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CYTOKININ PRIMING AS A TOOL TO INDUCE *IN VITRO* GROWTH AND BIOMASS PRODUCTION OF SOME SOIL FUNGI

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Abstract

Effect of different concentrations of cytokinin on growth of 4 species of soil fungi viz., *Aspergillus oryzae, A. terreus, A. niger* and *Alternaria alternata* was studied. The hormone was applied singly in various concentrations. Increased growth rate and biomass production revealed significant values when treated with dilute solutions of cytokinin at 15, 30 and 45 mgL^{-1}. For all test fungi fresh weights and dry weight values dropped significantly when 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 39.9% increase when treated with 45 mgL^{-1} concentration of hormone solution in comparison to control, whereas an increase of 43.75% was obtained in the case of dry weight. At 60 mgL^{-1}, a significant fresh biomass suppression of 17.9% and 17.64% was observed for *Aspergillus niger* and *A. oryzae*, respectively. The highest loss for dry biomass was noticed in *Alternaria alternata* (18.75%).

Introduction

Cytokinins are known to involve in various processes in the growth and development of higher plants such as cell division, apical dominance, root formation, leaf senescence, stomatal behavior and chloroplast development (Davies 1995; Brault & Maldney, 1999). Studies have also suggested that cytokinins modulate developmental processes in plants under stressful environment through interacting with other plant hormones and environmental signals (Brault & Maldney, 1999; Rashotte *et al*., 2005). cytokinins have been reported as antagonists of ABA (Drüge & Schönbeck 1992), while they work synergistically with auxins to enhance several physiological processes in plants (Brault & Maldney 1999; Swarup *et al*., 2002; Nordström *et al*., 2004). As priming agents plant growth regulators have been employed to initiate positive growth reponses in a number of species (Gadallah 1999; Debez *et al*., 2001; Iqbal & Ashraf 2005a). Seed priming with cytokins have been reported to have beneficial effects on wheat under salt stress (Gadallah 1999; Angrish *et al*., 2001; Iqbal & Ashraf 2005b; Iqbal *et al*., 2006).

However the exogenous application of cytokinin to stimulate the growth and sporulation of the microbes has only been reported scarcely in the literature (Nasim *et al*., 1995; Nasim *et al*., 2004a & b). Fungi have many traditional roles in biotechnology. They have been assigned some novel role and hence there is major scope for their commercial development (Wainwright, 1992). The present study was, therefore designed with a primary objective to evaluate the effect of exogenous application of cytokinins on the growth of four fungal species viz., *Aspergillus oryzae, A. terreus, A. niger* and *Alternaria alternata*.

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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 of kinetin 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 Petri plates 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 Petri plates containing filter papers moistened with 10ml of various concentrations of the cytokinin 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 & 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 cytokinin (Fig. 1). Fresh biomass production revealed an increase in growth along the concentration gradient of hormone solution of cytokinin from 0 to 45 mgL^{-1}. Maximum growth was induced by 45 mgL^{-1} conccentration causing an increase in the fresh biomass production of *A. oryzae* (Fig. 1). Percentage increase of 25% 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 17.64% 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 cytokinin were found to be least effective for fungal growth stimulation (Fig. 1). Maximum enhancement in growth was induced by 45 mgL^{-1} concentration of cytokinin. Percentage increase of up to 49% was obtained in 45 mgL^{-1} concentrations as compared to control. The concentration of 60 mgL^{-1} seemed to be less effective and had a percentage loss of 17.64% (Fig. 1).

Biomass productivity assays of *Aspergillus terreus*: The data on fresh biomass production revealed an increase in growth of *Aspergillus terreus* uptill 45 mgL^{-1} followed by a slight decline at 60 mgL^{-1}.concentration. Maximum growth was obtained at 45 mgL^{-1} concentration showing percentage increase of 38.46% and was statistically highly significant in comparison to control. Fig. 2 shows a gradual increase along the increasing concentrations of hormone solution except for 60 mgL^{-1}. The data for dry weight produced by *Aspergillus terreus* followed the same general pattern. The production of dry weight was markedly high, in 45 mgL^{-1} concentrations. A very sharp stimulus in growth, which was

significantly higher than control was recorded in this treatment. The 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 cytokinin. 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 45 mgL^{-1} (Fig. 1).

Biomass productivity assays of *Aspergillus niger*: Fresh biomass production by *Aspergillus niger* revealed a rather erratic pattern of growth 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 (Fig. 3). The concentration of 45 mgL^{-1} showed 17.9% increase in fungal growth as compared to control set. A statistically significant depression in growth was evidenced at higher concentration viz., 60 mg L^{-1} in comparison to control and 45 mg L^{-1} in case of both dry and fresh weight. Fresh biomass yield of *A. niger* treated with 45mg L^{-1} concentration of Kinetin showed 17.9% increase whereas at 60mgL^{-1} resulted in a percentage decrease of 6% as compared to 45mg L^{-1}.

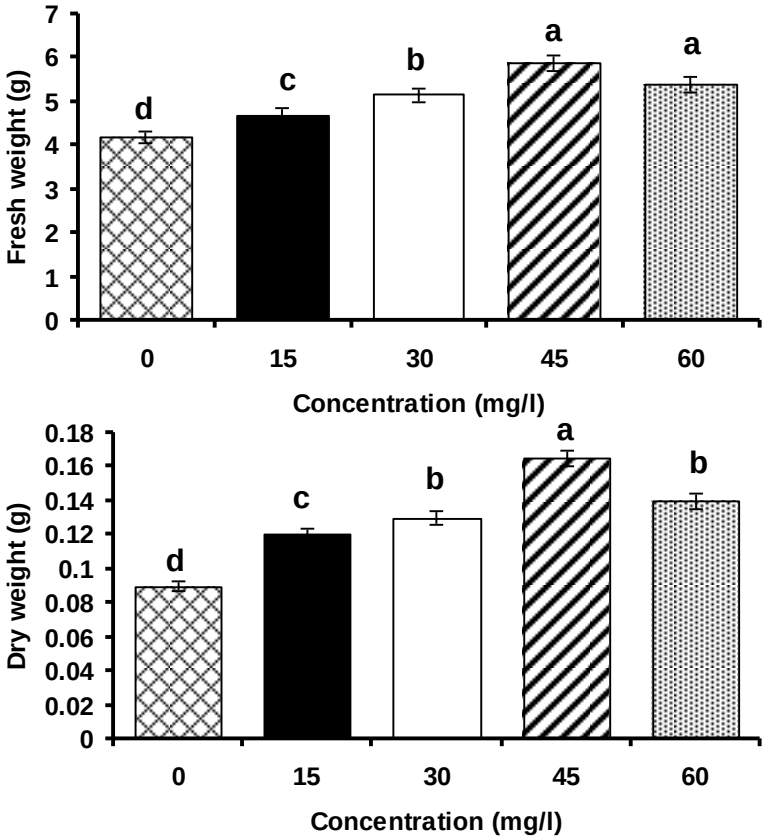

Fig. 1. Effect of Kinetin on fresh (A) and dry (B) weight of *Aspergillus oryzae.*
Line on data bars show SE of the mean while data bars with different letters show significant difference (p=0.05) as determined by DMRT.

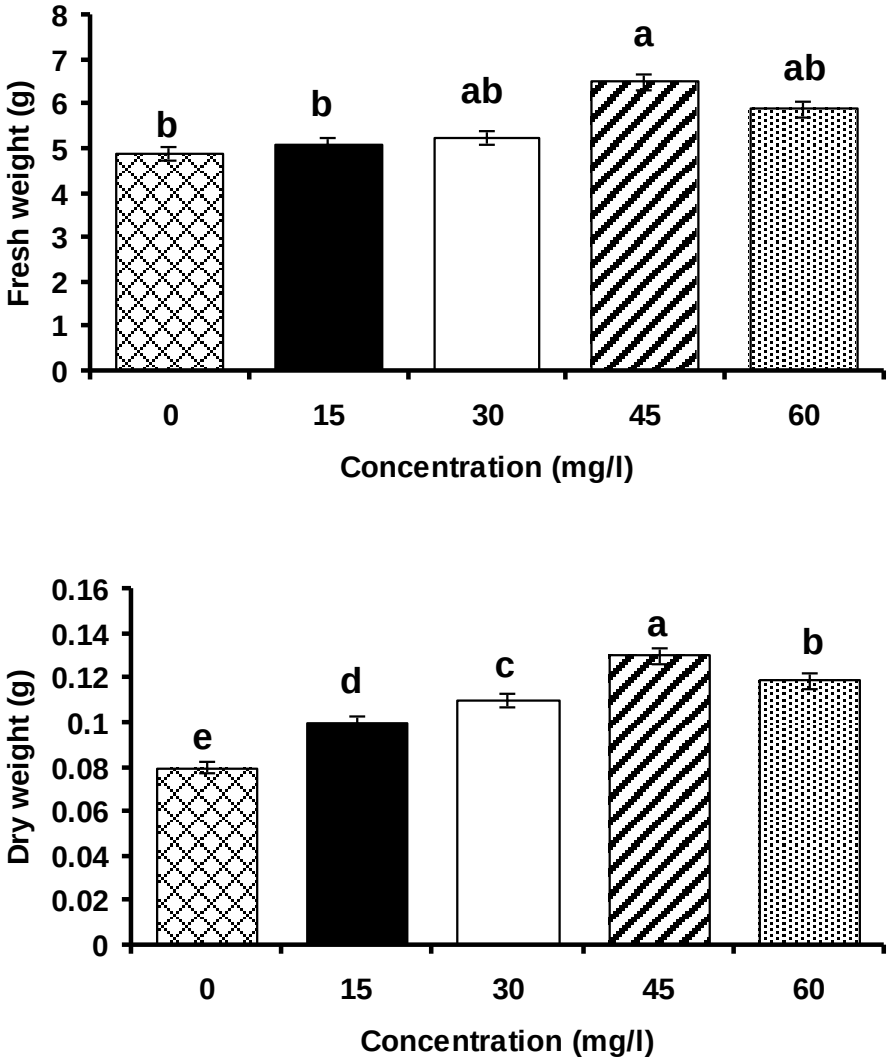

Fig. 2. Effect of Kinetin on fresh (A) and dry (B) weight of *Aspergillus terreus*.
Line on data bars show SE of the mean while data bars with different letters show significant difference (p=0.05) as determined by DMRT.

The dry biomass observed in 45mg L^{-1} concentration was significantly high as compared to control. The growth enhancement in terms of percentage increase in 45mg L^{-1} in comparison to control was 48%.

In case of dry mass the same trend in the results was observed. The biomass increased steadily from 0 to 45mg L^{-1}. The dry biomass values dropped at 65mg L^{-1} but the values were greater than those in remaining concentrations.

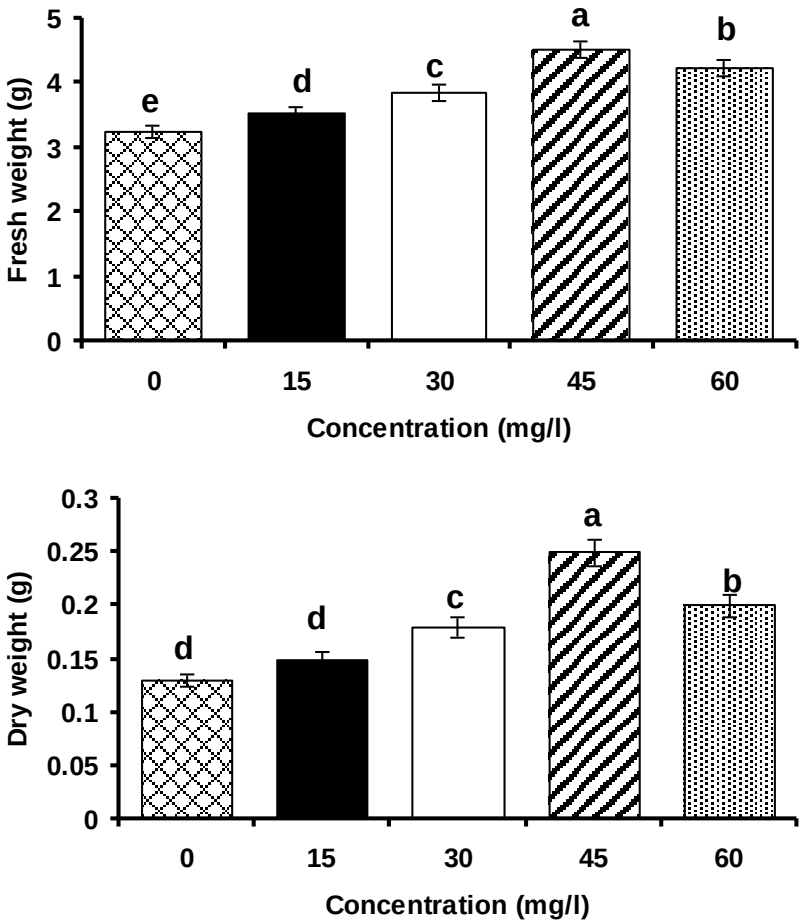

Fig. 3. Effect of Kinetin on fresh (A) and dry (B) weight of *Aspergillus niger*.
Line on data bars show SE of the mean while data bars with different letters show significant difference (p=0.05) as determined by DMRT.

Biomass productivity assays of *Alternaria alternate*: Effect of different concentrations of cytokinin on biomass production of *Alternaria alternata* after seven days of growth is shown in Fig. 4A-B. Biomass assay clearly reveals that this species displayed a significant pattern of growth in response to hormone solution. Fresh biomass production was enhanced at 45 mgL^{-1}concentration as compared to control. Percentage increase of 39.9% was observed at 45mgL^{-1} in comparison to control set whereas growth at 60 mgL^{-1} decreased to 11% as compared to 45 mgL^{-1} concentration of the hormone solution. Maximum growth was induced by 45mgL^{-1}causing an increase in the fresh and dry mass production of *Altenaria alternata*. Growth in 45mgL^{-1} concentrations increased to 43.75% from control. As compared to control the growth enhancement recorded in 15 and 30mgL^{-1} was 34.37 and 28.12 respectively. Whereas a negative response in 60mgL^{-1} was evident in the results. The percentage loss in dry mass at 60mgL^{-1} in comparison to that in 45mgL^{-1} was 18.75%. The effect of 15 and 30mgL^{-1} is statistically significant. The dry mass in 30mgL^{-1} concentrations has 8.69% enhancement over 15mgL^{-1}.

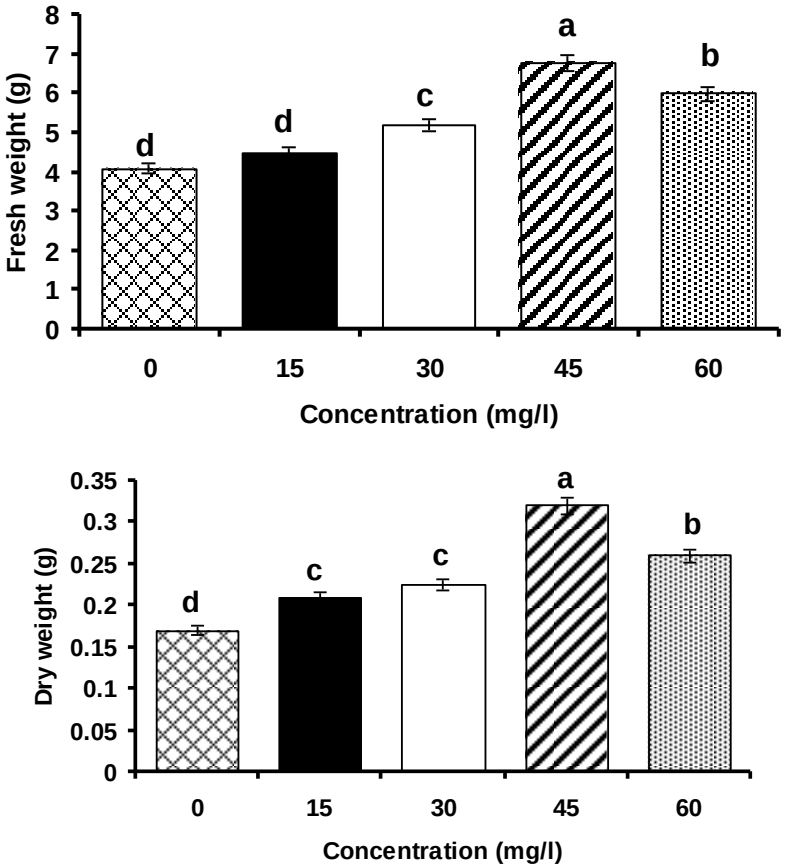

Fig. 4. Effect of Kinetin on fresh (A) and dry (B) weight of *Alternaria alternata*.
Line on data bars show SE of the mean while data bars with different letters show significant difference (p=0.05) as determined by DMRT.

Discussion

The data obtained in the present study has clearly indicated that dilute concentrations (60 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; Nasim *et al.,* 2004a & b). 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 $45gmL^{-1}$ concentrations in all test fungi treated with hormone solution. The growth in $45mgL^{-1}$ 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) & Nasim *et al.,* (2004a & b) 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 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 cytokinin caused a persistent negative impact on growth of all test fungi. The losses in fresh and dry biomass observed were 17.9% and 17.64% respectively. The lower concentrations of 30 and 45mgL^{-1} of hormone solution enhanced the fungal biomass productivity. Percentage increase of 39.9% and 43.75% was observed in fresh and dry biomass when treated with cytokinin. Maximum increased fungal biomass was obtained in *Alternaria alternata* as compared to other test species. Growth rate comparisons of control and treated fungal cultures in the study conducted by Nasim *et al*., (1995) and Nasim *et al*., (2004a & b) 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 & Tabassam 1982), Firdaus-e-Bareen *et al*., 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 cytokinin showed highest percent germination when treated with 20mgL^{-1} (Nayyer *et al*., 1995). Present behavior of test fungi in low concentrations of cytokinin 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 regarding the role of cytokinin on fungal growth. It has also helped us in refining the techniques. This investigation may successfully be extended into 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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