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RADIAL GROWTH OF *PENICILLIUM DIGITATUM* AND *FUSARIUM VERTICILIOIDES* 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 DEVELOPMENT OF ABELEEHI AND OBAATANPA

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ABSTRACT

Penicillium digitatum and Fusarium verticillioide (= F. moniliforme) were isolated from two recently-developed Ghanaian maize (Zea mays. L.) varieties Abeleehi and Obaatanpa under varying ambient Equilibrium Relative Humidity (ERH's). The humidity was provided by glycerol ; water mixtures at temperature of 28-30^oC for 36 days. The culture filtrate of the two fungal species raised in Maize Meal broth prepared from either Abeleehi or Obaatanpa and Potato Dextrose broth were tested on the germination capacity of the grain of Abeleehi and Obaatanpa using the blotter test method. The vegetative(radial) growth of the two fungal species were investigated on five solid media namely; Czapek-Dox. Malt Extract, Maize Meal (Abeleehi), Maize Meal (Obaatanpa), and Potato Dextrose at varying temperature of 18, 30, 35, and 40^oC to determine their optimum growth conditions. The best temperature for optimum growth of P. digitatum was 30^oC. The temperatures 35-40^oC were unsuitable and 18^oC was intermediate between the optimum 30^oC and minImum 40^oC. Radial growth of F. verticillioide followed characteristic sigmoid curves. The best temperature for growth in all the media tested was 30^oC. Vegetative growth at 18^oC was better than that of 35 and 40^oC. The fungus never grew significantly after 2 days at 40^oC such that the diameter of the colonies of the fungus remained nearly the same during the period of the investigation. The best temperature of the fungus was obtained on Maize Meal Agar and Malt Extract Agar for F.verticillioide while Czapek-Dox Agar was the best for P. digitatum. Seed germination was depressed by 50 to 70% at the highest concentration of the culture filtrate of F. verticillioide and P. digitatum. The inhibitory effect of the metabolites of the two fungi on seed germination was gradually removed with increasing dilution of the culture metabolite. Two-day old culture metabolite of F.verticillioide severely depressed by 40-90% radicle elongation of both Abeleehi and Obaatanpa. The

metabolites of P. digitatum generally exerted severer depressive effect on the development of the radicle of Obaatanpa than that of Abeleehi variety. The maize varietal differences in response to germination and radicle elongation in the presence of the metabolites in vitro could be attributed to the intrinsic genotypic differences in the seeds and also the possible variation in the composition of the metabolites from the two fungal species.

Keywords: *Fusarium verticillioide*, *Penicillium digitatum*, Abeleehi, Obaatanpa, relative equilibrium humidity.

INTRODUCTION

Maize (*Zea mays*. L.) is an important cereal grains in the world ranking as high as rice as a staple food. In Ghana, maize is an important crop cultivated throughout the country with varying degree of success depending on edaphic and climatic factors. The areas of maize cultivation in Ghana include the whole of the Southern Ghana, Ashanti, Brong Ahafo, the Northern regions part of the Upper regions. Intensive commercial production of maize is however, found in the (a) Somanya District of the Eastern region (b) the midland maize belt of Ashanti and Brong Ahafo regions. (c) The Ho-Kpando District of Volta region and Central region. Elsewhere, substantial amounts may be produced but are mainly used for home consumption.(Minamor 1995)

Maize is used industrially as an adjuvant in the production of some alcoholic beverages, the production of small-scale alcoholic and non-alcoholic beverages, in the poultry industry as feeds for livestock and many diversified use in human cuisines.

Globally, there are numerous report on maize mycoflora ; Moshiur et al., (2016); Eiham and Modhi, (2015); Tanova et al., (2015); Ishrat and Shahnaz, (2009). According to Nazar et al (2013) maize is infected and affected by different microbes which not only impede its growth but also cause low yield. In another related work, Chhokar 2001 reported that microbial infestation of maize is responsible for 30% total food loss globally. Of these, the greater part of the loss is due to mycofloral infection and deterioration (Agrios, 1997; Chandler, 2005).

Healthy seeds, that is free from seed-borne pathogens is a prerequisite for high crop production and agricultural success (Moshiur et. al., 2016). In Ghana, in order to produce drought resistant, high yielding, disease resistant, early maturing varieties of crops to feed the ever-increasing human population, there has been breeding programme that require crossing of local varieties with imported grain varieties which are not indigenous to Africa. The attendant problem is that man has to introduce new species and varieties into territories where they are not indigenous, to grow the same crop over wide areas to facilitate its hauling and harvesting of the produce and to produce maximum yields by crop husbandry. All these factors have contributed to the incidence and spread of seed-borne disease and the growing need for further investigation and control of seed-borne pathogens, particularly for the genetically modified cereal/grains.

Recently, the Crop Research Institute of the Council for Scientific and Industrial Research (CSIR) of Ghana has through its grains and Legumes Programmes developed high Lysine content maize grains

including Abeleehi and Obaatanpa which are being sold as seed grains for the next planting seasons to the local farmers.

Thirty different fungal species were isolated from Abeleehi variety as compared to twenty-eight from Obaatanpa variety stored under the same equilibium relative humidity condition and temperture.(Minamor 1995). The culture filtrate of three *Paecilomyces* species(*P. carneus, P. puntoni*, and *P. varioti*) and three *Aspegillus* species(*A. flavus, A.ochraceous*(= *A, alutaceous*), and *A. giganteous*) isolated from Abeleehi and Obaatanpa were found to depress significantly the radicle elongation and seed germination of both Abeleehi and Obaatanpa; (Minamor and Odamtten, 2016; Minamor and Odamtten, 2017).

The aim of the current study is to test the culture filtrate raised from maize meal broth either from Abeleehi or Obaatanpa of three other fungal species *Penicillium digitatum, P. expansum* and *Fusarium verticillioides* (= *F. moniliforme*) isolated from Abeleehi and Obaatanpa on the germination capacity of the two maize varieties and also determine the optimal radial growth of the fungal isolates on five mycological media at varying temperatures.

2. MATERIALS AND METHODS

2.1 Materials:

The maize varieties used Abeleehi and Obaatanpa were purchased from Aglow Seed Company, Accra. The fungal species, *Penicillium digitatum, Penicillium expansum*, and *Fusarium verticilioides* (= *F. moniliforme*), used in these investigations were isolated from the Abeleehi and Obaatanpa maize varieties.

2.2 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 5mins 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, *Penicilium digitatum, P. expasum,* and *Fusarium verticilioides(=F.moniliforme),* were maintained on slopes of Potato Dextrose Agar, slants in MacCartney tubes and sub-cultured every two weeks.

Preparation of Media

(i) 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.

(ii) 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 inoculated 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 Development**

Liquid static culture filtrate of the local isolates of *Penicilium digitatum, P. expasum and Fusarium verticilioides,* 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 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 (Kimura et. al., 1992a).

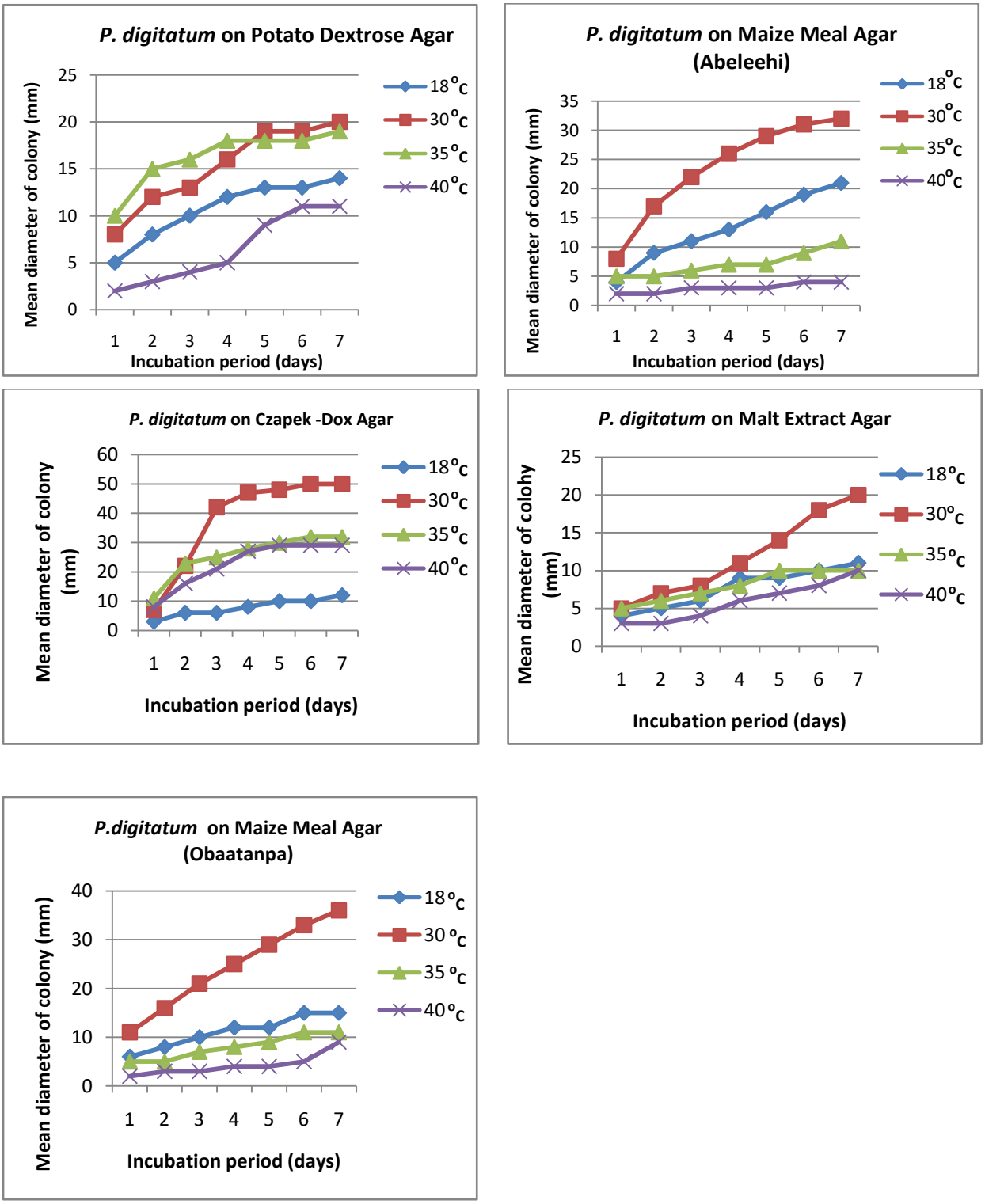

Fig.1 Radial growth of *Penicillium digitatum* on five different indicated media.

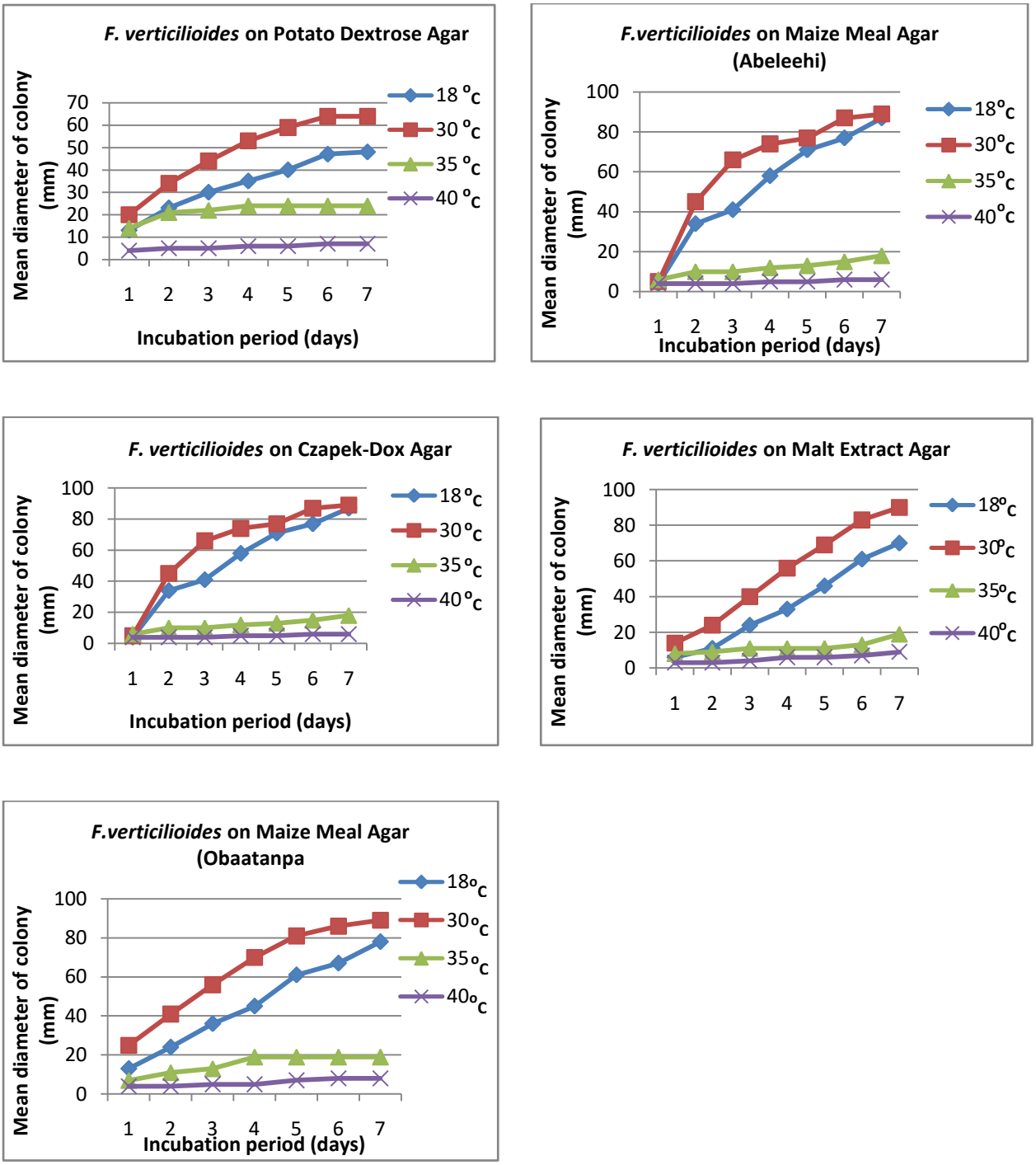

Fig.2 Radial growth of *Fusarium verticillioides* (= *F. moniliforme*) on five different indicated media.

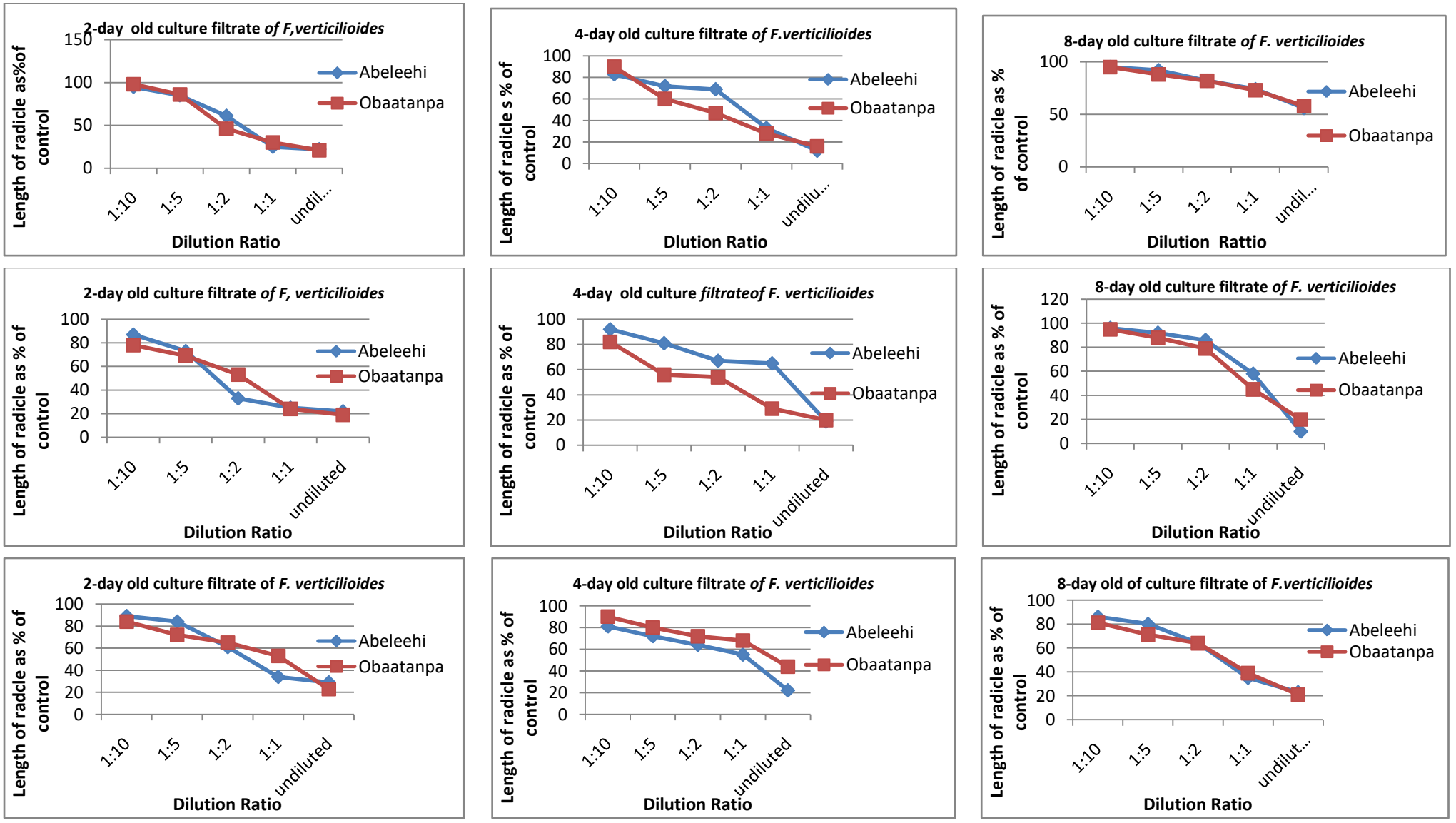

Fig 3 Development of radicle of two maize varieties Abeleehi and Obaatanpa growing on filter paper moistened with culture filtrate of *Fusarium verticillioides*(=*F. moniliforme.*

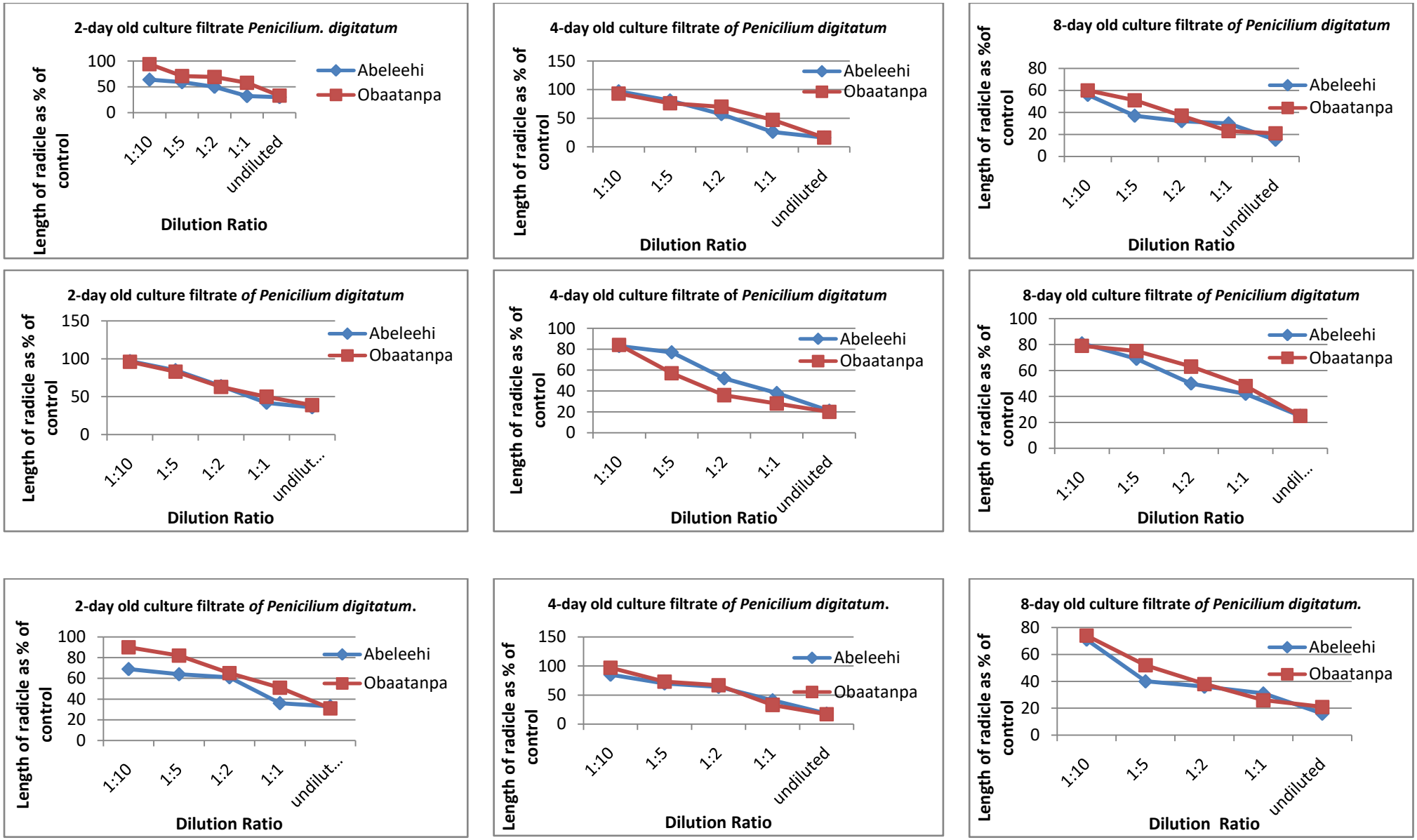

Fig.4 Development of radicle of two maize varieties Abeelehi and Obaatanpa growing on a filter paper moistened with culture filtrate of *Penicillium digitatum*.

DISCUSSION

Maize (Zea mays L.) is an important cereal grain after wheat and rice (Ishrat and Shahnazar 2009). According to (Anon 2007) maize is grown on 0.9355 million hectares annually with a production of about 1.7371 metric tonnes with average yield of 1857 kg/hectares. Maize has diversified use in many cuisines and also serve as feed for poultry.

Unfortunately, maize suffer attack from bacteria , viruses and worst of all fungi on the field and in storage due to poor agronomic and postharvest practices. (Bankole and Adebanjo 2003) reported a colossal loss of about 670 million US dollars in trade of cereals, dried fruits and nuts per year due the rejection of these commodities from African countries on the International market.

In an attempt to produce hybrid seeds that is drought resistance, disease resistance, early maturing, high varieties among others, breeding programmes have been encouraged by various countries particularly in Africa. In Ghana, new maize varieties such as Abeelehi, Obaatanpa, Okomasa, Dobidi to mention but a few have been produced. Unfortunately, not much in terms of the mycobiota of these new varieties are known.

(Minamor 1995) isolated thirty (30) and twenty-eight (28) species of fungi belonging to the genera *Aspergillus, Penicillium, Curvularia, Chaetomiun, Cladosporium, Emericella, Eurotium, Fusarium, Paecilomyces, Mucor, Neurospora* and *Rhizopus* were isolated from Abeelehi and Obaatanpa varieties at various equilibrium relative humidities (ERH'S), 55 - 95% provided by glycerol, water mixtures.

(Minamor and Odamtten 2016) revealed that culture filtrate of three *Paecilomyces* species, *P. carneus, P. puntuni, P. varioti* isolated from Abeelehi and Obaatanpa severely depressed length of the emerging radicles of Abeelehi and Obaatanpa by 45- 95% at the highest concentration applied. The radial growth of the three species on five mycological agar was influenced by the media and the temperature of incubation; each *Paecilomyces* species behaved differently on the agar. Interestingly, the three *Paecilomyces* species produced their inhibitory metabolites in 2-day culture filtrate in Maize Meal Broth and Potato Dextrose Broth used to culture the species. There were varietal differences in the response of the germinating maize grains to the active ingredients in the culture metabolites of the three *Paecilomyces* species.

In a related work, (Minamor and Odamtten 2017) reported that, *A. flavus* among two other *Aspergillus* species that is *A. ochraceus* (= *A. alutaceus*) and *A. giganteus* also isolated from Abeelehi and Obaatanpa depressed seed germination of Abeelehi and Obaatanpa at the highest concentration by 50 to 70% and adversely depressed 60 to 90% of the germinating maize grains. The mycological media as well as the temperature of incubation influenced radial growth of the three *Aspergillus* species tested.

In the current study, two fungal species namely, *Fusarium verticillioide* (= *F. moniliforme*) and *Penicillium digitatum* also isolated from Abeelehi and Obaatanpa were tested for their optimal radial growth condition on five 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.The culture

filtrates of the two fungal species raised from Maize meal Broth either from Abeleehi or Obaatanpa were used to test the germinating capacity of Abeleehi and Obaatanpa maize varieties.

Vegetative growth of *P. digitatum* (fig.1) on all the five media was inferior to what existed for the *Paecilomyces* and the *Aspergillus* species growing on the same media (Minamor and Odamtten 2016 and 2017). The best temperature for optimum growth of *P. digitatum* in this investigation was 30^{o}C; 35-40^{o}C was unsuitable and 18^{o}C was intermediate between 30^{o}C and 40^{o}C.

Radial growth of *Fusarium verticillioide* (= *F. moniliforme*) followed a characteristic sigmoid curves with the best temperature for growth in all media at 30^{o}C (fig.2).Vegetative growth *F. verticillioide* at 18^{o}C was better than that at 35^{o}C and 40^{o}C. The fungus grew significantly after 2 days at 40^{o}C such the diameter of the colony remained the same for 7days. The best growth was obtained on Maize Meal agar and Malt Extract agar (figs.2).

The metabolites of *F. verticillioides* and *P.digitatum* had similar deleterious effect on germination and radicle elongation of the two maize varieties as in (Minamor and Odamtten 2016 ; 2017) (figs. 3,4)

Seed germination was depressed by 50 to 70% at the highest concentration of culture filtrate of *F. verticillioides* and *P. digitatum* (figs.3,4). The inhibitory effect of the metabolites of the two fungi on seed germination was gradually removed with increasing dilution of the culture filtrate. Two -day old culture metabolite of F. *verticillioides* severely depressed by 40 to 90% radicle elongation of both Abeleehi and Obaatanpa at the highest concentration applied. In most instances the effect was severer on Abeleehi than Obaatanpa (fig.3) except in the case of 4-day old culture of *F. verticillioides* raised in maize meal broth prepared from Obaatanpa. In all instances the inhibitory effect was gradually reduced with increasing dilution of the culture filtrate such that the 1:10v/v dilution nearly approximated hat of the control (untreated).

The metabolites of *P. digitatum* generally exerted severer depressive effect on the development of the radicles of Obaatanpa than that of Abeleehi variety (fig.4). Again the inhibitory principle was gradually removed by increasing dilution of the filtrate used as the germinating medium for the seeds. The general conclution is that culture metabolites of both *F.verticillioides* and *P. digitatum* have adverse effect on the germination and radicle elongation of Abeleehi and Obaatanpa varieties.

The maize varietal differences in response to germination and radicle elongation in the presence of the metabolites in vitro could be attributed to the intrinsic genotypic differences in the seeds and also the possible variation in the composition of the metabolites from the two species; *F.verticillioides* (= *F. moniliforme*), *Penicillium digitatum*. (Dua- Yentumi and Ahiabor 1992) reported that genotypic differences in plants accounted for the quantitative differences in the rhizosphere microflora of maize and cowpea.

Penicillium digitatum forms *patulin* in culture. This might have played a role in the depression of germination and development of maize seedlings. A work by (Debnarth et. al., 2012) on the effect of

fungi on germination of six maize cultivars revealed that the effect of germination of maize seeds cultivar significantly differ from cultivar to cultivar with the highest germination failure recorded in a locality in which maximum prevalence of various fungi were recorded. Some of the fungal species recorded in that locality were *Aspergillus niger*, *Curvularia lunata*, *Rhizopus stolonifer* and *Penicillium oxalicum*. These findings agreed with the one reported by (Marley and Gbenga 2004).

Apart from the pathological implication of the effects of the metabolites on maize plant growth and development, many other fungal flora encountered in Abeleehi and Obaatanpa produce mycotoxins in food and may have serious Public Health implications if ingested by consumers.

The findings in this paper and that of (Minamor and Odamtten 2016; 2017) point to another important role of *Paecilomyces carneus, P, puntoni, P. varioti, Aspergillus flavus, A. ochraceous (= A.alutaceous), A. giganteous, F. verticillioides (= F. moniliforme*) and *Penicillium digitatum* may play as seed-borne pathogen during storage of the newly developed maize varieties Abeleehi and Obaatanpa earmarked as seed maize for the next planting season.

CONCLUSION

This paper contains data which indicates that mycological media as well as the temperature of incubation influenced radial growth of *Fusarium verticillioides* (= *F.moniliforme*) and *Penicillium digitatum* isolated from two Ghanaian maize varieties Abeleehi and Obaatanpa.

The best temperature for optimum growth of *P. digitatum* in this investigation was 30^{o}C ; 35-40^{o}C was unsuitable and 18^{o}C was intermediate between 30^{o}C and40^{o}C. Radial growth of *Fusarium moniliforme* followed a characteristic sigmoid curves with the best temperature for growth in all media at 30^{o}C. Vegetative growth of *F. verticillioides* at 18^{o}C was better at 35^{o}C and 40^{o}C. The fungus grew significantly after 2 days at 40^{o}c such that the diameter of the colony remained the same for 7 days. The best growth of *F. verticillioides* was obtained on Maize Meal Agar and Malt Extract Agar.

Seed germination was depressed by 50 to 70% at the highest concentration of the culture filtrate of *F. verticillioides* and *P. digitatum*, The inhibitory effect of the metabolites of the two fungi on seed germination was gradually removed with the increasing dilution of the culture filtrate. Two-day old culture metabolite of *F. verticillioides* severely depressed by 40-90% radicle developmentof both Abeleehi and Obaatanpa at the highest concentration applied. In most instances the effect was severer on Abeleehi than Obaatanpa.The metabolite of *P.digitatum* generally exerted severer depressive effect on the development radicle of Obaatanpa than that of Abeleehi variety.

The maize varietal differences in response to germination and radicle elongation in the presence of the metabolites *in vitro* could be attributed to the intrinsic genotypic differences in the seeds and also the possible variation in the composition of the metabolites from the two species; *Fusarium verticillioides* (= *F. moniliforme*) and *Penicillium digitatum*.

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REFERENCES

1 Minamor A.A. (1995). Influence of the metabolitee of three Paecilomyces species on the germination and seedling development of two Ghanaian maize varieties (Abeleehi and Obaatanpa). M Phil. Thesis, Department of Plant and Environmental Biology, School of Biological Sciences, University of Ghana Legon.

2.Akonda, Md. M,R., Yasmin, M.,Hossain, I. (2016). Incidence of seedborne mycoflora and their effects on germination of maize seeds. International Journal of Agronomy and Agricultural Research. Vol. 8, No. 1. p. 87-92.

3. Elham,S.D., and Modhi, K. E,(2015). Mycoflora Of Maize (Zea mays L.) At Different Locations In Hail Area-Saudi Arabia. International Journal of Scientific & Technology Research. Vol.4 Issue 6. pp 227-230.

4.Tanova, K., Raikov, S., Momchylova, P., Marinova, Z. (2015). Mycoflora of maize from Novi Pazar. International Journal of Agronomy and Agricultural Research. Vol. 7, No. 3. p. 9-13.

5, Ishrat, N. and Shahnaz, D. (2009). Detection of Seed-borne Mycoflora in Maize (Zea mays. L.). Pak J.Bot., Vol.41 Issue 1: 443-451.

6. Hussain, N., Hussain, A., Ishtiaq, M., Azam,S., and Hussain, T., (2013). Pathogenicity of two seed-borne fungi commonly involved in maize seeds of eight districts of Azad Jammu and Kashmir, Pastistan. African Journal of Biotechnology Vol. 12(12),pp.1363-1370.

7.Chhokar, R.S. (2001). Development and use of herbicides. Pest. Inf. 27: 25-27.

8. Agrios, G.N. (1997). Control of plant diseases. Plant pathology. 4thedition. California: Academic Press.

9.Chandler, J.(2005). Cost reduction in SIT programmes using exosect auto-disseminations part of area wide integrated pest management. Inter. J. Pest Control, 47: 257-260.

10.Minamor, A.A. and Odamtten.., G.T. (2016).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. 5(11):604-617.

11, Minamor, A.A. and Odamtten., G.T.(2017). Radial growth of three Aspergillus species isolated from two Ghanaian maize varieties Abeleehi and Obaatanpa. International Journal of Advances in Pharmacy, Biology and Chemistry.Vol. 6(1):52-61.
12. Kimura, Y., Shiojima, K., Nakajima, H.andHamasaki, T. (1992b).Altechromes A and B, new plant regulators produced by Alternaria sp. Bio. Sci. Biochem. 56(10): 1664 -1665.
13. Anonymous, (2007). Agricultural Statistics of Pakistan, Ministry of Food, Agriculture & Livestock. Economic Wing, Govt. of Pakistan. Islamabad. 189pp.
14. Bankole, S.A., and Adebanjo, A.(2003). Mycotoxins in food in West Africa: current situation and possibilities of controlling it. Afr. J. Biotechnol. 2:254-263.
15.Dua-Yentumi and Ahiabor, E.K. (1992). Rhizosphere Microorganisms in the root regions of maize (Zea mays. L.) and cowpea (Vigna unguiculata) In: Soil Resource Management towards sustainable Agriculture in Ghana. The role of the soil scientist Proc. Soil Sci. Soc. Gh. 139-143.
16. Debnath, M., Sultana, A., and Rashid, A.Q.M.B. (2012). Effect of seed-borne Fungi on the Germinating Seeds and their Bio-control in Maize. J. Environ. Sci. & Natural *Resources, 5(1): 117-120.*
*17.*Marley, P.S. and Gbenga, *O. (2004).* Fungicide control oif *Stenocarpella maydis* in the Nigerian Savanna. Archives of Phytopathology and Plant Protection. 37(1):19-28.