IJBPAS, July, 2016, 5(7): 1707-1716

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

AUXIN LIKE ACTIVITY OF ENDOPHYTIC FUNGI ASSOCIATED WITH BAMBOO IN RICE (Oryza sativa L.)

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ABSTRACT

The present study evaluated the potential auxin-like activity of six endophytic fungi associated with bamboo namely: *Aspergillus flavus, Aspergillus niger, Aspergillus ochraceus, Cladosporium cladosporoides, Monascus ruber* and *Penicillium cirtrinum.* Crude and ethanol extracts of the aforementioned fungal endophytes were used to test their influence in the growth of decapitated rice coleoptiles and in the formation and lengthening of roots. Results in showed that *Penicillium citrinum* crude extract had the longest coleoptiles means of 11.10mm while *Cladosporium cladosporoides* had the least of 7.10mm. Similarly, *Penicillium citrinum* crude extract is the best among the fungal extracts used in terms of lengthening of the roots and initiating root formation. It recorded the longest roots of 21.68 mm and the most numerous mean number of roots initiated of 10.52. Meanwhile, inhibitory activity of *Aspergillus niger* and *Monascus ruber* crude extracts in root elongation while *Aspergillus flavus, Aspergillus niger, Aspergillus ochraceus, Monascus ruber* and *Penicilium citrinum* ethanol extracts in root initiation was observed. Hence, the potential inhibitory and promoting activity of the fungal endophytes.

Keywords: Auxin, Endophytic fungi, Growth promoters, Phytohormone, Root initiation

INTRODUCTION

Fungal endophytes are fungal organisms the reside in the tissues between living plant cells, forming a mutually beneficial relationship with plants from symbiotic to bordering pathogens which always exist in natural ecosystems (Rodriguez et al. 2009; Maggio et al. 2010; Zhao et al. 2012). Accordingly, they are considered as the gold mines of life, due to the massive production of bioactive compounds which are beneficial for growth and development of host plants and with great importance in the fields of agriculture, medicine and food industry (Rai 2003; Liu et al 2010; Gangwar et al. 2012).

Nassar et al. (2005) mentioned that fungal endophytes are capable of producing phytohormones which enhance the growth of the host plants. Endophytes synthesize plant growth hormones such as indole-3-acetic acid, abscisic acid, cytokinines and gibberellins which are considered as the most common growth promoters associated with the induced growth of plants as a response to endophytic association (Shi et al. 2009; Van Loon 2007; Zhang 2008; Contreras-Cornejo 2009).

Auxins are known to stimulate cell division, resulting into increase in root mass and accelerated formation of root hairs, while cytokinins induce root elongation, thereby also increasing root mass (Daly and Inman 1958). Auxins play essential roles in cell division, cell elongation, vascular tissue differentiation, rhizogenesis and root formation, embryogenesis and inhibition of axillary shoot growth (Chawla, 2002; Lee et al. 2004; George et al. 2008; Khan et al. 2012; Waqas et al. 2012; Hoffman et al. 2013).

The study primarily aimed to determine the auxin like activity of ethanol and crude extracts of six fungal endophytes which could potentially promote or inhibit growth of decapitated coleoptiles, root formation and elongation.

MATERIALS AND METHODS

The Auxin like activity of crude and ethanol extracts of six endophytic fungi accosiated with bamboo (*Aspergillus flavus, Aspergillus niger, Aspergillus ochraceus, Cladosporium cladosporoides, Monascus ruber* and *Penicillium citrinum*) were evaluated through the increment in decapitated coleoptiles and the formation and lengthening of roots of the test plant.

Ethanol extraction

Fungal isolates was cultivated in Potato Dextrose Broth for 7 days. Ten grams (10 g) of dried mycelial mat was submerged and extracted in one hundred milliliter 95% ethanol for 48 hours at room temperature and was filtered using filter paper. The solvent was then be separately evaporated in

rotary evaporator under reduced pressure at 50°C to yield ethanol extracts.

Crude Extraction

Ten grams of fungal mycelia together with the 100ml supernatant was osteorized and it was placed in an amber bottle and refrigerated.

Evaluation of the Auxin-like Activity

Auxin-like activity of the endophytic fungus was evaluated using rice seeds as test seeds. The number and length of roots initiated and the growth of coleoptiles was recorded (Nitsch & Nitsch, 1955).

Growth of decapitated coleoptiles

Rice seeds were soaked in water for two hours and were placed in petri dish lined with filter paper and was incubated 35 cm 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. Then, nine (9) mm sections of the coleoptiles and 3 mm from the tip was cut and was placed in test tube with1.5 ml of different treatments under green light for 24 hours. Then, the coleoptiles were allowed growing for 20 hours in the dark at 25°C. Coleoptiles were measured using a vernier caliper.

Root initiation and elongation

For root initiation, rice seeds were soaked in different treatments for 24 hours and were placed in petri dish lined with filter paper. It was allowed to germinate for 5 days. Roots were cut 10 mm from the stem. It was placed in a bottle with 9 ml of test and control solutions. The number of roots initiated and the length of roots was measured using a digital vernier caliper after five days of incubation.

Statistical Analysis

The study was laid out using Completely Randomized Design (CRD). Data was analyzed using Analysis of Variance (ANOVA) and Comparison Among Means by Duncan's Multiple Range Test (DMRT). All tests of significance were done at 5% probability levels.

RESULTS AND DISCUSSIONS

Endophytic fungi can promote host plant growth and protect the host from microbes and insects (Strobel et al. 1996; Kawaguchi & Sydn 1996; Keith, 1998; Khan et al. 2011) Endophytes promote the growth and developments in crops and plants through different ways which includes the production of phytohormones like auxin (Lee et al. 2004; Verma et al. 2009; Gangwar et al. 2012). In the present study, the influence of crude and ethanol extracts of *Aspergillus flavus, Aspergillus niger, Aspergillus ochraceus, Cladosporium cladosporioides, Monascus ruber* and *Penicillium citrinum* was evaluated.

Elongation of Coleoptiles

As indicated in Table 1, *Penicillium citrinum* crude extract

registered the longest coleoptiles of 11.10 mm which is far significantly higher than those treated with commercial auxin of 9.67 mm, while coleoptiles treated with *Penicillium citrinum* ethanol extract with 9.08mm is comparable to later. This greatly suggests the auxin like promoting activity of crude and ethanol extracts of *Penicillium citrinum*. Additionally, the length of coleoptiles treated with crude and ethanol extracts of *Aspergillus flavus, Aspergillus niger, Aspergillus ochraceus, Cladosporium cladosporioides* and *Monascus ruber* were comparable with the untreated coleoptiles,therefore no inhibitory nor promoting activity was denoted. For the % increase in the length of coleoptiles, *Penicillium citrinum* crude extracts registered the highest of 85.00% followed by the commercial auxin of 61.17% and *Penicillium citrinum* ethanol extracts of 51.33%. Meanwhile, as compared to the untreated coleoptiles, the least % increase was recorded in *Aspergillus flavus, Cladosporium cladosporioides* and *Monascus ruber* ethanol extracts of 21.33%, 1833% and 25.50%, respectively.

This coincides with Magdalene et al. (2012) wherein *Penicillium* showed growth promoting activity in the elongation of the coleoptile with the used to TLC. According to Mei et al. (2010) endophytes secretes plant growth regulators (PGR) which include *Aspegillus* and *Penicillium* species producing auxin. Also, study of Seyis Biskay et al. (2008) and Hasan (2002) showed that *A. niger* was produce secondary metabolites such as auxin and gibberellic acid which influence the growth of the plant.

Root Initiation

Root promoting properties of auxin is one of their vital roles in growing plants which stimulate the occurrence of adventitious roots in the majority of plant species, and they are commonly used in vegetative propagation. Additionally, application of exogenous auxin increases concentration of natural auxins which increases in the number and quality of roots. In lower concentrations do they aid the coleoptile's elongation, that of the shoot and the roots. If the concentration becomes higher, the effect reverses and elongation of root and shoot is inhibited (Tonon et al., 2001).

Table 2 shows the effect of crude and ethanol extracts of fungal endophytes on root initiation. *Penicillium citrinum* crude extract, had the most numerous roots initiated with the mean average of 10.52 which is comparable to plants treated with commercial auxin of 10.43, indicating their potential in the production of auxin like

activity in rice plants. Meanwhile, only one (1) root per plant was recorded in rice plants treated with *Aspergillus flavus, Aspergillus niger, Aspergillus ochraceus, Cladosporium cladosporoides Monascus ruber* and *Penicillium citrinum* ethanol extracts. Also, the rest of the test fungal endophytes crude extracts signify their potential action in increasing the number of roots initiated. Based from the change in % number of roots initiated, *Penicillium citrinum* crude extract had 120% increase in number of roots, followed by the commercial gibberellic acid of 118.20% and 37.45% by *Monascus ruber* crude extract. Also, in reference to the untreated rice plants, 79.08% reduction in number of roots initiated was computed in plants treated with all ethanol extracts of fungal endophytes. Thus, both promoting and inhibitory activity of the crude and ethanol extracts of fungal endophytes can be noted.

Species of genus *Penicillium* are potent plant growth-promoting fungus, they interact with plants' roots and secrete the plant hormones (auxin and gibberellin) and is also involved in phosphate solubilization, which may be a reason to increase the plant growth (Hyakumachi, 1994; Shivanna et al. 1994; Khan et al. 2008: Khan et al. 2008; Khan et al. 2011; Radhakrishnan et al. 2013). Auxin has positive effect on root growth because auxin promotes root initiation (Kim et al., 2006).

Inhibitory effects of ethanol extracts of fungal endophytes can be attributed to high concentration of ethanol extracts used (Noguchi et al. 2001; Islam et al. 2012). Meanwhile, as stated by Rahman et al. (2007), auxin has the ability to inhibit root growth primarily through reducing the length of the growth zone rather than the maximal rate of elemental elongation and they do not reduce cell production rate.

Root Elongation

Among all fungal extracts used, *Penicillium citrinum* crude extracts is the most superior in terms of its influence in the lengthening of roots. The aforementioned obtained mean average of 22.39mm, followed by *Aspergillus niger* ethanol extract of 21.63mm, which are both comparable to the commercial auxin of 22.39mm, thus the indication of the potential auxin like activity which promote lengthening of roots (Table 3). Whereas, the shortest roots were recorded in rice plants treated with *Monascus ruber* and *Aspergillus niger* crude extracts of 12.91mm and 13.71mm. In comparison to the untreated plants, only *Aspegillus niger, Aspergillus ochraceus* and *Penicillium citrinum* obtained % increase in the length of roots of 29.64%, 17.29% and 20.19% respectively. Meanwhile inhibitory action of

both ethanol and crude extracts of *Monascus ruber* and *Aspergillus flavus;* crude extracts of *Aspergillus niger, Aspergillus ochraceus* and *Cladosporium cladosporioides* ranging from 1.78% to 27.99% lower when compared to untreated plants.

Accordingly, Yadav et al. (2011) and Nasim et al. (2012) observed increment in shoot length and root length of checkpea when treated with *P. citrinum*. Also, Radhakrishnan et al. (2013) revealed *Penicillium* sp. as potent plant growth promoting fungus that had ability to produce indole-3-acetic acid). Futhermore, Ganga Mani et al. (2016) revealed that *P. citrinum* significantly increased the leaves, root and the shoot length and the leaf surface area in the chili plant thus, the *P. citrinum* exhibited plant promoting characters such as production of IAA. Fauda et al. (2015), fungal isolates had a considerable impact on plant growth parameters including root elongation as a result of ammonia and IAA production.

Table 1: Length of coleoptiles treated with crude and ethanol extracts of different endophytic

Treatments	Length of Coleoptiles (mm)	% increase in coleoptiles
***Aspergillus flavus* (crude extract)**	**8.07^{def}**	**34.5**
***Aspergillus flavus* (ethanol extract)**	**7.28^{ef}**	**21.33**
***Aspergillus niger*(crude extract)**	**7.98^{def}**	**33.00**
***Aspergillus niger*(ethanol extract)**	**7.85^{def}**	**30.83**
***Aspergillus ochraceus*(crude extract)**	**8.70^{cd}**	**45.00**
***Aspergillus ochraceus*(ethanol extract)**	**7.67^{ef}**	**27.83**
***Cladosporium cladosporoides*(crude extract)**	**8.21^{cde}**	**36.83**
***Cladosporium cladosporoides* (ethanol extract)**	**7.10^{f}**	**18.33**
***Monascus ruber*(crude extract)**	**7.79^{def}**	**29.33**
***Monascus ruber*(ethanol extract)**	**7.53^{ef}**	**25.50**
***Penicillium citrinum*(crude extract)**	**11.10^{a}**	**85.00**
***Penicillium citrinum*(ethanol extract)**	**9.08^{bc}**	**51.33**
Positive control (Auxin)	**9.67^{b}**	**61.17**
Negative control (distilled water)	**7.70^{def}**	**28.33**

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

Table 2: Mean of number of roots initiated in crude and ethanol extracts of fungal endophytes

Treatments	Roots Initiated	% change in mean number of roots initiated
***Aspergillus flavus*(crude extract)**	**5.55^{d}**	**+16.11**
***Aspergillus flavus* (ethanol extract)**	**1.00^{f}**	**-79.08**
***Aspergillus niger*(crude extract)**	**6.25^{bc}**	**+30.75**
***Aspergillus niger*(ethanol extract)**	**1.00^{f}**	**-79.08**
***Aspergillus ochraceus*(crude extract)**	**5.82^{cd}**	**+21.76**
***Aspergillus ochraceus*(ethanol extract)**	**1.00^{f}**	**-79.08**
***Cladosporium cladosporoides*(crude extract)**	**6.40^{bc}**	**+33.89**
***Cladosporium cladosporoide*(ethanol extract)**	**1.00^{f}**	**-79.08**
***Monascus ruber*(crude extract)**	**6.57^{b}**	**+37.45**
***Monascus ruber*(ethanol extract)**	**1.00^{f}**	**-79.08**
***Penicillium citricum*(crude extract)**	**10.52^{a}**	**+120.00**
***Penicillium citricum*(ethanol extract)**	**1.00^{f}**	**-79.08**
Positive control (Auxin)	**10.43^{a}**	**+118.20**
Negative control (distilled water)	**4.78^{e}**	

***Treatments that have different letters are significantly different.**
***+ increase: -decrease**

Table 3: Length of roots initiation treated with different endophytic fungi in crude and ethanol extract

Treatments	Root Elongation (mm)	% change in the length of the roots
Aspergillus flavus **(crude extract)**	**14.43^{cd}**	**-19.52**
Aspergillus flavus**(ethanol extract)**	**17.61^{c}**	**-1.78**
Aspergillus niger **(crude extract)**	**13.71^{d}**	**-23.53**
Aspergillus niger**(ethanol extract)**	**21.63^{a}**	**+29.64**
Aspergillus ochraceus **(crude extract)**	**14.42^{cd}**	**-19.57**
Aspergillus ochraceus**(ethanol extract)**	**21.03^{ab}**	**+17.29**
Cladosporium cladosporoides **(crude extract)**	**14.53^{cd}**	**-18.96**
Cladosporium cladosporoides**(ethanol extract)**	**16.35^{cd}**	**-8.81**
Monascus ruber **(crude extract)**	**12.91^{d}**	**-27.99**
Monascus ruber**(ethanol extract)**	**15.82^{cd}**	**-4.92**
Penicillium citrinum **(crude extract)**	**21.68^{a}**	**+20.91**
Penicillium citrinum**(ethanol extract)**	**15.40^{cd}**	**-14.11**
Positive control (Auxin)	**22.39^{a}**	**+24.87**
Negative control (distilled water)	**17.93^{bc}**	

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

***+ increase: -decrease**

CONCLUSION

Auxin like activity of fungal endophytes was revealed in the study, which influenced growth of decapitated coleoptiles, root formation and lengthening. Among which, *Penicillium citrnum* exhibited the most superior growth promoting activity in the growth of rice plants.

ACKNOWLEDGMENT

To God be the Highest Glory! Thy Will Be Done

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