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Influence of the Culture Filtrate of Three *Paecilomyces* Species on Some Growth Parameters, Chlorophyll Content, and Root Anatomy of Two Ghanaian Maize Varieties (Abeleehi and Obaatanpa) and on Germination Capacity of Tomato and Pepper Seeds

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Abstract Three xerophillic fungal species, *Paecilomyces carneus, P. puntoni, P. variotii* were for the first time isolated from two Ghanaian maize (*Zea mays* L.) varieties Abeleehi and Obaatanpa. The fungal species were isolated under varying ambient humidity of 55-65% provided by glycerol, water mixtures in both grain varieties. The cultural filtrate of the *Paecilomyces* raised in Maize Meal Broth prepared from either Abeleehi or Obaatanpa and Potato-Dextrose broth. The cultural filtrate prepared in varying dilutions (undiluted - 1:10v/v) were used as germinating medium to test the germinating capacity and radicle development of hot pepper (*Capsicum annuum* L.) variety Legon 18 and tomato (*Lycopersicon esculentum* M.) varieties Owusu -Bio and Wosowoso using the blotter test method. The metabolites and mycelium/conidia of the three fungal species were tested on vegetative growth and dry matter accumulation of the plants under both greenhouse conditions and in the field. Dry weights of the root and shoot systems were determined at varying intervals by the oven-dry weight method. The metabolites of the three fungal species significantly ($P \leq 0.05$) depressed seed germination and radicle development of the two vegetables. Each culture filtrate from the test fungi was unique in its effect on the germination and radicle development of the seedlings of tomato and pepper. There were also varietal differences in response to the tomato and pepper seeds to the same metabolites. The inhibitory effect was highest on var. Owusu-Bio than var. Wosowoso. The inhibitory principle was still potent even at 1:10v/v dilution level. The reduction of plant height, leaf length and leaf width of the two maize varieties in pots in the greenhouse by the culture filtrate of the fungal species was marginal and, did not differ significantly ($P \leq 0.05$) from the control seedlings. The plant height, leaf length and leaf width were significantly ($P \leq 0,05$) depressed by the metabolites of the fungal species in the field. Each plant part responded differently to the inhibitory effect of the culture metabolites. The chlorophyll content of the leaves of the growing seedlings under the influence of the three fungal metabolites were affected both in the field and in the greenhouse Duncian's Multiple Range Test showed that chlorophyll content were significantly ($P \leq 0.05$) different from the untreated. Varietal differences in chlorophyll content were recorded in the leaves of the seedlings in the field and the greenhouse depending on the fungal metabolites used on the growing seedlings. Photosynthetic apparatus of the seedlings of the maize varieties were affected by the metabolites of the test fungi. The roots of the untreated seedlings was 2-3 times wider in diameter than those seedlings exposed to the three fungal metabolites. However, the endodermis and pericycle were clearly formed and demarcated in both the treated and the untreated samples. The pith parenchyma was sclerified and 2-3 times narrower in diameter in the treated plants. The phloem and xylem regions of the root were also reduced in number and size. Practical implications of findings are discussed.

Keywords: *Paecilomyces, Maize (Abeleehi, Obaatanpa), Chlorophyll content, Pericycle, Pith parenchyma, tomato, pepper*

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1. Introduction

According to FAO [1] maize (*Zea mays* L.) is the world's top most cereal crop in terms of total production after wheat and rice. Unfortunately, this important cereal grain suffer spoilage mainly from fungi both in the field and in storage resulting in colossal loss of the grain to the farmer, warehouse agents and pose a threat to food security, particularly, in sub-Sahara Africa'. In his study, [2] reported that microbial infestation is responsible for a huge loss of 30% of total food production in the world. Of this, the predominant share of the loss is due to microbiota infestation and deterioration. In a related work, [3] stated that more than sixty (60) different diseases caused by various pathogens affect maize crop, fungi is the worst offender. Pathogenic fungi deteriorate food grains by producing mycotoxins such as *aflatoxin*, *ochratoxin* to mention but a few during storage posing a serious health hazard to the consumer.

Fungal infestation of maize grains not only cause economic loss and health implications, but also affect the germination capacity and the seedling development of the grain. In vitro seed inoculation test, revealed that *Fusarium verticillioides* (= *F. moniliforme*) and *A. niger* had significant effect on the seed germination and seedling health due to high infection and production of mycotoxin [4]. Several other studies [5,6,7] reported that *F. verticillioides* on maize grains produce mycotoxins that are involved in retarding seed germination and seedling growth. In a recent study, [8] stated that the culture filtrate of three xerophilic fungal species; *Paecilomyces carneus*, *P. puntonii*, and *P. variotii* isolated for the first time from two recently-developed Ghanaian maize varieties (Abeleehi and Obaatanpa) significantly ($P \leq 0.05$) depressed the percentage germination of the two maize varieties by 10-75% by the undiluted, 2, 4 and 8 days old culture filtrate of the three fungal species. The same culture filtrate severely depressed length of emerging radicle of the two maize varieties by 40-90% at the highest concentration of the culture filtrate applied [8].

The current study investigated the effect of the culture filtrate of the three *Paecilomyces* species on the vegetative growth, dry matter accumulation, chlorophyll content, and the root anatomy of Abeleehi and Obaatanpa growing under greenhouse conditions and in the field with the view to elucidating their pathological significance in potential reduction of crop yield.

2. Materials and Methods

2.1. Materials

The maize varieties used Abeleehi and Obaatanpa were purchased from Aglow Seed Company, Accra. The fungal species, *Paecilomyces carneus, P. puntoni, and P. varioti* used in these investigations were isolated from the Abeleehi and Obaatanpa maize varieties using the conventional isolation techniques.

2.2. General Methods

Maize Sample kept in simulated 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.

Maintenance of stock Cultures

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

Influence of Fungal Metabolites on Vegetative Growth and Dry Matter Accumulation on Maize Seedlings

Field Studies

Healthy surface-sterilized (with 1% sodium hypochlorite) grains of Abeleehi and Obaatanpa were inoculated 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 x 10^3 spores /mL applied directly into the slit. The inoculated grains were kept covered in Petri dishes for 24 hrs at 30^oC 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, 21, 35, 42 and 56 days growth under the normal day/light regime in the tropics.

Greenhouse Studies

Black polythene bags (35 x 20cm) served as pot for soil which **were** seeded with five grains of either Abeleehi or Obaatanpa varieties and then thinned to two per bag after germination. The soil in each bag was moistened with either 30mL undiluted culture filtrate of either *P. carneus. P. puntoni* or *P. varioti* initially and at two days intervals for 2 weeks 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^oC. Measurement 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 plants parts were determined by keeping them at 80°C for 48 hrs. and weighed after cooling.

Chlorophyll content

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

$$\begin{aligned}&\text{Chlorophyll a}\\&=12.7 \text{ x Absorption at 663nm}\\&-2.69 \text{ x Absorption at 645 } (\text{mg/L}).\end{aligned}$$

$$\begin{aligned}&\text{Chlorophyll b}\\&=22.2 \text{ x Absorption at 645nm}\\&-4.67 \text{ x Absorption at 663 } (\text{mg/L}).\end{aligned}$$

$$\begin{aligned}&\text{Total Chlorohyll}\\&=20.2 \text{ x Absorption at 6.45nm}\\&+8.02 \text{ x Absorption at 663 } (\text{mg/L}).\end{aligned}$$

Anatomical Studies

The structure of the root of seedlings growing under the influence of the metabolites of the three *Paecilomyces* species in the field was studied. Sliding microtome (Riechert Nr. 15917, Austria) sections were stained temporarily in aniline chloride and permanently with safranin and fast green following the procedure of [9].

3. Results

3.1. Results

The three *Paecilomyce*s species under greenhouse conditions did not significantly ($P \geq 0.05$) affect the seedling height, leaf length and width of the seedlings of Abeleehi (Figure 1). The same was true for the leaf length of Obaatanpa seedlings. The effect of the three *Paecilomyces* species on Obaatanpa seedling height and leaf length can be described as marginal (Figure 1).

Under field conditions, the effect of the fungal metabolites showed some significant difference ($P \leq 0.05$) in their effect on leaf width, seedling height, and leaf length of both maize varieties. For example, in most instances the depressive effect of the three *Paecilomyces* species was significantly ($P \leq 0.05$) severer on the seedlings of Obaatanpa and Abeleehi than on the control (Figure 2).

Figure 3 summarizes the inhibitory effect of the fungi of the same genera on the dry weight, dry weight of stem and dry weight on seedlings of Abeleehi and Obaatanpa maize varieties under Greenhouse conditions. The effects of the *Paecilomyces* species on the measured parameters were variable. For example, there was no statistical difference between the effect of the three *Paecilomyces* species on the dry weight of Abeleehi maize variety raised under greenhouse conditions (Figure 3) for 56 days whereas there was a significant ($P \leq 0.05$) decline on the dry weight of Obaatanpa leaves treated with the fungi and metabolites of *P. varioti* followed by *P. carneus* (Figure 1) as compared to the control. The effect of the fungi and the metabolites was most potent on dry matter accumulation by both Abeleehi and Obaatanpa in the order *P. varioti* < *P carneus* < *P. puntoni*.

Therefore, the fungus and the metabolites of the *Paecilomyces* species under greenhouse condition affected the development of the plant parts differently.

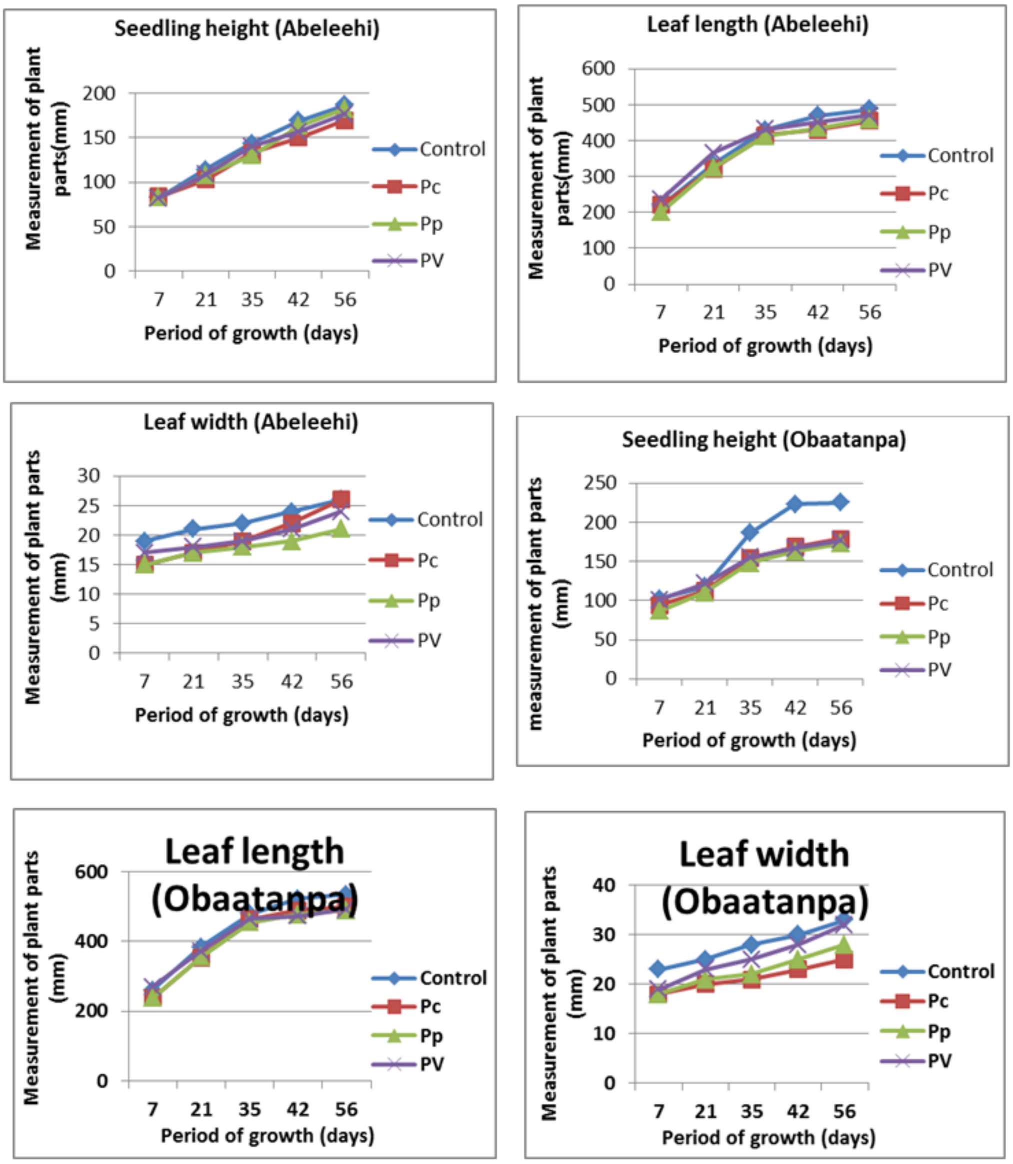

Figure 1. Influence of metabolites of the indicated *Paecilomyces* species on height, length and width of leaf of maize varieties in the Greenhouse at 28-31°C (**Key**: ***P.c : Paecilomyces carneus, P.p: P. puntoni, P.v: P. varioti***)

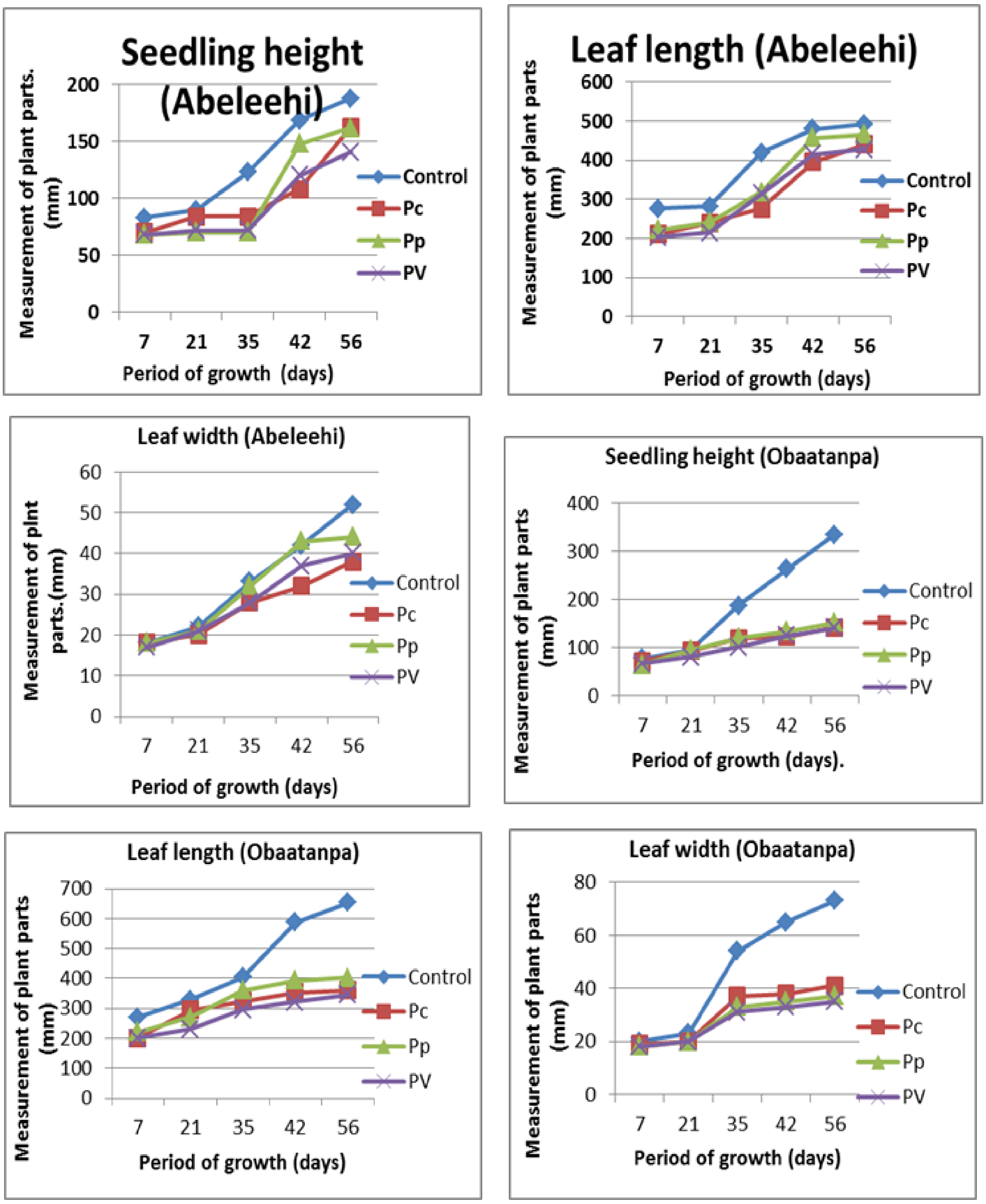

Figure 2. Influence of metabolites of the indicated *Paecilomyces* species on height, length and width of leaf of maize varieties in the field at 28-31°C

Table 1. Chlorophyll content of seedlings of indicated maize varieties growing in the Field or in Pots under normal day/night regime at 28 - 32°C for 56 days

Maize variety	Fungus inoculated	Chlorophyll content (mg/L).					
		Chlorophyll a		Chlorophyll b		Total chlorophyll	
		Field	Pot	Field	Pot	Field	Pot
Abeleehi	Untreated (control)	11.28	15.59	19.23	24.51	31.27	41.49
	P, carneus	8.44	13.81	15.15	18.73	24.79	40.13
	P. puntoni	10.41	9.85	17.88	15.11	29.14	29.06
	P. varioti	11.06	11.09	18.61	16.89	30.88	31.95
Obaatanpa	Untreated (control)	16.43	16.01	29.83	20.82	41.42	33.12
	P. carneus	9.69	9.07	17.81	15.19	27.46	26.66
	P. puntoni	13.43	8.67	22.51	14.40	35.99	25.74
	P. varioti	15.92	8.84	26.98	14.69	41.01	26.11

Table 2. Influence of the metabolites of indicated *Paecilomyces* species on germination and radicle development of tomato (*Solanum esculentum* = *Lycopersicum esculentum*) vars. Owusu-Bio, Woswoso and pepper (*Capsicum annuum*) var. Legon 18

Seed variety	Dilution ratio of filtrate (v/v)	Germination (%) in culture filtrate of			Radicle length (mm) in culture filtrate of		
		P. carneus	*P. puntoni*	*P. varioti*	*P. carneus*	*P. puntoni*	*P. varioti.*
Solanum esculentum var. Owusu -Bio.	Undiluted	30	15	17	4.0 ± 1.9	1.0 ± 0.0	1.0 ±0.1
	1:1	40	30	34	5.0 ± 0.9	1.0 ± 0.6	2.0 ± 0.9
	1: 2	45	50	38	7.0 ± 2.0	1.0 ± 0.3	3.0 ± 1.8
	!: 5	50	55	42	7.0 ± 2.3	4.0 ± 1.7	8.0 ± 1.5
	1:10	70	67	53	13.0 ± 1.8	10.0 ± 1.2	11.0 ± 1.2
	Control (distilled water).	85			25.1 ± 3.1		
Solanum esculentum var. Wosowoso	Undiluted	70	65	55	3.0 ± 0.8	3.0 ± 0.8	2.0 ± 0.3
	1:1	75	78	68	9.0 ± 1.9	4.0 ± 2.0	4.0 ± 1.6
	1:2	80	88	85	12.0 ± 2.1	4.0 ± 1.5	6.0 ± 1.5
	!:5	94	90	95	19.0 ± 2.8	6.0 ± 1.5	15.0 ± 2.0
	1:10	97	93	95	26.0 ± 2.7	9.0 ± 1.6	18.0 ± 2.1
	Control (distilled water)	100			40.4 ± 4.0		
Capsicum annuum var. Legon18.	Undiluted	0	10	0	-	1.0 ± 0.0	-
	1:1	13	17	3	2.0 ± 1.1	1.0 ± 0.7	1.0 ± 0.8
	1:2	10	40	17	3.0 ± 0.8	1.0 ± 0.0	2.0 ± 0.9
	!:5	33	48	37	3.0 ± 0.9	3.0 ± 1.0	2.0 ± 1.2
	1:10	47	52	50	4.0 ± 0.9	4.0 ± 0.7	4.0 ± 1.1
	Control (distilled water	78			5.6 ± 1.8		

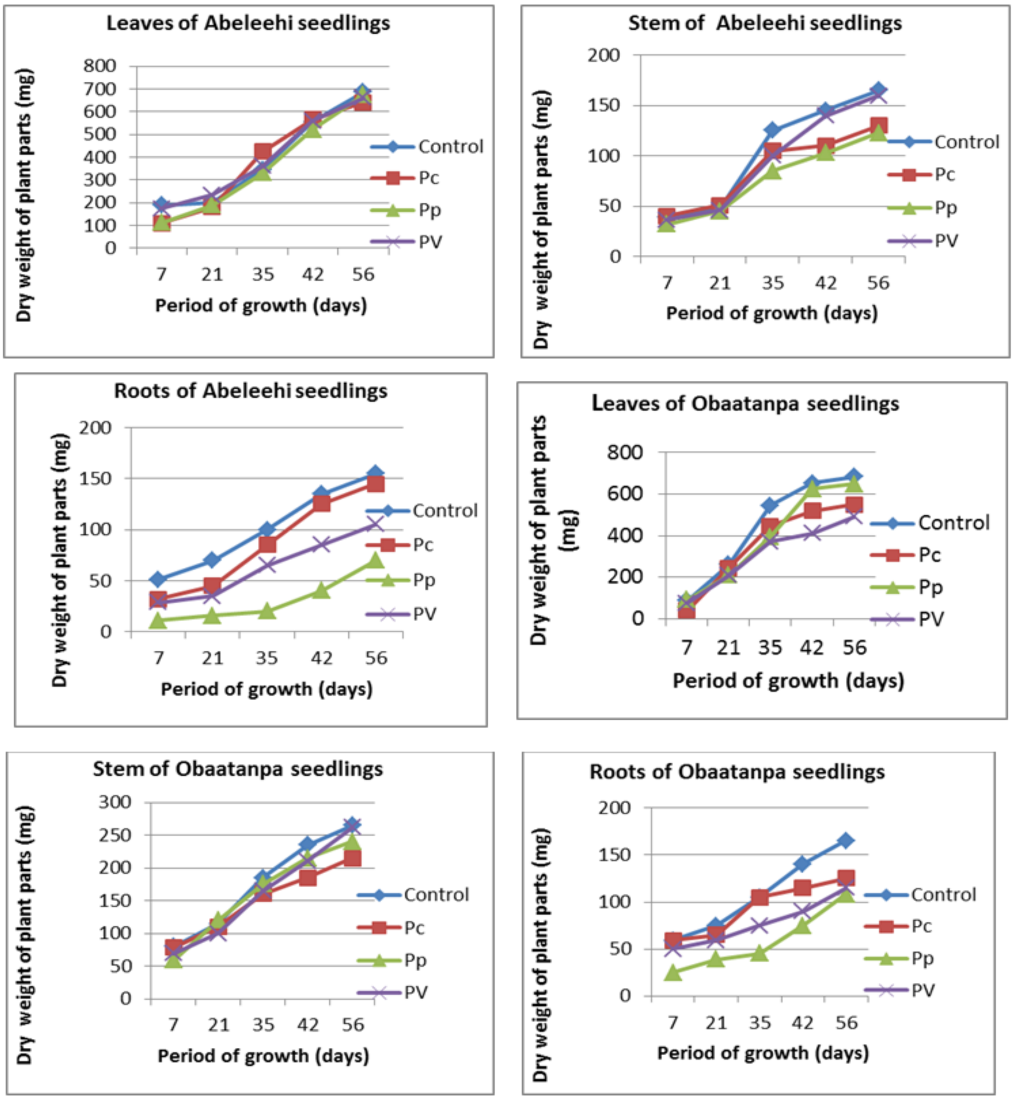

Figure 3. Influence of metabolites of the indicated *Paecilomyces* species on dry matter accumulation by leaves, shoots, and roots of maize varieties in the Greenhouse at 28-31°C

Influence of Metabolites of Three *Paecilomyces* species on Germination and Radicle Development of Pepper (*Capsicum annuum* L.) and tomato (*Solanum esculentum* Mill)

The inhibitory effect of the metabolites of *Paecilomyces carneus*, *P. puntoni* and *P. varioti* on germination and radicle development cannot be confined to maize grains only [8,10,11]. These same metabolites in the culture filtrates of the respective *Paecilomyces* species significantly ($P \leq 0.05$) depressed seed germination and radicle development of two tomato varieties (CV's Owusu-Bio and Wosowoso) and hot pepper (Var., Legon 18). Each culture filtrate from the test fungi was unique in its effect on the germination and radicle development of the seedling of tomato and pepper. There were also varietal differences in the response of the tomato and pepper seed to the same metabolites (Table 2). The inhibitory effect of the metabolites was highest on var. Owusu-Bio than var. Wosowoso. The inhibitory principle was still potent even at 1:10v/v dilution level. Clearly, tomato and pepper seeds are also adversely affected by the culture metabolites of the *Paecilomyces* species.

Studies on the Root Anatomy of Maize Seedling (Abeleehi and Obaatanpa Varieties). Growing under the Influence of *Paecilomyces* species.

Figure 4 & Figure 5 show the effect of the metabolites on the root anatomy of maize seedlings. The root of the untreated seedlings (control) was 2-3 times wider in diameter than seedlings exposed to the metabolites of *P. carneus*, *P. puntoni* and *P. varioti*. However, the endodermis and pericycle were clearly formed and demarcated in both the treated and the untreated (control) samples. The pith parenchyma was sclerified and 2-3 times narrower in diameter in the treated plants exposed to the metabolites of the *Paecilomyces* sp; the protoxylem and metaxylem vessels were about 2 times wider in the control plants. The phloem and xylem regions of the root treated with the fungal metabolites of *Paecilomyces* were thus reduced in number and size.

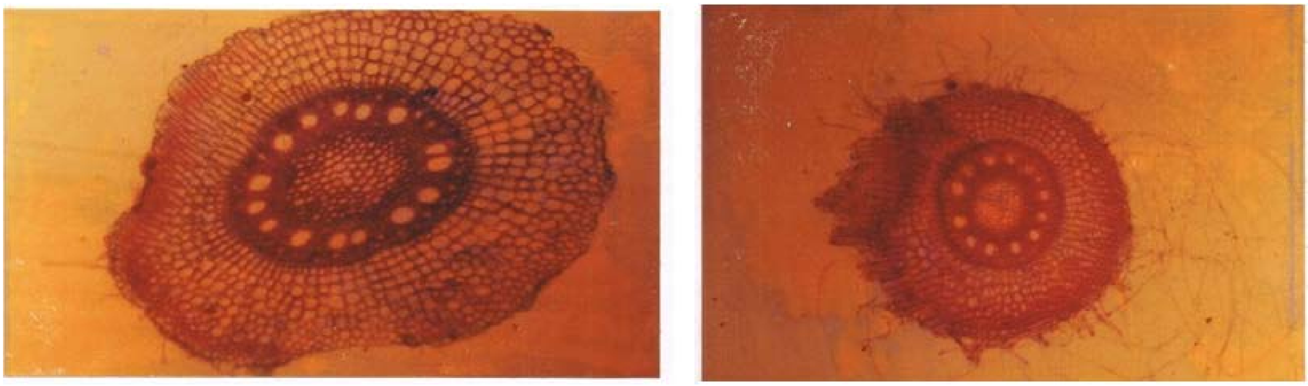

Figure 4. Transverse section of roots of 'Obaatanpa' seedlings (left : Untreated (control) ; right: Treated with 4-day old culture metabolites of *P. carneus* growing in pots in the Greenhouse. (x 400))

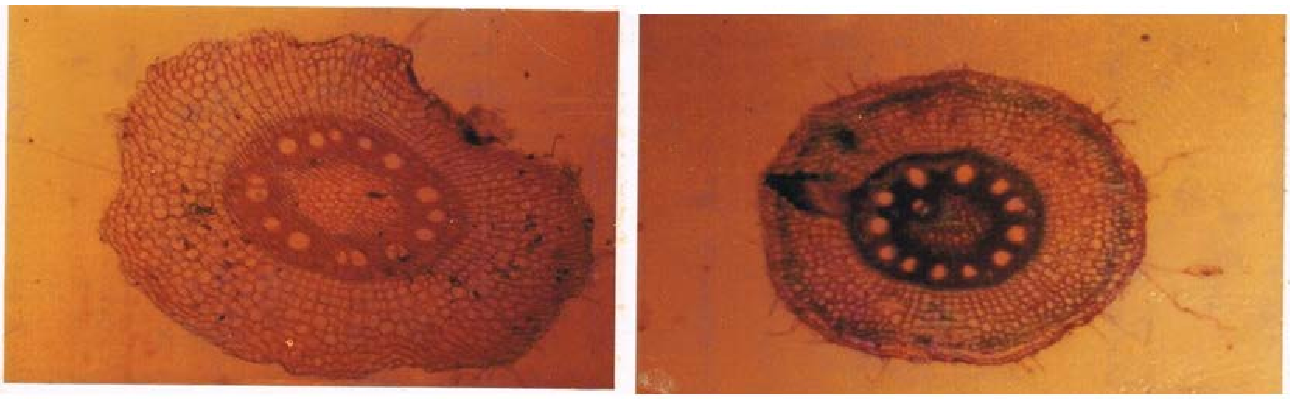

Figure 5. Transverse section of roots of 'Abeleehi' seedlings (left: Untreated (control); right: Treated with 4-day old culture metabolites of *P.varioti* growing in pots in the Greenhouse. (x 400))

3.2. Discussion

Metabolites of non-pathogenic fungi have been reported to have adverse or beneficial effect on plants. Important observations include suppression on seed germination [12,13,14,15,16], malformation and retardation of growth of seedlings [15,17,18,19] and root growth promoters [20,21]. In this paper, the culture filtrate of three *Paecilomyces* similarly depressed germination and radicle development of two Ghanaian maize varieties Abeleehi and Obaatanpa [8] and two Ghanaian tomato varieties, Owusu-Bio and Wosowoso and one Ghanaian pepper (*Capsicum annuum* L.) variety Legon 18. This investigation and earlier ones [8,10,11] extends the list of fungi whose metabolites have adverse effect on seed germination and radicle development of maize, pepper and tomato which are of economic importance in Ghana. Interestingly, the laboratory observations of the inhibitory effect of the fungal metabolites of the three *Paecilomyces* species on Abeleehi and Obaayanpa maize varieties were reproduced in the field (Figure 2). The height of plants, leaf length and leaf width of the growing seedlings in the field were variably depressed by the metabolites of *Paecilomyces carneus*, *P. puntoni* and *P. varioti*. However, the toxic effect of the same fungal metabolites in culture before being used in moistening soil in the Greenhouse was marginal. Presumably, the application of the culture metabolites directly to the soil in pots in Greenhouse rendered them less potent or antibiosis and rhizoshere effect interacted to decrease potency.

There has been some failures in some instances to duplicate in soil admirable results of antagonism tests obtained in cultures. The reasons for these failures include obvious differences between growth of the fungus on agar, liquid culture and in soil. Secondly, the known toxins are either too unstable to persist in soil or if stable, will be rapidly inactivated by adsorption on soil colloids. For instance, [22] found that *Aspergillus niger* failed to depress vegetative growth of cocoa seedlings in non-sterile native cocoa farm soil in contrast with severe depression of growth reported by [15,16] using sterile vermiculite. In this paper, however, the severe depression of vegetative growth of Abeleehi and Obaatanpa by metabolites of *Paecilomyces* species could be reproduced when the seeds were directly inoculated with fungus prior to sowing. The photosynthetic apparatus of the growing seedlings of the two maize varieties was also affected (Table 1). Chlorophyll 'a' and 'b' contents were lower in Abeleehi and Obaatanpa plants growing in the field and inoculated with *P. carneus* followed by grains treated with *P. puntoni* (Table 1). Seedlings raised in pots in the Greenhouse and moistened with the culture filtrates of *Paecilomyces* species behaved differenly although chlorophyll 'a' and 'b' contents of both Abeleehi and Obaatanpa were significantly reduced by *P. puntoni* and *P. varioti.*

The process of photosynthesis occurs in two stages. The first is the light reaction which is that part in which light and chlorophyll 'a' and 'b' are involved. It is during the light reaction that light energy is converted to chemical energy. This chemical energy is used to synthesize carbohydrate from carbon (iv) oxide in the air entering through the stomata during the second part of photosynthesis called 'dark reaction' because it does not require light. It therefore, stands to reason that reduction in the chlorophyll 'a' and 'b' content of leaf may presumably affect the efficiency of the light reaction stage of photosynthesis involving the two pigments and subsequently the formation of photosynthates in the dark reaction stage. This presumably explains the variable adverse effects of the metabolites of especially *P. carneus* and *P. varioti* on vegetative growth and dry matter accumulation by the shoot and root systems of Abeleehi and Obaatanpa maize varieties in the field (Figure 1 - Figure 3 and Table 2).

Culture filtrate of the three *Paecilomyces* species affected the root system of Abeleehi and Obaatanpa in various ways (Figure 3). The root of maize seedlings growing in the field were thinner with reduced width of pith parenchyma tissue; narrower protoxylem and metaxylem and phloem cells were seen (Figure 4 and Figure 5). This could adversely affect rate and efficiency of translocation of nutrients and photosynthates to and from roots. A fortuitous condition is thus created in which the cultural metabolites from *Paecilomyces* species depress seed germination, reduced radicle elongation, decrease chlorophyll 'a' and 'b' contents of leaf, and depressed total dry matter accumulation by shoot and root systems of the seedlings. Consequently, there was reduction in yield of the treated seedlings resulting in smaller and narrower cobs with fewer grain as compared to the control. Although information on mycotoxin production by *Paecilomyces* is scanty, *P. varioti* produces patulin [23] the same as *Penicillium expansum* this may play a role in the severity of *P varioti* on the test plants.

Interestingly, the metabolites of *P. carneus,P. puntoni* and *P. varioti* affected the chlorophyll content of the leaves of the growing seedlings of Abeleehi and Obaatanpa varieties both in the field and in greenhouse. Chlorophyll a and b contents were lowest in Abeleehi and Obaatanpa plants growing in the field whose seeds were inoculated with *P. carneus* followed by seeds treated with *P. puntoni* (Table1). Duncian Multiple Range Test showed that the data recorded were significantly ($P \leq 0.05$) different from the untreated (control). Total chlorophyll content of Abeleehi seedlings was also significantly ($P \leq 0.05$) lower in seedlings whose seeds were inoculated with *P. carneus* and *P. puntuni* but not with *P. varioti*. Seedlings of Abeleehi and Obaatanpa in the greenhouse which were treated with metabolites of the three *Paecilomyces* species behaved differently. Metabolites of *P. carneus,P; puntoni* and *P. varioti* severely reduced chlorophyll a and b contents of both Abeleehi and Obaatanpa. *P. puntoni* metabolite was more potent. Total chlorophyll contents of the leaves of both maize varieties treated with the metabolites of the three *Paecilomyces* species were also lower (Table 1). Therefore the metabolites of *Paecilomyces* species also affected the photosynthetic apparatus of the maize varieties tested

It will be interesting in future experiments to elucidate the differences in the composition of the metabolites produced by the *Paecilomyces* species and their specific effect on the photosynthetic apparatus of the developing maize seedlings This paper has provided evidence of the potential risk of long-term storage of maize seeds infected with *Paecilomyces* species and also provide data which could assist in formulating policies for safe long-term storage of Abeleehi and Obaatanpa under Ghanaian tropic conditions.

4. Conclusion

This paper and previous ones [8,10,11] contain data that suggest that the two newly developed Ghanaian maize varieties Abeleehi and Obaatanpa harbour many field and storage fungi some of which are of pathological importance such as *Fusarium verticillioides* (= *F. moniliforme*), *Aspergillus alutaceus* (= *A ochraceus*), *A niger*, *A. fumigatus*, *Penicillium digitatum*, *P.expansum*, *Paecilomyces* species (*P. carneus*, *P. puntuni*, *P. varioti* among others. Metabolites of these pathogens especially *Paecilomyces* spp. depressed seed germination, reduced radicle development, decreased chlorophyll 'a' and 'b' content of leaf and depressed total dry matter accumulation by shoot and root systems, reduced yield of the crop, the length and the number of grains on the cob. Long-term storage of seeds (maize, tomato, pepper) infected with these fungi can result in drastic reduction in germination capacity. A better understanding of the dynamics and phenology of microorganisms in the rhizosphere and rhizoplane of the crop as well as the nature of antagonism at play would help formulate effective biological control of these potential pathogens in the field.

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