

Influence Of The Metabolites Of Three *Paecilomyces* Species On The Germination And Seedling Development Of Two Ghanaian Maize (*Zea Mays* L) Varieties (Abeleehi And Obaatanpa)

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Abstract

Three *Paecilomyces* species (*P. carneus*, *P. puntoni* and *P. varioti*) isolated from two Ghanaian maize varieties 'Abeleehi' and 'Obaatanpa' at varied ambient Equilibrium Relative Humidities (ERH) (55-95%), representative of the Ghanaian ambient conditions were used for the study. The three *Paecilomyces* species produced their toxic metabolites in two days and their undiluted culture filtrate depressed seed germination of Abeleehi and Obaatanpa, by 10-75% depending on fungal species and period of incubation. This inhibitory effect was gradually removed with increasing dilution up to (1:10v/v). There were varietal differences in the response of the germinating grains to the toxic metabolites of the three *Paecilomyces* species. Undiluted culture filtrate of the three *Paecilomyces* species also severely depressed length of the emerging radicles of Abeleehi and Obaatanpa by 45-90% but this inhibition was gradually removed by increasing dilution of the culture filtrates up to (1:10v/v) dilution. There were varietal differences between the three *Paecilomyces* species in their effect on the vegetative growth and dry matter

accumulation of Abeleehi and Obaatanpa maize varieties. Metabolites of the three fungi variably depressed plant height, leaf width, leaf length, dry matter accumulation (dry weight of root and shoot systems) as well as chlorophyll 'a' and 'b' content of Abeleehi and Obaatanpa varieties cultivated in the field and under greenhouse conditions. The maize cobs obtained from the field plants infected with *Paecilomyces* species were diminutive with fewer and smaller grains in the cob as compared to the control. Culture metabolites of *P. carneus*, *P. puntoni*, and *P. varioti* reduced by 2-3 times the diameter of roots of the seedlings of Abeleehi and Obaatanpa although the endodermis and pericycle were clearly formed and demarcated in both the control and treated seedlings. The pith parenchyma was thinly lignified and 2-3 times narrow in diameter in the treated plants exposed to the three *Paecilomyces* species; pro- and metaxylem vessels were about two times wider in the control seedlings and the phloem xylem regions of the roots of the treated plants were reduced in number and size

Key words; *Paecilomyces* species, 'Abeleehi', 'Obaatanpa', Metabolites, Pericycle, Metaxylem,

INTRODUCTION

The role of fungi in quality deterioration of grains is well-documented in developed

countries Bothast *et al.* (1979); Christensen and Kaufmann, 1965; 1969) but there is

limited information regarding fungal flora of maize in West Africa and the role such fungi play. Broadbent, *et al.* (1969) provided an extensive list of fungi associated with maize stored in Nigeria. Later, Oyeniran (1973 a,b) extended the list by eleven fungal species belonging to the genera *Aspergillus* and *Penicillium*.

In Ghana, forty-two (42) fungal species belonging to twelve (12) genera (*Aspergillus*, *Chaetomium*, *Cladosporium*, *Curvularia*, *Emericella*, *Eurotium*, *Fusarium*, *Mucor*, *Neurospora*, *Paecilomyces*, *Penicillium* and *Rhizopus*) have been isolated from different local maize varieties (Danquah, 1973; Odamtten, 1986) *Aspergillus* species predominated followed by *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 seed pathogens fungi (e.g. *Fusarium moniliforme* and *Verticillium albo-atrum*). Storage fungi are those that contaminate stored products. Most of them are able to grow without free water. Most storage flora are species of *Aspergillus* and *Penicillium*. Some fungi *Aspergillus*, *Penicillium* etc can survive in seeds as long as 4-8 years (Neergaard, 1983).

Most of the seed-transmitted pathogens are fungi. Some are easily detected, others occur but cannot be revealed by conventional testing procedures. To survive in seeds most fungi must be able to

withstand dehydration. Xerophilic or xerotolerant fungi are characteristically capable of producing abundant xerotolerant propagules such as chlamydospores conidia or other dormant structures, including dormant mycelium, sclerotium etc. Examples include species of *Paecilomyces*, *Alternaria*, *Cercospora*, *Curvularia*, *Dreschlera* and *Stemphylium* (Neergaard, 1983).

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 (Leelavathy, 1969; Narain and Prakash, 1968; Odamtten and Clerk, 1985, 1988) root growth promoters (Kimura *et al.*, 1992 a,b). Fungi involved are members of the *Aspergillus*, *Penicillium*, *Alternaria* etc. The association of such seed-borne fungi with stored grains may result in serious reduction in crop yield in the field by seeds infected with pathogenic seed-borne fungal species.

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 exotic grain varieties which are not indigenous to Africa. The attendant problem is the production of new varieties whose versatilities in terms of drought tolerance, yield and susceptibility to local indigenous diseases have not been thoroughly investigated prior to introduction of the crop to the farmers.

An exhaustive search through the pertinent literature revealed that no detailed study has ever been made in West Africa to elucidate the adverse effect of the *Paecilomyces* species associated with stored maize grains including the two newly developed Ghanaian maize varieties 'Abeleehi' and 'Obaatanpa'. Furthermore, the effect of the metabolites of these species on seeds and other economic crops such as pepper and tomato earmarked for long term storage has hitherto received limited attention.

This paper reports the effect of some of the seed-borne fungi (*Paecilomyces carneus*, *P. puntoni*, *P. varioti*) isolated from Abeleehi Obaatanpa on the seed germination, radicle elongation and field and greenhouse performance of Abeleehi and Obaatanpa.

MATERIALS AND METHODS

MATERIALS

The fungal species, *Paecilomyces carneus*, *P. varioti* and *P. puntoni* used in these investigations were isolated from maize grains and the air at Kaneshie warehouse and Supreme warehouses at Tema.

The maize varieties used are Abeleehi and Obaatanpa were purchased from Aglow Seed Company Accra.

The soil used for the greenhouse experiment were obtained from the Botanical gardens, University of Ghana,

Legon. Black polythene bags (35cm x 20.3cm) were used for sowing the maize.

GENERAL METHODS

MAINTENANCE OF CULTURE

Stock cultures of *Paecilomyces carneus*, *P. puntoni*, *P. varioti*, were maintained on slopes of Potato Dextrose Agar slants in MacConkey tubes and sub-cultured every two weeks.

PREPARATION OF MEDIA

POTATO DEXTROSE AGAR

Two hundred grams (200g) of peeled potato were boiled in 500ml of water, strained and made up to 1000ml; twenty grams(20g) of glucose and twenty grams(20g) of agar were added.

MAIZE MEAL AGAR PREPARED FROM EITHER ABELEEHI OR OBAATANPA

Two hundred grams (200g) maize blended and 500ml of 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 twenty grams Agar were added and made up to 1litre with sterile distilled water.

ASSESSMENT OF GROWTH

FUNGAL CULTURES

Vegetation growth in liquid medium was assessed by estimating the dry weight of harvested mycelium at the end of the incubated period. Mycelium collected on a

previously weighed and dried Whatman No.1 filtered paper was dried at 80°C for 24 hours. The filter paper carrying the dried mycelium was then weighed after it has been allowed to cool in a desiccator.

Growth of cultures on solid media in Petri dishes was assessed by measuring width of culture along two diameters for 7 days.

SEED VIABILITY TEST

Maize seeds completely free from fungal attacks were used in the viability test. Fifty seeds of Abeleehi and Obaatanpa varieties were cut longitudinally to expose the germ regions 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 characteristics pinkish colour in the germ region were counted and percentage viability calculated.

Length of culture filtrates and period of growth (2, 4, 8 days) of the respective fungi. Percentage germination was calculated after 5 days incubation at 28-31°C and the

INFLUENCE OF FUNGAL METABOLITES ON VEGETATIVE GROWTH AND DRY MATTER ACCUMULATION BY SEEDLINGS.

FIELD STUDIES

Healthy surface-sterilized grains of Abeleehi and Obaatanpa were inoculated

IN VITRO STUDIES ON THE EFFECT OF FUNGAL METABOLITES ON GERMINATION AND RADICLE ELONGATION.

Liquid static culture filtrate of the local isolate of *Paecilomyces carneus*, *P. puntoni*, *P. varioti*, were obtained by raising the fungi (aliquot of $1.2-1.8 \times 10^5$ spores/ml per flask) in either 30ml of Potato Dextrose Broth (PDB), Maize Meal Broth (MMB) prepared from both Abeleehi and Obaatanpa. The mycelium was harvested after 2, 4 and 8 days at 28-31°C. Vegetative growth of fungi was assessed by the conventional dry weight method and the cultural filtrate stored separately in 500ml Erlenmeyer flasks covered with black polythene bags for immediate use.

The pH of the filtrate were taken before and after each pre-determined incubation period 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 1:10 v/v) as above. There were 250 grains for each dilution level

length of radicle noted. The length of the radicle (hypocotyl) are given as ratio (%) to those of the control seedlings in distilled water (Kimura *et al*, 1992).

with mycelium/ spore suspension of the respective 7 days old culture of *P. carneus*, *P. puntoni* and *P. varioti*. A

small superficial slit was made in the germ region of the grain and the inoculum (about $1.8-2.8 \times 10^5$ spores/ml) applied directly into the slit. The inoculated grains were incubated in Petri dishes for 24 hour at 30°C to allow the grains to take the fungus. The grains were then sown in the field plot at the recommended spacing of holes 25cm apart in rows 90cm apart. There were 50 replicates per treatment. Records of plant height, leaf width (at the broadest point) and leaf length were taken after 7, 14, 21, 35, 42 and 56 days growth.

GREENHOUSE STUDIES

Black polythene bags(35cm X 20.3cm) served as pot for soil which were with five grains of either Abeleehi and Obaatanpa varieties and then thinned to two per bag after germination. The soil in each bag was moistened with either 20-30ml undiluted culture filtrate of either *P. carneus*, *P. puntoni* or *P. varioti* initially and at two days intervals for 2weeks and thereafter at weekly intervals. There were 50 replicates per treatment. The germinating seedlings were kept in the greenhouse exposed to the normal day/night regime at 28-31°C. Measure of stem height, leaf width, leaf length, dry weight of shoot, leaf and root systems were made after 7, 21, 35, 42 and 56 days. the dry weight of plant parts were determined by keeping them at 80°C for 48 hours and then weighed after cooling

Chlorophyll content

About 100ml of 80% acetone extract of maize leaf (2g) was filtered through Whatman No 1 filter paper in Buchner funnel. The colour of the resultant liquid was read on Shimadzu Spectrophotometer at 663 and 645nm using 80% acetone as blank. Chlorophyll concentration was calculated as follows:

Chlorophyll a = $12.7 \times \text{Absorption at } 663\text{nm} - 2.69 \times \text{Absorption at } 645$ (mg/l)

Chlorophyll b = $22.2 \times \text{Absorption at } 645\text{nm} - 4.67 \times \text{Absorption at } 663$ (mg/l)

Total chlorophyll = $20.2 \times \text{Absorption at } 645\text{nm} + 8.02 \times \text{Absorption at } 663$ (mg/l).

Effect of Culture Filtrates on Germination and Radicle Elongation of Maize Grains (Blotter Test Method)

Ten surface-sterilized grains were placed on sterile Whatman's No. 1 filter paper in 9cm Petri dishes. The filter papers were moistened with 5ml of the appropriate dilutions of the culture filtrate. There were 25 replicates (250 grains) for each treatment. Sterile distilled water served as control. The plate were incubated in darkness for 5days at 28-31°C. The two maize varieties Abeleehi Obaatanpa were used for the germination tests. The length of the emerging radicles were measured

and dry weight determined at 80°C for 24h

Preparation of Polythene Bags and Field for Growing Seeds.

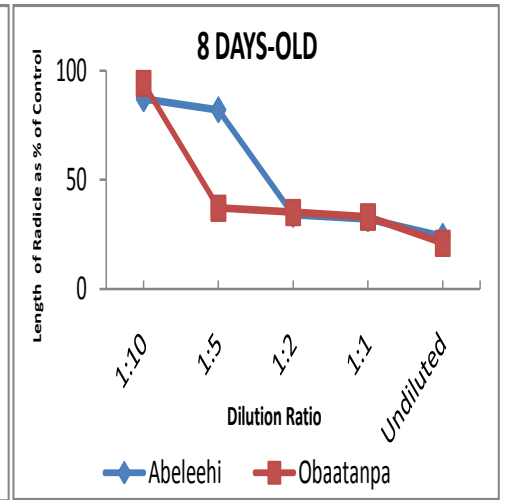
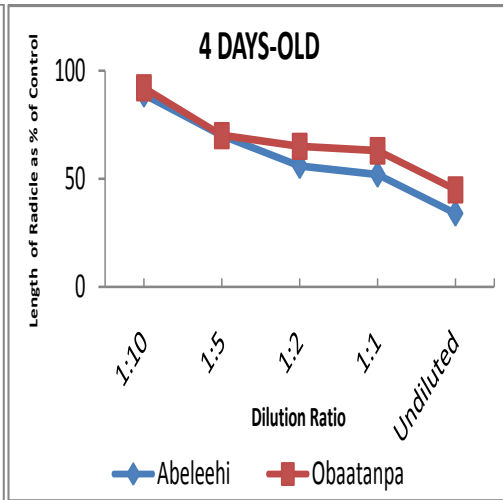
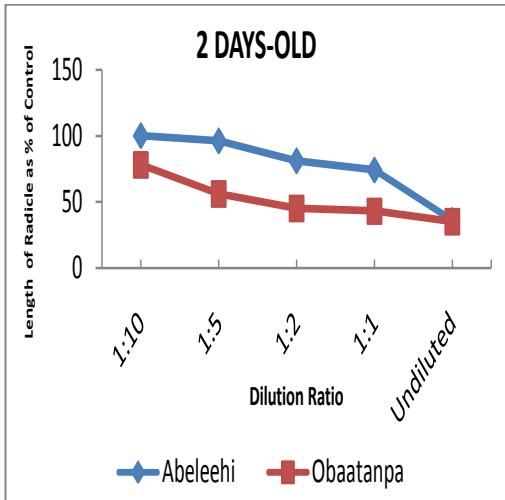
The soil used in this investigation is a sandy loam. The soil was mixed thoroughly, air-dried sieved ($\leq 2\text{mm}$) to collect unwanted particle before transferring into polythene bags(35 x20.3cm) provided with two holes for drainage. Forty polythene bags were filled to about 3/4 full making sure that the same amount of soil was found in each polythene bag. Washed coarse sand was spread over the surface of the soil to prevent compaction of the soil surface during watering. Thereafter, the soil was moistened daily with tap water before planting and placed in the greenhouse.

A plot of land was weeded and ploughed. The plot was left for three (3)days before sowing the grains at the recommended spacing for maize grains. (seed holes 25cm apart in rows 90cm apart).

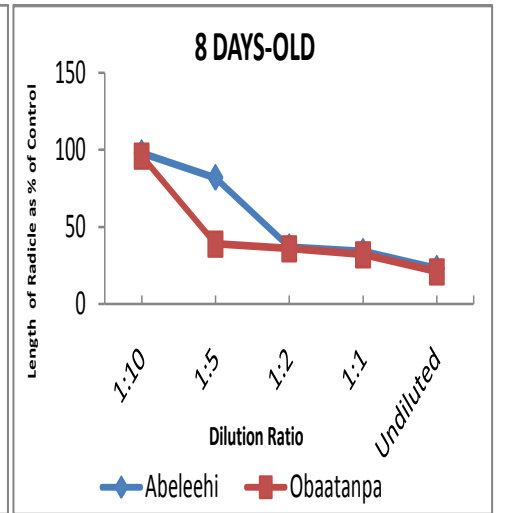
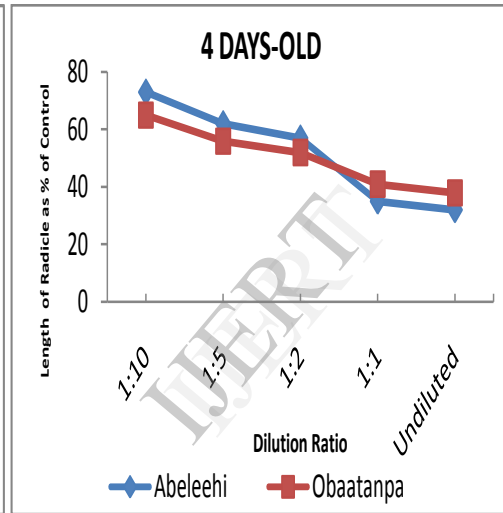
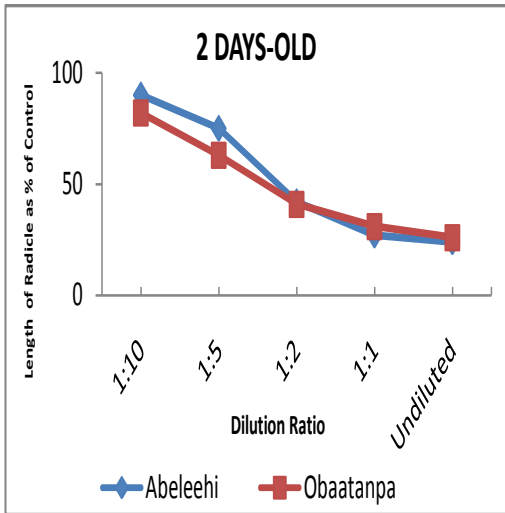
for each treatment.

RESULTS AND DISCUSSION.

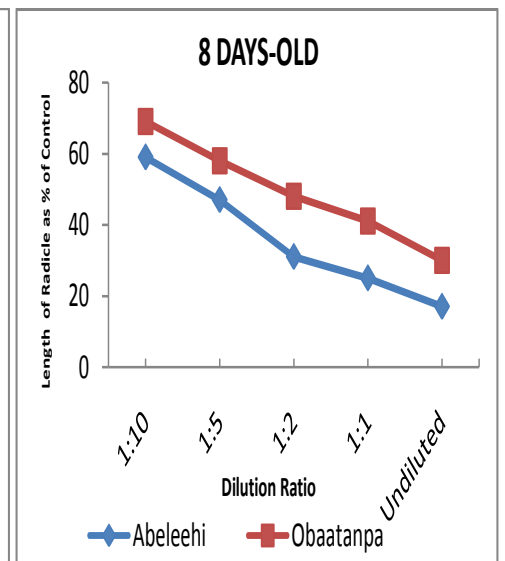
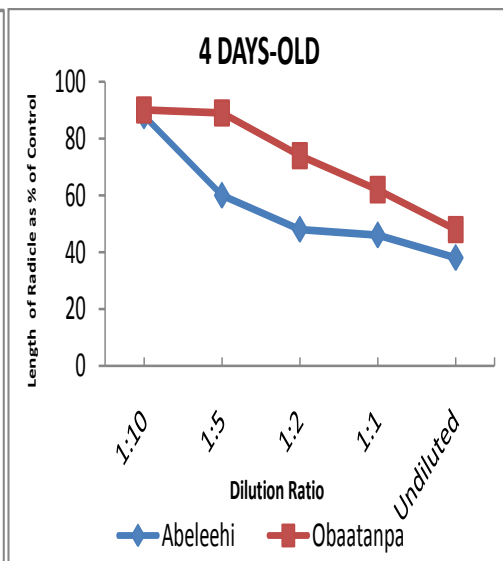
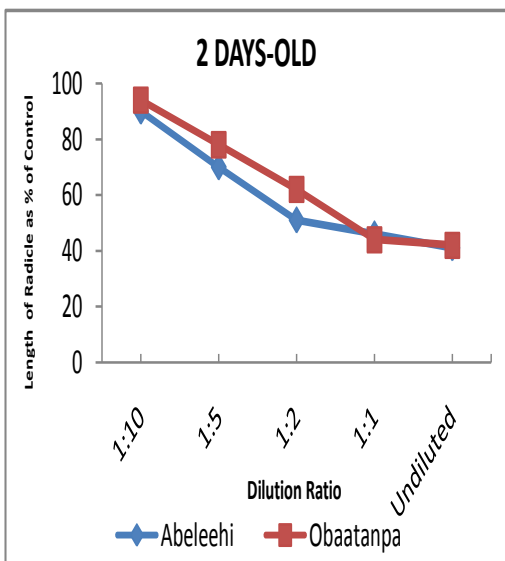
POTATO DEXTROSE BROTH



MAIZE MEAL BROTH-ABELEEH

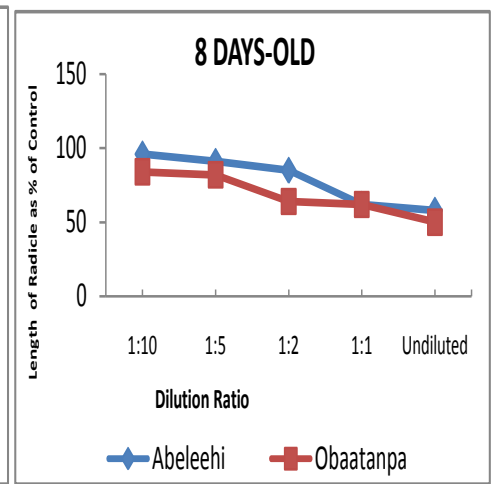
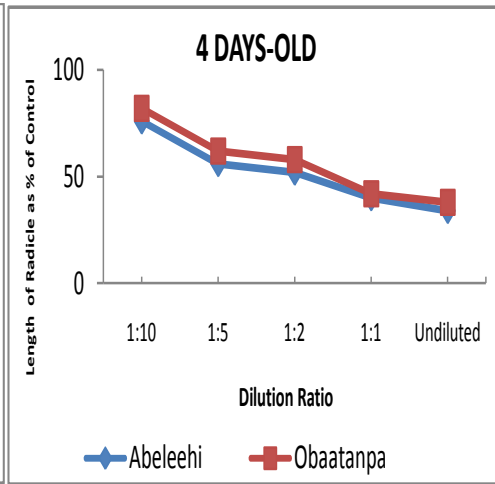
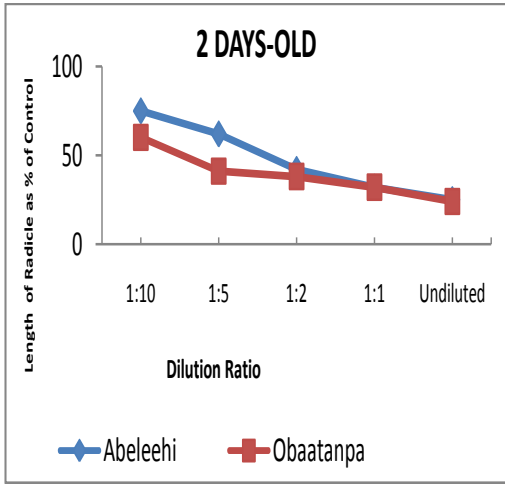


MAIZE MEAL BROTH-OBAAATANPA

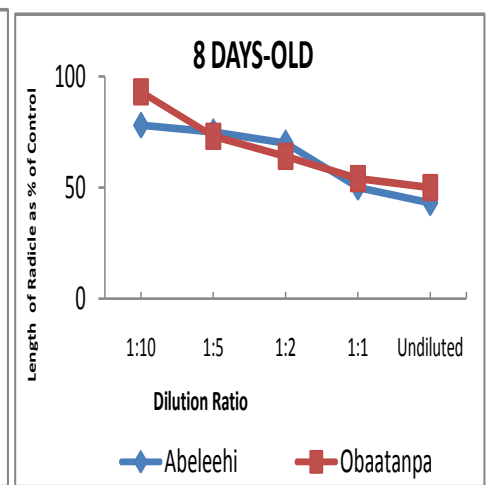
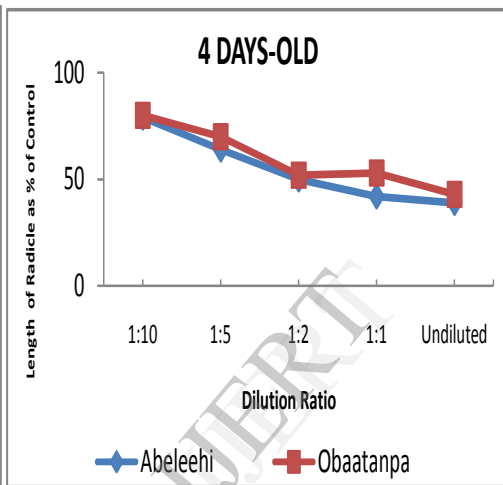
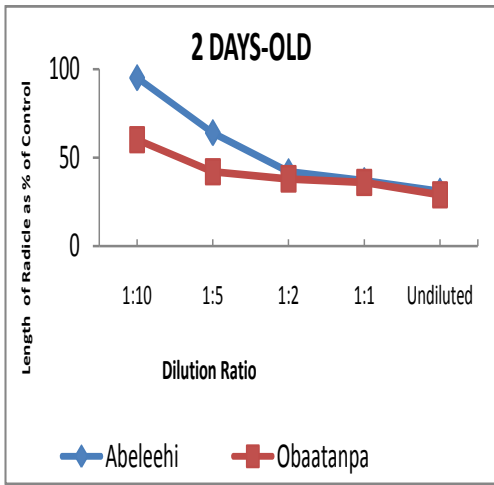


Development of Radicle of two maize varieties (Abeleehi and Obaatanpa) growing on filter paper moistened with culture filtrate of *Paecilomyces carneus*

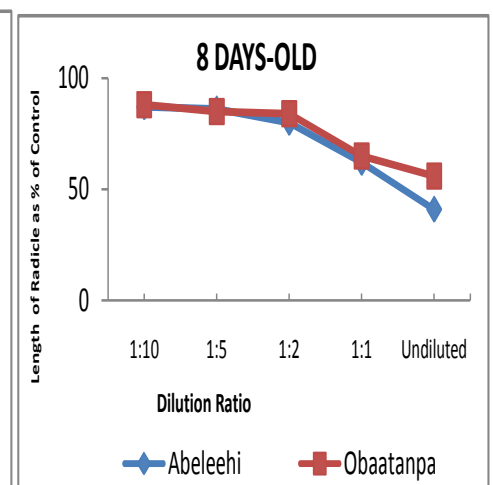
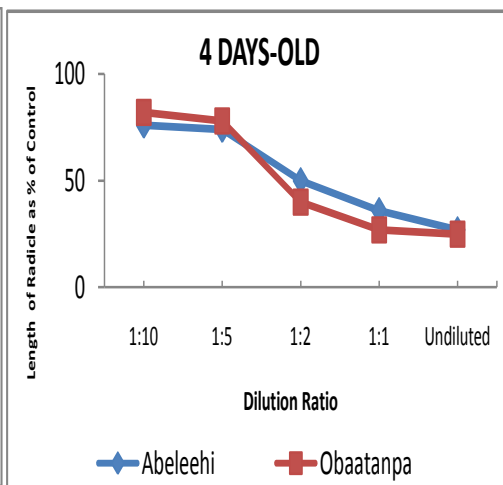
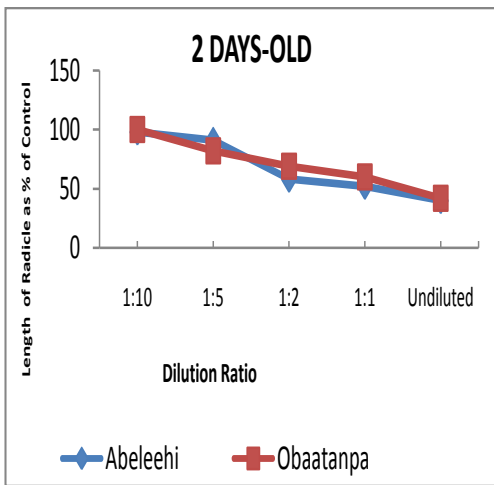
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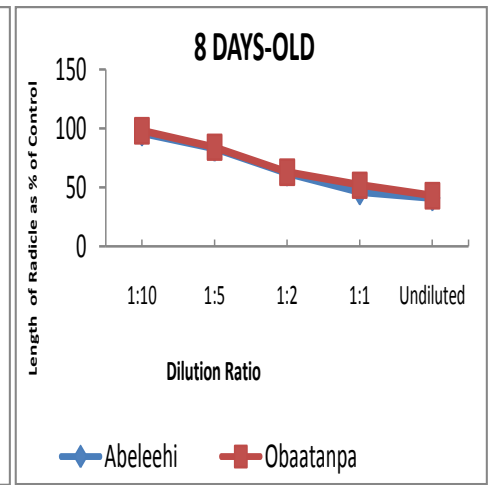
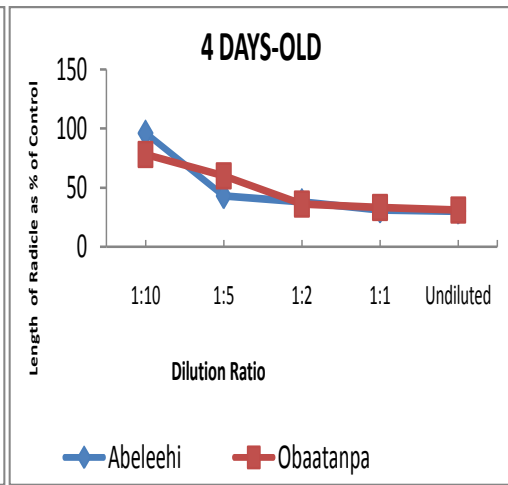
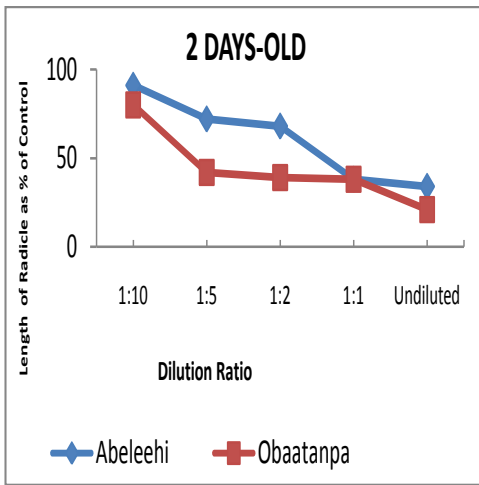


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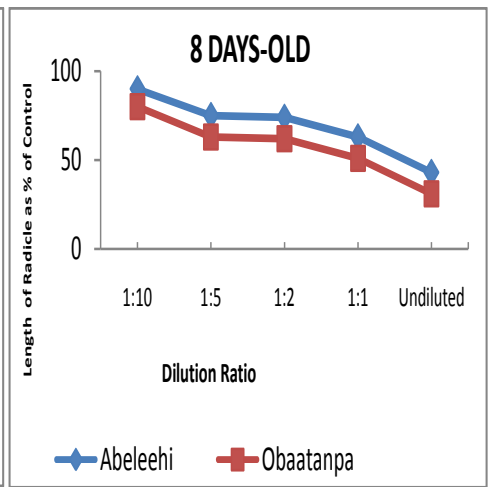
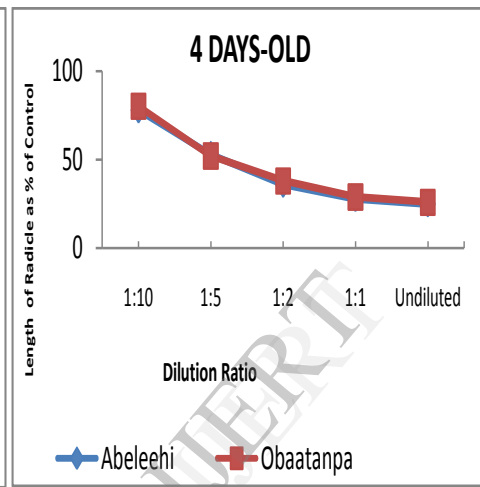
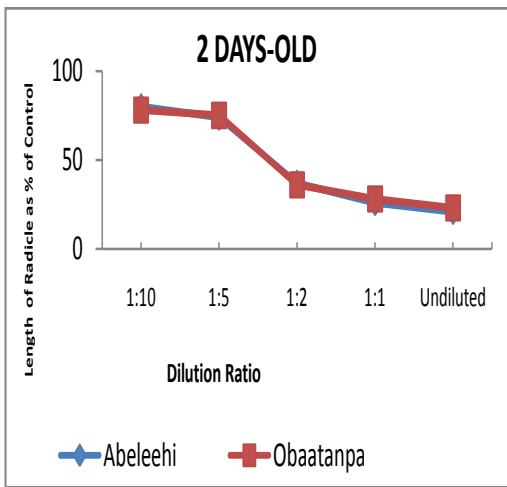


Development of Radicle of two maize varieties (Abeleehi and Obaatanpa) growing on filter paper moistened with culture filtrate of *Paecilomyces puntoni*

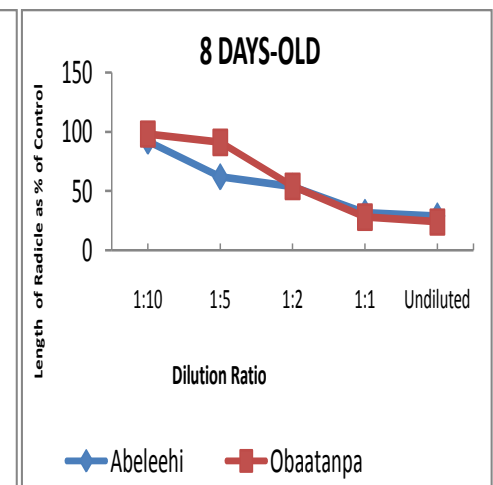
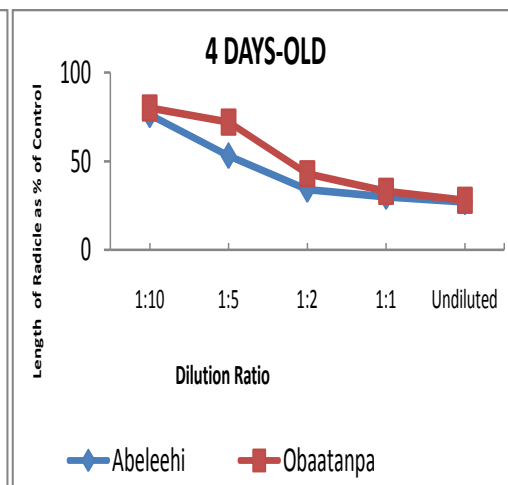
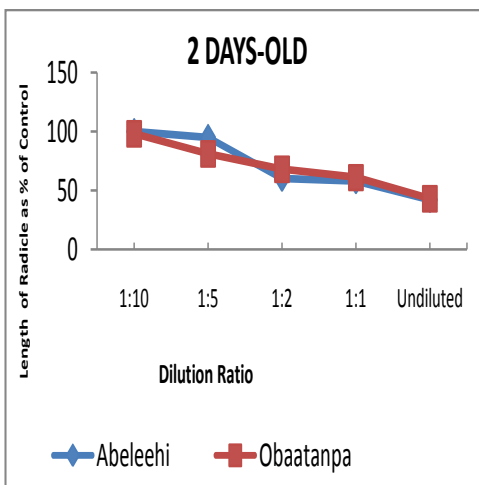
POTATO DEXTROSE BROTH



MAIZE MEAL BROTH-ABELEEHII



MAIZE MEAL BROTH-OBAAATANPA



Development of Radicle of two maize varieties (Abeleehi and Obaatanpa) growing on filter paper moistened with culture filtrate of *Paecilomyces varioti*



Left: Influence of 4 days old culture metabolites of *P.puntoni* on radicle length of germinating seeds of "Abeleehi" Top: left (control); Middle (1:10v/v); Right (1:5v/v) Bottom: left (1:2v/v); Middle (1:1v/v); Extreme right (undiluted)



Right: Influence of 4 days old culture metabolites of *P. varioti* on radicle length of germinating seeds of "Abeleehi". Top: left (control); Middle (1:10v/v); Right (1:5v/v). Bottom: left (1:2v/v); Middle (1:1v/v); Extreme right (undiluted).



Left: Influence of 4 days old culture metabolites of *P.puntoni* on radicle length of germinating seeds of "Obaatpana" Top: left (control); Middle (1:10v/v); Right (1:5v/v). Bottom: left (1:2v/v); Middle (1:1v/v); Extreme right (undiluted)



Right: Influence of 4 days old culture metabolites of *P. varioti* on radicle length of germinating seeds of "Obaatpana" Top: left (control); Middle (1:10v/v); right (1:5v/v). Bottom: left (1:2v/v); Middle (1:1v/v); Extreme right (undiluted)



Left: Influence of 4 days old culture metabolites of *P. carneus* on radicle length of germinating seeds of "Abeleehi". Top: left (control); Middle (1:10v/v); Right (1:5v/v). Bottom: left (1:2v/v); Middle (1:1v/v); Extreme right (undiluted)

Right: Influence of 4 days old culture metabolites of *P. carneus* on radicle length of germinating seeds of "Obaatanpa". Top: left (control); Middle (1:10v/v); Right (1:5v/v). Bottom: left (1:2v/v); Middle (1:1v/v); Extreme right (undiluted).



Left: Photograph showing cobs form on the Abeleehi maize plant after 3 months (90days) exposure to the indicated *Paecilomyces* in the field (x1/4)

Right: Photograph showing cobs with husks removed (1/4)

Discussion

Three *Paecilomyces* species namely; *Paecilomyces carneus*, *P. puntoni*, *P. varioti* were isolated from two recently-developed Ghanaian maize varieties Abeleehi and Obaatanpa under varying ambient equilibrium relative humidities 55-95% representative of the Ghanaian ambient conditions, The pathogenicity of the three *Paecilomyces* species were tested under laboratory, field and greenhouse conditions.

The inhibitory active ingredients in the metabolites of the three *Paecilomyces* species were produced in two days on the various media used. These metabolites when undiluted depressed seed germination of Abeleehi and Obaatanpa by 10-80% and drastically reduced by 45-85% length of emerging radicles.

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 due to the difference in shift of pH during vegetative growth of these fungal species in culture.

The culture filtrate of the *Paecilomyces* species similarly depressed germination and radicle development of two tomato (*Lycopersicon esculentum* L. vars. Owusu-Bio and Wosowoso) and pepper (*Capsicum annum* L. var. Legon 18).

Works done by other researchers revealed that metabolites of non-pathogenic fungi had adverse or beneficial effect on plants. Important observations made include suppression on seed germination (Leelavathy, 1969 a,b; Narain and Prakash, 1968; Odamtten and Clerk, 1985, 1988) mal-formation and retardation of growth of seedlings (Bowen and Rovira 1961; Odamtten and Clerk 1985) and root growth promoters (Kimura et al 1992 a,b).

Interestingly, the laboratory observations of the inhibitory effect of the fungal metabolites of the three *Paecilomyces* species on Abeleehi and Obaatanpa maize varieties were reproduced in the field. The heights of plants, leaf length and leaf width of the growing seedlings were significantly depressed by the metabolites of the three *Paecilomyces* species used in the study. However, the toxic effects of the same fungal metabolites produced in culture before being used in moistening soil in greenhouse was marginal. Presumably, the application of the cultural metabolites directly to soil rendered them less potent.

The severe depression of vegetative growth of maize (Abeleehi and Obaatanpa) by metabolites of the *Paecilomyces* species was reproduced when the seeds were directly inoculated with fungus prior to sowing on the field. The photosynthetic apparatus of the growing seedlings was affected. Chlorophyll "a" and "b" contents were lower in Abeleehi and Obaatanpa plants growing in the field inoculated with

P. carneus followed by grains treated with *P. puntoni*.

The plants raised in pots in the greenhouse and moistened with the culture filtrate of *Paecilomyces* species behaved differently although chlorophyll "a" and "b" contents of both maize varieties were significantly reduced by *P. puntuni* and *P. varioti*.

The culture filtrate of the *Paecilomyces* species affect the system of Aeleehi and Obaatanpa in various ways. The root of the maize seedlings growing in the field thinner with reduced width of pith parenchyma tissue; narrower proto- and metaxylem phloem were seen. This could adversely affect rate and efficiency of translocation of nutrients and photosynthates to and from the roots.

In summary, the cultured metabolites from three *Paecilomyces* species; *P.carneus*, *P. puntoni*, *P. varioti* depressed seed germination, reduced radicle elongation, decreased chlorophyll "a" and "b" contents of leaf and depressed total dry matter accumulation of shoot and root systems the seedlings.

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