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The effect of fungal extracts on the growth of decapitated coleoptiles and root development of rice and corn

Arlyn G. Linsangan, Emmanuel E. Gandalera*, Mary Jhane G. Valentino

Department of Biological Sciences, College of Arts and Sciences, Central Luzon State University, Science City of Munoz, Nueva Ecija 3120, Philippines

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Abstract

This study aimed to evaluate the growth of decapitated coleoptiles and root development of rice and corn plants as affected by fungal extracts (crude, ethanol and fungal of spent) of *Aspergillus niger, Aspergillus fumigatus, Rhizopus stolonifer* and *Rhizomucor pusillus* by evaluating their effect on decapitated coleoeptiles and root elongation. Forthe elongation of decapitated coleoptiles of the rice and corn seeds, results showed the lack of auxin like activity of fungal isolates, and their inability to influence growth of the decapitated coleoptiles. For the length of roots and the number of roots in germinated rice, among the fungal treated seeds, *A. niger* crude extract registered the highest mean length of 14.81 mm and the least of 10.58mm was observed in *R. stolonifer* spent. For the number of roots, among the fungal treated seeds, the most number of roots were observed in *A. fumigatus* ethanol extract. Whereas, reduction in the number of roots when treated with *R. pusillus* crude extract and spent, and *A. fumigatus* crude extract were recorded.

***Corresponding Author:** Emmanuel E. Gandalera ✉ maryjhanevalentino@yahoo.com.ph

Introduction

Vermicomposting utilizes the earthworm's metabolic potential inconverting biodegradable wastes to biomass and its wastes becomes cast (Suthar, 2007; Pereira *et al*, 2014). During the process, earthworms' microflora aids up by using their degradative enzymes, promote plant growth by secreting growth hormones such as auxins, cytokinins, and gibberellins (Prabha *et al*, 2007; Ijayabharati *et al*, 2014; Carr, 2016).Accordingly, Plant–microorganism interactions could lead to crop productivity by improved nutrient uptake, and increased plant growth (Yang *et al*, 2009; De Zelicourt *et al*, 2013; Grover *et al*, 2013).

Auxin is a plant hormone involved in a broad variety of biological mechanisms. It is responsible for cell elongation and differential growth. They are involved in the formation of meristems, apical dominance and mediation of tropisms, stimulate formation of callus and they can also act as rooting hormone (Friml, 2003; Sauer *et al*, 2013).

The study primarily aimed to determine the potential auxin-like activity of fungi associated with vermicast which could promote root formation and elongation and promote the growth of decapitated coleoptiles. Determining the hormone-like activities could be helpful on the growth of developing plants and it will have a high demand as it will be safer to use, and it is an excellent soil additive made up of digested compost that contains much higher in nutrients and microbial life and therefore, are considered as a higher value product.

Materials and methods

Preparation of Fungal Inoculants

The fungal inoculants were sub-cultured into Potato Dextrose Agar (PDA) plates and incubated to allow mycelial growth.

After seven days of incubation, mycelial discs were prepared using sterile cork borer and used as culture inoculants for the liquid media for mycelial biomass production.

Production of Mycelial Biomass

Twenty-four grams (24g) of Potato Dextrose Broth (PDB) was suspended on one liter of distilled water and heated to completely dissolve the medium. After which, it was sterilized in an autoclave at 121°C at 15 psi for 30 minutes. On 10ml of PDB, mycelial discs of fungal isolates were aseptically inoculated and incubated for seven days which allowed fungal growth. After incubation, mycelial mats were removed using sterile forceps and it was placed in Petri dish for overnight drying at room temperature.

Crude Extraction

Thirty grams (30g) of mycelial mat was grounded using a blender. The grounded mycelia were squeezed using a clean cheese cloth to obtain the liquid portion. Using a filter paper, filtrate was separated from the residue, placed in a sterile amber bottle and it was refrigerated.

Ethanol Extraction

Ethanol extracts of the different fungal isolates were extracted following the extraction protocol of Maiquez *et al* (2016). About thirty grams (30g) of powdered dried mycelial mat was submerged and extracted in a sterile Erlenmeyer flask containing 300ml of 95% ethanol for 48 hours at room temperature. This was filtered using a filter paper. The filtrates were separately evaporated in rotary evaporator under reduced pressure at 50˚C to yield ethanol extracts. The extracts were then placed in a sterile amber bottle and it was refrigerated prior to use.

Collection of Fungal Spent

The ramified mycelia in the Potato Dextrose Broth was removed, then the liquid spent in the Potato Dextrose Broth was transferred and filtered using filter paper in sterile flask to remove the residue. The spent was placed in a sterile amber bottle and refrigerated.

Source of Test Plants

Rice seeds (NSIC Rc 222) was obtained from Philippine Rice Research Institute (PhilRice) and

corn seeds (IES) from Nueva Ecija Fruits and Vegetables Seed Center, Science City of Muñoz, Nueva Ecija.

Evaluation of the Auxin-like Activity

Auxin-like activity of the fungi was evaluated using rice and corn seeds. The number and length of roots initiated, and the growth of coleoptiles were recorded following the procedure described by Nitsch and Nitsch(1955) with modifications.

Growth of Decapitated Coleoptiles on Rice and Corn Seeds

Rice and corn seeds were soaked in water for two hours. Ten seeds of rice and corn were placed in a microwavable dish lined with filter paper wet with distilled water in three replications. Seeds were incubated 35cm below white fluorescent light for 3 to 4 hours, then the seeds were incubated in a dark room maintained at room temperature for three to four days. After incubation 9mm sections of the coleoptiles, 3mm from the tip was cut, then placed in sterile test tubes containing 1.5 ml of different treatments under green light for 24 hours.

Then the coleoptiles were allowed to grow for 20 hours in the dark at 25˚C. Growth of coleoptiles was measured using a digital Vernier caliper.

Root Initiation on Rice and Corn Seeds

Rice and corn seeds were soaked indifferent treatments for 24 hours then it was placed in a microwaveable dish lined with filter paper wet with distilled water in three replications. Seeds were allowed to germinate for five days.

The roots were cut 10mm from the stem then germinated seeds were placed in a bottle with 9ml of test and control solutions. After five days of incubation, the number of roots initiated and the length of roots was measured using a digital Vernier caliper.

Statistical Analysis

The study was laid out using Completely Randomized Design (CRD). Data gathered was analyzed using Analysis of Variance (ANOVA) in Special Package for Social Sciences (SPSS) version 17 software and the treatment means was compared using Tukey's HSD (Honest Significant Difference). All tests of significance were done at 5% probability levels.

Results and discussion

Elongation of Decapitated Coleoptiles

The elongation of coleoptiles of the rice and corn seeds in different treatments with their corresponding means are presented in Table 1.

Table 1. Length of coleoptiles germinated in rice and corn seeds treated with crude extract, ethanol extract and spent of fungi.

Treatments	Elongation of coleoptiles (mm) Rice	Corn
R. stolonifer crude extract	6.46^{bcde}	6.53^{b}
R. stolonifer ethanol extract	6.42^{bcde}	6.29^{b}
R. stolonifer spent	6.49^{bcde}	6.41^{b}
A. fumigatus crude extract	6.74^{b}	6.48^{b}
A. fumigatus ethanol extract	6.36^{de}	6.32^{b}
A. fumigatus spent	6.38^{cde}	6.37^{b}
*A. niger*crude extract	6.72^{bc}	6.67^{b}
*A. niger*ethanol extract	6.62^{bcd}	6.39^{b}
*A. niger*spent	6.24^{e}	6.60^{b}
R. pusillus crude extract	6.41^{bcde}	6.51^{b}
R. pusillus ethanol extract	6.23^{e}	6.32^{b}
R. pusillus spent	6.58^{bcde}	6.69^{b}
Auxin	8.05^{a}	11.21^{a}
Distilled water	6.43^{bcde}	6.36^{b}

*Treatments in column with different letters are significantly different.

Results showed the lack of auxin like activity of fungal isolates, and their inability to influence growth of the decapitated coleoptiles. Among the fungal treated decapitated coleoptiles, the highest mean was recorded in *A. fumigatus* crude extract with 6.74mm, followed by *A. niger* crude extract of 6.72mm and *A. niger* ethanol extract with 6.62mm. As for decapitated coleoptiles of corn, *R. pusillus* spent registered 6.69mm, followed by *A. niger* crude extract and *R. stolonifer* crude extract with 6.67mm and 6.53mm, respectively.

While the least length of coleoptiles was observed in *R. pusillus* ethanol extract with 6.23mm for rice and *R. pusillus* and *A. fumigatus* ethanol extracts both with 6.32mm for corn. Statistically, the length of fungal treated with decapitated coleoptiles were significantly lower than those treated with auxin.

Table 2. Length of roots and number of roots initiated in rice.

Treatments	Length of roots (mm)	Number of roots
R. stolonifer crude extract	12.18^{cd}	3.66^{defg}
R. stolonifer ethanol extract	10.89^{e}	4.90^{bc}
R. stolonifer spent	10.58^{e}	3.63^{efg}
A. fumigatus crude extract	10.77^{e}	3.33^{fg}
A. fumigatus ethanol extract	11.65^{de}	5.00^{b}
A. fumigatus spent	11.00^{e}	3.80^{defg}
*A. niger*crude extract	14.81^{b}	4.60^{bcd}
*A. niger*ethanol extract	12.84^{c}	4.43^{bcde}
*A. niger*spent	10.64^{e}	3.23^{fg}
R. pusillus crude extract	11.03^{e}	3.00^{g}
R. pusillus ethanol extract	10.94^{e}	3.96^{cdef}
R. pusillus spent	10.59^{e}	2.93^{g}
Auxin	21.21^{a}	9.93^{a}
Distilled water	12.51^{cd}	4.40^{bcde}

*Treatments in column with different letters are significantly different.

The coleoptile serves to protect true leaves against soil pressures and other physical constraints and also provides nutrients for the developing tissues. Accordingly, decapitation of coleoptile will cause growth inhibition and it can be restored upon application of auxin (Frohlich and Kutschera, 1995; Kawai and Uchimaya, 2000).In addition, Ismaiel and Papenbrock (2015) revealed that secondary metabolites of *Aspergillus*, *Penicillium* and *Fusarium* affect the seed quality, germination, viability, seedling vigour, and the growth of coleoptile.

Root Elongation and Root Initiation in Rice

The length of roots and the number of roots in germinated rice are presented in Table 2. Among the fungal treated seeds, *A. niger* crude extract registered the highest mean length of 14.81 mm followed by *A. niger* ethanol extract 12.84mm and the least of 10.58mm by *R. stolonifer* spent. For the number of roots, among the fungal treated seeds, the most number of roots were observed in *A. fumigatus* ethanol extract, *R. stolonifer* ethanol extract and *A. niger* crude extract with 5.00mm, 4.90mm and 4.60 mm, respectively. Meanwhile, the least number of roots were recorded in *R. pusillus* spent with 2.93 and *R. pusillus* crude extract of 3.00.

For the length of roots and the number of roots formed, statistical analysis showed that the length and the number of roots in rice when treated with fungal isolates were significantly lower than those treated with commercial auxin. Also, a significant

reduction was noted in *R. stolonifer* ethanol extract and spent, *A. fumigatus* crude extract and spent, *A. niger* spent, *R. pusillus* crude extract, ethanol extract and spent. Thus, the inhibitory activity of the aforementioned fungi. Similarly, reduction in the number of roots when treated with *R. pusillus* crude extract and spent, and *A. fumigatus* crude extract.

Table 3. Length of roots and number of roots initiated in corn.

Treatments	Length of roots (mm)	Number of roots
R. stolonifer crude extract	11.03^{d}	4.53^{bcd}
R. stolonifer ethanol extract	11.75^{cd}	3.36^{e}
R. stolonifer spent	11.06^{d}	4.86^{bc}
A. fumigatus crude extract	11.70^{cd}	9.00^{a}
A. fumigatus ethanol extract	10.98^{d}	3.50^{e}
A. fumigatus spent	11.33^{cd}	8.43^{a}
*A. niger*crude extract	11.12^{cd}	3.60^{de}
*A. niger*ethanol extract	11.17^{cd}	3.23^{e}
*A. niger*spent	11.39^{cd}	4.90^{bc}
R. pusillus crude extract	12.30^{c}	3.96^{cde}
R. pusillus ethanol extract	11.21^{cd}	3.26^{e}
R. pusillus spent	11.24^{cd}	5.10^{b}
Auxin	24.36^{a}	9.26^{a}
Distilled water	15.16^{b}	3.96^{cde}

*Treatments in column that have different letters are significantly different.

Root Elongation and Root Initiation in Corn

The length of roots and the number of roots initiated in corn are presented in Table 3. Among the fungal treated corn seeds, *R. pusillus* crude extract had the highest mean of 12.30mm followed by *R. stolonifer* ethanol extract of 11.75mm and *A. fumigatus* crude extract of 11.70mm. For the number of roots initiated, *A. fumigatus* crude extract and spent had the highest mean of 9.00 and 8.43, whereas the least number of roots were formed when treated with *A. niger* ethanol extract with 3.23.

Statistically, the length of roots as influenced by the fungal extracts were significantly lower than those treated with auxin and distilled water, therefore the inhibitory activity of all fungi which influence the length of roots was noted. Meanwhile, for the number of roots initiated, statistical analysis revealed that those treated with *A. fumigatus* crude extract and spent were comparable with those treated with commercial auxin, thus their ability to increase the number of roots and their auxin like activity.

According to Khokhar *et al* (2013) and Haikal (2008), several fungi produce toxic metabolites which causes plant growth and development inhibition, reduce seed germination. Similarly, *A. niger* can produce mycotoxins such as oxalic acid crystals, kojic acid and malformins which inhibits seed germination and root-shoot elongation (TSCA, 2012; Jalander and Gachande, 2012).

Similarly, Zakiah *et al* (2017) and Brown *et al* (2001) stated that secondary metabolite compounds including terpenoids, flavonoids, alkaloids and phenolic compounds have a potential as stimulants or inhibitors of plant growth. Also, according to Besseau *et al* (2007), deregulation of flavonoid synthesis caused inhibition of auxin transport and has severe consequences for plant growth and development. Also, triterpenoid is reportedly have a growth

inhibitory effect which cause the wheat rootlet and shoot growth inhibition by interfering with mitotic cell division (Shinoda *et al*, 1985; Shahid-Ud-Daulaand Basher, 2009). Furthermore, concentration ethanol could also pose inhibitory effects to growing plants (Islam and Kato-Noguchi, 2012).

Conclusion

Based from the results of the study, *A. niger, R. pusillus, R. stolonifer and A. niger*ethanol extract and mycelial spent posed inhibitory activity in coleoptile elongation and root development of rice and corn, thus fungal isolates did not exhibit any auxin-like activity.

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