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(RESEARCH ARTICLE)

Comparative growth of three *Paecilomyces* species on natural media and the possible pathological effects of their cultural metabolites on seed germination and radicle development of six economic crops in Ghana

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Abstract

Three *Paecilomyces* species from Cabi Science (UK) and *P. carneus* GHL 001 isolated from a local shrimps (Ghana) were tested for their radial growth characteristics on four different natural media, at temperatures of 15 °C, 18 °C, 20 °C and 25 °C using the culture plate radial method. The ability of culture metabolites of the test fungi raised in Yam Dextrose and Potato Dextrose Broths were tested on the germination capacity of six economic crops, using the conventional seed blotter test method. Radial growth of the fungal species showed similar growth pattern with optimum growth at 25 °C in most instances. The local *P. carneus* GH001 grew better and faster on all the media tested than *P. carneus* IMI133119 from the UK, underscoring the influence of environmental adaptation to physiological performance. The varietal differences in the response of the germinating seeds to the toxic metabolites can be ranked in decreasing order as follows: *P. carneus* IMI GHL 001 > *P. carneus* IMI 133119 > *P. varioti* IMI 40025 > *P. puntoni* IMI 58415. To the best of our knowledge, this is the first report on the metabolites of *P. carneus*, *P. puntoni* and *P. varioti* adversely having pathological influence on germination and radicle development of cowpea, groundnut, soybean, okra, sorghum and cucumber in Ghana. Since these *Paecilomyces* species are used in some agricultural jurisdictions as entomopathogens for biocontrol of pests and diseases, environmental impact assessment is encouraged with the view of monitoring the soil bank for conidia of *Paecilomyces* which may affect the performance of crops in the field.

Keywords: *Paecilomyces* species; Metabolites; Fungi; Radial growth; Economic crops

1. Introduction

Seed-borne fungi are the major causes of seed-transmitted pathogens of economic importance [1]. Some classes of fungal genera are frequent in seeds, others occur occasionally or not at all. The extents to which fungi reside in seed depend on their ability to survive under dry conditions. Xerophilic or xerotolerant fungi produce chlamydospores, conidia, sclerotia, etc in order to survive under adverse conditions. Fungi are ubiquitous; their spores and infective reproductive structure can be airborne, soil-borne or seed-borne. Fungi infecting crops in the field are called field fungi and those residents in harvested and stored seeds are called storage fungi [2]. In the tropics, *Aspergillus*, *Penicillium* and *Fusarium* species are frequently encountered, and some *Paecilomyces species* have been isolated from stored seeds [3, 4-6] in Ghana. The role of fungi in quality deterioration of food crops and viability of seeds has been well-documented

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in cereals, legume and other field crops in the USA, South America, Pakistan, India, Asia and Africa [7]. In Ghana, fungal development and seed contamination of some agricultural crops have been well-documented [8-13].

Microbial contamination is responsible for about 30% food losses in the world [14], and is mainly due to mycofloral infection [15, 16]. Seed-borne fungi are one of the major causes of reduction in germination capacity and field development of cultivated field crops [17]. Information in pertinent literature indicate that, fungi belonging to the genera *Alternaria, Aspergillus, Bipolaris, Cephalosporium, Fusarium, Mucor, Rhizopus, Penicillium, Paecilomyces* and some members of the *Deuteromycota* (Fungi Imperfecti) have been shown to have adverse effect on seed viability and crop development [4-6, 17-20]. Metabolites of non-pathogenic fungi may have adverse effects on plants in the field. Important observations include suppression of seed germination [21, 22] and malformation and retardation of growth of seedling [23, 24]. Recently, Minamor and Odamtten [4, 5] showed that 2-day old metabolites of *Penicillium digitatum* and *Fusarium verticillioides* isolated from two maize varieties in Ghana severely depressed seed germination by 50-70% and radicle elongation by 40-90% depending on the maize variety. Xerophilic fungal species namely; *Paecilomyces carneus, P. puntoni* and *P. varioti* also depressed maize seed germination by 10-75% and there were varietal differences in the response of the maize to the same metabolites [25]. Minamor [26] also showed that the cultural metabolites of *P. carneus*, *P. puntoni* and *P. varioti* adversely affected seed germination and radicle development of the seedlings of pepper and tomato, which are vegetables of economic importance in Ghana.

A fortuitous condition is thus created whereby the cultural metabolites of the three *Paecilomyces* species depressed seed germination, reduced radicle development, decreased chlorophyll content of leaf, depressed dry matter accumulation by shoot and root system of plants [4, 5, 26]. Clearly, the pathological effect of *Paecilomyces* species on seed germination and growth development of economic crops cannot be overlooked.

This study therefore compared the radial growth of three foreign *Paecilomyces* species, namely; *P. carneus* IMI 133119, *P. puntoni* IMI 58415, *P. varioti* IMI 40025 (all from CaBi Bioscience UK), and a local *P. carneus* GH 001 (isolated from dry shrimp). The radial growth comparison was made on natural mycological media prepared from yam (*Dioscorea rotundata*), plantain (*Musa parasidicus*), potato (*Solanum tuberosum*) and cassava (*Manihot esculentum*). The influence of the metabolites of the test *Paecilomyces* species on seed germination and radicle development was also studied. This was done in order to ascertain their potential pathological effects on six locally cultivated economic crops namely: Cowpea (*Vigna unguiculata*), groundnut/peanut (*Arachis hypogeae*), soybean (*Glycine* max), okra (*Abelmoschus esculentus*), sorghum (*Sorghum bicolor*), and cucumber (*Cucumis sativus*).

2. Material and methods

2.1. Acquisition of isolates and seeds

The *Paecilomyces species namely; P. carneus* (IMI 133119), *P. puntoni* (IMI 58415) and *P. variotii* (IMI.40025) used in this study were obtained from Cabi Bioscience in the UK. The local *Paecilomyces carneus* (GH 001) was isolated from local shrimps bought from Kasoa market, in the Central region of Ghana. The seeds of soybeans (*Glycine* max), cowpea (*Vigna unguiculata*), okra (*Abelmoschus esculentus*), cucumber (*Cucumis sativus*), groundnuts/peanuts (*Arachis hypogeae*), and sorghum (*Sorghum bicolor*) were purchased from Aglow Seed Company in Accra, Ghana and authenticated at the Department of Plant and Environmental Biology, School of Biological Sciences, University of Ghana.

2.2. Preparation of solid media

Four solid media were used for the study, namely: Potato Dextrose Agar (PDA), Cassava Dextrose agar (CDA), Yam Dextrose Agar (YDA), and Ripe Plantain Extract Agar (RpDA). The formation of the media used was as follows:

For Potato Dextrose Agar, about 200 g of Irish potatoes was peeled and boiled in an aluminum sauce pan with 500 ml distilled water. After boiling for about 20min, the supernatant liquid was strained with cheese cloth and poured into beakers. The extract was made up to 1 litre in a measuring cylinder. Exactly 20g of glucose and 15g of agar were added and warmed to dissolve the agar. The resultant uniform solution of Potato Dextrose agar was dispensed into 250 ml culture tubes and sterilized at 121°C for 15 min in an autoclave (Touchclave-ecotech Autoclave®, LTE Scientific Ltd, Oldham, United Kingdom).

Similarly, Cassava Dextrose Agar (CDA), Yam Dextrose Agar (YDA), and ripe Plantain Extract Agar (RpDA) were prepared with 200 g of the substrate mixed with 20 g glucose and 15 g agar. Chloramphenicol was added at the recommended concentration of 100 mg/L to each of the medium prepared to suppress bacterial contamination [27]. When a broth was needed 20 g/L agar was not added.

2.3. Determination of radial growth

About 20 ml of each medium (Potato Dextrose Agar, Cassava Dextrose Agar, Yam Dextrose Agar, and Ripe plantain Dextrose Agar) was poured into separate sterilized Petri dishes and left to solidify. Five (5) replicates were made for each treatment.

Each plate was inoculated with one of the four fungal species. The fungus was placed at the intersection point of the lines drawn at the bottom of the plates. The inoculated Petri plates were incubated at different temperatures, namely; 15 °C, 18 °C, 20 °C and 25 °C for eight (8) days.

2.4. Preparation of cultural filtrate

A flame-sterilized stem-borer (5 mm) was used to punch disc of the pure cultures of *Paecilomyces* species which were picked with a flame-sterilized inoculation pin and dropped into 250 ml conical Erlenmeyer flasks containing 30 ml of media (Potato Dextrose broth or Yam Dextrose Broth). There were fifteen replicates for each *Paecilomyces* species for each media. The flasks were incubated at 30 °C for seven (7) days and the filtrate harvested and stored in 500 ml Erlenmeyer flasks covered with black polythene bags and stored for use at 10^{o}C.

2.5. In-vitro studies on the influence of fungal metabolites on germination and radicle development of the five economic crops used (Blotter Test Method)

The culture metabolites were used either undiluted or diluted (1:1, 1:2, 1:5 and 1:10 v/v) to test the germination capacity of the six economy crops. Sterilized distilled water served as the control.

Ten (10) seeds of cowpea, cucumber, soybeans, sorghum, okra, and groundnuts were placed each into Petri dishes (9 cm) moistened with 10 ml distilled water as the control and 10 ml of appropriate dilution of the culture filtrate of the listed fungi (undiluted, 1:1, 1:2, 1;5, 1:10 v/v). There were 250 seeds for each dilution level of the culture metabolites. The plates were incubated at 28 °C – 31 °C. Percentage germination was determined after 7 days. A seed was considered germinated when radicles had emerged and attained a length of not less than 1.5 mm. Photographs of the effects of the metabolites on radicle development were recorded after 7days.

3. Results

Radial growth patterns of the three *Paecilomyces* species on the media tested were similar on all the media. In all instances, best growth of the three species was attained at 25 °C (Figures 1, 2, 3 and 4).

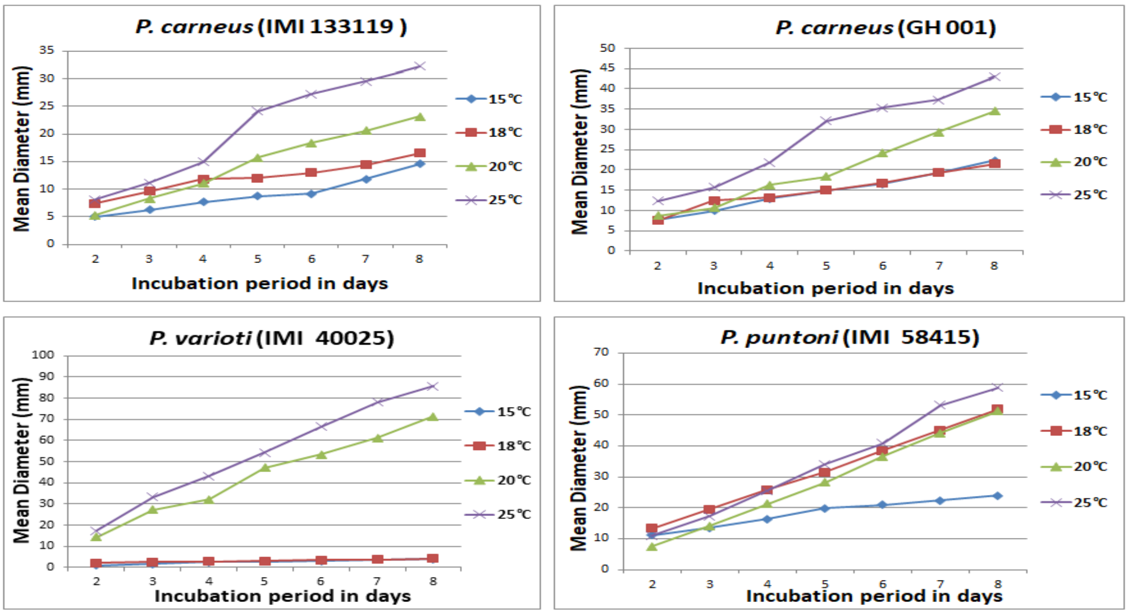

Figure 1 Radial growth of *Paecilomyces* species at different temperatures on Potato Dextrose agar

Growth at temperature of 20 °C was intermediate between 25 °C and 18 °C in most instances. Clearly, radial growth at 15 °C and 18 °C lagged behind and therefore, were inferior for growth in most instances. In all cases, the local *P. carneus* GH 001 grew significantly (P = 0.031) better than the foreign *P. carneus IMI 133119* on all the media tested (Figures 1, 2, 3 and 4). The differences observed between the local *P. carneus* GH 001 and *P. carneus* IMI 133119 was also statistically significant (P = 0.034).

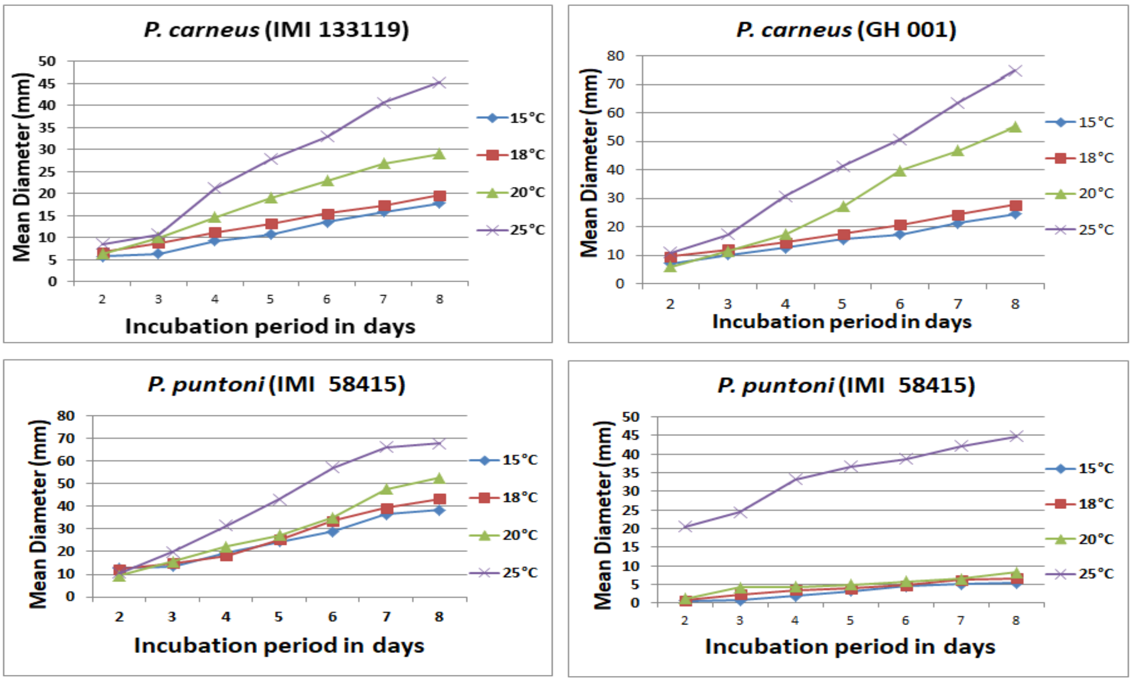

Figure 2 Radial growth of *Paecilomyces* species at different temperatures on Cassava Dextrose agar

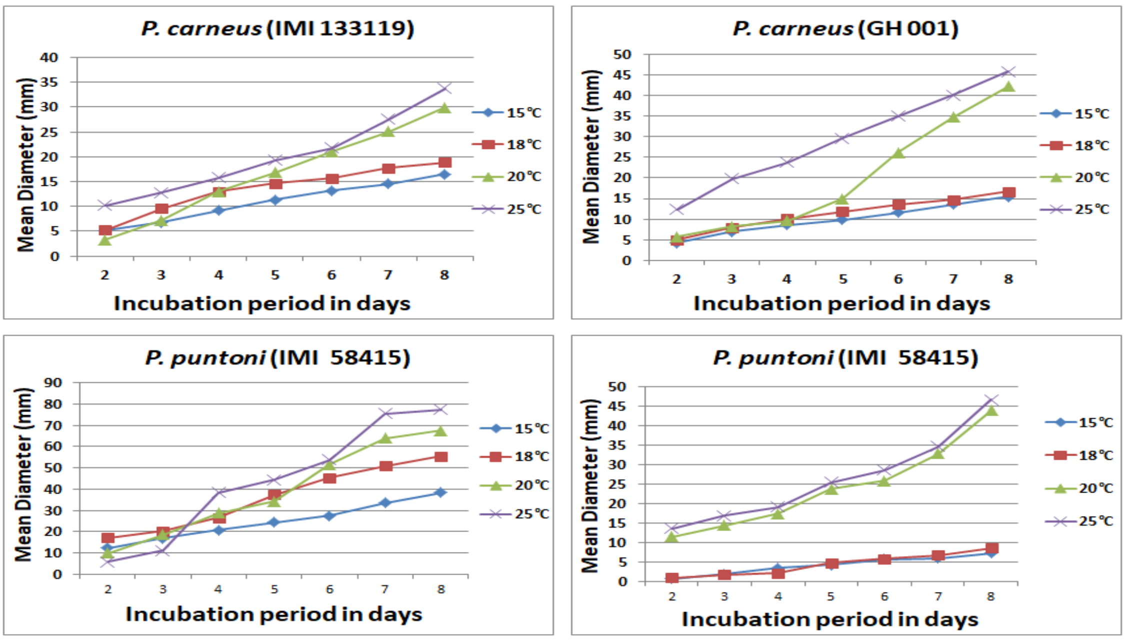

Figure 3 Radial growth of *Paecilomyces* species at different temperatures on Plantain Extract agar

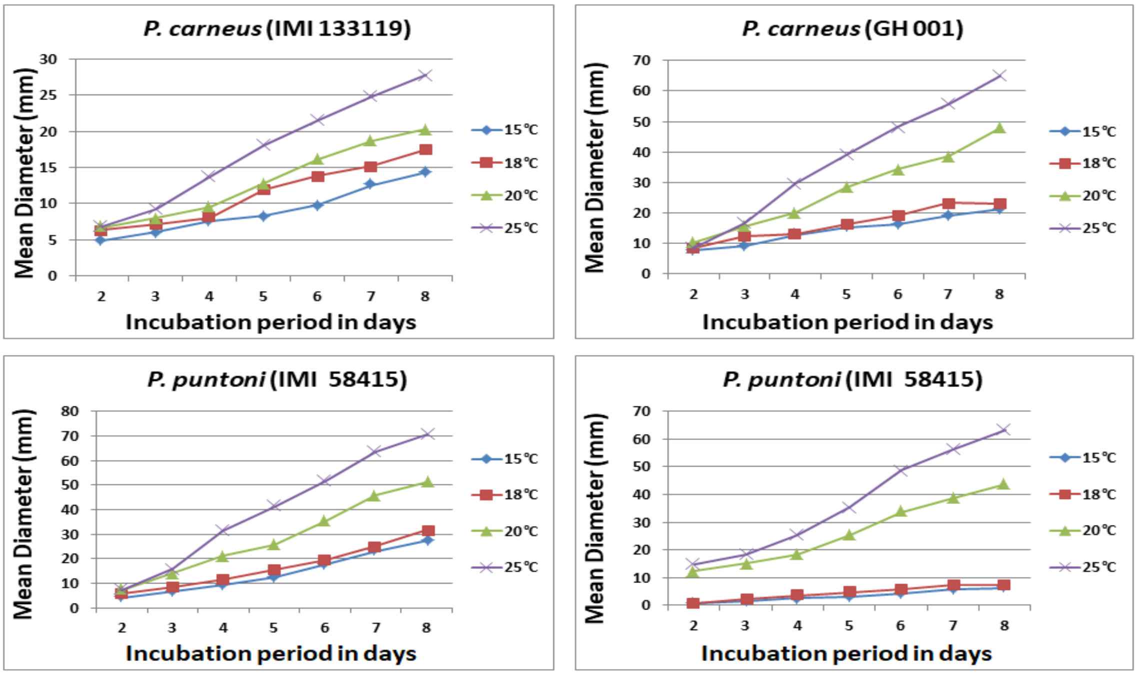

Figure 4 Radial growth of *Paecilomyces* species at different temperatures on Yam Dextrose agar

The influence of the cultural metabolites of the four *Paecilomyces species* on seed germination and radicle development is summarized in Table 1. Depression in the germination of seeds of cowpea and cucumber commensurate with the concentration of the culture filtrate used (Figure 5 A and B). Seed germination was directly proportional to the concentration of filtrate applied. For example, the higher the concentration, the greater the depressive effect on seed germination across board. Germination increased as the inhibitory effect was diluted up to 1:10 v/v but never approximated the control in all instances (Table 1).

The same trend was observed on the influence of the culture metabolites of *Paecilomyces* on the seed germination of sorghum, okra, soybean and groundnuts (Table 1).There was clear high inhibitory effect on seed germination at the highest concentration applied (undiluted, 1:1 v/v) which was reduced with increase in the dilution of the cultural filtrate up to 1:10 dilution. Again, germination of seeds placed in petri plates moistened with 1:10 v/v of the cultural metabolites never approximated that of the control (Table 1). Germination of all the test seeds placed in Petri dishes moistened with the culture filtrate of the local *Paecilomyces carneus* GH 001 was higher than what existed in plates moistened with culture filtrate of the *P. carneus* IMI 133119 (Table 2). Thus the culture metabolites of *P. carneus* GH 001 was significantly (P <0.05) more depressive than seed germination of all the others (Table 1).

Another important observation was that, at the highest concentration of the metabolites applied (undiluted, 1:1 v/v), the emerging radicles were diminutive but the radicle lengths increased as the culture filtrate concentration was decreased by dilution up to 1:10 v/v dilution (Figure 5).

The varietal differences in response of the germinating seeds to the toxic metabolites of the *Paecilomyces species* can be ranked as follows in decreasing order: *P. carneus* GH 001 > *P. carneus* IMI 133119 > *P. varioti* IMI 40025 > *P. puntoni* IMI 58415.

Table 1 Influence of the metabolites of the *Paecilomyces species* on germination of the six economic crops used

Seed variety	Dilution ratio of filtrate (v/v)	Percentage (%) germination			
		***P. carneus* (GH 001)**	***P. carneus* (IMI 133119)**	***P.puntoni* (IMI 58415)**	***P. varioti* (IMI 40025)**
Cowpea (*Vigna unguiculata*)	Undiluted	29	32	43	41
	1 : 1	35	37	46	45
	1 : 2	39	46	51	52
	1 : 5	57	62	68	57
	1 : 10	86	87	71	67
	Distilled water	100	100	100	100
Cucumber (*Cucumis sativus*)	Undiluted	32	35	37	43
	1 : 1	37	38	42	46
	1 : 2	46	48	54	54
	1 : 5	62	71	68	64
	1 : 10	76	81	86	76
	Distilled water	96	96	96	96
Soybeans (*Glycine max*)	Undiluted	28	32	36	38
	1 : 1	35	41	45	43
	1 : 2	41	52	56	57
	1 : 5	61	65	64	68
	1 : 10	82	85	84	85
	Distilled water	90	90	90	90
Sorghum (*Sorghum biscolor)*	Undiluted	27	31	29	28
	1 : 1	38	41	34	32
	1 : 2	46	49	44	38
	1 : 5	58	61	56	48
	1 : 10	73	78	75	76
	Distilled water	98	98	98	98
Okra (*Abelmoschus esculentus*)	Undiluted	26	28	31	29
	1 : 1	32	34	33	32
	1 : 2	38	41	45	43
	1 : 5	54	56	58	52
	1 : 10	73	78	77	78
	Distilled water			85	
Groundnut (*Arachis hypogeae*).	Undiluted	32	34	36	27
	1 : 1	37	39	39	31
	1 : 2	46	45	42	38
	1 : 5	68	64	56	53
	1 : 10	79	83	71	69
	Distilled water	97	97	97	97

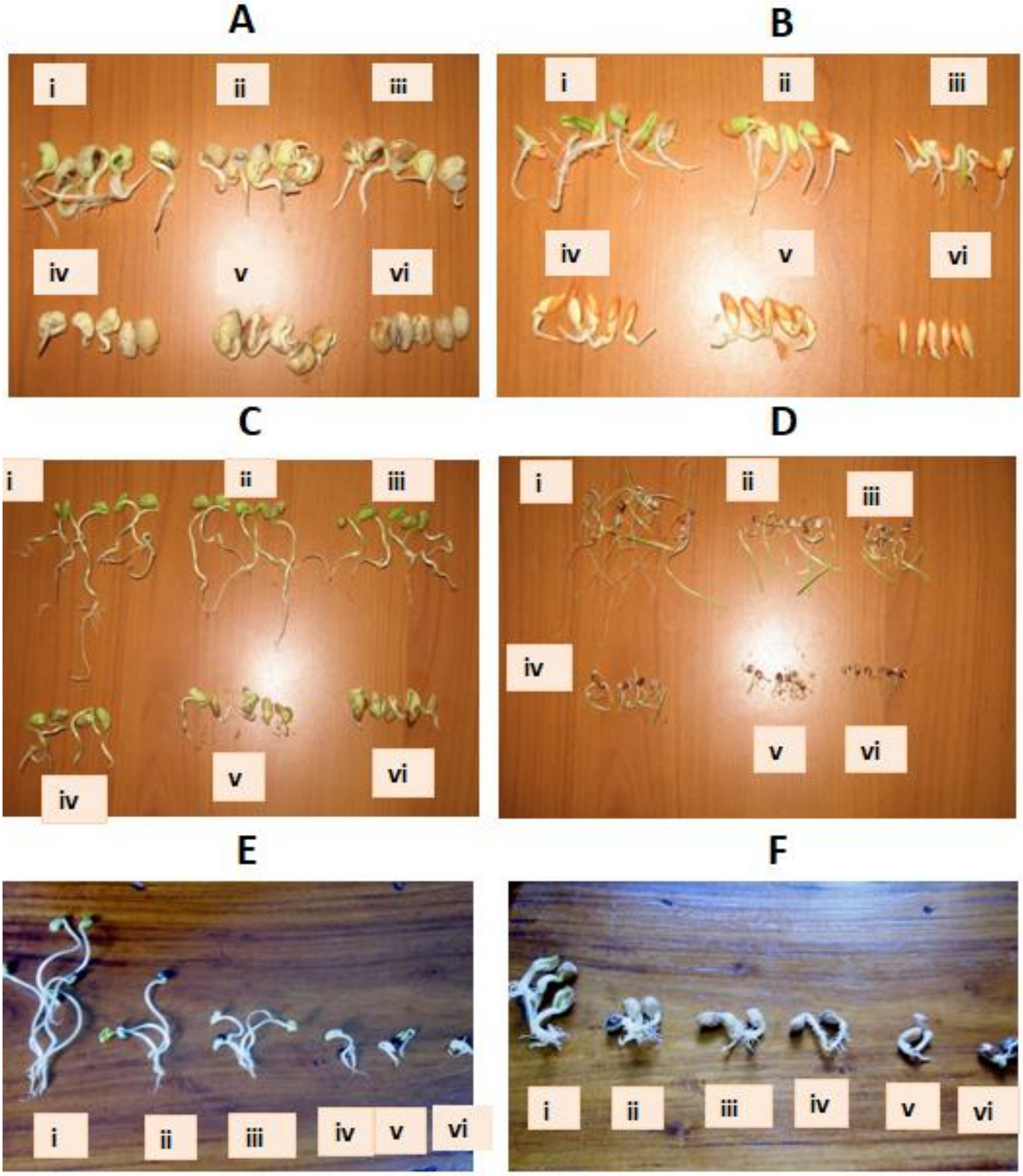

Figure 5 Influence of 7-days old culture metabolites on radicle length of germinating seeds of the economic crops (A) metabolites of *P. carneus* GH 001 on Cowpea (*Vigna unguiculata*), (B) metabolites of *P. carneus* GH 001 on Cucumber (*Cucumis sativus*) var. Darina mix., (C) metabolites of *P. puntoni* IMI 58414 on Soybeans (*Glycine max*), (D) metabolites of *P. puntoni* IMI 58414 on Sorghum *(Sorghum bicolor),* (E) metabolites of *P. varioti* IMI 40025 on okra (*Abelmoschus esculentus*) (F) metabolites of *P. varioti* IMI 40025 on groundnuts (*Arachis hypogeae*). (i) control; (ii) 1 : 10 v/v (iii) 1 : 5 v/v (iv) 1 : 2 v/v (v) 1 : 1 v/v (vi) undiluted.

4. Discussion

This study compared the growth of three *Paecilomyces* species (*P. carneus, P. puntoni* and *P. varioti*) on four natural media (Potato Dextrose agar, Cassava Dextrose agar, Plantain Extract agar and Yam Dextrose agar) and the possible pathological effects of their cultural metabolites on seed germination and radicle development of six economic crops in Ghana.

Seed and soil-borne fungi have been identified as the major cause of reduction in germination capacity, field development and yield of cultivated field crops world-wide [28]. Fungi belonging to many genera such as *Alternaria, Aspergillus, Cephalosporium, Fusarium, Mucor, Rhizopus, Penicillium*, *Paecilomyces* and some members of the *Deuteromycota* can be mentioned as examples [18, 20]. Recently, Minamor and Odamtten [25] showed that three *Paecilomyces* species (*P. carneus , P. puntini*, and *P. varioti*) isolated from two Ghanaian maize varieties (Abeleehi and Obaatanpa) and grown in five different media contained active inhibitory properties that depressed the highest concentration percentage of the maize varieties. Cultural filtrate of those three *Paecilomyces* species (at the highest concentration) also severely depressed length of emerging radicles of the two maize varieties [25]. Subsequent studies by Minamor and Odamtten [6] showed similar pathological effect of the three *Paecilomyces* species on tomato and pepper seeds and seedlings, both in the field and in the greenhouse. The photosynthetic apparatus of seedlings of these crops were also adversely affected [6]. Thus the pathological effects of *Paecilomyces* on crops cannot be overlooked and this warrants the need investigations such as the current work.

In the current study, best growth of the three species was attained at 25°C and this is in accordance with our earlier study [25] where the best growth of *P. carneus, P. puntoni* and *P. varioti* were also around 30^{o}C. It was mentioned in that study [25] that, the nutritional status of media or its richness can influence growth response to ambient temperature by *Paecilomyces species*. Our data from this paper cannot fully explain the differences of growth at different temperatures observed. However, future studies can look at the efficacy of the active inhibitory ingredients of the metabolites produced in different media at different ambient temperatures.

Also, the local *P.carneus* GH 001 grew significantly ($P \leq 0.05$) better than the *P. carneus* IMI 133119 from Cabi Science (UK). This underscores the physiological differences between same species coming from different environmental conditions. Interestingly, the cultural metabolites of the four *Paecilomyces* species (*P. carneus* GH 001, *P. carneus* IMI 133119, *P. puntoni* IMI 58415 and *P. varioti* IMI 40025) raised in Potato Dextrose Broth and Yam Dextrose Broth significantly depressed ($P \leq 0.05$) seed germination of cowpea, cucumber, okra, groundnut, sorghum and soybeans, commensurate with the dilution applied. The higher the concentration, the greater the depressive effect on seed germination. At the higher concentrations of metabolites applied, the emerging radicles were diminutive but increased in length with increasing dilution. Again, the cultural metabolites of *P. caneus* GH 001 was significantly more depressive on seed germination and radicle length of all the six vegetables and bean seeds tested than on those treated with the culture metabolites of *P. carneus* IMI133119. This also highlights the earlier observation of the superior growth of the former (*P. carneus* GH 001) on the test media.

To the best of our knowledge, this is the first report on the inhibitory effect of the metabolites of *P. carneus, P. puntoni* and *P. varioti* adversely having pathological influence on the germination and radicle development of cowpea, groundnut, soybean, okra, sorghum, and cucumber seeds in Ghana.

It is well known that species of *Paecilomyces* for example, *P. carneus, P. farinosus* and *P. fumosoroseus* are common entomogenous fungi, geographically wide spread and can also be isolated from soil samples [29]. *Paecilomyces* spp infect more than 40 insect species [30], and produce blastospores in liquid media [31, 32] and conidia on solid media [33]. Isolate of *P. carneus* PC1 and PC2 caused the highest correct mortality of 93.8% at the concentration of 1 x 10^{7} CFU ml^{-1} to 1st instar Palm Bagworm (*Pteroma pendula Joannis*) [34]. This fungus together with other *Paecilomyces* are being developed for use against a range of agricultural pests such as insects, where bio-control may offer an effective alternative for the control of such insects which have increasingly become resistant to chemical pesticides [34]. It has not been established whether the active metabolites from *P. carneus* killing insects is the same as what inhibited seed germination and radicle development. But in the event of the increase of the soil bank of *P. carneus* during bio-control application, this may make the fungus presumably affect agricultural production if not monitored.

Paecilomyces species are also saprophytic and are uncommon pathogens that can produce serious infectious in immunocompromised patients. *P. variotii* is a common occurring species in compost, air, food and is also associated with many types of human infections, such as fungemia, endocarditis peritonitis and osteomyelitis [35-37]. Recently a patient with refractory lymphoma who underwent allogenic haematopoletic cell transplant developed pneumonia due *P. variotii* [38]. This emphasizes the need for monitoring use of such fungal species due to the demand for environmentally friendly agricultural procedures [39, 40].

5. Conclusion

This paper provides novel information which gives credence to the ability of the culture metabolites of *P. carneus* GH 001, *P. carneus* IMI 133119, *P. puntoni* IMI 58415, and *P*. *variotii* IMI 40025 to significantly depress seed germination and radicle development of cowpea, cucumber, soybean, sorghum, okra and groundnuts *in vitro*. Therefore, the current study adds on the list of new plants whose seed germination capacity and radicle development are adversely affected by *Paecilomyces* species to include cowpea, cucumber, soybean, sorghum, okra and groundnut. The metabolites from the local *P. carneus* GH 001 was more potent compared to the imported *P. carneus* IMI 133119, an indication of how ecological and environmental conditions can have influence the on physiological processes of the same species. Currently, sustainable agriculture is demanding innovative and environmentally friendly procedures to protect plants against biotic stress. It is therefore, important that Environmental Impact Assessment (EIA) is intensified on farms cultivated with non-target crops using Quality Index principle EQI. This will help in identifying whether or not accumulation of conidia of *Paecilomyces* (at soil bank) in the field will adversely affect the cultivation of economic crops such as reported in this paper.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that they have no conflict of interest.

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