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Effect of Gibberellin on *in vitro* growth and biomass production of some soil fungi

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Abstract

Effect of different concentrations of Gibberellin was studied on growth of four species of soil fungi namely, *Aspergillus oryzae, A. terreus*, *A. niger* and *Alternaria alternata*. The hormone was applied singly in various concentrations. Increased growth rate and biomass production revealed significant values when treated with dilute solutions of Gibberellin at 15, 30 and 45 mgL^{-1} except *A. terreus*. Fresh weights and dry weight values were observed appreciably high when *Aspergillus terreus* was treated with 60 mgL^{-1} concentration of the hormone solution. The data on fresh and dry biomass revealed that the highest biomass increase was obtained for *Alternaria alternata*. Fresh biomass of *Alternaria alternata* showed 75% increase when treated with 45 mgL^{-1} concentration of hormone solution in comparison to control, whereas an increase of 77.8% was obtained in the case of dry weight. At 60 mgL^{-1},a significant fresh biomass suppression of 16.3% and 7.43% was observed for *Alternaria alternata* and *Aspergillus* oryzae, respectively. The highest loss for dry biomass was noticed in *Alternaria alternata* (33.33%).

Key words: Growth hormone, Gibberellin, in-vitro growth, biomass, fungi

Introduction

Five major types of hormones regulate plant development namely: Auxins, Gibberellin, Cytokinin, Ethylene and Abscisic acid. Gibberellins are diterpenoid acids, which have the same basic ent- gibberellane ring structure (Wilkins, 1984). Gibberellins were first isolated from the fungus *Gibberella fujikuroi* in which they occur in large quantities as secondary metabolites (Adams and Ross, 1983). Its application to dwarf or rosette plants, dormant buds or dormant and germinating seeds can result in dramatic and diverse effects on growth (Jones 1980). Gibberellins have stimulatory effect on the growth of adventitious buds reported by (Bates et al. 1992). Gibberellins application has been reported to be helpful in enhancing wheat growth under saline conditions (Parasher and Varma 1998). Diseased plants grow tall and spindly and tend to fall over their own weight (Jones and Moll, 1983). The effect of exogenous application of gibberellins on fungus is not known yet. (Kapulnik and Douds, 2000).

Alternaria and *Cladosporium*, as well as many other species, are much more common in outdoors than indoors. Houseplants, moldy carpets and stored food products can all be a potential source of allergens in the home or indoor work place (Lacey, 1991). *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 (Del Prado and Christenen, 1952), 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 (Contois, 1953).

The black *Aspergilli* are probably more common than any other group within this genus. The large spored *Aspergillus niger*, 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 substrate. *Aspergillus oryzae* exists in wild.

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 Gibberellin 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*. Media plates were prepared with 2% Malt Extract Agar (MEA) and were incubated at 25±1°C for 24 hours. Plates showing no sign of contamination were inoculated. 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 $0mgL^{-1}$, $15mgL^{-1}$, $30mgL^{-1}$, $45mgL^{-1}$ and $60mgL^{-1}$were prepared. The medium prepared 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 10ml of various concentrations of the hormone solution. 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 a 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 the hormone. 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 six hours and reweighed to determine the weights.

Statistical analysis of all the data recorded for fungal dry biomass was carried out by using Duncan's New Multiple Range (DMR) test (Steel and Torrie, 1980) at $P< 0.05$ to detect the significant difference among the treatments.

Results

Biomass productivity assays of *Aspergillus oryzae*

Biomass productivity response of *Aspergillus oryzae* was variable when treated with different concentrations of gibberellic acid (Table 1). Fresh biomass production revealed an increase in growth along the concentration gradient of hormone solution of gibberellic acid from 0 to 45 mgL^{-1}. Maximum growth was induced by 45 mgL^{-1} conc. causing an increase in the fresh biomass production of *A. oryzae* (Table 1). Percentage increase of 21% was obtained in 45 mgL^{-1} concentration as compared to control set. The decrease in growth of *A. oryzae* treated with 60 mgL^{-1} was 7.43% as compared to the set exposed to 45 mgL^{-1}. In case of dry biomass production assays the lower concentrations of 0 and 15 mgL^{-1} of gibberellic acid were found to be least effective for fungal growth stimulation (Table 1). Maximum enhancement in growth was induced by 45 mgL^{-1} concentration of gibberellic acid. Percentage increase of up to 47.6% was obtained in 45 mgL^{-1} concentration as compared to control. The concentration of 60 mgL^{-1} seemed to be less effective and had a percentage loss of 23% (Table 1).

Table 1: Effect of Gibberellin on *Aspergillus oryzae*

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Sr. no	Concen-trations (mgL^{-1})	Fresh weights (g)	Dry weights (g)
1.	0	6.21 (±0.10)	0.11 (±0.009)
2.	15	6.8 (±0.06)	0.13 (±0.003)
3.	30	6.98 (±0.003)	0.17 (±0.003)
4.	45	7.89 (±0.15)	0.21 (±0.01)
5.	60	7.43 (±0.11)	0.16 (±0.003)

Biomass productivity assays of *Aspergillus terreus*

The data on fresh biomass production revealed an increase in growth of *Aspergillus terreus*. Maximum growth was obtained at 60 mgL^{-1} concentration showing percentage increase of 54.4% and was statistically highly significant in comparison to control. Table 2 shows a gradual increase along the increasing concentrations of hormone solution. The data for dry weight produced by *Aspergillus terreus* followed the same general pattern. The production of dry weight was markedly high, in 60 mgL^{-1} concentration. A very sharp stimulus in growth, which was significantly higher than control was recorded in this treatment where as concentration of 45 mgL^{-1} showed 35.20% increase in fungal biomass in comparison to control and 5.88% in comparison to 30 mgL^{-1}concentration of gibberellin. The difference in dry biomass yield of *Aspergillus terreus* treated with 15, 30 and 45 mgL^{-1} concentrations was found to

be statistically insignificant as compared to control set of experiment. At the same time these treatments were found to be statistically significant in comparison to 60 mgL^{-1} (Table 1).

Table 2: Effect of Gibberellin on *Aspergillus terreus*

Sr. no	Concen-trations (mgL^{-1})	Fresh weights (g)	Dry weights (g)
1.	0	5.06 (±0.19)	0.11 (±0.003)
2.	15	6.27 (±0.14)	0.14(±0.003)
3.	30	6.72 (±0.023)	0.16 (±0.003)
4.	45	7.26 (±0.10)	0.17 (±0.003)
5.	60	7.99 (±0.45)	0.24 (±0.03)

Biomass productivity assays of *Aspergillus niger*

Fresh biomass production revealed a steady increase in growth of *A. niger* from control to 45 mgL^{-1}. A highly significant enhancement in growth was observed in 45 mgL^{-1} concentration in comparison to control (Table 3). The concentration of 45 mgL^{-1} shown 68.19% increase in fungal growth as compared to control set. On the other hand growth declined at 60 mg L^{-1} and was reduced to 4.7% as compared to 45 mgL^{-1}. There was a gradual increase in fungal biomass by increasing the concentration of gibberellic acid from control to 45 mgL^{-1}. The effect was significant in all treatments except between 45 mgL^{-1} and 60 mgL^{-1} (Table 3). Dry biomass revealed a gradual increase in fungal biomass by increasing the concentration of gibberellic acid from control to 45 mgL^{-1} (Table 3). Maximum growth was obtained in 45 mgL^{-1}. Percentage increase in 45 mgL^{-1} as compared to control was 38.09%. Whereas at 60 mgL^{-1} followed by 45 mgL^{-1} concentration, growth declined and 23.80% of loss in fungal biomass was obtained. However the fungal biomass in 60 mgL^{-1} was statistically high in comparison to control. The concentration of 15 mgL^{-1} has shown a slight increase in fungal biomass that was non-significant in comparison to 45 mgL^{-1} (Table 3).

Biomass productivity assays of *Alternaria alternata*

Effect of different concentrations of Gibberellic acid on biomass production of *Alternaria alternata* after seven days of growth is shown in (Table 4). Fresh biomass production revealed enhanced growth at 45 mgL^{-1} concentration. Percentage increase of 75% was observed in comparison to control set. Where as growth 60 mgL^{-1} decrease to 16.3% as compared to 45 mgL^{-1} concentration of the hormone soluion. At the end of growth period there was a gradual increase in fungal biomass productivity in control, 15 and 60 mgL^{-1} concentrations. A sudden increase in growth of 50% was evident in 45 mgL^{-1} concentration of GA and a gradual decline of 33.33% was obtained in 60 mgL^{-1} concentration (Table 4). Incase of dry weight maximum fungal growth was obtained when *A. alternaria* was treated with 45 mgL^{-1} concentration of the hormone solution. Fungal biomass revealed an increase of 77.8% in comparison to control. The GA concentration of 60 mgL^{-1} followed by 45 mgL^{-1} treatment suppressed fungal biomass production by 33.33% (Table 4). Concentrations of 15 and 30 mgL^{-1} have shown an increase of 42% and 55% in fungal biomass.

Table 3: Effect of Gibberellin on *Aspergillus niger*.

Sr. no	Concen-trations (mgL^{-1})	Fresh weights (g)	Dry weights (g)
1.	0	2.29 (±0.05)	0.13 (±0.006)
2.	15	5.53 (±0.13)	0.15 (±0.003)
3.	30	6.38 (±0.15)	0.17 (±0.003)
4.	45	7.37 (±0.45)	0.21 (±0.015)
5.	60	7.02 (±0.13)	0.16 (±0.003)

Table 4: Effect of Gibberellin on *Alternaria alternata*.

Sr. no	Concen-trations (mgL^{-1})	Fresh weights (g)	Dry weights (g)
1.	0	1.87 (±0.31)	0.04 (±0.006)
2.	15	2.98 (±0.03)	0.0 (±0.008)
3.	30	3.80 (±0.41)	0.09 (±0.003)
4.	45	7.53 (±0.43)	0.18 (±0.01)
5.	60	6.30 (±0.31)	0.12 (±0.006)

Discussion

The data obtained in the present study has clearly indicated that dilute concentrations (30 and 45 mgL^{-1}) to be most effective. This trend is in line with the previous investigations conducted in higher plants as well as fungi (Firdaus *et al.*, 1987; Nasim *et al.*, 1995). 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.

Maximum growth was observed in 45gmL^{-1} concentration in all test fungi treated with

hormone solution expect in the case of *Aspergillus terreus*. In this case highest biomass production was observed in 60mgL^{-1} concentrations. The growth in 60mgL^{-1} was highly stimulated by hormone solution and was statistically significant in comparison to control. Lowest value was found in untreated (control) culture disc and it seemed to be least effective. The results are in line with those shown by the earlier researchers. Nasim *et al.,* (1995) in other fungal species due to Indole Acetic Acid (IAA) have reported similar result. In the treated discs of *F. oxysporium* in early stages peak was at 50 mgL^{-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 60mgL^{-1} of hormone solution except *A. terreus* suppressed the fungal biomass productivity in all the test fungal species. In contrast, the lower concentration of 30 and 45 mgL^{-1} of hormone solutions enhanced the fungal biomass production in all the three test species. The concentration of 60mgL^{-1} of hormone solution of gibberellins caused a persistent negative impact on growth of *Aternaria alternata and Aspergillus niger*. The losses in dry biomass were observed 33.3% and 23.8% respectively .The lower concentrations of 30 and 45mgL^{-1} of hormone solution enhanced the fungal biomass productivity by increasing the concentrations of solution. Percentage increase of 75% and 77.8% was observed in fresh and dry biomass when *Alternaria alternata* was treated with Gibberellin. Maximum increased fungal biomass was obtained in this case as compared to other test species. Growth rate comparisons of control and treated fungal cultures in the study conducted by Nasim *et al.* (1995) has also shown that the difference was appreciably significant. Among the treatments comparisons have shown that low concentrations (50, 100 & 150 mgL^{-1}) were more stimulatory as compared to high concentrations (200 & 1000 mgL^{-1}).

Similar stimulatory effects of low concentrations of 2,4,D (Khan *et al.,* 1982), Firdaus-e- Bareen, 1987) have been reported in higher plants, fungal flora also shows its 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 20mgL^{-1} (Nayyer *et al.* 1995) fresh weight in segments of morning glory. Present behavior of test fungi in low concentrations of gibberellins is very similar to higher plants; these results are also in line with previous investigations

As a pilot study this investigation has founded with some basic information's regarding the role of gibberellins 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.

References

Adams PA, Ross MA, 1983. Interaction of Indole acetic acid and gibberellic acid in short term growth kinetics of oat stem segments. *Plant Physiol.,* **73:** 566-568.

Bates S, Preece JF, Navarrete NE, Van Sambeek JW, Gaffrey GR, 1992. Thiadiazuron stimulates shoot organogensis in white ash (*Frexinus americana* L.). *Plant Cell Tiss. Org. Cult.,* **31:** 21-29.

Basalah MO, Mohammad S, 1999. Effect of salinity and plant growth regulators on seed germination of *Medicago sativa. Pak. J. Biol. Sci.,* **2**(3): 651-653.

Contois DE, 1953. Microflora of the rhizosphere of the pineapple plant. *Soil Sci.,* **76:** 259-272.

Del Prado FA, Christensen CM, 1952. Grain storage studies, XII. The fungus flora of stored rice seed. *Cereal Chem.,* **29:** 456-462.

Firdaus B, Iqbal SH, Zain A, 1987. Effect of IAA and VA Mycorrhiza treatment on growth in *Allium sativum* L. *Biologia.,* **34:** 113-122.

Jones RL, 1980. The physiology of gibberellin induced elongation. In: *Plant Growth Substances (1979)* (Skoog, F., ed.). Springer Verleg, Berlin, Heidelberg, New York, pp. 188.

Jones RL, Moll C, 1983. Gibberellin-induced growth in excised lettuce hypocotyles. In: *The Biochemistry and Physiology of Gibberellins* (Crozier, A. ed.). Pregamon Press, New York, pp. 95.

Kapulnik Y, Douds DD Jr, 2002. Arbuscular Mycorrhizas: Physiology and Function. Kluwer, Dortrecht, pp.384.

Khan ZD, Tabassum Z, 1982. Effect of IAA, GA_3 and adenosine on the growth of isolated epicotyl segments of *Zea mays* seedlings. *Biologia.,* **28:** 91-100.

Klick MA, 2000. Identification of common *Aspergillus* species, Centreal bureau voor schimmel culture. The Nether Land, pp. 315-320.

Kurtzman CP, Smiley MJ, Robnett CJ, Wicklow DT, 1996. DNA relatedness among wild and domesticated species of *Aspergillus flavus* group. *Mycologia* **78**(6): 955-959.

Lacey J, 1991. Aerobiology and health: the role of air borne fungal spores in respiratory disease. In: *Frontiers in Mycology* (D.L. Hawksworth, ed.).AB International, Walling ford, United Kingdom, pp. 157-158.

Nasim G, Haider ST, Zahra T, Zahoor R, 1995. Effect of IAA on growth, Biomass and antifungal exudates of *Trichoderma viride*. *Biota*, **1**(1 & 2): 25-40.

Nayyer H, Walia DP, Kaistha BL, 1995. Performance of bread wheat (*Triticum aestibun* L.) under different saline conditions, *Ind. J. Biol. Sci.*, **26**(6): 473-475.

Parasher A, Varma SK, 1998. Effect of wheat (*Triticum aestivum* L.) under different saline conditions. *Ind. J. Biol. Sci.*, **26**(6): 473-475.

Pincus G, Thiamann KV, 1948. The Hormones, New York, Acad. Press Inc. I. Chap. 2 & 3.

Reid JB, Potts WC, 1986. Internode length in *Pisum.* Two further mutants lh and Le with reduced gibberellin synthesis and a gibberellin insensitive mutant lk. *Physiol. Plant.,* **66:** 417-426.

Steel RGD, Torrie JH, 1980. Principles and procedures of statistics. A Biometrical Approach (2nd ed.) Mc. Graw Hill Book Co. Inc. NewYork, U.S.A.

Taiz L, Zeiger E, 1998. Plant Physiology (2nd ed.), Sinaver Associates, Inc. Publishers SunderLand, Massachusetts. **623:** pp.462-560.

Wilkins MB, 1984. The advanced plant physiology, Ditman Publishing Inc. Plain Street, Marsh field, Massachusetts, pp. 110-130.