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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 7 (12): 2039-2044, 2004
ISSN 1028-8880

Effect of Indole Acetic Acid on *in vitro* Growth and Biomass Production of Some Soil Fungi

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Abstract: Effect of different concentrations of Indole Acetic Acid (IAA) was studied on growth of four species of soil fungi namely; *Aspergillus oryzae*, *A. terreus*, *A. niger* and *Alternaria alternata*. The hormone was applied in various concentrations. Increased growth rate and biomass production revealed significant values when treated with dilute solutions of hormone i.e. 15, 30 and 45 mg L^{-1}. Fresh weight and dry weight values were observed appreciably high when these fungi were treated with 45 mg L^{-1} concentration of the hormone solution. The data on fresh biomass revealed that the highest increase in biomass was obtained for *Alternaria alternata*. It was noted that there was a percentage increase of 56% in this case. Where as the data on dry biomass revealed that the highest significant increase was obtained for *Aspergillus oryzae* that was 66%. A significant suppressed growth in case of fresh biomass was obtained for *Alternaria alternata* after treated with IAA followed by *Aspergillus terreus* the percentage losses were 31.43 and 8.78%, respectively. The highest percentage loss for dry biomass of *Aspergillus niger* was 15.3%. Whereas dry biomass of *Aspergillus terreus* remained unchanged in comparison to 45 mg L^{-1}.

Key words: IAA, Biomass production, soil fungi, *Aspergillus oryzae*, *A. terreus*, *A. niger*, *Alternaria alternata*

INTRODUCTION

Growth hormones are a organic substances produced naturally in higher plants, controlling growth or physiological functions and are able to mediate intercellular communications in minute amounts[1]. Five major types of hormones regulate plant development namely: Auxins, Gibberellin, Cytokinin, Ethylene and Abscisic acid. Indole Acetic Acid (IAA) has been found to be of universal occurrence and is a major plant hormone. It is one of the most common and extensively used auxin[2].

In the modern biotechnological methods i.e., especially tissue culturing, IAA stimulates differentiation of root/shoot in an undifferentiated callus[3]. Plants propagated by cuttings are difficult to root. IAA initiates and hastens the root development and often results in larger and more vigorous roots. A significant increase in root length, root number and mycorrhizal infections has also been reported by IAA application[4-6]. Seedless fruits of full marketable size have also been successfully prepared by exogenous application of various hormones including IAA in the case of cucumber and watermelons[7]. Improvement of fruit set in almonds using growth regulators have been reported by Sotomayer and Castro[8].

Alternaria and *Cladosporium*, as well as many other species, are much more common in outdoors than in indoors. Houseplants, moldy carpets and stored food products can all be a potential source of allergens in the home or indoor work place[9]. *Alternaria alternata* is the commonest species of tropics and is responsible for diseases of wheat and other economically important crops. *Aspergillus terreus* is especially wide spread in warm arable soils and has been isolated in great abundance from these soils. *A. terreus* frequently occur, however, upon a great variety of materials useful to man, including grains in storage[10], straw and forage products, cotton and other fibrous materials not adequately protected from excessive moisture. It has been found to be the prominent fungus in the rhizospheres of the Pineapple plant[11] and some of Egyptian cotton varieties[12].

As cited in "The Genus *Aspergillus*", the black *Aspergilli* are probably more common than any other group within this genus. They are world wide in distribution and occur upon the greatest variety of substrata, including grains, forage products, spoiled fruits and vegetables, exposed cotton textiles and fabrics, leather, dairy products and decaying vegetation in the field. A large spored member of the *Aspergillus niger*

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group, has been reported to cause grape rot in India. *A. niger* and *A. wentti* are used to produce citric acid for the soft drinks industry. In conditions of normal pH, *A. niger* is also used to produce gluconic acid (as a dietary supplement) by the direct enzymatic oxidation of glucose supplied as the substrata. *Aspergillus oryzae* exists in wild. Some researchers[13] indicate that *A. oryzae* can be isolated in nature.

Fungi have many traditional roles in biotechnology. They have also been assigned some novel roles and hence there is major scope for their commercial development[14]. Presently the data on exogenous application of fungi with various plant hormones is scarce. The present study has therefore been designed with the objective to investigate and enhance the basic knowledge about the effect of Auxin on the growth of four fungal species viz., *Aspergillus oryzae, A. terreus, A. niger* and *Alternaria alternata.*

MATERIALS AND METHODS

The fungi selected to be treated with hormonal solutions were *Aspergillus oryzae*, *Aspergillus terreus*, *Aspergillus niger* and *Alternaria alternata*. Petriplates, forceps, needles, corkborer, conical flasks, measuring cylinders were washed with detergent and tap water, then autoclaved at 121°C temperature and 15 lb/inch^{-2} pressure for 15 min. The autoclaved apparatus was then dried and resterilized in oven at 150°C for 30 min.

Media plates were prepared with 2% Malt Extract Agar (MEA) and were incubated at 25±1°C for 24 h. Plates showing no sign of contamination were inoculated with pure cultures of fungal species obtained from First Fungal Culture Bank of Pakistan, Department of Mycology and Plant Pathology, University of the Punjab, Lahore. These fungal cultures were mass multiplied on fresh media plates as per requirement of the experiment. Sub-culturing, mass multiplication and all steps of experiments were done in Laminar Air Flow Chamber aseptically. Four different concentrations like 0, 15, 30, 45 and 60 mg L^{-1} were prepared as follows:

To prepare 250 mL of stock solution of IAA, 15 mg of the hormone powder was carefully and aseptically weighed on a piece of glossy paper. The suspension was initially raised in 2 mL of rectified spirit. Final volume was made adding sterilized distilled water in separate pre-sterilized labeled bottle. Stock solution was kept at 4°C. Freshly prepared (3-4 days) solution was used for further experimental work. Stock solution was subsequently diluted aseptically to prepare 15, 30 and 45 mg L^{-1} concentrations. About 100 mL of each of dilution were prepared. Sterilized distill water was taken as control.

Two percent aqueous Malt Extract (ME) medium was prepared by dissolving 1 g of malt extract in 50 mL of distilled water. Then the media flasks were autoclaved at 121°C temperature and 15lb/inch pressure for 15 min. The media were left to cool down to approximately 45°C then the antibiotic Chloromycetin, was added @1 Capsule/200 mL into each medium flask. Two percent Malt Extract Agar (MEA) medium was prepared by dissolving 20 g of malt extract and 20 g of Agar in 1 L of water. The medium was autoclaved and then poured in pre-sterilized, oven-dried petriplates under sterilized conditions.

To proceed with the hormonal treatment, discs (1 cm in diameter) from 7-days old petri plates of pure cultured test fungi were removed with the help of a sterilized corkborer. These discs were transferred to petriplates containing filter papers moistened with 10 mL of various concentrations of the hormones. These discs were exposed to hormonal treatments for a period of one and a half hour.

Disc method was used for inoculations. These discs were removed after one and half hour and two such discs for each treatment were used to inoculate liquid media flasks. The flasks were incubated at 25±1°C. Flasks were prepared in triplicates for each concentration of all three hormones. Growth analysis of fungal species was carried out in terms of fresh and dry weight after 7 days. Fungal biomass from replicate flasks was filtered on pre-weighed Whatman No. 1.The materials were oven dried at 60°C for 6 h and reweighed to determine the oven-dried weights.

Statistical analysis of all data recorded for fungal dry biomass was carried out by using Duncan's New Multiple Range test[15] at $p<0.05$ to detect the significant difference among the treatments.

RESULTS

Biomass productivity assays of *Aspergillus oryzae*: The growth assays of *Aspergillus oryzae* in terms of fresh and dry biomass production, against exposure to hormone solution of indole acetic acid were carried out after seven days of incubation. The data on fresh biomass production revealed an increase in growth of *A. oryzae* from control to 45 mg L^{-1} concentration (Table 1). Maximum growth was induced by 45 mg L^{-1} concentration causing an increase in the fresh biomass production of *A. oryzae*. The growth enhancement of *A. oryzae* in 45 mg L^{-1} concentration was 21% in comparison to control set. Whereas significantly negative response at higher concentration i.e. 60 mg L^{-1} was evident in this experiment. The decrease in growth of *A. oryzae* treated with 60 mg L^{-1} IAA was 6.26% as compared to the set exposed to 45 mg L^{-1} IAA concentration. The effect of

Table 1: Effect of IAA on *in vitro* growth of *Aspergillus oryzae*

IAA concentration (mg L^{-1})	Fresh weights (g)	Dry weights (g)
0	2.76±0.080	0.09±0.006
15	2.89±0.003	0.11±0.003
30	3.0±0.0500	0.12±0.006
45	3.35±0.028	0.15±0.002
60	3.14±0.008	0.14±0.002

Table 2: Effect of IAA on *in vitro* growth of *Aspergillus terreus*

IAA concentration (mg L^{-1})	Fresh weights (g)	Dry weights (g)
0	2.44±0.14	0.07±0.003
15	2.86±0.03	0.09±0.003
30	3.04±0.02	0.11±0.003
45	3.53±0.21	0.13±0.006
60	3.22±0.03	0.13±0.003

Table 3: Effect of IAA on *in vitro* growth of *Aspergillus niger*

IAA concentration (mg L^{-1})	Fresh weights (g)	Dry weights (g)
0	2.95±0.34	0.15±0.002
15	3.87±0.25	0.17±0.008
30	5.41±0.10	0.20±0.003
45	6.68±0.29	0.26±0.003
60	6.22±0.05	0.22±0.003

IAA treatment at intermediate concentration i.e., 15 and 30 mg L^{-1} was insignificant and negligible.

The data on dry biomass production after 7 days revealed (Table 1) an appreciable interference of hormone solution with the growth of fungal species at 60 mg L^{-1} concentration whereas a significantly positive effect on biomass production was obtained at concentration of 45 mg L^{-1} (Table 1). Fungal growth in terms of dry weight of *A. oryzae* treated with 45 mg L^{-1} IAA show an increase of 66% in comparison to the control set. The growth in 60 mg L^{-1} concentration of IAA decreased as compared to in 45 mg L^{-1} treatment. The decrease of growth in 60 mg L^{-1} was 6.6% in comparison to 45 mg L^{-1}. The influence of 15 and 30 mg L^{-1} concentration is insignificant and found to have negligible difference, as was the case with fresh mass.

The fungal biomass of *A. oryzae* was markedly high in 30 and 45 mg L^{-1} concentration of IAA. A very sharp stimulus in growth, significantly higher than the control set was recorded in these treatments. However, the difference in biomass production by *A. oryzae* was not statistically significant in 30 mg L^{-1} as compared to 15. Minimum growth in both fresh and dry weights was recorded in the set of treatment labeled as control. Visibly the hyphal growth on the medium was more profuse in 45 mg L^{-1} concentration.

Biomass productivity assays of *Aspergillus terreus*: Fresh and dry biomass assays of *Aspergillus terreus* after a period of 7 days revealed a rather erratic pattern of growth (Table 2). Except for 60 mg L^{-1} concentration all concentrations of indole acetic acid caused considerable increase in biomass production by the fungus. The trend in results obtained for this test fungus was inline with the results obtained for *A. oryzae*. At higher concentration viz., 60 mg L^{-1}, statistically significant depression in growth was evidenced as comparison to control treatment. The fresh mass reported in the case of 45 mg L^{-1} was greater than the mass obtained in the case of 60 mg L^{-1}. The difference between the two was however not statistically significant at $p<0.05$ (Table 2). Percentage loss in growth of the test fungus exposed to 60 mg L^{-1} concentration of IAA was found to be 8.78% in comparison to 45 mg L^{-1} IAA concentration. An appreciable growth enhancement of *A. terreus* was reported in 45 mg L^{-1}, which was calculated to be 44.6% greater as compared to growth in 0 mg L^{-1} IAA treatment.

The data for dry weight produced by *A. terreus* showed a same general trend as was reported for fresh mass production by the fungus. The growth slightly enhanced from low to high concentrations viz., 0.45 mg L^{-1}. Percentage increase of 46.15 was observed at 45 and 60 mg L^{-1} concentrations as compared to control. Although at $p<0.05$, statistically insignificant results for dry weight were obtained in case of 15 and 30 mg L^{-1} concentration of IAA. However, a percentage increase in growth of *A. terreus* treated with 30 mg L^{-1} IAA as compared to 15 mg L^{-1} was computed to be 6.2%. The percentage increase of fungal biomass was computed to be same in case of 45 and 60 mg L^{-1} concentrations as compared to control. The concentration of 60 mg L^{-1} has shown no decrease as compared to 45 mg L^{-1}.

Biomass productivity assays of *Aspergillus niger*: The values for fresh and dry weight of *Aspergillus niger* recorded at the end of 7-days incubation period in various treatments of IAA (Table 3) clearly represent a distinct pattern of growth in response to hormone solution of indole acetic acid. It is evident from the fresh biomass assessment that growth of the fungal species was low in control set. In contrast to that all applied concentrations of hormone solution of indole acetic acid, except 60 mg L^{-1}, yielded a considerably high fresh mass. Statistically significant stimulation in fresh weight yield was achieved in all treatments of IAA. *Aspergillus niger* given in the treatment of 45 mg L^{-1} yielded an enhancement in growth up to 55.83% as compared to control.

It was noted, that there was percentage loss of 6.68% in fresh weight and 15.3% in dry weight in 60 mg L^{-1} concentration in comparison to 45 mg L^{-1}. In 60 mg L^{-1} concentration, the fresh weight values were low but insignificantly different at $p<0.05$ as compared to those obtained in 45 mg L^{-1} concentration of IAA. In contrast

Table 4: Effect of IAA on *in vitro* growth of *Alternaria alternata*

IAA concentration (mg L^{-1})	Fresh weights (g)	Dry weights (g)
0	2.29±0.14	0.10±0.003
15	2.57±0.03	0.11±0.003
30	3.18±0.09	0.12±0.003
45	5.28±0.28	0.16±0.003
60	3.62±0.13	0.14±0.006

to that biomass assay at 60 mg L^{-1} concentration was statistically significant in comparison to control medium (Table 3). The dry mass production was significantly higher and showed a percentage increase of 17.6% in 30 mg L^{-1} in comparison to 15 mg L^{-1}. A decline in dry weights of *A. niger* recorded in 60 mg L^{-1} concentration, was statistically significant to those recorded in 45 mg L^{-1} concentration (Table 3).

A steady increase in dry weights yielded by the fungus was recorded from control set to 45 mg L^{-1} through 15 and 30 mg L^{-1} concentrations of IAA. The differences among various concentrations in terms of dry mass production were statistically significant. *A. niger* given the treatment of 45 mg L^{-1} yielded an enhancement in growth of up to 42.3% as compared to the control set.

Biomass productivity assays of *Alternaria alternata*: The growth biomass assays of *Alternaria alternata*, in terms of fresh and dry biomass production after treatment with various dilutions of IAA were carried out after seven days of incubation. The data on fresh and dry biomass production by *Alternaria alternata* was significantly high for 45 mg L^{-1} concentration of IAA as compared to rest of the concentrations (Table 4).

The data on fresh biomass production revealed an increase in growth of *Alternaria alternata* from control to 45 mg L^{-1} concentration (Table 4). Maximum growth was induced by 45 mg L^{-1} concentration causing an increase in the fresh biomass production of *A. alternata* (Table 4). The growth enhancement of *A. alternata* in 45 mg L^{-1} was 56% in comparison to control set. On the other hand significant suppression in fresh mass production at higher concentration i.e., 60 mg L^{-1} was observed. The decrease in growth of *A. alternata* treated with 60 mg L^{-1} was 31% as compared to the set exposed to 45 mg L^{-1} IAA concentration. The effect of IAA treatment at lowed levels of IAA concentration i.e., 15 and 30 mg L^{-1} showed a significant drop. However, the results for these two treatments at $p<0.05$ were insignificantly different with each other (Table 4).

Dry biomass production of *Alternaria alternata* in response to various concentrations of IAA revealed an excessive increase of growth of fungal test species at 45 mg L^{-1} concentration. Percentage increase of growth in 45 mg L^{-1} was 60% in case of dry weight and 56.6% in case of fresh weights. Percentage loss of 12.5% was computed for *Alternaria alternata* given in the treatment of 60 mg L^{-1} as compared to 45 mg L^{-1} concentration. It is evident from the dry biomass assessment data that growth of *A. alternata* was least in control medium (Table 4) and it was statistically significant in comparison to rest of the treatments. In contrast to that all applied concentrations of IAA except in 60 mg L^{-1} provided a considerable increase in biomass productivity and statistically significant stimulation in growth yield was achieved in all treatments.

DISCUSSION

Plant hormones are a group of naturally occurring, organic substances, which influence physiological processes at low concentrations. The processes influenced consist mainly of growth, differentiation and development, through other processes such as stomatal movement, may also be affected. Plant hormones are also referred to as phytohormones[16]. The use of phytohormones to improve the growth and yield of economically important plant is not new in the history of agriculture.

In the present study, however, it has been attempted to summarize the effects of exogenous application of an important plant hormone i.e. indole acetic acid on some soil fungi i.e., *Aspergillus oryzae, Aspergillus terreus, Aspergillus niger* and *Alternaria alternata.* There are indeed very few references available on this kind of studies in the past[17]. Nasim *et al.*[18] have summarized the results of exogenous application of *Aspergillus flavus, Fusarium oxysporium, Fusarium moniliformeans* and *Trichoderma viride* with variable concentrations of IAA. The present study is thus a continuation of the investigation conducted by Nasim *et al.*[18]. It attempts to describe the effects of various concentrations of hormone solution on the growth and biomass production of a group of soil fungi. The data obtained in the present study has clearly indicated that dilute concentrations (30 and 45 mg L^{-1}) to be most effective. This trend is in line with the previous investigations conducted in higher plants as well as fungi[18,19]. The difference was statistically significant between control and treated cultures of all the test fungi as far as biomass production in terms of fresh/dry weight was concerned.

Nasim *et al.*[18] in other fungal species due to indole acetic acid have reported similar result. In the treated discs of *F. oxysporium* in early stages peak was at 50 mg L^{-1}. However, the results deviated at later stages of the growth.

The results obtained in the present study revealed that generally the highest concentration of 60 mg L^{-1} of the hormone suppressed the fungal biomass productivity in all the test fungal species. In contrast, the lower concentration of 30 and 45 mg L^{-1} of hormone solutions enhanced the fungal biomass production in all the four test species. However, the fungal growth response to lower and higher concentrations of solutions was variable in different test species at corresponding growth stages. The concentrations of 30 and 45 mg L^{-1} of hormone solution enhanced the fungal biomass productivity by increasing the concentrations of solution. Percentage increase of 55% was observed when *A. oryzae* was treated with IAA maximum increased fungal biomass was obtained in this case as compared to other test species and hormone solutions. Growth rate comparisons of control and treated fungal cultures in the study conducted by Nasim *et al.*[18] also shown that the difference was appreciably significant. Among the treatments comparisons have shown that low concentrations (50, 100 and 150 mg L^{-1}) were more stimulatory as compared to high concentrations (200 and 1000 mg L^{-1}). Biomass comparisons between control and treated cultures of test fungi in liquid medium showed significant difference. Fresh and dry weights were maximum in concentrations 100 and 200 mg L^{-1} among the discs of test fungi treated with IAA. Lowest growth parameters were in very high 1000 mg L^{-1} concentrations[18].

Similar stimulatory effects of low concentrations of 2,4,D[20,6] has been reported in higher plants, fungal flora also shows responses to the plant growth hormones. In another study, wheat seeds after treatment with various growth regulators including gibberellic acid showed highest percent germination when treated with 20 mg L^{-1} GA3[21] fresh weight in segments of morning glory. Present behavior of test fungi in low concentrations of IAA is very similar to higher plants, these results are also in line with previous investigations

Mycorrhizal plants pretreated with IAA (low conc.) showed significant increase in fresh and dry weights[6] increase in fresh weight may probably be to the increased absorption activity of cells. According to the[22], dry matter accumulation of bean plants is directly proportional to the amount of IAA. Like the higher plants, soil born fungi may probably have adopted the similar enhanced mechanisms of nutrient absorption from the medium supplied for growth resulting in increased fresh and dry weights. Hormones concentration pattern of effectiveness shown that in terms of growth rate and biomass production low concentrations (15, 30, 45 mg L^{-1}) in all test fungi seen to be more effective.

As a pilot study this investigation has founded with some basic information's regarding the role of IAA on fungal growth. It has also helped us in refining the techniques. This investigation may successfully be extended in to a comprehensive project encompassing other fungi of commercial importance. The newly discovered plant hormones may be included in the list for future studies.

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