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Gibberellin-like activity of fungi associated with vermicast in corn and rice

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Abstract

The study determined the potential of four fungi associated with vermicast namely *Aspergillus niger*, *Rhizopus stolonifer, Aspergillus fumigatus* and *Rhizomucor pusillus* in producing gibberellin-like activity that can promote plant growth. Coleoptile elongation and length of second leaf sheath of rice seeds and corn seeds were evaluated in the study. Coleoptile of rice seeds treated with fungal extracts were significantly lower than those treated with gibberellic acid. Whereas in corn, the gibberellin-like activity was observed at 24 hours of incubation in coleoptiles treated with *A. fumigatus* fungal spent and *R. pusillus* in ethanol extract (5.56mm and 5.49mm), at 48 hours of incubation, *R. pusillus* fungal spent and crude extract (11.65mm and 11.63mm), and at 72 hours, *R. stolonifer* ethanol extract and fungal spent (20.10mm and 19.38mm).Moreover, the second leaf sheath of rice seeds with the highest mean length was observed with *R. stolonifer* ethanol extract (39.15mm). Whereas, the longest second leaf sheath of corn was treated with *A. niger, R. stolonifer* and *A. fumigatus* crude extracts with mean values of 53.58mm, 51.88mm and 48.33mm, respectively. Thus, all fungi associated with vermicast exhibited growth promoting activity which influence the elongation in test plants.

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Introduction

Majority of fungal species most likely occur in soil as their environment, thereby exhibiting functions in soils which include both active roles like degradation of dead plant materials and inactive roles wherein propagules are present in the soil at resting states (Bridge and Spooner, 2001).

During the process of vermicomposting, interaction of earthworm and microorganisms promotes bio-oxidation and stabilization of organic materials producing vermicast which contains higher nutrient contents such as vitamins like B12 and plant growth regulators (Joshi and Kelkar, 1952; Canellas *et al,* 2002; Ibrahim *et al,* 2013).

In addition, microorganisms speed up the vermicomposting process by using their degradative enzymes, promote plant growth by secreting phytohormones like auxin, gibberellins, cytokinins and inhibit phytopathogens by secreting antibiotics and enzyme (Sathya and Vijayabharathi, 2014).

Plant growth hormones such as auxin, cytokinins, gibberellins, ethylene and abcisic acid are produced by cells in one part to be distributed to different organs which then will triggers response. In this study, gibberellins or known as gibberellic acids will be determined from the fungal isolates found in vermicast namely *Aspergillus niger, Aspergillus fumigatus, Rhizopus stolonifer, and Rhizomucor pusillus*. This plant hormone was first discovered in Japan because of the fungal disease called foolish seedlings (Cho *et al,* 2005).

They function to stimulate plant growth and development, seed germination, trigger transitions from meristem to shoot growth, juvenile adult leaf stage, vegetative to flowering, determines sex expression and grain development along with an interaction of different environmental factors like light, temperature and water (Chahrabarty and Gupta, 2013). The study elucidate the gibberellin-like activity of fungi associated with vermicast which could potentially promote growth in plant.

Materials and methods

Evaluation of the gibberellin-like activity

The study evaluated the gibberellin-like activity of the ethanolic extracts, crude extracts and fungal spent of *A. niger, A. fumigatus, R. stolonifer and R. pusillus* using rice and corn test plants. The length of coleoptile and second leaf sheath of the germinated rice and corn seeds were measured in millimeter.

Preparation of fungal inoculants

The fungal inoculants were sub-cultured into Potato Dextrose Agar (PDA) plates and were incubated to allow mycelial growth. After seven days of incubation, mycelial discs were prepared using sterile cork borer and these served as culture inoculants in bottle with Potato Dextrose Broth (PDB) for mycelial biomass production.

Production of mycelial biomass

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

Coleoptile length

The seeds were subjected to viability to ensure that all seeds will germinate. After which, thirty (30) viable rice and corn seeds were soaked in different treatments for 24 hours and the seeds were transferred in dish lined with filter paper flooded with five milliliter of different treatments. Treated seeds were incubated at room temperature and allowed to germinate. The length of coleoptile was measured at 24, 48 and 72 hours of incubation using digital vernier caliper.

Leaf sheath elongation

For the elongation of the second leaf sheath, corn and rice seeds were allowed to germinate for 2 to 4 days.

Then germinated seeds with one millimiter coleoptiles were transferred to a filter paper immersed in nine milliliters of fungal extracts and control solutions. The germinated seeds were allowed to grow under ordinary daylight conditions at 25-28°C. In each treatments, 0.5 ml of distilled water were added every 24 hours. The second leaf sheath was measured using a vernier caliper after seven days of incubation.

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

Coleoptile elongation as influenced by fungal extracts

Rice: Elongation of coleoptile in rice after 24 hours, 48 hours and 72 hours of incubation treated with different treatments were presented in Table 1 and Fig. 1.

Table 1. Coleoptile Elongation in rice after 24, 48 and 72 hours of incubation.

TREATMENTS	LENGTH OF COLEOPTILES (mm)		
	24 HOURS	48 HOURS	72 HOURS
A. niger Crude Extract	4.03 ^{de}	7.46 ^{cd}	8.04 ^{cd}
A. niger Ethanol Extract	3.01^{f}	5.98^{e}	7.45^{d}
A. niger Fungal Spent	5.07^{bc}	6.87 ^{de}	7.72 ^{cd}
A. fumigatus Crude Extract	3.88 ^{def}	9.03^{b}	9.83 ^{b}
A. fumigatus Ethanol Extract	4.59 ^{cd}	7.14 ^{cde}	7.75 ^{cd}
A. fumigatus Fungal Spent	3.95^{ef}	6.64 ^{de}	7.40^{d}
R. stolonifer Crude Extract	3.16 ^{ef}	7.51 ^{cd}	8.03 ^{cd}
R. stolonifer Ethanol Extract	5.09 ^{bc}	7.05 ^{cde}	7.29 ^{d}
R. stolonifer Fungal Spent	3.83^{def}	7.07 ^{cde}	8.04 ^{cd}
R. pusillus Crude Extract	4.62 ^{cd}	7.11 ^{cde}	7.70 ^{cd}
R. pusillus Ethanol Extract	6.01^{b}	7.16 ^{cde}	7.54 ^{cd}
R. pusillus Fungal spent	5.19 ^{bc}	8.09 ^{bc}	8.27 ^{cd}
Gibberellic acid	7.44^{a}	11.57 ^{a}	12.22 ^{a}
Distilled Water	4.76^{cd}	8.09 ^{bc}	8.26 ^{cd}

* Treatment means in column with the same letter are not significantly different.

Among the coleoptiles treated with fungi, at 24 hrs of incubation *R. pusillus* ethanol extract and spent had the highest coleoptile length with 6.01mm and 5.19 mm, respectively while *A. niger* ethanolic extract had the least with 3.01mm. At 48 and 76 hrs, the longest coleoptiles were recorded in *A. fumigatus* crude extract (9.03mm and 9.83mm), followed by *R. pusillus* spent (8.09mm and 8.27mm). Lastly, the shortest coleoptiles were recorded in *A. niger* ethanolic extract with 5.98mm (at 48hrs) and *R. stolonifer* ethanolic extract with 7.29mm (at 72 hrs). Statistically, all fungal treated coleoptiles were significantly lower than those treated with gibberellic acid and only those treated with *R. pusillus* ethanol extract (at 24hrs) and *A. fumigatus* crude extract (at 48 hrs and 72 hrs) were significantly higher than those treated with distilled water.

Corn: Elongation of corn coleoptiles were presented in Table 1 and Fig. 2. Among the fungal treated coleoptiles, at 24 hours of incubation, the longest coleoptiles of corn were observed in *A. fumigatus*

fungal spent followed by *R. pusillus* in ethanol extract with means of 5.56mm and 5.49mm while the least length of coleoptile were recorded in *R. stolonifer* fungal spent with 3.92mm. Meanwhile, after 48 hours of incubation, corn seeds treated with *R. pusillus* fungal spent and crude extract registered the highest mean with 11.65mm and 11.63mm while the least of 6.87mm was measured in coleoptiles treated with *A. niger* fungal spent. Lastly, after 72 hours, coleoptiles treated with *R. stolonifer* ethanol extract followed by *R. stolonifer* fungal spent showed the highest mean of 20.10mm and 19.38mm, respectively. Whereas, corn seeds treated with *A. niger* in crude extract showed the smallest coleoptiles of 10. 35mm. Statistically, *A. fumigatus* crude extract and fungal spent and *R. pusillus* ethanol and crude extract, *A. niger* crude extract, *R. pusillus* crude and ethanol extracts, and fungal spent, and *A. fumigatus* fungal spent were comparable to the effect of gibberellic acid on the coleoptile elongation of corn at different incubation periods. Thus, the gibberellin like activity of the aforementioned fungal extracts.

Table 2. Coleoptile Elongation in corn after 24, 48 and 72 hours of incubation.

Treatments	Length of coleoptiles (mm)		
	24 hours	48 hours	72 hours
A. niger Crude Extract	5.00^{abc}	10.35 ^{ab}	10.35 ^{e}
A. niger Ethanol Extract	4.67 ^{abcde}	7.73 ^{bc}	15.53 ^{abcd}
A. niger Fungal Spent	5.07^{bc}	6.87^{de}	7.72^{cd}
A.fumigatus Crude Extract	5.17 ^{ab}	8.41 ^{bc}	13.11 ^{cde}
A. fumigatus Ethanol Extract	4.35^{bcde}	7.40 ^{bc}	15.09 ^{bcd}
A. fumigatus Fungal Spent	5.56 ^{a}	8.43^{bc}	17.4 ^{abc}
R. stolonifer Crude Extract	5.19 ^{ab}	8.47 ^{bc}	14.20 ^{cde}
R. stolonifer Ethanol Extract	4.11^{cde}	10.10 ^{ab}	20.10 ^{a}
R. stolonifer Fungal Spent	3.92^{de}	9.39 ^{abc}	19.38 ^{ab}
R. pusillus Crude Extract	4.91^{abcd}	11.65 ^{a}	17.52 ^{abc}
R. pusillus Ethanol Extract	5.49 ^{a}	10.27 ^{ab}	13.54 ^{cde}
R. pusillus Fungal spent	4.74 ^{abcde}	11.63 ^{a}	14.21 ^{cde}
Gibberellic acid	5.33^{ab}	9.34 ^{ab}	17.17 ^{abc}
Distilled Water	3.77 ^{e}	6.56^{c}	12.66 ^{de}

* Treatment means in column with the same letter are not significantly different.

Table 3. Length of second leaf sheath of rice and corn treated with fungal extracts.

Treatments	Length of second leaf sheath (mm)	
	Rice	Corn
A. niger Crude Extract	25.64 ^{c}	53.58 ^{a}
A. niger Ethanol Extract	24.85 ^{cd}	18.83^{de}
A. niger Fungal Spent	18.56^{de}	18.56^{e}
A. fumigatus Crude Extract	34.88 ^{ab}	49.33 ^{a}
A. fumigatus Ethanol Extract	29.93 ^{bc}	17.93 ^{e}
A. fumigatus Fungal Spent	28.64^{bc}	22.77^{cde}
R. stolonifer Crude Extract	26.24 ^{c}	51.88 ^{a}
R. stolonifer Ethanol Extract	39.15^{a}	24.83 ^{c}
R. stolonifer Fungal Spent	23.35^{cd}	22.46 ^{cde}
R. pusillus Crude Extract	28.77 ^{bc}	18.55 ^{e}
R. pusillus Ethanol Extract	24.48^{cd}	24.68 ^{cd}
R. pusillus Fungal spent	25.70 ^{c}	17.84 ^{e}
Gibberellic acid	33.58^{ab}	42.49 ^{b}
Distilled Water	15.94^{e}	20.70^{cde}

Elongation of second leaf sheath of rice and corn as influenced by fungal extracts

Rice: The length of second leaf sheath of rice treated with crude, ethanol and fungal spent of *A. niger*, *A. fumigatus*, *R. stolonifer* and *R. pusillus* are presented in Table 2 and Fig. 3. Second leaf sheath treated with *R. stolonifer* ethanol extract measure the longest with a mean of 39.15mm, followed by the gibberellic acid with 33.58 mm and *A. fumigatus* ethanol extract with 29.93mm. Whereas, leaf sheath treated with *A. niger* spent had the lowest mean length of second leaf sheath of 18.56mm. Statistically, *A. fumigatus* crude extract and *R. stolonifer* ethanol extract were comparable to the gibberellic acid, and all except *A. niger* fungal spent were significantly higher than those treated with distilled water. Therefore the ability of the fungal extracts to lengthen the second leaf sheath of rice.

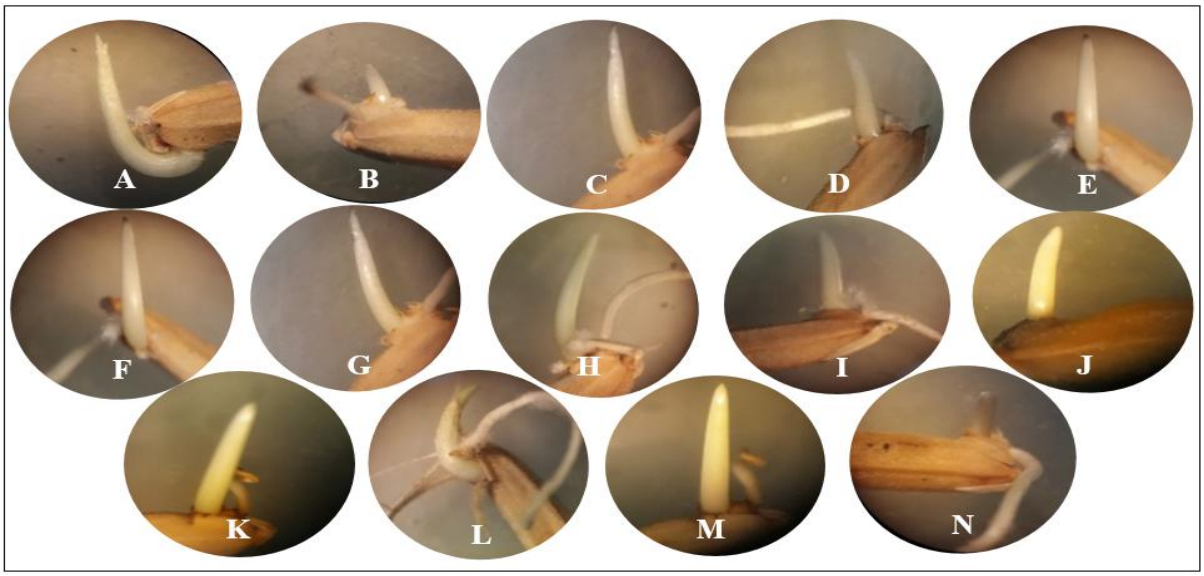

Fig. 1. Coleoptile of rice under dissecting microscope: A. niger (A) crude extract; (B) ethanol extract; (C) fungal spent; A. fumigatus (D) crude extract; (E) ethanol extract; (F) fungal spent; R. stolonifer (G) crude extract; (H) ethanol extract ; (I) fungal spent ; (J) crude extract; (K) ethanol extract; (L) fungal spent; (M) gibberellic acid; (N) distilled water.

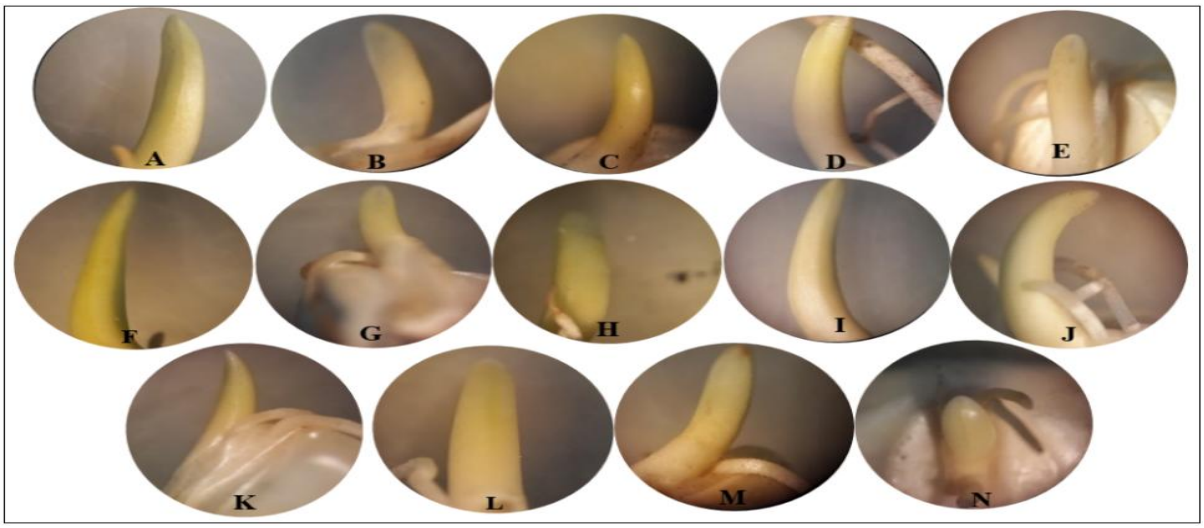

Fig. 2. Coleoptile of corn under dissecting microscope: A. niger (A) crude extract; (B) ethanol extract; (C) fungal spent; A. fumigatus (D) crude extract; (E) ethanol extract; (F) fungal spent; R. stolonifer (G) crude extract; (H) ethanol extract ; (I) fungal spent ; (J) crude extract; (K) ethanol extract; (L) fungal spent; (M) gibberellic acid; (N) distilled water.

Corn: The corresponding means in the length of second leaf sheath of corn seeds treated with crude, ethanol and fungal spent of *A. niger*, *A. fumigatus*, *R. stolonifer* and *R. pusillus* are shown in Table 3 and Fig. 4. *A. niger*, *R. stolonifer* and *A. fumigatus* crude extracts treated corn had the longest leaf sheath with 53.58mm, 51.88mm, and 48.33mm, respectively. Meanwhile, the least length of coleoptiles were recorded in *A. niger* fungal spent, *A. fumigatus* ethanol extract, *R. pusillus* crude extract and fungal spent of 18.56mm, 17.93mm, 18.55mm, and 17.84mm, respectively. Statistically, *R. stolonifer*, *A. niger* and *A. fumigatus* crude extracts were significantly higher than the gibberellic acid, therefore they have the growth promoting activity which influence the second leaf elongation of corn.

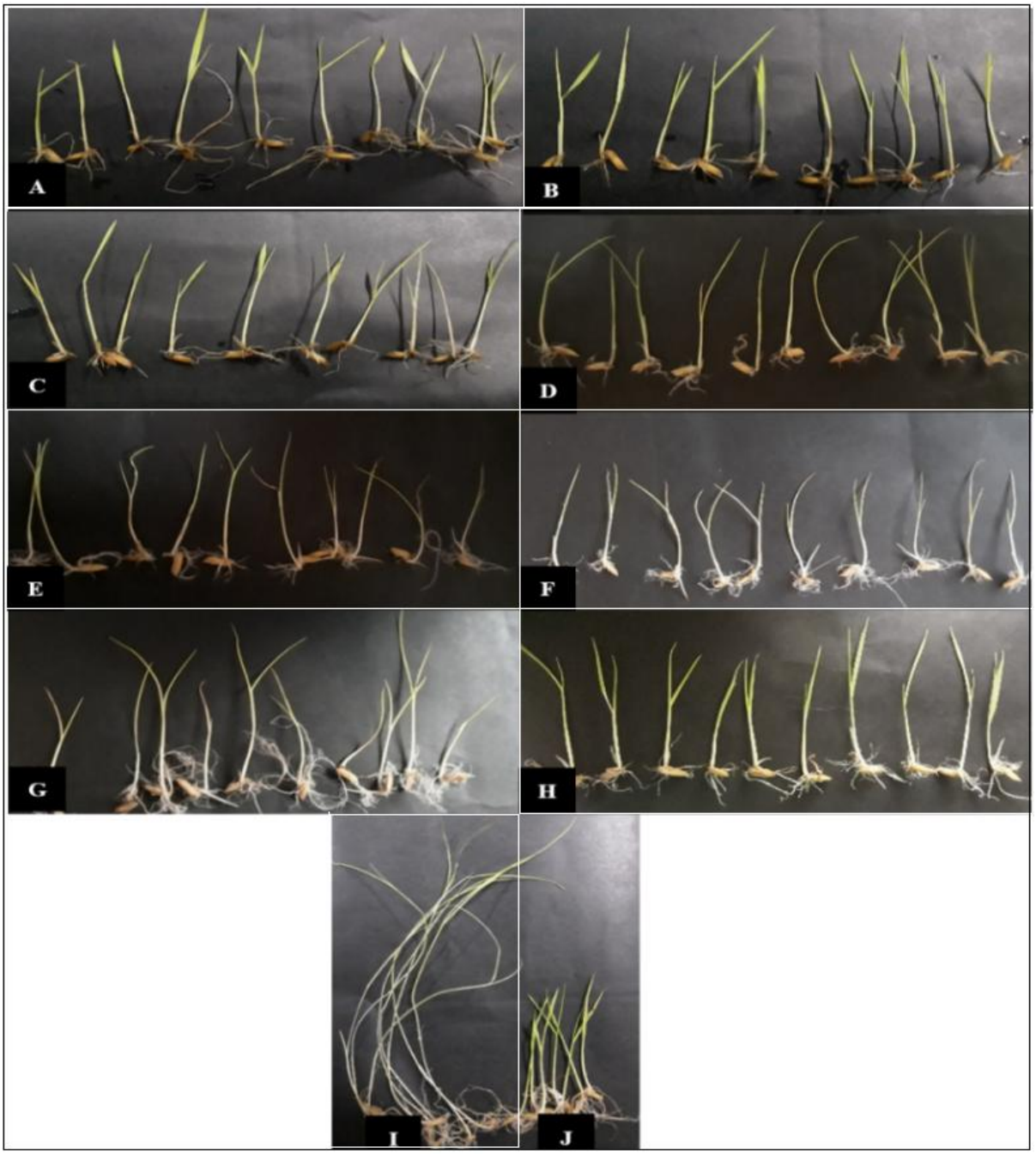

Fig. 3. Rice treated with ethanol extract: (A) *A. niger*; (B) *A. fumigatus*; (C) *R. stolonifer*; (D) *R. pusillus*; with mycelial spent : (E) *A. niger*; (F) *A. fumigatus*; (G) *R. stolonifer*; (H) *R. pusillus;* with ethanol extract(I) gibberellic acid (J) distilled water, crude extract: (K) *A. niger*; (L) *A. fumigatus*; (M) *R. stolonifer*; (N) *R. pusillus*.

Discussion

Fungal organisms are potential sources of plant growth hormone which they secrete as secondary metabolites (Aksoz *et al,* 2008). It was also revealed in the previous studies that species of fungi such as *A. niger*, *A. flavus*, *F. oxysporum*, *P. funiculosum*, *P. corylophilum*, *R. stolonifer*, and *P. formosus* produce phytohormones including gibberellins and auxins(Khan *et al,* 2015; Deng and Cao, 2017).

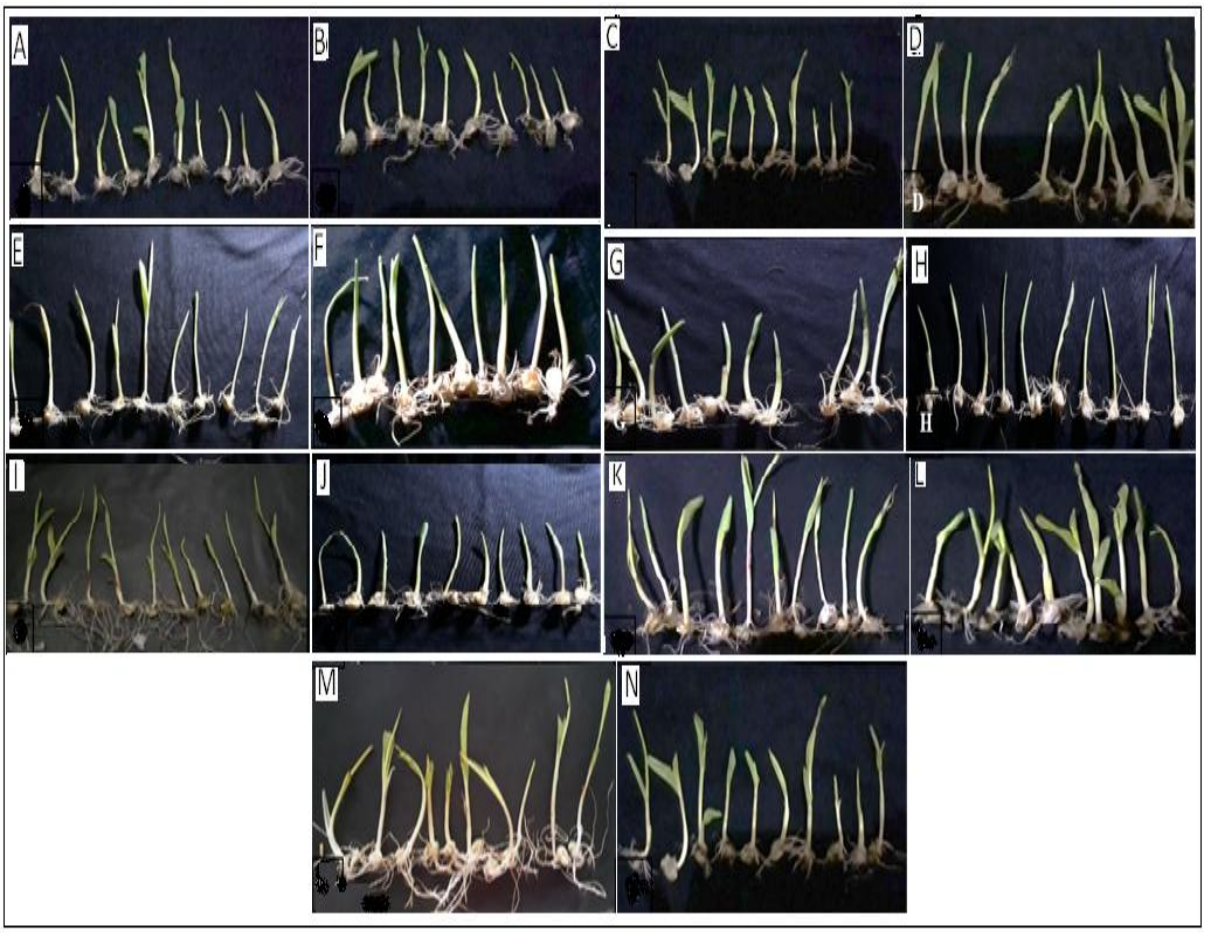

Fig. 4. Corn treated with ethanol extract: (A) *A. niger*; (B) *A. fumigatus*; (C) *R. stolonifer*; (D) *R. pusillus*; with mycelial spent : (E) *A. niger*; (F) *A. fumigatus*; (G) *R. stolonifer*; (H) *R. pusillus;* with ethanol extract(I) gibberellic acid (J) distilled water, crude extract: (K) *A. niger*; (L) *A. fumigatus*; (M) *R. stolonifer*; (N) *R. pusillus*..

Results of the present study are in accordance with the study of Khan *et al* (2015), wherein *A. fumigatus* was proven to produce gibberellins. Also, Silva *et al* (2005) revealed that *R. pusillus* can produce a highly active dextrinogenic and saccharogenic enzymes which is present in an inactive form prior to seed germination. Additionally, many microbialorganisms produce amylase which degrade extracellular starches. Furthermore, *A. niger* produce phytase which increases plant's shoot (Gujar *et al,* 2012). Fungi of genera *Fusarium*, *Aspergillus* and *Penicillium* were characterized as gibberellin producers (Tudzynski, 2005; Bomke *et al,* 2009).Accordingly, in the study of Hamayun *et al* (2011), *A. fumigatus* significantly increased the shoot length, shoot fresh and dry biomass, leaf area, chlorophyll contents and photosynthetic rate of plants. Foliar application of GAs has been known for its role in plant stem elongation and mitigation of abiotic stress (Yang *et al*, 1996; Kaur *et al,* 1998). Gibberellins are synthesized starting from geranyl diphosphate (GDP), farnesyl diphosphate (bFDP) and geranylgeranyl diphosphate (GGDP), which is a precursor for gibberellins and some carotenoids and ubiquinones (Leitao and Enguita, 2016).

Conclusion

Based from the findings it can be concluded that *A. fumigatus* crude extract and fungal spent and *R. pusillus* ethanol and crude extract, *A. niger* crude extract, *R. pusillus* crude and ethanol extracts, and fungal spent, and *A. fumigatus* fungal spent showed gibberellin-like activy in terms of coeleoptile elongation. Whereas, *A. fumigatus* crude extract and *R. stolonifer* ethanol extract and crude extracts, and *A. fumigatus* crude extracts influenced lengthening of second leaf sheath.

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