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E: ISSN NO.: 2455-0817 Remarking An Analisation

Effect of Different Carbon Sources on The Antifungal Efficacy of Trichoderma Species against Macrophomina

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Abstract

Trichoderma is an effective biocontrol agent against *Macrophomina phaseolina* causing dry rrot rot in blackgram. But their growth and antifungal activity depends the nutrient sources they utilize. In the present study different carbon sources were used as nutrient source to investigate their effect on the growth of *Trichoderma* strains as well as to study their efficacy against *Macrophomina phaseolina.* Glucose*, sucrose, cellulose chitin and laminarin were used as carbon source. Trichoderma harzianum 1 T. harzianum-1* possessed maximum growth with all the carbon sources used in the study in comparison to other strains. Cellulose was next preferred carbon source for maximum radial growth of *T. harzianum-1* (8.2 cm) while glucose followed by laminarin was preferred by *T. atroviride* (7.4 cm and 7.2 cm, respectively). Maximum per cent inhibition of *M. phaseolina* by all selected *Trichoderma* strains occurred when glucose was used as sole carbon source in medium. Cellulose was the next preferred carbon source for maximum per cent inhibition (79.4%) of *M. phaseolina* by *T. harzianum-1*while glucose followed by laminarin was preferred by *T. atroviride* for maximum per cent inhibition (82.6 and 77.6%, respectively). When chitin was used as sole carbon source, it had no marked effect on per cent inhibition of *M. phaseolina* when grown with *T. atroviride* and *T. viride*.

Keywords: *Trichoderma*, Glucose, Laminarin.

Introduction

Macrophomina phaseolina is a soil borne fungus. It has a worldwide distribution and is more competitive for nutrients than most other soil microorganisms during hot and dry periods. There have been efforts to control it since long but its extreme prevalence, wide host range and extreme environmental adaptability has forfeited all the attempts. However, efforts are now made to control this pathogen *M. phaseolina* through biological control. *Trichoderma* species are well known biocontrol agent. Mycoparasitism and antibiosis are the common mechanism employed by *Trichoderma* strain against the pathogen. The nutrient present in the rhizosphere play an important role in antifungal activity possessed by *Trichoderma*. In the present investigation different carbon sources were used to access their effect on antifungal potential of *Trichoderma* strain.

Review of literature

Tropical crop plants are seriously affected by *Macrophomina phaseolina* (Malaguti, 1990). Biological control is an attractive approach to control this pathogen. The genus of *Trichoderma* is widely applied for the biocontrol of phytopathogenic fungi in agriculture sector (Khalid et al., 2017). *Trichoderma* spp. are free-living soilborne fungi that are highly interactive in root, soil and foliar environments and have ability to produce a wide range of antibiotic substances as well as parasitized on several soil borne phytopathogenic fungi (Skaptsov et al, 2018). The fungus is also a decomposer of cellulosic waste materials.. The biocontrol activity involving mycoparasitism, antibiotics and competition for nutrients, also induces defence responses or systemic resistance responses in plants. Trichoderma have been shown to act, and are commercially applied as biological control agents against fungal pathogens (Naher et al, 2014).

Competition is one of the mechanisms of biological control activity of *Trichoderma* spp. against phytopathogenic fungi. *Trichoderma* species generally considered to be aggressive competitors (Samuels, G.J, 1996). Starvation is the most common cause of death for microorganisms, so that competition for limiting nutrients results in biological control of fungal phytopathogens (Chet I *et al* 1997).

Material and Methods

Effect on radial colony growth of the selected *Trichoderma* strains

10 ml of M9 medium amended with different carbon and nitrogen sources were taken in 90 mm diameter Petri plates in triplicate set and were allowed to solidify. The Petri plates containing solidified medium were centrally inoculated with 5 mm blocks from actively growing cultures of the selected *Trichoderma* strains. The plates were incubated for 6 days at 25±2^{0}C. The radial growth of the colony was measured after 6 days of incubation.

Effect of different carbon sources on the antagonistic capability of *Trichoderma Strains*

Assay of influence of different carbon and nitrogen sources on possible antagonistic capability was done by dual culture plate method.

For studying effect of different carbon sources on the Production of enzymes, glucose, laminarin, chitin cellulose and sucrose @ 20g/l were used.

10 ml of PDA amended with different carbon and nitrogen sources were taken in 90mm diameter petriplates. 5mm agar block of freshly grown *M. phaseolina* and 5mm agar block of *Trichoderma* spp .cut from the margin of pure culture and were placed 3cm apart from each other on PDA medium containing Petri plates and incubated at 25^{0}C for 5 days. % inhibition of radial growth was assayed by using the following formula (Fokkema1976).

$$R_1\text{-}R_2/R_1\times100$$

R_1 denotes diameter of the radial growth of the pathogen toward the opponent antagonist.

Observation:

Table1. Effect of different carbon sources on the radial colony growth of selected strains of *Trichoderma*

***Trichoderma* strains**	**Radial colony growth (cm)** * **C-sources**				
	Glucose	Sucrose	Cellulose	Chitin	Laminarin
T. virens-1	$6.8^{a} \pm 0.75$*	$5.2^{a} \pm 0.15$	$6.4^{bc**} \pm 0.62$	$3.7^{ab} \pm 0.68$	$4.3^{a} \pm 0.80$
T. harzianum-1	$8.9^{a} \pm 0.26$	$6.5^{ab} \pm 0.40$	$8.2^{c} \pm 0.75$	$5.1^{b} \pm 0.51$	$6.5^{ab} \pm 0.87$
T. harzianum-2	$7.2^{a} \pm 0.36$	$5.8^{ab} \pm 0.47$	$6.9^{bc} \pm 0.95$	$4.5^{a} \pm 0.3$	$5.6^{ab} \pm 0.42$
T. pseudokoningii	$6.2^{a} \pm 0.92$	$5.4^{ab} \pm 0.36$	$3.8^{a} \pm 0.87$	$3.2^{ab} \pm 0.25$	$5.9^{ab} \pm 0.52$
T. atroviride	$7.4^{a} \pm 0.61$	$6.7^{b} \pm 0.56$	$5.1^{ab} \pm 0.15$	$4.9^{ab} \pm 0.20$	$7.2^{b} \pm 0.61$
T. viride	$7.0^{a} \pm 0.52$	$6.1^{ab} \pm 0.20$	$4.2^{a} \pm 0.30$	$3.9^{ab} \pm 0.90$	$6.3^{ab} \pm 0.70$

*Average of three replicates; ± SEM

Table 2. Effect of different carbon sources on the antagonistic capability of some selected *Trichoderma* strains

Trichodema strains	**Inhibition (%)***				
	Glucose	**Sucrose**	**Chitin**	**Cellulose**	**Laminarin**
T. virens-1	$70.8^{b} \pm 0.45$	$45.8^{b} \pm 0.17$	$67.9^{c} \pm 0.32$	$52.3^{b} \pm 0.24$	$46.8^{a} \pm 0.36$
T. harzianum-1	$87.9^{d} \pm 0.37$	$63.5^{d} \pm 0.20$	$81.4^{d} \pm 0.27$	$79.4^{e} \pm 0.23$	$64.4^{b} \pm 0.19$
T. harzianum-2	$77.2^{bc} \pm 0.23$	$52.8^{bc} \pm 0.12$	$70.1^{c} \pm 0.17$	$64.8^{d} \pm 0.22$	$54.4^{a} \pm 0.23$
T. pseudokoningii	$61.1^{a} \pm 0.19$	$36.9^{a} \pm 0.11$	$7.9^{a} \pm 0.03$	$42.2^{a} \pm 0.19$	$62.4^{bc} \pm 0.16$
T. atroviride	$82.6^{d} \pm 0.24$	$58.1^{cd} \pm 0.31$	$29.2^{b} \pm 0.14$	$64.2^{cd} \pm 0.17$	$77.6^{d} \pm 0.22$
T. viride	$74.4^{bc} \pm 0.21$	$36.2^{ab} \pm 0.29$	$28.4^{b} \pm 0.12$	$54.1^{bc} \pm 0.15$	$68.9^{c} \pm 0.21$

*Average of three replicates; ± SEM

Result

Effect of five different carbon sources namely glucose, sucrose, cellulose, chitin, and laminarin on the radial colony growth of *Trichoderma* strains were studied (Table 1).. It is evident from table 1 that carbon sources had noticeable effect on the colony diameter of *Trichoderma* strains. Out of 5 carbon sources, the growth of *Trichoderma strains* was more restricted when chitin was used as sole carbon source except *T. harzianum-1*, whereas maximum growth was found when glucose was used as sole carbon source. Result showed that *T. harzianum-1*possessed maximum growth with all the carbon sources used in the study in comparison to other strains. Cellulose was next preferred carbon source for maximum radial growth of *T. harzianum-1* (8.2 cm) while glucose followed by laminarin was preferred by *T. atroviride* (7.4 cm and 7.2 cm, respectively).

Table 2 showed that different carbon sources also had noticeable effect on the antagonistic capability of *Trichoderma.* Result depicted that maximum per cent inhibition of *M. phaseolina* by all selected *Trichoderma* strains occurred when glucose was used as sole carbon source in medium. Cellulose was the next preferred carbon source for maximum per cent inhibition (79.4%) of *M. phaseolina* by *T. harzianum-1*while glucose followed by laminarin was preferred by *T. atroviride* for maximum per cent inhibition (82.6 and 77.6%, respectively). When chitin was used as sole carbon source, it had no marked effect on per cent inhibition of *M. phaseolina* when grown with *T. atroviride* and *T. viride*. Other strains also showed inhibition of *M. phaseolina* with different carbon sources but not so vigoursly as *T. harzianum-1*or *T. atroviride*

Discussion

Mechanisms which contribute to the successful colonization of the rhizosphere by certain strains could involve utilization of energy and nitrogen sources present in root exudates (Arotupin *et al*., 2011**,** Grayston *et al*., 1996). Plant root exudates are a major source of carbon and energy for microorganisms. Other carbon sources are cells detached from old parts of the root, or the root itself after plant death (Cook and Baker, 1983). Competition for nutrients primarily carbon, nitrogen and iron may results in biological control of soil-borne plant pathogens (Upadhyay *et al*., 2003). The strains which are able to utilize these nutrients more efficiently than the indigenous rhizosphere microflora may have an ecological advantage.

Trichoderma has a superior capacity to mobilize and take up soil nutrients compared to other organisms. The efficient use of available nutrients is based on the ability of *Trichoderma* to obtain ATP from the metabolism of different sugars, such as those derived from polymers wide-spread in fungal environments: cellulose, glucan and chitin among others, all of them rendering glucose (Chet I *et al*,1997).

Papavizas (1985) has stated that different species of *Trichoderma* have their own ecological preferences.The growth and sporulation of *Trichoderma* was greatly influenced by various carbon and nitrogen sources as they show ability to utilize a variety of nutritional factors.

In the present investigation effect of different carbon and nitrogen sources on the radial growth, antagonistic capability of *Trichoderma strains* were studied.

It is evident from Table 1 and 2 that *T. harzianum-1* preffered glucose followed by cellulose as sole carbon source for maximum radial growth as well as for maximum antagonistic capability. Whereas in case of *T atroviride*, glucose followed by laminarin was preferred for maximum radial growth as well as for maximum antagonistic capability.

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