tested. Growth on Czapek-Dox agar and Malt Extract agar lagged behind the remaining media. A temperature of 40^{o}C was unsuitable for *A. giganteus* as radial growth remained stationary after 2-3 days. *A. alutaceus* (=*A. ochraceus*) grew best at 35^{o}C on Czapek-Dox agar and Malt Extract agar at 30^{o}C on Maize Meal agar. Radial growth of the fungus was considerably depressed at 40^{o}C for all media tested except on Czapek-Dox agar. The optimum growth of *A. alutaceus* was between 30 and 35^{o}C. The culture filtrate of *A. alutaceus* depressed seed germination at the highest concentration by (50 to 70%) and adversely depressed (60 - 90%) radicle elongation of the germinating maize grains

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REFERENCES

1. Rawnsley J. Crop Storage Rep. Food Agric. Organ.,1969. No. PLSF/GH, 7 IX.U. N., FAO.
2. Adams JM. A review of the Literature concerning losses in stored cereals and pulses

published since 1964. Tropical Science, 1977; 19 (1): 1-28.
3. Bothast RJ, Rogers RF and Hesseltine, CW. Fungal deterioration of bags during International transport of corn soya milk: a test shipment. J. Food Sci.1979; 44(2):411-415.
4. Christensen, CM. and Kaufmann HH. Deterioration of stored grains by fungi Ann Rev. Phytopathology1965; 3: 69-84.
5. Christensen, CM. and Kaufmann HH. Grain storage. The role of fungi in quality loss. University of Minnesota Press (1969), Minneapolis, USA.
6. Broadbent JA, Oyeniran JO, and Kuku FO. A list of fungi associated with stored products In Nigeria. Nigeria stored Products Research Institute Technical Report, 1969; No.9: 57-66.
7. Oyeniran, JO. Microbiological studies on maize as poultry and livestock feed at two research farms at Ibadan, Western State, Nigeria Report. Nigeria stored Prod. Res. Inst. Tech, 1973a ;(6): 47-56.
8. Oyeniran, J.O. Microbiological examination of maize from various sources soon after harvest Rep. Nigerian Stored Prod. Res. Inst, Tech, 1973b; (3): 27-32.
9. Neergaard P. Seed Pathology. The MacMillian Press Ltd. London and Basingstoke Company 1983;(1): 283-297.
10. Tyler, P. Heat discolouration of bagged maize. World Grain, Sept.1992; 14-16.
11. Minamor AA, Odamtten GT. Radial Growth of three *Paecilomyces* Species Isolated from Two Ghanaian Maize Varieties Abeleehi and Obaatanpa on Five Different Media and the Effects of their Culture Filtrate on Seed Germination and Radicle Elongation of Abeleehi and Obaatanpa. Int.J. Curr. Microbiol. App. Sci., 2016; 5(11): 604-617.
12. Kimura, Y. Shiojima, K., Nakajima H., and Hamasaki, T.. Altechromes A and B, new plant growth regulators produced by the fungus *Alternaria* sp. Bio. Sci. Biotech, Biochem,1992b; 56(10): 1664-1665.
13. Odamtten GT. and Clerk GC. Studies on the possible use of two fungi, *Aspergillus niger* and *Trichoderma viride* as biological control agent of *Phytophthora palmivora* and the effect of their metabolites on cocoa seedlings In: " Proceedings, 9th International Cocoa Research Conference", Cocoa Producers Alliance Lagos 1985; 293-301.
14. Odamtten GT. and Clerk GC. Effect of metabolites of *Aspergillus niger* and *Trichoderma viride* on development and structure of radicle of cocoa. (*Theobroma cacao* L.) Seedlings. Plant and Soil, 1988; 106: 285-288.
15. Mushtaq SNM, Hashmi MH. Seed-borne mycoflora of Sunflower (Helianthus annuus L.). Pak. J. Bot., 2005; 37(2): 451-457.
16. Fakhrunnisa M, Hashmi H, Ghaffar A. Seed-borne mycoflora of wheat, sorghum and barley. Pak. J. Bot., 2006; 38(1): 185-192.
17. Elham SD, Modhi KL. Mycoflora of Maize (Zea mays. L) at different locations in Hail Area-Saudi Arabia. Int. J. Scientific& Technology Research, 2015; 4(6).
18. Sreenivasa RS, Dassand RS, and Jamardhana GR, Survey of Post Harvest Fungi Associated with Sorghum Grains Produced inKarnastaka. J. Plant Protec. Res. 50(3): 335-339.
19. Bashir M, Mani MA. and Kutama, AS. Seed-Borne Mycoflora of Local and Improved Wheat (Triticum aesativum L.) Cultivars in Kano, Nigeria. Bayero J. of Pure and App. Sci., 2012; 5(2): 101-103.
20. Debnath M, Sultana A, Rashid AQMB. Effect of Seed-borne Fungi on the Germinating Seeds and their Bio-control in Maize. J. Environ. Sci. & Natural Resource, 2012; 5(1): 117-120.
21. Marley PS and Gbenga O. Fungicide control oif *Stenocarpella maydis* in the Nigerian Savanna. Archives of Phytopathology and Plant Protection, 2004; 37(1): 19-28.
22. Nazar H, Altaf H, Muhammad I, Shehzad A and Tanveer H. Pathogenicity of two seed-borne fungi commonly involved in maize seeds of eight districts of Azad Jammu and Kashmir, Pakistan. African Journal of Biotechnology. 2013; 12(12): 1363-1370.
23. Sreenivasa, MY, Dass RS, ChairthRaj, AP and Janardhana GR. Mycological Evaluation of Maize Grains Produced in Karnataka (India) for the post Harvest Fungal Contamination. World App.. Sci. J., 2011; 13(4) : 688-692.
24. Abedi-Tizaki M, Sabbagh sk. Fungi associated with harvested corn grains of Golestan province in Iran. Annals Biol. Res., 2011; 2(5): 681-688.