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EFFICACY OF *TRICHODERMA HARZIANUM* AFTER MUTIPLICATION ON DIFFERENT SUBSTRATES IN THE CONTROL OF ROOT ROT FUNGI

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ABSTRACT

Population of *T.harzianum* after multiplication on different substrates viz., rice grain, sorghum grain, millet grain and saw dust was determined at 0 day and after 15, 30, 60,90 and 180 days of storage. Growth of *T.harzinum* increased with the increase in time on sorghum grain, rice and millet grains, while saw dust was not suitable substrate for the multiplication of *T. harzianum*. The inoculum multiplied and stored in plastic bags remained viable for up to 180 days at room temperature. Soil amendment with *T. harzianum* after multiplication on rice, millet and sorghum showed significant increase in height and weight of mash bean and chick pea plants, and significantly reduced the infection of *Macrophomina phaseolina, Rhizoctonia solani* and *Fusarium* spp. at different storage intervals on chick pea and mash bean. *T.harzianum* after multiplication on sorghum grain reduced the infection of *M.phaseolina* whereas inoculum on rice grain reduced the infection of *Fusarium* spp. *R.solani* infection was completely inhibited by *T.harzianum* multiplied on rice, sorghum and millet after 15, 30, 60 and 90 days of storage.

Key Words: Biological control, *Trichoderma harzianum*, root infecting fungi, shelf life, mashbean, chickpea

INTRODUCTION

Of the plant diseases causing organisms, the soil borne pathogens like fungi, bacteria and nematodes infect plant roots with the result that plant is unable to absorb nutrients from soil and often results in death of the crop plant. The genus *Fusarium* contains a number of species, which have been recognized for a long time as being important plant pathogens (Booth, 1971; Nelson *et al*., 1983). An average yield loss of 2.2 ha in pea was observed in Ontario due to root rot diseases caused by *F.solani* and *F.oxysporum*, with complete loss in many cases. (Tu, 1987). Similarly, *M.phaseolina* causes seedling blight charcoal rot, root rot, stem rot, pod rot on more then 500 plants (Dhingra and Sinclair, 1978). *R.solani* caused seed rot, damping off of seedling, wilt and root rot of over 2000 plant species (Parmeter, 1970). Due to the cost of chemicals and their hazardous effect, use of microorganism in the control of root rot fungi is an other alternate method (Lumsden and Locke, 1989). Several fungi and bacteria have received considerable attention in the control of soil borne root-infecting fungi. Like *M.phaseolina*, Rhizoctonia solani and *Fusarium* spp and root rot nematodes (Ghaffar, 1978, 1988 and 1992). Experiments were therefor carried out for the mass multiplication of *T.harzianum* and its efficacy in the control of root rot fungi. *T.harzianum* protects the root system against *F.solani, R.solani* and *M.phaseolina* infection on a number of crops. (Malik and Dawar, 2003).

MATERIALS AND METHODS

Biological antagonist: *Trichoderma harzianum*, used as antagonistic agent for the control of root infecting fungi, was obtained from Karachi University culture collection (KUCC 115).

In vitro experiment

Multiplication of *Trichoderma harzianum*

Four different organic substrates were used for the growth of *T.harzianum:*. rice grain, sorghum grain, millet grain and saw dust, which have the different composition of nutrition, utilized by *T.harzianum.* The organic substrates were soaked in water containers for 15 min., subsequently dried and transferred in polyethylene bags. The bags were sealed and then sterilized in an autoclaved by injecting with a syringe a conidial suspension of *T.harzianum* at 2ml/100g substrate. The inoculated substrates were stored at room temperature for 6 months and their shelf life observed at 0 and after 15, 30, 60, 90 and 180 days intervals. Population of *T.harzianum* on different substrates 0 and after 15, 30, 60, 90 and 180 days was observed by the use of counter chamber, or haemocytometer.

In vivo experiment

Soil used for experiment

Non-sterilized sandy-loam soil (Sand: Silt: Clay, 70:19:11) of pH 8.1 with moisture-holding capacity of 40% and 0.6% organic matter was obtained from the experimental field of the Department of Botany, University of

Karachi and used to fill plastic pots (8cm diam.) at 250gm per pot. The soil had a natural population of 3-9 sclerotia of *M.phaseolina* /gm soil as determined by a wet sieving method (Sheikh and Ghaffar, 1975); 6% of *R.solani* (Wilhelm, 1955) and *Fusarium* spp 3000 cfu/gm of soil, as assessed by a soil dilution technique (Nash and Snyder, 1962).

Efficacy of *T.harzianum* inoculum on different substrates at different storage time

Efficacy of *T.harzianum* after multiplication on different substrates viz rice grain, sorghum grain, millet grain and saw dust was tested at 0 day and after 15, 30, 60 and 90 days, soil amendments with *T.harzianum* on different substrates was carried out @ 1% w/w, non treated soil served as control Each treatment was replicated four times and the pots were kept in a randomized complete block design on the greenhouse bench where soil was adjusted to 50% moisture-holding capacity. Mash bean and chickpea are used as test plants. The plants were uprooted after 30 days of growth. Plant height and fresh weight of the shoot was recorded, to determine the infection of root-infecting fungi, the roots were washed in running tap water, surface- sterilized in 1% $Ca(OCl)_2$ and five, 1cm long root pieces were inoculated on to PDA plates containing penicillin (100 000 units/liter) and Streptomycine (0.2 g/liter). The plates were incubated at room temperature and the incidence of root-infecting fungi was recorded.

Analysis of data

Data were subjected to analysis of variance (ANOVA) or factorial analysis of variance (FANOVA) using STATISTICA software. Following ANOVA or FANOVA, a least significant difference (LSD) was calculated in order separate the treatment means (Gomez and Gomez, 1984).

Table 1. Population of *T.harzianum* multiplied on different substrates stored at room temperature, for 180 days storage Time.

Days **Substrates**	**0** **cfu/gm**	**15** **cfu/gm**	**30** **cfu/gm**	**60** **cfu/gm**	**90** **cfu/gm**	**180** **cfu/gm**
Rice grains	2×10^6	3×10^7	10^8	8×10^8	1.6×10^8	6×10^8
Sorghum grains	7.3×10^6	3.6×10^7	6×10^7	4.6×10^8	8×10^8	7×10^8
Millet grains	3.1×10^6	3×10^7	10^8	4.4×10^8	1.66×10^8	1.5×10^9
Saw dust	2×10^6	2.4×10^7	6.4×10^7	2.2×10^7	1.6×10^7	1×10^7

RESULTS AND DISCUSION

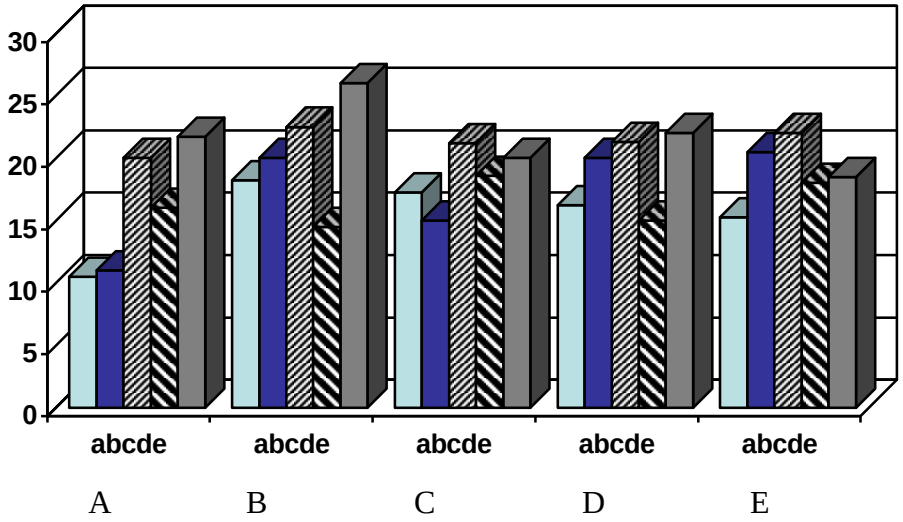
Papulation of *T.harzianum* on different substrates

Trichoderma harzianum @ 10^7 conidia / ml was inoculated on rice grain, sorghum grain, millet grain and saw dust. Growth of *T.harzianum* was good on all four substrates. However, *T.harzianum* exhibited variable growth pattern on various substrates. Population of biocontrol agent on sorghum rice and millet grain increased with the increase in time, while it declines steadily on saw dust (Table-1). Millet grain supported highest population of *T.harzianum* through out the incubation period followed by sorghum grain, rice grain and saw dust. (Table 1).

Efficacy of *T.harzianum* after multiplication on different substrates at different storage time

Efficacy of *T.harzianum* after multiplication on different substrates like rice grain, sorghum grain millet grain and saw dust was tested at different intervals 0, 15, 30, 60 and 90 days. Soil amendment with *T.harzianum* after multiplication on different substrates at different intervals showed that *T.harzianum* inoculum on millet, sorghum and rice grain significantly increased ($p<0.01$) the height of chick pea plant as compared to control. (Fig. **1**). Similarly *T.harzianum* inoculum on various growth substrates showed a significant ($p<0.001$) effect on height of mash bean plant (Fig. **1**).

Plant Height of Chickpea LSD0.05=6.09.



Plant Height of Mashbean LSD0.05=0.57

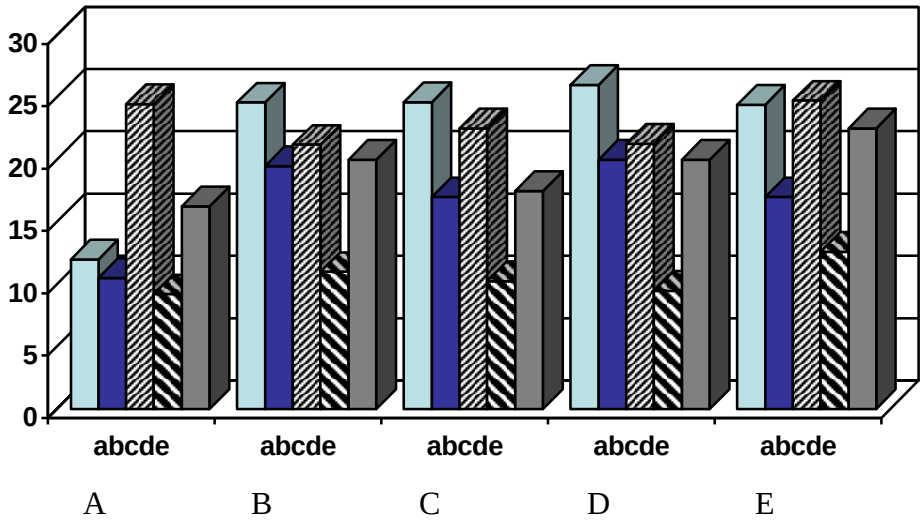


Fig. 1. Effect of *Trichoderma harzianum* inoculum multiplied on different substrates and stored for different intervals and check the growth of plant Chickpea and Mashbean.
A= Control, **B**=Rice, **C**=Millet**, D**=Sorghum, **E**=Sawdust.
a=0day, **b**=15days, **c**= 30days, **d**= 60, **e**=90days.

Significant reduction in *Fusarium solani* infection was observed in chickpea plant, when soil was amended with *T.harzianum* inoculum after 90 days of storage (Fig. **2**). Significant ($p<0.001$) reduction in *M.phaseolina* infection was observed in chickpea plant when *T.harzianum* inoculum on, millet grain was used after 30, 60 and 90 days for amendment of soil, (Fig. **2**). *T.harzianum* inoculum on all substrate showed significant ($p<0.001$) suppression of *Rhizoctonia solani* infection as compared to control chick pea plants. (Fig. **2**). Inoculum on millet grain after 15, 30, 60 & 90 days storage completely controlled the *R.solani* infection (Fig. **2**).

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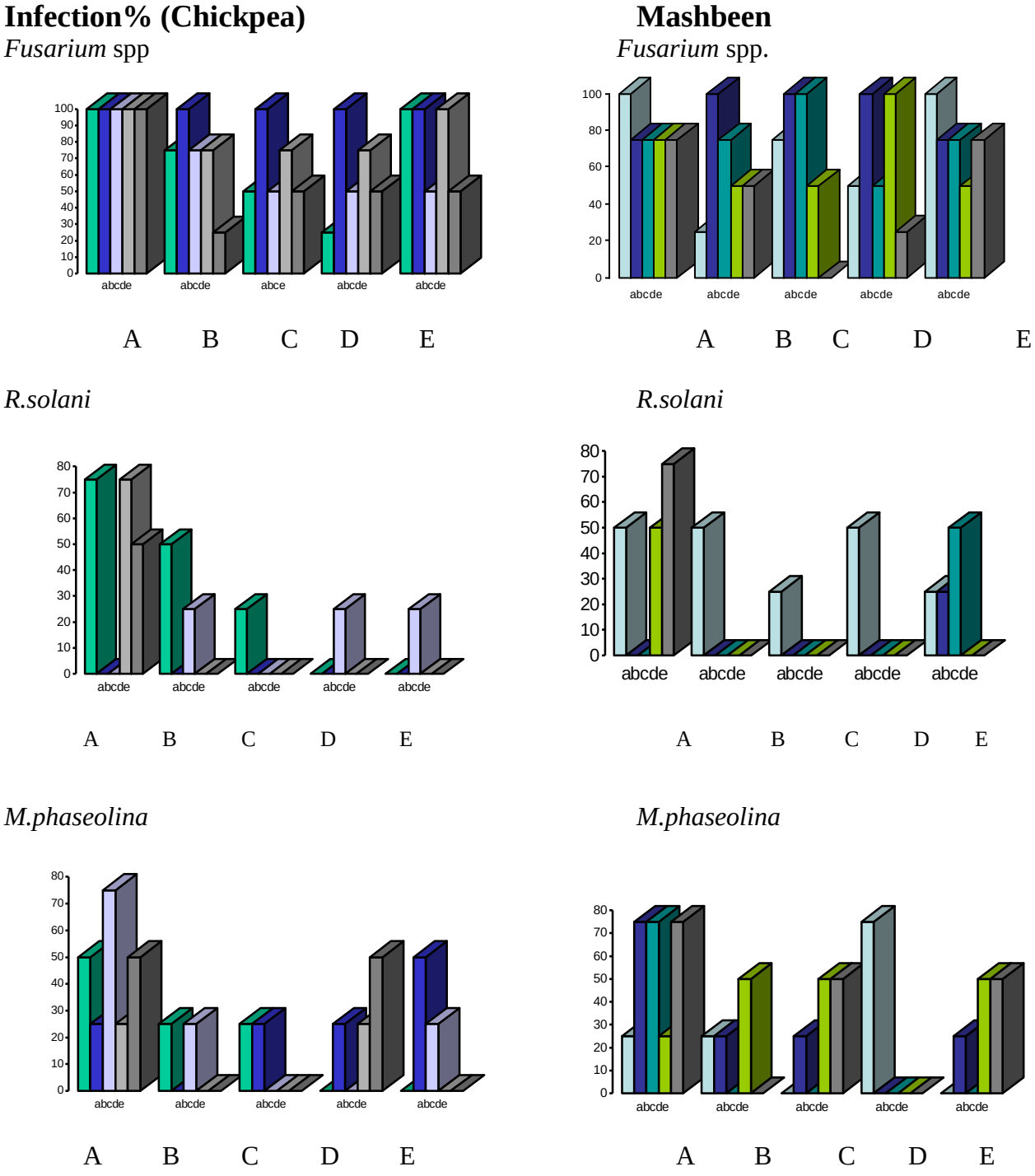

Fig 2. Effect of *Trichoderma harzianum* inoculums multiplied on different substrates and stored for different intervals in the control of root infecting by *F.solani*, *M.phaseolina* and *R.solani* on chickpea and mash bean.
A= Control**, B=**Rice, **C=**Millet**, D=**Sorghum**, E**=Sawdust; **a**=0 day, **b**=15 days, **c**= 30 days, **d**= 60 days, **e**=90 days.

LSD0.05=Treatment=4.88, Pathogen=3.78, Time=4.88.(Chickpea); **LSD0.05**=Treatment= 2.07 , Pathogen= 1.6 , Time=2.07.(Mashbean)

Trichoderma harzianum multiplied on rice grain (P<0.001) and sorghum grain (P<0.001) significantly suppressed the *Fusarium* infection on mash bean as compared to control. *T. harzianum* multiplied on rice grain after 30, 60 & 90 days storage steadily decline the (p<0.001) *Fusarium* spp infection. *T. harzianum* multiplied on millet grain at 0, 30, and 90 days intervals showed a significant (P<0.001) reduction in *Fusarium* infection (Fig. **2**). Similarly rice grain inoculum showed significant (p<0.001) reduction of *M.phaseolina* infection as compared to control. *T. harzianum* inoculum on rice grain after 30 and 60 days storage significantly (P<0.001) reduced the *M. phaseolina* infection. *T. harzianum* inoculum on sorghum grain and millet grain after 15, 30, 60 and 90 days of storage completely suppressed (P<0.001) the *M. phaseolina* infection as compared to the control mash bean. (Fig. **2**). Present results showed that rice grain, millet grain and sorghum grains were the best substrates for the growth of *T.harzianum* as compared to saw dust, (Lewis and Papavizas 1985) also reported that grain seeds and meals bagasse, straw, saw dust individually or in combination were found suitable for multiplication of *Trichoderma spp*. Such similar results have been made by Dawar and Ghaffar (2003). Population of *T.harzianum* inoculum on millet grain, sorghum grain and rice grain increased with the increased in time. An other attribute of biocontrol agent is the consistent efficacy for plant disease control. Actively growing hyphae of *T.harzianum* in bran culture have been more effective against *R.solani* as compared to conidial preparation (Lewis and Papavizas, 1985).

Trichoderma harzianum after multiplication on sorghum grain and millet showed good growth of plant, but saw dust did not show the significant enhancement of plant growth (chickpea). Such similar results have been obtained by Papavizas (1985). The biocontrol product, which control living organisms when formulated must also have an acceptable shelf life without special storage requirements so that their viability is maintained. Population of biocontrol agents increased with maximum population of *T.harzianum* on millet grain, sorghum grain and rice grain after 180 days intervals. Not much difference was observed in the population of *T.harzianum* on sawdust. Such similar result has also been obtained by Dawar and Ghaffar (2003). Another attribute of biocontrol products is the consistent efficacy for plant disease control. Actively growing hyphae of *T.harzianum* in bran culture have been more effective against *R.solani* as compared to conidial preparation (Lewis and Papavizas, 1985). In the present study on efficacy of biocontrol agent after multiplication on different substrates showed significant (P< 0.001) correlation between reduction in infection and population of microbial antagonists. *T.harzianum* after 90 days multiplication on sorghum grains, millet grains and rice grains showed highest cfu and was significant by (P< 0.001) effective against *R.solani* and provides complete protection against the *R.solani* infection on chickpea and effective also against *M.phaseolina* infection.

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