

Original Research Article

<http://dx.doi.org/10.20546/ijcmas.2016.511.071>

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

Andrew A. Minamor^{1*} and G.T. Odamtten²

¹Department of Science Laboratory Technology, Accra Technical University,
P. O. Box GP 561, Accra- Ghana

²Department of Plant and Environmental Biology, School of Biological Sciences,
University of Ghana, P. O. Box LG 55 Legon

*Corresponding author

ABSTRACT

Three xerophilic fungal species, *Paecilomyces carneus*, *P. puntoni*, and *P. varioti* were for the first time isolated from two newly-developed Ghanaian maize varieties; Abeleehi and Obaatanpa. The fungal species were isolated under varying ambient humidity of 55-65% in both grain varieties. The culture filtrate of the *Paecilomyces* species raised in Maize Meal broth prepared from either Abeleehi or Obaatanpa and Potato-Dextrose broth were tested on the germination capacity of grains of Abeleehi and Obaatanpa using the blotter test method. The physiological condition for optimal growth of the *Paecilomyces* species were tested on Czapek-Dox Agar, Maize Meal Agar (Abeleehi), Maize Meal Agar (Obaatanpa), Malt Extract Agar and Potato Dextrose Agar at (18, 30, 35, and 40) °C. Radial growth of the *Paecilomyces* species was influenced by media and temperature of incubation. The optimum growth of *P. carneus* on all agar media used was obtained at a 30°C, a temperature of 40°C was clearly unsuitable for growth of *P. carneus* as the fungus remained static at this temperature after 2-4 days growth. The best radial growth of *P. puntoni* was obtained between 30-35°C. Growth at 18°C initially lagged behind those kept at 30°C but approximated their growth rate after 6 days incubation period. There was no statistical difference ($P \leq 0.05$) between growth of *P. puntoni* in all the media tested. Temperature of 35°C and 40°C depressed radial growth of *P. varioti* in all media tested. The best temperature for growth of *P. varioti* was 30°C. Temperature 18°C was almost as suitable as 30°C for *P. varioti*. Radial growth of *P. varioti* was slowest on Potato Dextrose agar as compared to the other media tested. Percentage germination of the maize grains was depressed by 10 -75% by the undiluted, 2, 4, and 8 days old culture filtrate of the three *Paecilomyces* species. However, there were varietal differences in the response of the germinating maize grains to the active ingredient in the culture metabolites of the three *Paecilomyces* species. The inhibitory effect was gradually removed with increasing dilution. The same culture filtrate severely depressed length of emerging radicles of the two maize varieties by 40 - 90% at the highest concentration applied. The reduction in radicle length was severer on Abeleehi variety as compared to Obaatanpa.

Keywords

Abeleehi,
Obaatanpa,
Maize Meal Agar,
Paecilomyces,
Radicle length.

Article Info

Accepted:
26 October 2016
Available Online:
10 November 2016

Introduction

Seeds of cereal grains and legumes harvested from the field harbour both field fungi - mycoflora contaminating seeds in the field before harvest- and storage fungi- those fungi that become resident during post-harvest storage of seeds-. These categories of fungi have been known to shorten the shelf-life of stored seeds; reducing seed viability, nutrient content and imparting toxic metabolites into the seed among others. During the first half of the twentieth century, knowledge of seed-borne diseases of crops has greatly increased and there is hardly any cultivated crop where at least one seed-borne fungal pathogen is not known (Malone and Musket, 1964). The extent of their occurrence and some idea of the damage they cause have been compiled by (Neergaard, 1983).

The reasons for this increased interest in seed-borne disease is due not only to technological advances which have made more detailed investigational work possible but also the increased attempt by man to cultivate new species and varieties of crops more suited to his needs.

Man has to introduce new species and varieties into territories where they 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 intensive 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 (Minamor, 1995).

The Crop Research Institute of the Council for Scientific and Industrial Research, (CSIR) of Ghana has through the Grain and Legumes Improvement Programme, developed high lysine content maize grains

including Abeleehi, Obaatanpa, Okomasa, Dobidi, to mention but a few which are being sold to the local farmers. However, there is hardly any information on the mycoflora associated with these grains which have to be stored for prolonged periods as seeds grains for the next planting seasons.

In this study, three *Paecilomyces* species, namely; *P. carneus*, *P.puntoni*, *P. varioti* isolated from Abeleehi and Obaatanpa among other genera and species at varying Environmental Relative Humidity provided by glycerol and water mixture. at 28-31°C for 6 days. These xerophilic species were isolated for the first time on stored Ghanaian maize varieties.

These fungi could be of pathological importance if their metabolites affect the viability and germination capacity of grains of Abeleehi and Obaatanpa used as seed maize in the next planting season. There is hardly any information in the pertinent literature on the physiological condition for optimal growth of these species isolated in Ghana. Five different natural and synthetic mycological media-Czapek- Dox Agar, Maize Meal Agar (Abeleehi), Maize Meal Agar (Obaatanpa), Malt Extract Agar and Potato-Dextrose Agar- were tested for ability to support optimal growth of the *Paecilomyces* species at different temperatures (18, 30, 35, 40, and 45)°C.

Materials and Methods

Materials

The maize varieties used Abeleehi and Obaatanpa were purchased from Aglow Seed Company, Accra. The fungal species, *Paecilomyces carneus*, *Paecilomyces pntoni* and *P.aecilomyces varioti* 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°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 Sabourauds 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°C until fungi grew (7-14) days.

Maintenance of stock Cultures

Stock cultures of, *Paecilomyces carneus*, *P.varioti*, and *P. puntonii*, 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°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°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°, 30°, 35° and 40°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 *Paecilomyces carneus*, *P. puntoni*, *P. varioti* were obtained by raising the listed fungi (aliquot of 1.2 - 1.8 x 10⁵ 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°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 was 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°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).

Results and Discussion

Maize (*Zea mays*. L.) serves as the basis of the diet of millions of Ghanaians due to its versatile food uses and storage characteristics. In Ghana, maize is an important crop cultivated throughout the country with varying degrees of success depending on edaphic and climatic factors. Being a seasonal crop, especially in sub-Saharan Africa, maize is stored as dry grains and forms an enormous reserve of food. The areas of maize cultivation in Ghana include the whole of Southern Ghana, Ashanti, Brong Ahafo, the three Northern Regions. Intensive commercial production of maize is however, found in the Somanya District of the Eastern Regions, the midland maize belt of Ashanti and Brong Ahafo Regions, the Ho - Kpandu District of the Volta Regions and Central Region. Unfortunately, maize is

subject to a wide range of pathogens including viruses, bacteria, nematode, fungi both in storage and on the field. (Shurtleff, 1980) reported that of all these organisms, fungi are the main cause of the majority of diseases on maize.

The increased 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 introduction of the crop to farmers. In this study, three *Paecilomyces* species namely; *P. carneus*, *P. puntoni*, and *P. varioti* were for the first time isolated from two recently developed Ghanaian maize varieties Abeleehi and Obaatanpa were investigated for their radial growth on five mycological media including Potato Dextrose agar, Czapek-Dox agar, Maize Meal agar prepared from either Abeleehi or Obaatanpa and Malt Extract agar. The culture filtrate of these xerophilic fungi raised on Maize Meal broth from either Abeleehi and Obaatanpa and Potato Dextrose broth were tested on the germination capacity and the effects of the culture filtrate on radicle development of the two maize varieties (Tables 1,2 & 3).

Seed-borne fungi isolated from Ghanaian maize varieties Abeleehi and Obaatanpa for the first time in Ghana. Twelve (12) *Aspergillus* species predominated over the other species encountered followed by *Penicillium* nine (9) species. The species diversity was influenced by grain variety and the Environmental Relative Humidity (ERH) at which the grains were incubated the xerophilic species of *Paecilomyces* (*P.*

carneus, *P. puntoni*, *P. varioti*) were isolated at 55-65% in both maize grain varieties (Minamor, A.A. 1995). *Paecilomyces* species are dominant in cereals stored in airtight condition by low ERH's. *Paecilomyces varioti* is a common contaminant in substrates from higher temperatures. The species is thermophilic growing even at 50 °C (Samson and Van Reenen Hoekstra, 1988). The local isolates of *P. varioti* grew best at 30-35°C (fig.2) but could still thrive at 40°C. The optimum growth for *P. puntoni* was between 35-40°C (fig.2). *P. carneus* grew at 30-35°C (fig, 1).

The striking feature of the local isolation of *Paecilomyces* species is that they continue to thrive even at 40°C. The optimum temperature for growth of *Paecilomyces* species isolated elsewhere is close to what obtain for the Ghanaian species. For instance *P.niveus* (Stock and Samson) has an optimum temperature 30-35°C, minimum about 10°C, maximum 40°C. *P. fulvus*, Stock and Samson, optimum 30-35°C, minimum about 10°C, maximum 45°C (Samson, 1974; Samson and Van Reenen-Hoekstra, 1988). In heated grains such as obtained in Stackburned grains, *Paecilomyces* species and other thermophilic species are likely to thrive and play a role in deterioration even under quiescent conditions in heated grains. Stackburn is the term used to describe maize grains stored in woven polypropylene sacks stacked in the warehouse which experience heated conditions of about 40°C up to 5 months and consequently become discoloured turning from white to varying shades of brown in the testa and germ regions. These grains are subsequently downgraded and disposed of cheaply - this constitute a loss to the farmer and the Warehousing agent, above all pose a threat to food security in sub-Saharan Africa.

The inhibitory active ingredient in the metabolites of the three *Paecilomyces* species were formed even in two-day old culture filtrate on the various media used. The difference in shift of pH during vegetative growth of these fungal species in culture may reflect the varying chemical composition of the metabolites of the fungal species. These metabolites when used undiluted depressed seed germination of the Ghanaian maize variety Abeleehi and Obaatanpa by 10-80% and drastically reduced (≥ 45 -85%) length of the emerging

radicles at the highest concentration applied. The reduction in length of radicle was severer on Abeleehi variety as compared to Obaatanpa where culture metabolites of *P.carneus*, *P. puntoni*, and *P.variotti* were applied. The inhibitory effect of the metabolites was however, gradually removed with dilution such that the radicle lengths of the germinating grains in the presence of 1:10v/v dilution of the filtrate nearly approximated that of the control (distilled water) in some instances.

Table.1 Culture filtrate of *Paecilomyces puntoni* growing in Potato Dextrose Broth used as germination medium for maize varieties - Abeleehi and Obaatanpa.

Dilution Ratio	Radicle Length AS % of Control	Dilution ratio	Radicle Length as % of Control
2 - DAY OLD			
	(ABELEEH)		(OBAATANPA)
UNDILUTED	36	UNDILUTED	40
1 : 1	46	1 : 1	56
1 : 2	53	1 : 2	67
1 : 5	91	1 : 5	80
1 : 10	97	1 : 10	100
4 - DAY OLD			
UNDILUTED	26	UNDILUTED	24
1 : 1	33	1 : 1	24
1 : 2	48	1 : 2	38
1 : 5	65	1 : 5	73
1 : 10	76	1 : 10	76
8 - DAY OLD			
UNDILUTED	40	UNDILUTED	54
1 : 1	58	1 : 1	65
1 : 2	78	1 : 2	81
1 : 5	85	1 : 5	84
1 : 10	96	1 : 10	94

Table.2 Culture filtrate of *Paecilomyces puntoni* growing in Maize Meal Broth (Abeleehe) used as germination medium for maize varieties - Abeleehe and Obaatanpa

Dilution Ratio	Radicle Length AS % of Control	Dilution ratio	Radicle Length as % of Control
2 - DAY OLD			
	(ABELEEHI)		(OBAATANPA)
UNDILUTED	25	UNDILUTED	23
1 : 1	30	1 : 1	30
1 : 2	39	1 : 2	34
1 : 5	61	1 : 5	40
1 : 10	94	1 : 10	61
4 - DAY OLD			
UNDILUTED	29	UNDILUTED	39
1 : 1	35	1 : 1	50
1 : 2	47	1 : 2	49
1 : 5	62	1 : 5	67
1 : 10	80	1 : 10	79
8 - DAY OLD			
UNDILUTED	40	UNDILUTED	48
1 : 1	48	1 : 1	52
1 : 2	66	1 : 2	62
1 : 5	69	1 : 5	69
1 : 10	92	1 : 10	74

Table.3 Culture filtrate of *Paecilomyces puntoni* growing in Maize Meal Broth (Obaatampa) used as germination medium for maize varieties - Abeleehi and Obaatampa

Dilution Ratio	Radicle Length AS % of Control	Dilution ratio	Radicle Length as % of Control
----------------	--------------------------------	----------------	--------------------------------

2 - DAY OLD

(ABELEEH)		(OBAATANPA)	
UNDILUTED	25	UNDILUTED	23
1 : 1	30	1 : 1	30
1 : 2	39	1 : 2	34
1 : 5	61	1 : 5	40
1 : 10	94	1 : 10	61

4 - DAY OLD

UNDILUTED	23	UNDILUTED	21
1 : 1	29	1 : 1	26
1 : 2	45	1 : 2	54
1 : 5	50	1 : 5	61
1 : 10	85	1 : 10	83

8 - DAY OLD

UNDILUTED	55	UNDILUTED	45
1 : 1	63	1 : 1	63
1 : 2	92	1 : 2	63
1 : 5	93	1 : 5	83
1 : 10	95	1 : 10	85

Fig.1 Radial growth of *Paecilomyces carneus* on five different mycological media (Indicated).

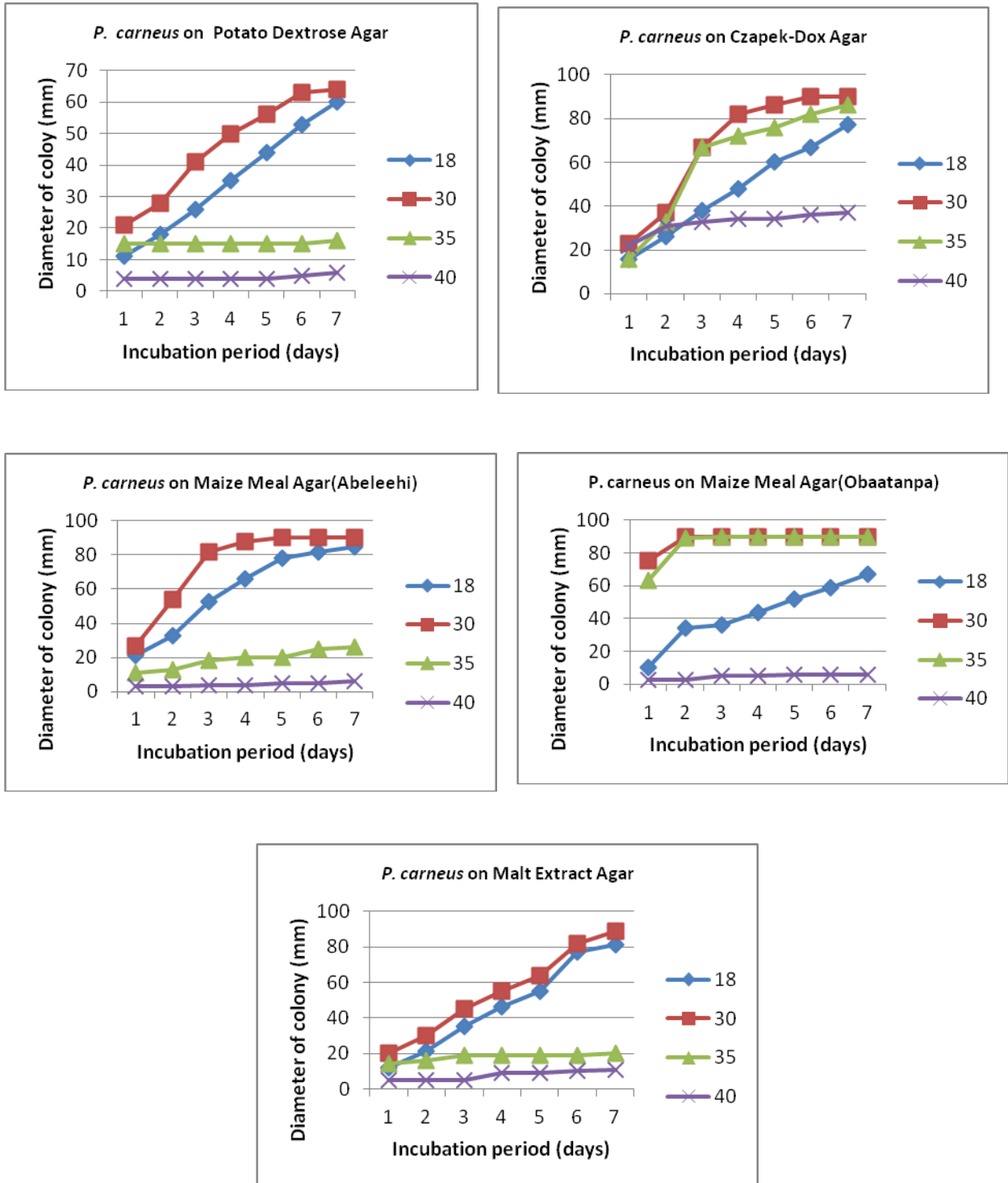
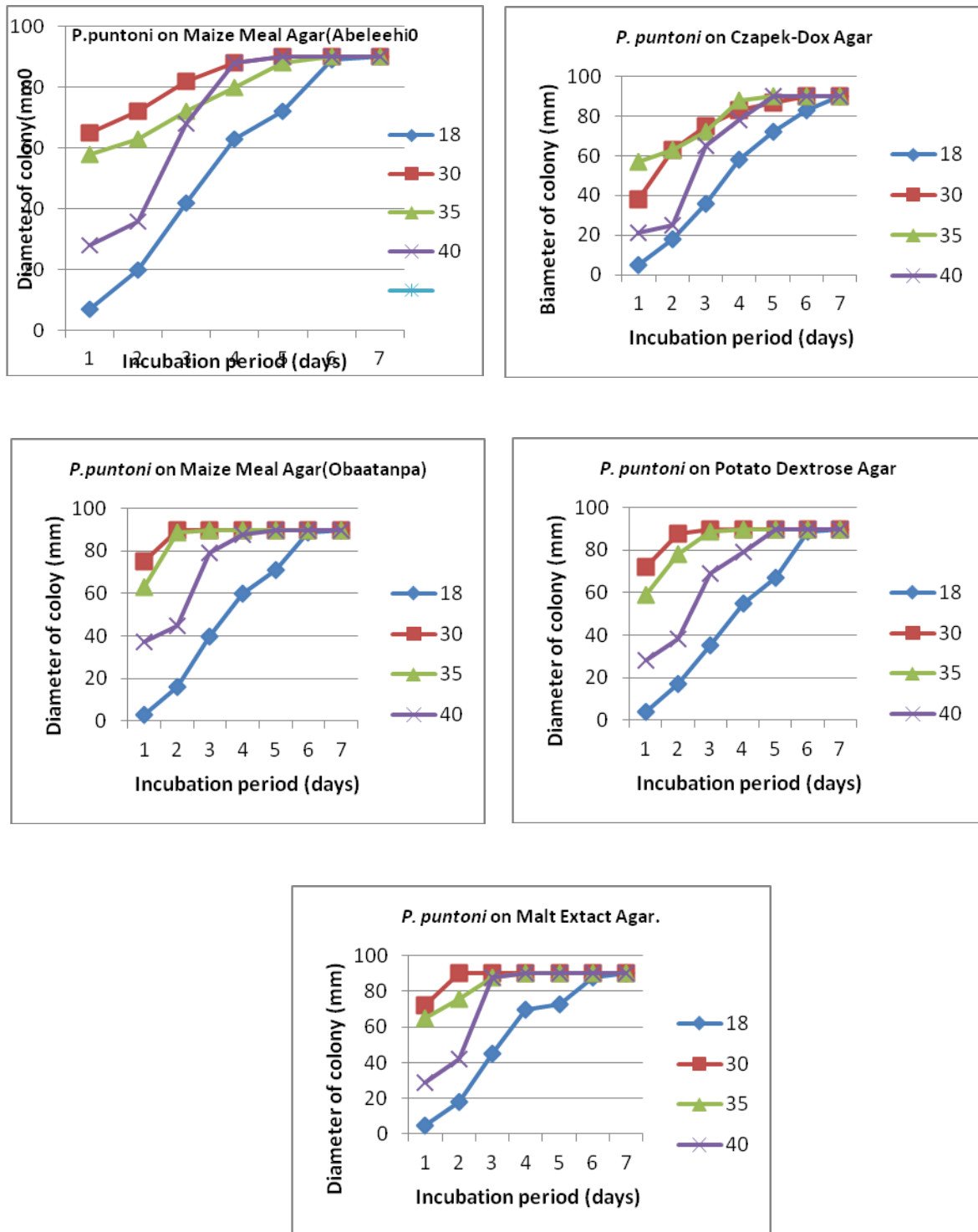
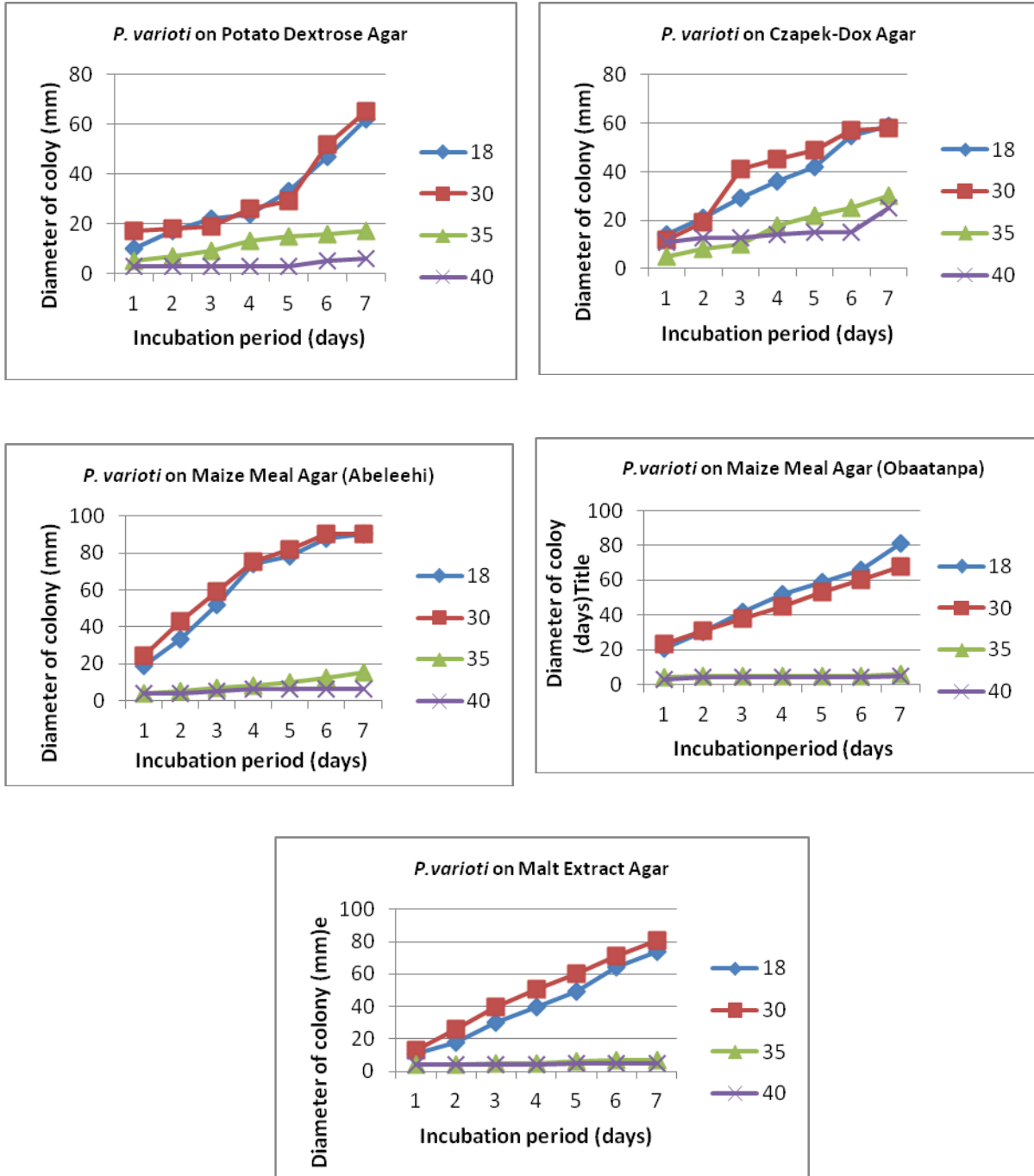


Fig.2 Radial growth of *Paecilomyces puntoni* on five different mycological media. (Indicated)



Note: The growth at 18°C initially lagging behind the remaining temperatures.

Fig.3 Radial growth of *Paecilomyces varioti* on five different mycological media (Indicated).



Note: The slow growth of fungus on PDA

Fig.4 Influence of 4-day old culture filtrate of *P.carneus* on radicle length of germinating seeds of Abeleehi(left) and Obaatanpa (right). Top: Left (Control); Middle (1:10v/v); Right (1:5v/v)
Bottom: Left (1:2v/v); Middle (1:1v/v); Extreme right (undiluted)



Fig.5 Influence of 4-day old culture filtrate of *P.puntoni* on radicle length of germinating seeds of Abeleehi(left) and Obaatanpa(right). Top: Left (Control); Middle (1:10v/v); Right (1:5v/v)
Bottom: Left (1:2v/v); Middle (1:1v/v); Extreme right (undiluted)



Fig.6 Influence of 4-day old culture filtrate of *P.variotti* on radicle length of germinating seeds of Abeleehi(left) and Obaatanpa(right). Top: Left (Control); Middle (1:10v/v); Right (1:5v/v)
Bottom: Left (1:2v/v); Middle (1:1v/v); Extreme right (undiluted).



The maize varietal differences in response to germination and radicle development in the presence of the metabolite *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 three *Paecilomyces* species. (Figs. 4, 5 & 6). *Paecilomyces varioti* produces *patulin*, a mycotoxin (Frisvad, 1988) but the nature of the mycotoxins from *P. carneus*, *P. puntoni* have not been clearly elucidated to date.

In conclusion, the data in this paper indicates that radial growth of *Paecilomyces* species on agar was influenced by the media and temperature of incubation; each *Paecilomyces* species behaved differently. The optimum growth of *P. carneus* on all agar used was obtained at a 30°C, a temperature of 40°C was clearly unsuitable for growth of *P. carneus* as the fungus remained static at this temperature after 2 - 4 days growth.

The best radial growth of *P. puntoni* was obtained between 30 - 35°C. Growth at 18°C initially lagged behind those kept at 30°C but approximated their growth rate after 6 days incubation period. This was no statistical difference ($P \leq 0.05$) between growth of *P. puntoni* in all the media used. Temperature of 35°C and 40°C depressed radial growth of *P. varioti* in all media tested. The best temperature for growth of *P. varioti* was 30°C, 18°C was almost as suitable as 35°C for *P. varioti*. Radial growth of *P. varioti* was slowest on Potato Dextrose agar as compared to the other media tested. The three *P.* species produced their inhibitory metabolites in 2 days in the media used to culture the species. Percentage germination of maize grains was depressed by 10 - 75% by culture filtrates of the species. The same culture filtrate 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 compare to Obaatanpa. However, the inhibitory effect was gradually removed with dilution such that the radicle lengths of the germinating grains in the presence of 1: 10v/v dilution of the filtrate nearly approximated that of the control in some instances.

Acknowledgement

The authors are grateful to the University of Ghana for laboratory facilities for this work at the Department of Plant and Environmental Biology. All the technicians at the department deserve our warmest gratitude for their various technical assistance. Finally, we thank Mr. Bonah a Senior Laboratory Technologist of the Department of Science Laboratory Technology, Accra Technical University for kindly arranging the graphs and the tables.

References

- Frisvad, J.C. 1988. Fungal species and their specific production of mycotoxin. In: Samson, R.A., and Reenen-Hoekstra E.S. Chapter 4. Introduction to food-borne fungi, CBS. Institute of the Royal Netherlands Academy of Arts and Sciences Pp. 249-249.
- Kimura, Y. Shiojima, K., Nakajima H., and Hamasaki, T. 1992b. Altechromes A and B, new plant growth regulators produced by the fungus *Alternaria* sp. *Bio. Sci. Biotech. Biochem.*, 56(10): 1664-1665.
- Malone, J.P. and Muskett, A.E. 1964. Seed-borne fungi. Description of 77 fungus species, *Proc. Int. Test Ass.*, 29: 179-384.
- Minamor, A.A. 1995. Influence of the metabolites 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.
- Neergaard, P. 1983. Seed Pathology Vol 1. The MacMillian Press Ltd. London and Basingstoke Company 283-297.
- Samson, R.A. 1974. *Paecilomyces* and some allied *Hyphomycetes* Stud. Mycol., Baarn 6: 119pp. In; Introduction to food-borne fungi 3rd Edition CMS. Institute of the Royal Netherlands Academy of Arts and Sciences.
- Samson, R.A., and Van Reenen-Hoekstra, E. 1988. Introduction to food-borne fungi 3rd Edition Centraalbureau Voor Schimmel Cultures Baar. Institute of the Royal Netherlands Academy of Arts and Sciences.

How to cite this article:

Andrew A. Minamor and G.T. Odamtten. 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.
doi: <http://dx.doi.org/10.20546/ijcmas.2016.511.071>