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Effect of Urea on the Production of Chitinase by *Trichoderma Viride*

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ABSTRACT

Trichoderma spp. is proved to be an efficient bio-control agent. It is one of the most frequently studied genera in relation to biological control of plant diseases. The bio-control activity of *Trichoderma* spp depends on the production of large number of hydrolytic enzymes. *Trichoderma* spp. produces variety of antimicrobial substances or biologically active substances those are inhibitory to plant pathogens. *Trichoderma* is one of the groups of beneficial fungi, that have been shown to act, and are commercially applied as biocontrol agents against fungal pathogens. *Trichoderma* Biocontrol agents can even exert positive effects on plants with an increase in plant growth and also in the stimulation of plant defense mechanism. Urea did not show any negative impact on the growth of *Trichoderma viride*. *Trichoderma viride* cannot be applied to crops along with fungicides or insecticides. While applying chemical fertilizers, care maybe taken by avoiding the place and time of application of both.

KEYWORDS: *Trichoderma viride, urea, chitinase assay*

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INTRODUCTION

Trichoderma spp. is proved to be an efficient bio-control agent. It is one of the most frequently studied genera in relation to biological control of plant diseases. The bio-control activity of *Trichoderma* spp depends on the production of large number of hydrolytic enzymes. *Trichoderma* spp. produces variety of antimicrobial substances or biologically active substances those are inhibitory to plant pathogens. *Trichoderma* is one of the groups of beneficial fungi, that have been shown to act, and are commercially applied as biocontrol agents against fungal pathogens. *Trichoderma* Biocontrol agents can even exert positive effects on plants with an increase in plant growth and also in the stimulation of plant defense mechanism. Urea did not show any negative impact on the growth of *Trichoderma viride*. *Trichoderma viride* cannot be applied to crops along with fungicides or insecticides. While applying chemical fertilizers, care maybe taken by avoiding the place and time of application of both.

METHODOLOGY

Isolation and identification of *Trichoderma*

Trichoderma spp. was isolated from soil samples collected from Thonnakkal area. The soil suspension was serially diluted to 10^{-6} dilution and was spread plated on potato dextrose agar (PDA) plate and incubated at room temperature for 5-6 days. The isolates were studied based on their morphological characteristics and microscopical structure by lactophenol cotton blue staining.

Effect of urea on the growth of *Trichoderma spp*

The effect of urea on *Trichoderma* was identified by the growth and sporulation of *Trichoderma* after the application of urea. The growth radius of the fungus was noted at specific intervals of 5 days and 10 days.

Chitinase Assay after addition of urea

The chitin flakes were grounded to powder and added slowly to 10N HCl and kept overnight at 4^0C. The suspension was then added to cold 50% ethanol with rapid stirring and kept overnight at 25^0C. The precipitation was collected by centrifugation at 7000 rpm for 20 minutes and washed with sterile distilled water until the colloidal chitin became neutral (pH 7). It was then freeze dried to powder form. 2ml of 1% chitin in potassium phosphate buffer (pH 6) and 0.5ml of enzyme extract was incubated at 25^0C for 2hrs with shaking. After incubation it was kept in boiling water bath for 2 minutes. The filtrate was collected by centrifugation and add 1ml of DNS, boiled for 5 minutes. Graph was plotted with concentration of glucose along X-axis and OD along Y-axis.

RESULTS

Isolation and identification of *Trichoderma*:

The culture was identified as *Trichoderma viride* by its dark green, smooth, fast growing and smooth mycelium, separated with branched chlamydospores that occurs as intercalary globose rarely ellipsoidal. Conidiospores arise in

compact loose tuft, main branches produced several side branches in groups of 2-3 and all branches stand at wide angles.

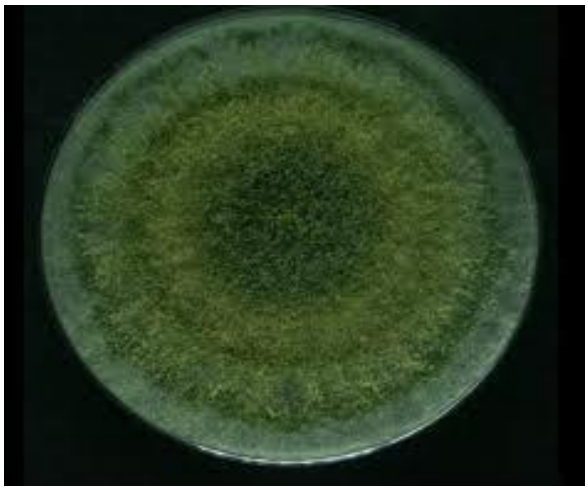

Fig:1

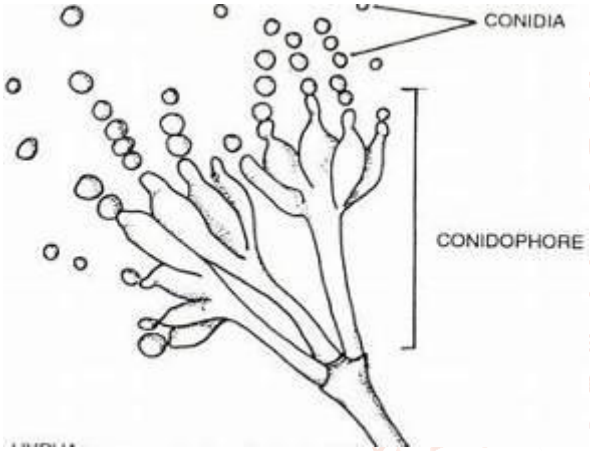

Fig:2

Fig:1- Growth of *Trichoderma viridae* on the plates.
Fig:2- *Trichoderma viride*

The lactophenol cotton blue (LPCB) preparation has three components: phenol, which will kill any live organisms; lactic acid which preserves fungal structures, and cotton blue which stains the chitin in the fungal cell walls.

Fig:3

Fig:3- Stained *Trichoderma viride* using LPCB

Effect of urea on the growth of *Trichoderma spp*

Without addition of urea	After 5 days	After 10 days
3.8 cm	4.25 cm	4.75 cm

From the results it can be observed that addition of urea increased the growth and biocontrol activity of *Trichoderma* after 5 days, which decreased after 10 days.

Chitinase production

Sample	Enzyme value µg/ml	
	5 days	10 days
Without Urea	210	210
With Urea	215	220

From the results it can be observed that addition of Urea altered the chitinase production efficiency of *Trichoderma.*

CONCLUSION

In the current study the effect of chemical fertilizer in biocontrol activity and enzyme secretion was determined. The result suggests that potent application of urea can effect adversely the biocontrol activity of *Trichoderma viride.* The biocontrol efficiency of *Trichoderma* was observed to be decreased with time in presence of fertilizer. The study also draws new insights regarding the indiscriminative use of chemicals along with biocontrol agents.

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