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Effect of Different Organic Substrates and Carbon and Nitrogen Sources on Growth and Shelf Life of *Trichoderma harzianum*

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ABSTRACT

Nine organic substrates *viz*., rice grains, sorghum grains, wheat grains, millet grains, wheat straw, rice husk, cow dung, sawdust, and poultry manure were used for mass multiplication of *Trichoderma harzianum*. Of these, sorghum grains followed by millet grains were the best substrates. The poultry manure appeared to be the most unsuitable substrate, whereas rice grains, wheat grains, wheat straw, and rice husk performed moderately well. Sucrose was the best carbon source and supported the highest colony growth of *T. harzianum* on Czapek's Agar plates. Similarly, ammonium nitrate at 3,000 ppm appeared to be the most suitable nitrogen source and produced the highest colony growth as well as abundant conidia. A combined use of sucrose at 30,000 ppm as carbon source, and ammonium nitrate at 3,000 ppm as nitrogen source significantly enhanced the mycelial growth and conidial production by *T. harzianum* in wheat straw, rice husk, and millet grains, whereas, in sorghum grains and rice grains, the addition of carbon and nitrogen sources showed negative effect on sporulation of *T. harzianum*. Studies on shelf life of the inocula multiplied on various substrates showed that the populations of *T. harzianum* on all the substrates achieved the peak at 60-75 days incubation period and declined gradually thereafter. However, even after 330 days, the populations were greater than the population at 0-day. At 345-360 days interval, population was found to be less than the initial population at 0-day.

Keywords: Biocontrol agent, Mass multiplication, Shelf life, *Trichoderma harzianum.*

INTRODUCTION

Plant diseases, especially soil-borne diseases inflict serious losses to crop plants and adversely affect the agriculture economy of a country. The soil-borne fungal pathogens play a major role in the development of root rot disease complexes on many important field and horticultural crops, which often result in death of the plants. Since soil applied pesticides are costly and produce environmental hazards (Saleem *et al*., 2000; El-Katatny *et al.,* 2000; Cook *et al.,* 1983), crop resistance to pathogens is the ideal means of controlling plant diseases. However, many crops have little or no resistance to certain plant pathogens. Thus, use of microbial antagonists in the biological control of plant disease is an alternative method for disease control that would also protect our environment from the hazardous effects of the chemicals (Harman *et al.,* 2004; Larena *et al*., 2002; Melo and Faull, 2000; Lumsden and Locke, 1989).

Several fungi and bacteria have received considerable attention in the control of soil-borne root infecting fungi like

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Macrophomina phaseolina, Rhizoctonia solani, Sclerotium rolfsii and *Fusarium* sp., and root knot nematodes (Ghaffar, 1978, 1988, 1992; Kucuk and Kivanc, 2003, 2004; Benitez *et al.,* 2004; Adekunle *et al*., 2001). *Trichoderma* species also have plant growth promoting capabilities that may or may not be integral to biological control (Benitez *et al*., 2004; Dubey *et al*., 2007; Khan and Shahzad, 2007). *Trichoderma* species suppressed *R. solani* and *M. phaseolina* infection on cotton, sugar beet, lentil and soybean (Akhter, 1977; Hashem, 2004; Tarek and Moussa, 2002; Etebarian, 2006; Dolatabadi *et al.*, 2012). The main hindrance in the large-scale application of biocontrol organisms is the lack of cost effective methods for mass multiplication of biocontrol inoculum. The aim of present studies was to evaluate the suitability of different organic substrates for mass multiplication of *T. harzianum* and effect of carbon and nitrogen sources on growth and sporulation of *T. harzianum* in order to get enhanced production of conidia of the biocontrol agent on less suitable but economically very cheap organic substrates.

MATERIALS AND METHODS

Evaluation of Different Substrates for Multiplication of *T. harzianum*

Cultures of *T. harzianum* present in the Pest and Disease Research Lab (PDRL), Department of Agriculture and Agribusiness Management, University of Karachi, were used during the present studies. Rice grains, sorghum grains, millet grains, wheat grains, wheat straw, saw dust, rice husk, poultry manure, and cow dung were used for mass multiplication of *T. harzianum*. The substrates were soaked in water for two hours in containers. Extra water was decanted and the substrates were pressed with hand in order to remove the excess moisture. Fifty g substrate was transferred into each polyethylene bag. The bags were sealed and then sterilized in an autoclave at 15 psi for 20 minutes and allowed to cool down at room temperature. Spore suspension of *T. harzianum* was prepared by adding 20 ml sterile water to one-week old culture of the fungus on potato sucrose agar (PSA) medium in a 9 cm diam Petri plate and rubbing the surface with a sterile spatula. The population was determined with the help of haemocytometer and adjusted to containing 1.2×10^{7} conidia ml^{-1}. Two ml of the suspension were inoculated with the help of a sterile syringe into each bag containing 50 gram substrate. There were three replicates for each treatment.

The wet weights were recorded and known quantities of the wet substrates were placed in an oven at 80°C for 24 hours to determine the dry weight and the amount of water per g substrate at the time of inoculation in order to see the effect of moisture content of the substrate on sporulation by *T. harzianum.*

The inoculated substrates were stored at 30±2°C and population of the biocontrol agent on each substrate was determined at 0-days and after 15-days intervals. One g sample of a substrate was air dried, crushed using a pestle and mortar, and, later, two ml sterile water along with two drops of Tween-20 were added to make a homogenized paste and then seven ml water was added to obtain 1:10 dilution. A serial dilution was then prepared to get 1:100, 1:1,000 and 1:10,000 dilutions (Waksman and Fred, 1922). The number of cfu per g substrate was determined using a haemocytometer as described by Somasegaran and Hoben (1985).

Effect of Carbon Sources on Growth of *T. harzianum*

Different carbon sources viz., Sucrose, Maltose, Dextrose, Glucose, Starch and Cellulose at 0, 10,000, 20,000, 30,000, 40,000 and 50,000 ppm were added to Czapek's Agar (CzA) medium without any nitrogen source before the medium was autoclaved. CzA without carbon and nitrogen sources served as control. The media were sterilized for 20 minutes at 15 psi. Penicillin at100,000 units L^{-1} and streptomycin at 0.2 g L^{-1} were added to the

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sterilized stock media just before pouring to inhibit the bacterial growth. The media were poured in 9 cm diam., Petri plates at 10 ml per plate. There were three replicates for each treatment. After solidification, a 5 mm diam. inoculum disc of T. harzianum was placed in the center of each Petri plate. The plates were incubated at 28±2°C and diameters of the growing colonies were recorded daily till the plates in any treatment were filled by the fungal growth.

Effect of Nitrogen Sources on Growth of *T. harzianum*

Different nitrogen sources viz., NPK (containing 8% nitrogen, 23% phosphorus and 18% potash), Urea, DAP (di-ammonium phosphate containing 18% ammonium and 48% phosphate), ammonium nitrate and sodium nitrate were used separately at 0, 10,000, 30,000 and 50,000 ppm to see their effect on *in-vitro* growth of *T. harzianum* using the methods described above. No carbon source was added to the medium. Since the growth of *T. harzianum* at these nitrogen concentrations was not good, another experiment was, therefore, carried out where the nitrogen sources were used at 0, 1,000, 3,000, 5,000, 7,000, 9,000 and 10,000 ppm to find out the best concentration for growth of *T. harzianum*.

Combined Effect of Selective Carbon and Nitrogen Sources on Growth of *T. harzianum*

CzA medium amended with most suitable concentrations of the carbon and nitrogen sources were poured in 9 cm diam Petri plates and, after solidification, a 5 mm diam. inoculum disc of *T. harzianum* was placed in the center of each Petri plate. The plates were incubated at 28±2°C and diameters of the growing colonies were recorded daily till the plates in any treatment were filled by the fungal growth.

Effect of Carbon and Nitrogen Sources on Growth and Sporulation of *T. harzianum* on Organic Substrates

Five selected substrates *viz.,* rice grains, sorghum grains, millet grains (the most suitable substrates) and wheat straw and rice husk (less suitable substrates) were used for mass multiplication of the fungus. The substrates were soaked in water for two hours in containers and 50 g of a substrate was transferred into a polyethylene bag. Sucrose at 1.5 g and ammonium nitrate at 0.15 g were mixed with 50 g of the substrate. Carbon and nitrogen sources were also used separately. Substrates without carbon and nitrogen sources served as control. Fifty g substrate in each bag was inoculated with two ml spore suspension of *T. harzianum* containing 1.2×10^7 conidia ml^{-1}. Growth and population of *T. harzianum* was determined using the methods described above.

Shelf-life of *Trichoderma harzianum*

Polyethylene bags filled with 50 g of each substrate *viz*., sorghum grain, millet grains, rice grains, wheat straw and rice husk were inoculated with 2 ml conidial suspension of the test antagonistic fungus containing 1.2×10^7 conidia ml^{-1}. In a comparable set of treatments, each substrate was also amended with the selected carbon and nitrogen sources i.e. Sucrose at 1.5 g per and ammonium nitrate at 0.15 g. The bags were stored at room temperature and populations of *T. harzianum* was determined at 0-days and then at 15-days intervals for up to 360 days using a haemocytometer.

RESULTS

Growth of *T. harzianum* on Different Substrates

Generally, cereal grains were found more appropriate for the mass production

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of antagonistic fungus T. harzianum as significantly higher populations of the fungus were recorded on cereal grains as compared to other substrates (Figure 1-A). However, the highest population of T. harzianum was observed on sorghum grains (100.3×10^8 cfu g^{-1}) followed by millet grains (75.23×10^8 cfu g^{-1}). The poultry manure appeared to be the most unsuitable substrate and produced the lowest *T. harzianum* population (1.03×10^8 cfu g^{-1}) followed by cow dung (2.07×10^8 cfu g^{-1} and saw dust (2.5×10^8 cfu $^{-1}$) (Figure 1-A). Rice grains, wheat grains, wheat straw, and rice husk performed moderately well and produced 27.27×10^8, 23.27×10^8, 20.4×10^8 and $17.3\ 5\times10^8$cfu g^{-1} of T. harzianum, respectively.

During soaking, grains absorbed the least moisture, whereas the highest moisture was absorbed by wheat straw followed by saw dust, rice husk, cow dung, and poultry manure (Figure 1-B). No correlation between the moisture content of the

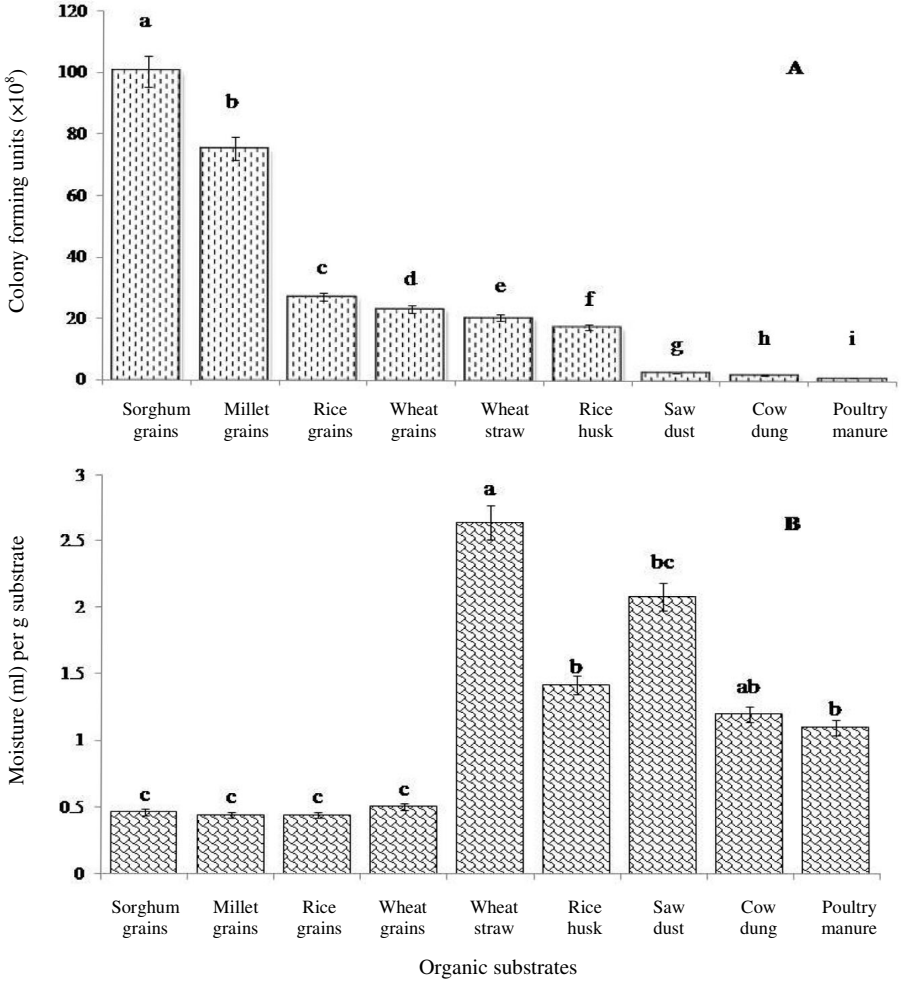

Figure 1. (A) Population of *Trichoderma harzianum* after 15 days incubation on different substrates. (B) Amount of moisture absorbed by one gram of different organic substrates. Bars with similar letters at the top are not significantly different from each other at $P< 0.05$ level as determined by DMRT.

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substrate and the amount of conidia produced was evident.

Effect of Different Carbon Sources on Growth of *T. harzianum*

In all carbon sources, the colony growth of *T. harzianum* increased with increasing concentrations, except in case of cellulose and starch where maximum colony growth occurred at lowest concentration (Figures 2-3). The significantly highest colony growth was recorded on sucrose followed by dextrose, glucose, and maltose amended media (Figures 2-3). However, Dextrose, glucose, and maltose amended media produced less conidia but more superficial mycelial growth of *T. harzianum* as compared to the sucrose amended medium, which supported abundant conidial production of the test fungus. Sucrose was, therefore, used as a selected carbon source in further experiments.

Effect of Different Nitrogen Sources on Growth of *T. harzianum*

When the nitrogen sources viz., DAP, NPK, ammonium nitrate, sodium nitrate, and urea were used at higher concentrations of 10,000, 30,000 and 50,000 ppm, the colony growth of *T. harzianum* showed a negative correlation with the concentration of the nitrogen source in the medium. The highest colony growth of *T. harzianum* was recorded at the lower concentrations, which gradually decreased with increasing concentrations (Figure 4). DAP followed by NPK amended medium produced higher colony growth as compared to the other nitrogen sources, however, the growth was scanty, the mycelium was submerged into

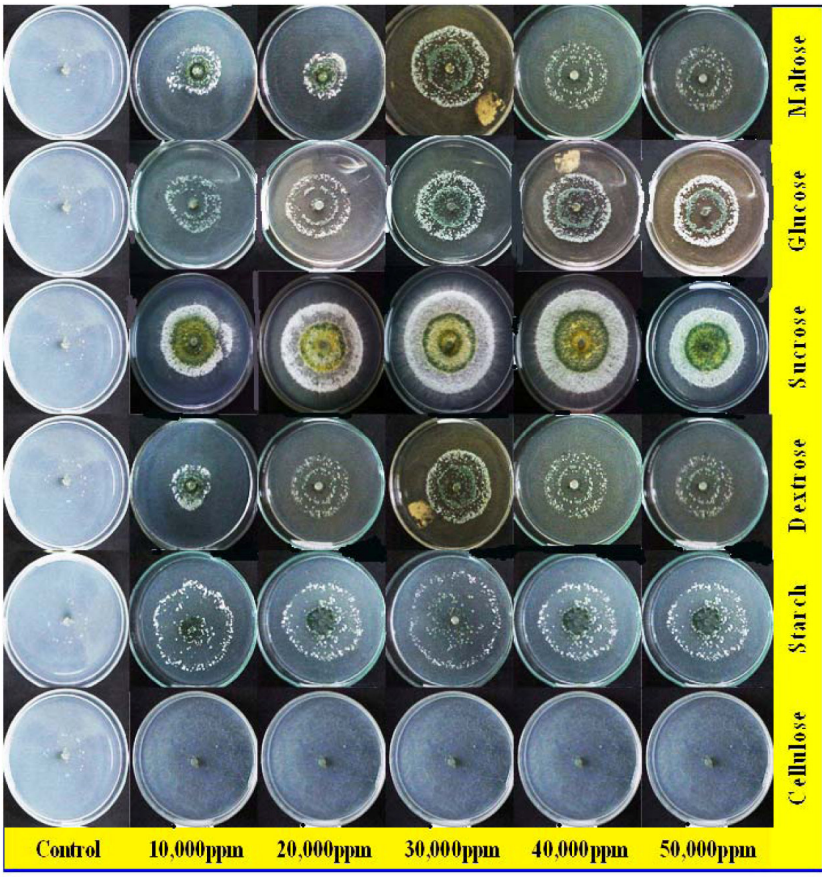

Figure 2. Growth of *T. harzianum* on media amended with different carbon sources.

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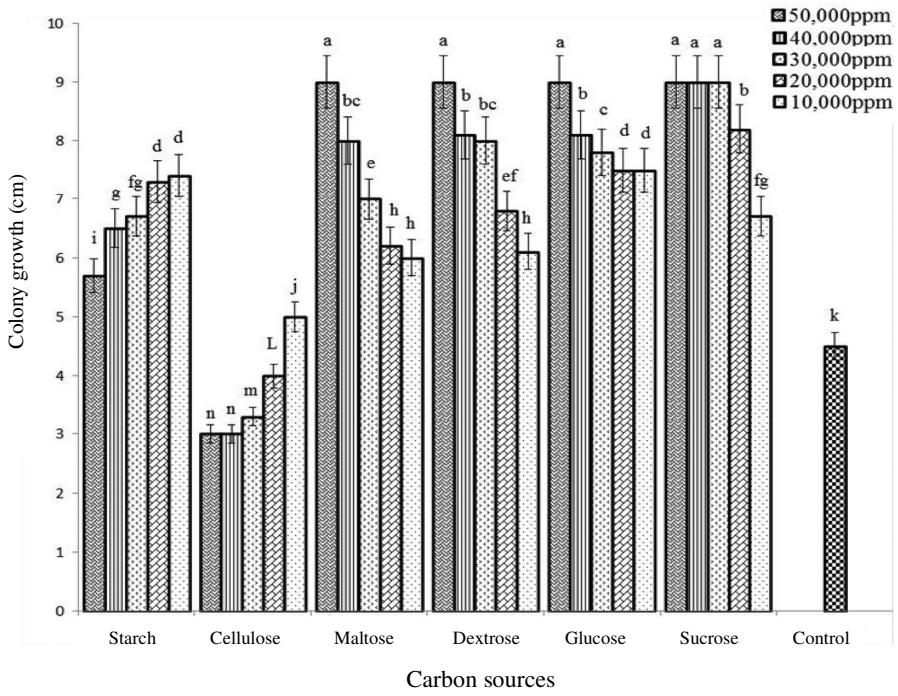

Figure 3. Effect of different carbon sources on *in vitro* growth of *Trichoderma harzianum.* Bars with similar letters at the top are not significantly different from each other at $P< 0.05$ level as determined by DMRT.

the medium, and without any sporulation. In view of this negative effect on growth and sporulation of *T. harzianum,* another experiment was conducted to test the effect of comparatively lower concentrations of N sources on the growth and sporulation of *T. harzianum.*

In the 2nd experiment, low concentrations of nitrogen sources i.e. 1000, 3,000, 5,000, 7,000, 9,000 and 10,000 ppm put the positive effect on the colony growth of *T. harzianum* and comparatively better growth of the test fungus was recorded, except in the case of urea, where no significance impact of decreasing concentrations was observed (Figures 5-6). Among all the nitrogen sources, the ammonium nitrate at lower concentrations appeared as the most encouraging and produced the highest colony growth as well as abundant conidial production (Figures 5-6). Therefore, ammonium nitrate at 3,000 ppm was selected for further experiments.

Combined Effect of Carbon and Nitrogen Sources

The combined use of the best carbon and nitrogen sources acted positively on the mycelial growth and conidial production of the test antagonistic fungus as significantly higher colony growth was recorded on medium amended with sucrose at 30,000 ppm+ammonium nitrate at 3,000 ppm as compared to the control (Figure 7-8).

Effect of Carbon and Nitrogen Sources on Sporulation of *T. harzianum* on Organic Substrates

In case of sorghum grains and rice grains,

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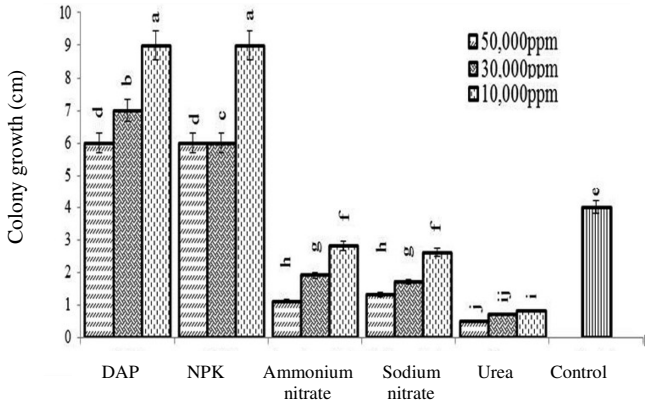

Figure 4. Effect of high concentration of different nitrogen sources on *in- vitro* growth of *Trichoderma harzianum*. Bars with similar letters at the top are not significantly different from each other at $P< 0.05$ level as determined by DMRT.

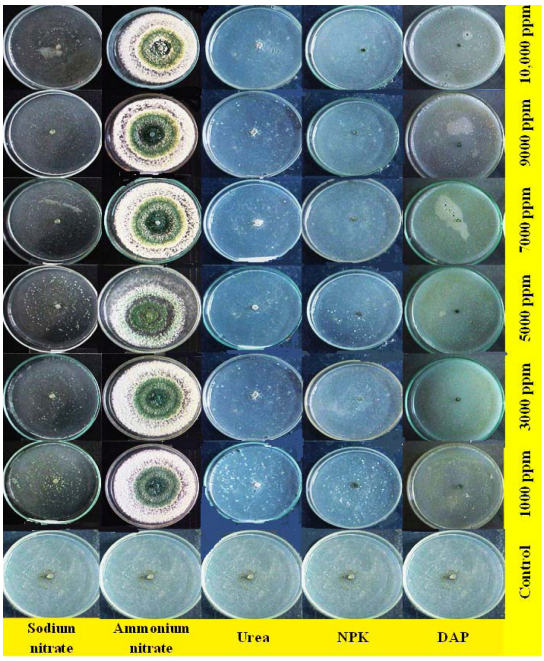

Figure 5. Growth of *Trichoderma harzianum* on media amended with different nitrogen sources.

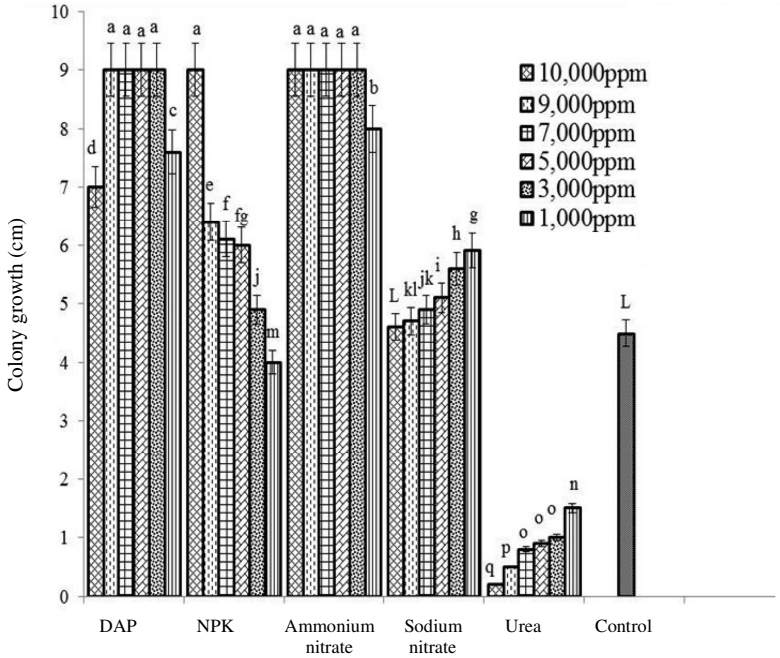

Figure 6. Effect of low concentration of different nitrogen sources on *in- vitro* growth of *Trichoderma harzianum.* Bars with similar letters at the top are not significantly different from each other at $P< 0.05$ level as determined by DMRT.

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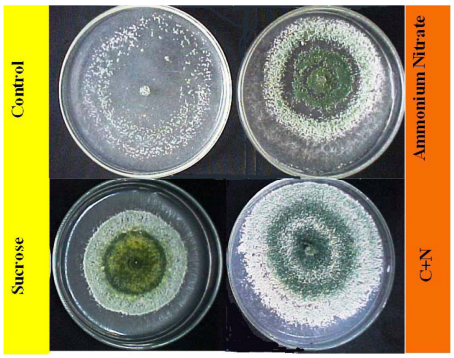

Figure 7. Growth of *Trichoderma harzianum* on media amended with selected carbon and nitrogen sources.

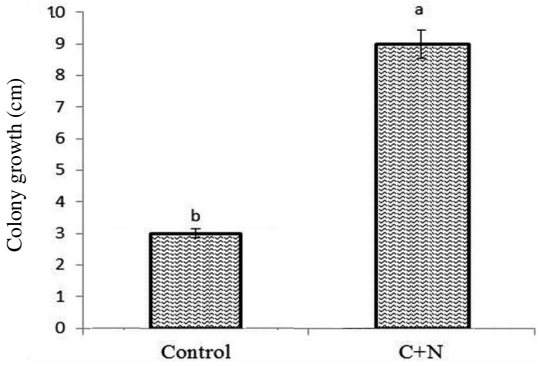

Figure 8. Effect of combined use of selected carbon and nitrogen sources on *in-vitro* growth of *Trichoderma harzianum*.Bars with similar letters at the top are not significantly different from each other at $P< 0.05$ level as determined by DMRT.

the addition of carbon and nitrogen alone or in combination acted negatively on the sporulation *T. harzianum* as in both substrates significantly high populations of *T. harzianum* were recorded on un-amended (control) substrate as compared to carbon-nitrogen amended substrate (Figure 9). Among all the treatments, the highest population of *T. harzianum* was recorded on un-amended sorghum grains (44×10^9 cfu g^{-1}) and rice grains (43×10^9 cfu g^{-1}). However, the addition of carbon and nitrogen significantly enhanced the conidial population of *T. harzianum* in wheat straw, rice husk, and millet grains (Figure 9). In case of wheat straw, the conidial population of test fungus was increased from 17×10^9 cfu g^{-1} in un-amended substrate to 33×10^9 cfu g^{-1} (in C+N amended substrate). Similarly, in rice husk the number of conidia of *T. harzianum* increased from 10×10^9 cfu g^{-1} in un-amended substrate to 25×10^9 cfu g^{-1} in C+N amended substrate (Figure 9).

Shelf Life of *Trichoderma harzianum*

Sporulation of *T. harzianum* on sorghum grains not amended with carbon and nitrogen sources was 55×10^9 cfu g^{-1} after 15 days of incubation and reached the maximum of 71×10^9 cfu g^{-1} after 60 days. On sorghum grains amended with carbon and nitrogen sources, the population was 37×10^9 cfu g^{-1} after 15 days and reached 53×10^9 cfu g^{-1} after 60 days. Thereafter, the populations of *T. harzianum* in both treatments declined gradually and, after 180 days, only 22×10^9 and 15×10^9 cfu g^{-1} of *T. harzianum* were recorded on un-amended and carbon and nitrogen amended sorghum grain, respectively. After 360 days of incubation, *T. harzianum* population in both, treatments was 0.01×10^9 and 0.005×10^9 cfu g^{-1}, respectively (Figure 10).

Similar results were observed on rice grain where after 15 days of incubation the population of *T. harzianum* in carbon and nitrogen un-amended and amended treatments were 51×10^9 and 31×10^9 cfu g^{-1}, respectively, and reached the maximum of 68×10^9 and 56×10^9 cfu g^{-1}, respectively, after 60 days. Thereafter, the populations of *T. harzianum* in both treatments declined gradually and, after 180 days, only 8×10^9 and 5×10^9 cfu g-1 of *T. harzianum* were recorded on un-amended and carbon and nitrogen amended rice grains, respectively. After 360 days of incubation, *T. harzianum* population in both the treatments reduced to 0.01×10^9 and 0.005×10^9 cfu g^{-1}, respectively (Figure 10).

Amendment of millet grain, wheat straw, and rice husk gave increased populations

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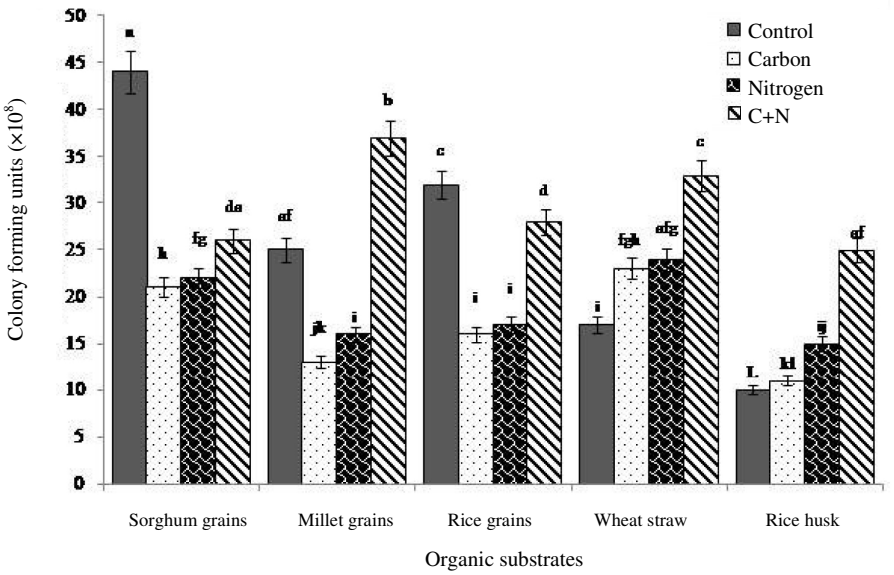

Figure 9. Effect of selected C+N sources on growth and sporulation of *Trichoderma harzianum* on organic substrates. Bars with similar letters at the top are not significantly different from each other at $P<$ 0.05 level as determined by DMRT.

over the un-amended substrates. In millet grains, the population of *T. harzianum* after 15 days of incubation was 14 $\times 10^9$ and 19×10^9 cfu g^{-1} on un-amended and amended substrates, respectively. The maximum conidial populations i.e. 14×10^9 and 38×10^9 cfu g^{-1}, respectively, were achieved after 60 days of incubation and declined to 5.2×10^9 and 15×10^9 cfu g^{-1}, respectively, after 180 days. The conidial production were reduced to 0.006×10^9 and 0.06 $\times10^9$ cfu g^{-1}, respectively, on un-amended and carbon and nitrogen amended millet grains after 360 days of incubation (Figure 10).

Similarly, population of *T. harzianum* after 15 days of incubation was 15×10^9 cfu g^{-1} on carbon and nitrogen amended wheat straw as compared to 11×10^9 cfu g^{-1} on un-amended substrate. The population reached the maximum (28×10^9 cfu g^{-1}) on carbon and nitrogen amended wheat straw after 60 days of incubation and on un-amended substrate (13.7×10^9 cfu g^{-1}). Thereafter, the populations on un-amended and amended substrates declined gradually to 0.62×10^9 and 1.8×10^9 cfu g^{-1}, respectively, after 180 days, and to 0.001×10^9 and 0.0012×10^9 cfu g^{-1}, after 360 days (Figure 10).

In case of rice husk, the conidia production by *T. harzianum* after 15 days of incubation was 14×10^9 cfu g^{-1} on carbon and nitrogen amended, and 0.09×10^9 cfu g^{-1} on un-amended substrate. The population of *T. harzianum* attained its peak on amended substrate after 60 days (24×10^9 cfu g^{-1}) and on un-amended substrate (12×10^9 cfu g^{-1}) in 45 days. After 180 days of incubation, the populations were reduced to 0.037 $\times10^9$ and 1×10^9 cfu g^{-1}, respectively, whereas, the viability of the conidia was lost completely after 285 days on un-amended and after 315 days on carbon and nitrogen amended substrate (Figure 10).

DISCUSSION

Besides their effectiveness, the main hindrance in the widespread application of bio-control agents like *T. harzianum* is their unavailability for large scale field use. To overcome the problem, different workers have tried a number of substrates such as rice grain, sorghum grain, millet grain, cotton cake, mustard cake, wheat straw, rice straw, saw dust, sugarcane bagasse,

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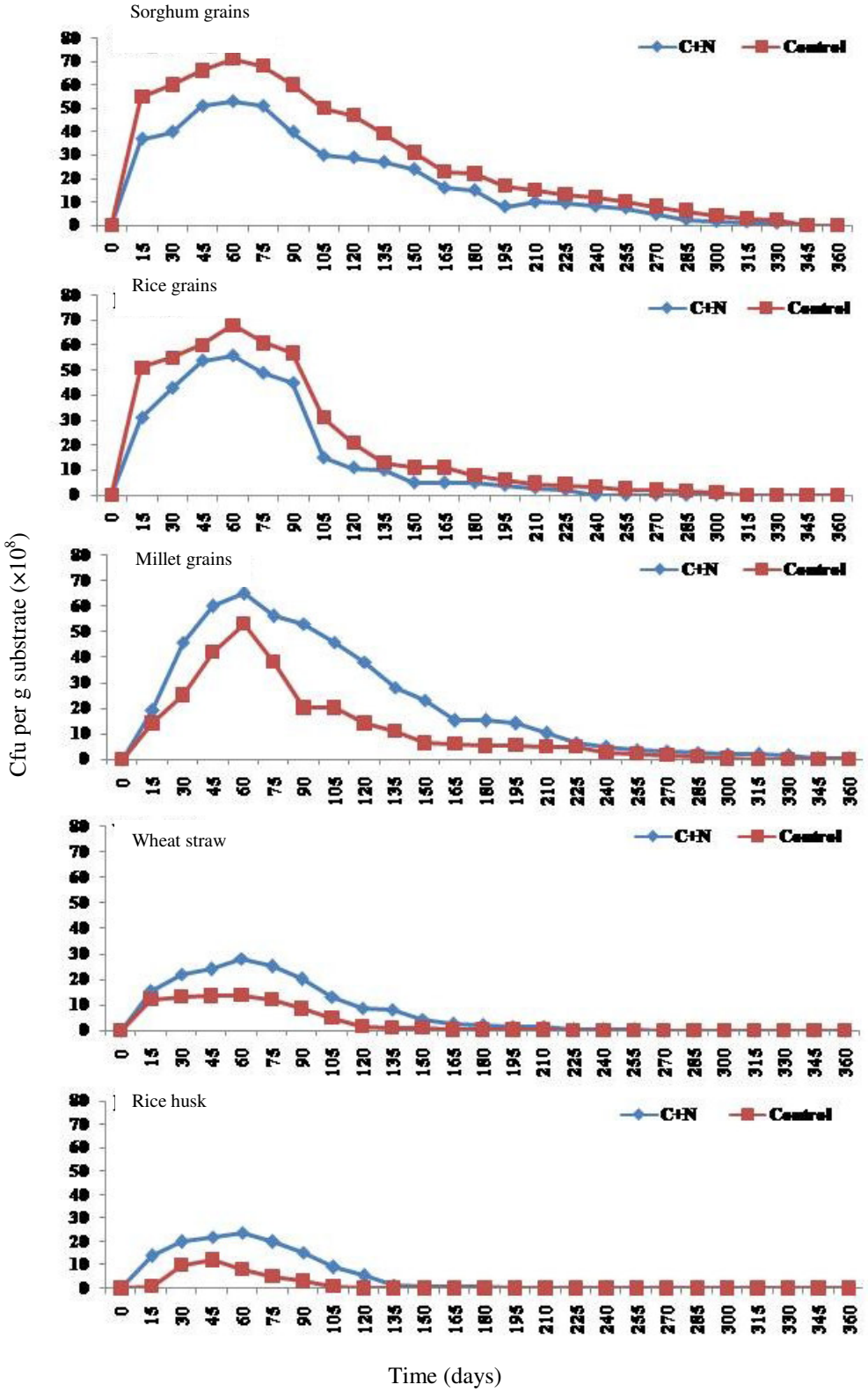

Figure 10. Effect of C and N amendment on shelf life of *Trichoderma harzianum* on different substrates.

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sugarcane ash, farmyard manure (FYM), and wheat bran for mass multiplication of bio-control agents (Saju *et al*., 2002; Sangle and Bambawale, 2003; Sharma *et al.,* 2005; Rettinassababady and Ramadoss, 2000; Sharma *et al.,* 2004). Similarly, different substrates have been tested by several scientists for the greater conidial production (Pfirter *et al.,* 1999; Prasad *et al.,* 2002a, b; Bailey *et al*., 2004).

In the present studies, sorghum grains, followed by millet grains, appeared to be the most effective substrate and gave highest population of *T. harzianum.* Our results are in confirmation to those reported by Masangkay *et al*. (2000), Malik and Dawar (2003), Dawar and Ghaffar (2003), and Rini and Sulochana (2007) who also found that sorghum grains produced significantly more population of *Trichoderma harzianum* and *T. viride* as compared to cow dung, neem cake, coir pith, saw dust, and rice bran, used either alone or in certain combinations. Likewise, Pandey (2009) observed that rice bran, rice husk, and sawdust when mixed with sorghum in a 1:1 ratio supported excellent growth and sporulation of *T. viride*, however, maximum spore concentration was observed in sorghum powder, followed by the rice husk and sorghum and sawdust and sorghum mixtures.

Attempts have also been made to enhance the conidial yield of bio-control agents by adding nutritional supplements to the growth substrates (Jackson *et al*., 1991; Naima *et al*., 2004). The present investigations revealed that, among different carbon sources, the maximum colony growth of *T. harzianum* was obtained on sucrose followed by dextrose, glucose, and maltose amended media. Ammonium nitrate at lower concentrations appeared as the best nitrogen source. The combined use of sucrose at 30,000 ppm and ammonium nitrate at 3,000 ppm highly favored the growth of *T. harzianum.* Our results are in accordance to those reported by Jayaswal *et al.* (2003) who also found that growth and sporulation of *T. viride* were greatly influenced by various carbon and nitrogen sources. They observed the best growth and sporulation of *T. viride* on sucrose, peptone, and trehalose supplemented medium; growth and sporulation both were favored by ammonium forms of nitrogen compared to nitrite or nitrate forms. Similarly, Gashe (1992) observed that nitrogen in the form of KNO_3 was better than NH_4Cl or urea for the growth of *Trichoderma* species. Li *et al*. (2004), while evaluating the effects of different carbon and nitrogen sources on the growth of *T. harzianum*, observed that sucrose, mannose, glucose, xylose, and starch were beneficial to its growth as compared to maltose and D-galactose. Peptide and ammonium sulfate were the best nitrogen sources for growth, but urea was the worst among the seven nitrogen sources. Younis *et al*. (2004) observed that best carbon source used was cellobiose, whereas malt extract and ammonium phosphate were the best nitrogen sources for *T. harzianum* growth. Syahiddin (2007) also found that maximum sporulation of *T. harzianum* occurred on glucose. Seyis and Aksoz (2005) evaluated the effects of sucrose, maltose, and lactose and observed maximum xylanase activity of *T. harzianum* in the presence of sucrose; ammonium sulfate was the most appropriate inorganic nitrogen source for xylanase production and urea increased xylanase activity. Similarly, Prasad and Rangeshwaran (2000c), while making an improved medium for mass production of *T. harzianum*, found that, among the 3 nitrogen sources tested, soya flour and sucrose as carbon source supported the highest biomass, number of viable propagules, and spore production.

During the present studies on shelf life, populations of *Trichoderma harzianum* on different substrates attained the peak at 60-75 days incubation period and declined gradually thereafter. However, even after 330 days, the population was greater than the population at 0-day. At the interval of 345-360 days, population was found less than the initial population at 0- day. The viability period recorded during the present

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studies on different substrates are much longer than reported by other workers. Prasad and Rangeshwaran (2000a) reported that the viability of *T. harzianum* propagules on talk and kaolin was reserved for up to 90 days, but declined below the optimum level after 120 days. They concluded that the talc and kaolin were best sources for multiplication and bio-efficacy of *T. harzianum* as compared to bentonite carrier material**.** Similarly, Prasad and Rangeshwaran (2000b) reported that storage of *T. harzianum* as a granular preparation at room temperature promoted initial growth of the bio-agent up to 30 days and the population declined after 90 days. Prasad *et al*. (2002b) observed that the conidial formulation engaged ideal amount of viable propagules after 180 days of storage at room temperature, but in case of chlamydospore formulation, viable propagules decreased after 150 days. According to Mev and Meena (2003), population of *T. harzianum* at room temperature increased up to 40 days and declined thereafter.

The results of the present study show that amendment of sucrose as carbon source and ammonium nitrate as nitrogen source significantly enhanced the conidial population of *T. harzianum* in wheat straw, rice husk, and millet grains, which without amendment of carbon and nitrogen showed poor performance. The population on carbon and nitrogen amended wheat straw was not significantly different from that on rice grains without additional carbon and nitrogen sources. The efficacies of rice grain and carbon and nitrogen amended wheat straw against soil-borne root infecting fungi on mungbean were also not significantly different (data not shown). It was interesting to note that addition of carbon and nitrogen sources to rice and sorghum grains resulted in significant reduction in conidia production. Presumably, the amount of nitrogen in these seeds was sufficient for growth and multiplication of the antagonist and additional dose of nitrogen proved to be toxic to the antagonist. It could, therefore, be suggested that substrates less suitable for growth and sporulation of biocontrol agents can be made suitable by addition of proper carbon and nitrogen sources to enhance growth and sporulation.

REFERENCES

1. Adekunle, A. T., Cardwell, K. F., Florini, D. A. and Ikotun, T. 2001. Seed Treatment with *Trichoderma* Species for Control of Damping Off of Cowpea Caused by *Macrophomina phaseolina. Biocon. Sci. Technol.,* **11**: 449-457.
2. Akhter, C. M. 1977. Biological Control of Some Plant Diseases Lacking Genetic Resistance of the Host Crops in Pakistan. *Ann. N. Y. Acad. Sci.,* **287**: 45-56.
3. Bailey, B. A., Hebbar, K. P., Lumsden, R. D., Oneill, N. R. and Lewis, J. A. 2004. Production of *Pleospora papaveracea* Biomass in Liquid Culture and Its Infectivity on Opium Poppy (*Papaver somniferum*). *Weed Sci*., **52**: 91–7.
4. Benitez, T., Rincon, A. M., Limon, M. C. and Codon, A. C. 2004. Biocontrol Mechanisms of *Trichoderma* Strain. *Int. J. Microbiol.,* **7(4):** 249-260.
5. Cook, R. J. and Baker, K. F. 1983. *The Nature and Practice of Biological Control of Plant Pathogens*. American Phytopathological Society of Minnesota, 539 PP.
6. Dawar, S. and Ghaffar, A. 2003. Screening of Substrates for Mass Production of Biocontrol Agents. *Pak. J. Bot.,* **35**: 409-414.
7. Dolatabadi1, H. K., Goltapeh1, E. M., Mohammadi, N. and Rabiey, M. 2012. Biocontrol Potential of Root Endophytic Fungi and *Trichoderma* Species against *Fusarium* Wilt of Lentil under *In vitro* and Greenhouse Conditions. *J. Agric. Sci. Technol.,* **14:** 407-420.
8. Dubey, S. C., Suresh, M. and Singh, B. 2007. Evaluation of *Trichoderma* Species against *Fusarium oxysporum* f. sp. *ciceris* for Integrated Management of Chick Pea Wilt. *Biological Control*, **40(1):** 118-127.
9. El-Katatny, M. K., Somitsch, W., Robra, K. H., El-Katany, M. S. and Gubitz, G. M. 2000. Production of Chitinase and 1,3-glucanase by *Trichoderma harzianum* for Control of the Phytopathogenic Fungus

[DOR: 20.1001.1.16807073.2014.16.4.19.9]

Sclerotium rolfsii. *Food Technol. Biotechnol*., **38(3):** 173-180.

10. Etebarian, H. R. 2006. Evaluation of *Trichoderma* Isolates for Biological Control of Charcoal Stem Rot in Melon Caused by *Macrophomina phaseolina. J. Agric. Sci. Technol.,* **8**: 243-250.
11. Gashe, B. A. 1992. Cellulase Production and Activity by *Trichoderma* sp. A-001. *J. Appl. Microbiol.,* **73(1)**: 79-82.
12. Ghaffar, A. 1978. *Biological Control of Sclerotial Fungi*: *Final Research Report*. Department of Botany, University of Karachi, Karachi-75270, Pakistan, 140 PP.
13. Ghaffar, A. 1988. *Soilborne Diseases Research Center*: *Final Research Report*. Department of Botany, University of Karachi, Karachi-75270, Pakistan.
14. Ghaffar, A. 1992. Use of Microorganisms in the Biological Control Soil Born Root Rot Diseases of Crop Plants: Final Research Report. Department of Botany. University of Karachi, Karachi-75270, Pakistan.
15. Harman, G. E., Howell, C. R., Viterbo, A., Chet, I. and Loreto, M. 2004. *Trichoderma* Species Opportunistic, Avirulent Plant Symbionts. *Nature Rev. Microbiol.,* **2(1):** 43-56.
16. Hashem, M. 2004. Biological Control of Two Phytopathogenic Fungal Species Isolated from the Rhizoplane of Soybean (*Glycine max*). *Czech Mycology,* **56**: 223-238.
17. Jackson R. B. and Caldwell, M. M. 1991. Kinetic Responses of *Pseudoroegneria* Roots to Localized Soil Enrichment. *Plant Soil*, **138(2)**: 231-238.
18. Jayaswal R. K., Singh, R. and Lee, Y. S. 2003. Influence of Physiological and Environmental Factors on Growth and Sporulation of an Antagonistic Strain of *Trichoderma viride* RSR 7. *Mycobiol.,* **31(1)**: 36-41.
19. Khan, M. O. and Shahzad, S. 2007. Screening of *Trichoderma* Species for Tolerance to Fungicides. *Pak. J. Bot.,* **39(3):** 945-951.
20. Kucuk, C. and Kivanc, M. 2003. Isolation of *Trichoderma* spp. and Determination of Their Antifungal, Biochemical and Physiological Features. *Turk J. Biol.,* **27**: 247-253.
21. Kucuk, C. and Kivanc, M. 2004. *In vitro* Antifungal Activity of Strains of *Trichoderma harzianum*. *Turk J. Biol.,* **28**: 111-115.
22. Larena, I., Melgarejo, P. and De Cal, A. 2002. Production, Survival, and Evaluation of Solid-substrate Inocula of *Penicillium oxalicum*, a Biocontrol Agent against *Fusarium* Wilt of Tomato. *Phytopathol*., **92**: 863-869.
23. Li, F., Kang, S. and Zhang, J. 2004. Interactive Effects of Elevated CO_2, Nitrogen and Drought on Leaf Area, Stomatal Conductance, and Evapotranspiration of Wheat. *Agricult. Water Management,* **67**: 221–233.
24. Lumsden, R. D. and Locke, J. C. 1989. Biological Control of *Pythium ultimum* and *Rhizoctonia solani* Damping-off with *Gliocladium virens* in Soil Less Mix. *Phytopathol.,* **79**: 361-366.
25. Malik, G. and Dawar, S. 2003. Biological Control of Root Infecting Fungi with *Trichoderma harzianum*. *Pak. J. Bot.,* **35**; 971-975.
26. Masangkay, R. F., Paulitz, T. C., Hallett, S. G. and Watson, A. K. 2000. Solid Substrate Production of *Alternaria alternata* f. sp. *sphenocleae* Conidia. *Bio-Con. Sci. Technol.,* **10:** 399-409.
27. Melo, I. S. and Faull, J. I. 2000. Parasitism of *Rhizoctonia solani* by Strains of *Trichoderma* spp. *Scientia Agricola.,* **57**: 55-59.
28. Mev, A. K. and Meena, R. L. 2003. Mass Multiplication of *Trichoderma harzianum* for Biocontrol of Rhizome Rot of Ginger. *J. Phytopathol. Res.,* **16(1):** 89-92.
29. Naima, K., Brahim, E., Latifa, L. and Abdallah, O. 2004. Effect of Nitrogen Fertilizers and *Trichoderma harzianum* on *Sclerotium rolfsii. Agronomie,* **24**: 281-288.
30. Pandey, K. K. 2009. Evaluation of Different Agricultural Based Substrate for Mass Multiplication of *Trichoderma viride*. *Indian Phytopathol*., **62(4):** 530-532.
31. Pfirter, H.A., Guntli, D., Ruess, M. and Defago, G. 1999. Preservation, Mass Production and Storage of *Stagonospora convolvuli*, a Bioherbicide Candidate for Field Bindweed (*Convolvulus arvensis*). *Biol. Control,* **44**: 437-47.
32. Prasad, R. D. and Rangeshwaran, R. 2000a. Shelf Life and Bioefficacy of *Trichoderma harzianum* Formulated in Various Carrier Materials. *Plant Dis. Res.,* **15(1):** 38-42.

[DOR: 20.1001.1.16807073.2014.16.4.19.9]

33. Prasad, R. D. and Rangeshwaran, R. 2000b. Effect of Soil Application of a Granular Formulation of *Trichoderma harzianum* on *Rhizoctonia solani* Incited Seed Rot and Damping-off of Chickpea. *J. Mycol. Plant Pathol.,* **30(2):** 216-220.
34. Prasad, R. D. and Rangeshwaran, R. 2000c. An Improved Medium for Mass Production of the Biocontrol fungus *Trichoderma harzianum. J. Mycol. Plant Pathol.,* **30(2):** 233-235.
35. Prasad, R. D., Rangeshwaran, R., Anuroop, C. P. and Phanikumar, P. R. 2002a. Bioefficacy and Shelf Life of Conidial and *chlamydospore* Formulations of *Trichoderma harzianum* Rifai. *J. Biol. Control.,* **16(2):** 145-148.
36. Prasad, R. D., Rangeshwaran, R. and Sunanda, C. R. 2002b. Jaggery: An Easily Available Alternative to Molasses for Mass Production of *Trichoderma harzianum. Plant Dis. Res.,* **17:** 363–365.
37. Rettinassababady, C. and Ramadoss, N. 2000. Effect of Different Substrates on the Growth and Sporulation of *Trichderma viride* Native Isolates. *Agricult. Sci. Digestion,* **20(3):** 150-152.
38. Rini, C. R. and Sulochana, K. K. 2007. Substrate Evaluation for Multiplication of *Trichoderma* spp. *J. Tropical Agricult.,* **45(1-2):** 58–60.
39. Saju, K. A., Anandaraj, M. and Sharma, Y. R. 2002. On Farm Production of *Trichoderma harzianum* Using Organic Matter. *Indian Phytopathol.,* **55**: 277–281.
40. Saleem, A., Hamid, K., Tariq, A. H. and Jamil, F. F. 2000. Chemical Control of Root and Collar Rot of Chilies. *Pak. J. Phytopathol.,* **12(1)**: 1-5.
41. Sangle, U. R., Bambawale, O. M., Nasim, A. and Singh, S. K. 2003. Substrate and Temperature Requirements for Sporulation of Sub-tropical isolates of *Trichoderma* spp. *Annals Plant Protect. Sci.,* **11(2)**: 192-195.
42. Seyis, I. and Aksoz, N. 2005. Effect of Carbon and Nitrogen Sources on Xylanase Production by *Trichoderma harzianum* 1073 D3. *Int. Biodeter. Biodegr*., **55**: 115-119.
43. Sharma, S., Aggarwal, A., Parkash, V. and Sharma, D. 2005. Mass Production of VAM Fungi Using Different Substrates and Hosts. *J. Mycopathol. Res.,* **43(1):** 51-56.
44. Sharma, S. K., Kalra, K. L. and Kocher, G. S. 2004. Fermentation of Enzymatic Hydrolysate of Sunflower Hulls for Ethanol Production and Its Scale up. *Biomass Bioenergy,* **27**: 399-402.
45. Somasegaran, P. and Hoben, H. J. 1985. *Methods in Legume-rhizobium Technology.* University of Hawaii, NiFTAL Project, Paia.
46. Syahiddin, D. S. 2007. Spore Production of Biocontrol Agent *Trichoderma harzianum*: Effect of C/N Ratio and Glucose Concentration. *J. Rekayasa Kimia dan Lingkungan,* **6(1):** 35-40.
47. Tarek, A. and Moussa, A. 2002. Studies on Biological Control of Sugar Beet Pathogen *Rhizoctonia solani* Kühn. *J. Biol. Sci.,* **2(12):** 800-804.
48. Waksman, S. A. and Fred, E. B. 1922. A Tentative Outline of the Plate Method for Determining the Number of Micro-organisms in the Soil. *Soil Sci.*, **14(1):** 27-28.
49. Younis M., Khalid, M., Rashid, A. and Ashiq, A. 2004. Effect of Carbon, Nitrogen Sources and Ascorbic Acid on the Colony Growth and Acervulus Production of *Pestalotia psydii. Int. J. Agricult. Biol.,* **6**: 1110–1112.

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اثر بستره های آلی مختلف و کربن و نیتروژن روی رشد و ماندگاری انباری *Trichoderma Harzianum*

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چکیده

به منظور تکثیر *Trichoderma harzianum*، تعداد نه بستره آلی شامل دانه برنج، دانه سورگم، دانه گندم، دانه ارزن، کاه گندم، سبوس برنج، کود گاوی،خاک اره، و کود مرغی به کار گرفته شد.از میان آنها، دانه سورگوم و سپس دانه ارزن بهترین بستره بودند در حالیکه کود مرغی ظاهرا از همه نامناسب تر بود ولی دانه برنج، دانه گندم ، کاه گندم و سبوس برنج نسبتا خوب بودند. بهترین منبع کربن سوکروز بود و بیشترین رشد کلنی *T. harzianum* را روی تشتک آگار Czapek فراهم کرد. نیز، چنین می نمود که نیترات آمونیوم در حد ۳۰۰۰ قسمت در ملیون مناسب ترین منبع نیتروژن بود که بالاترین رشد کلنی و فراوانی کنیدیوم ها را فراهم آورد. کار برد همزمان سوکروز در حد ۳۰۰۰۰ قسمت در ملیون به عنوان منبع کربن و نیترات آمونیوم در حد ۳۰۰۰ قسمت در ملیون به عنوان مبنع نیتروژن رشد میسلیوم و تولید کنیدیوم ها توسط *T. harzianum* را به طور معنی داری در کاه گندم، سبوس برنج و دانه ارزن فزود، در حالیکه در مورد دانه سورگوم و دانه برنج، اضافه کردن منابع کربن و نیتروژن اثرات منفی روی هاگ آوری *T. harzianum*داشت. پژوهش انجام شده روی ماندگاری انباری مایه های تکثیر شده روی بستره های مختلف نشان داد که جمعیت*T. harzianum* روی همه بستره ها، طی ۶۰ تا ۷۵ روز خواباندن به حداکثر رسید و بعد از آن به تدریج رو به کاهش گذاشت.با اینهمه، حتی بعد از ۳۳۰ روز، جمعیت مشاهده شده بیشترازجمعیت اولیه(روز صفر)بود.با گذشت ۳۴۵ تا ۳۶۰ روز، شمارجمعیت از جمعیت روز صفر کمتر شد.

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