ISSN 1682-296X (Print)
ISSN 1682-2978 (Online)

Bio Technology

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Biotechnology 5 (3): 280-283, 2006
ISSN 1682-296X

The Growth Kinetic of *Trichoderma harzianum* G-(432) in Green House Soil of Tataristan (Russia)

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Abstract: Presently it has been observed that every year the preparation of arsenical microorganisms are used in agriculture for the protection of plants. Although, it does not always provide positive results. Owing to the lack of knowledge about the kinetic of microbial population and the relationship between microbes in natural environment. Therefore the basic microorganisms that posses the useful characteristics were studied in laboratory. The pure cultures were isolated in the laboratory and their inter relationship with other microorganisms was studied in the green house substrate. The results of a single culture in laboratory showed high performance of growth and antagonestic activity, which is not the same as was found in normal soil.

Key words: *Trichoderma harzianum*, growth kinetic, green house soil

INTRODUCTION

The genus *Trichoderma* an anamorphic fungi was introduced almost 200 years ago. It was isolated primarily from soil and decomposing organic material. Trichoderma is ubiquitous in its distribution and is relatively easy to isolate and culture. In addition isolates grow rapidly on different substrates. Trichoderma produces metabolites that demonstrate antibiotic and mycoparasitic activity against a wide range of phyto pathogens. Mycoparasitic activity and antibiotic production in Trichoderma first demonstrated by Weindling (1932 and 1934) and many other modern biotechnical application of this fungi as biocontrol agent against plant pathogen and viral vectors (Chet, 1987; Chet and Inbar, 1994). Most species of this genus grow rapidly in artificial culture and produces large number of small green or white condia from conidiogenous cells situated at the end of widely branched conidiophores. To get constant positive results from inoculating soil microorganism, it is necessary to know about the additional knowledge of vital activities of microorganisms in concreate ecotypes or ecoclines at large. The am of this work to study the growth kinetic of natural population of Trichoderma harzianum G-432 in green house substrate depend upon basic requirement with ecotopic and anthropogenic factors. Trichoderma harzianum G-432 posses high antagonestic activity against the phytopathogens and there is no any phytotoxcity even to use to get trichodermin (Zakharova *et al*., 1991).

MATERIALS AND METHODS

Trichoderma harzianum G-432 was selected and isolated from vegetable soil of green house of Tataristan and studied in collaborated department of Microbiology, Kazan State University Tataristan, Russia. The stability of culture in soil environment occur many years and there is no effect on the viability and basic physiological characteristics of *Trichoderma* (Kojivin, 1989).

To get the trichodermin, fungi was grown in the potato dextrose broth medium and in the liquid food waste factory of Tataristan.

Experiment was carried out in the laboratory microecosystem on fresh green house substrate (Artificially composed from animal fertilizer and sand). Then the NPK fertilizer (nitrogen, phophrous, potassium) 0.25 g/100 g of green house soil and fungicide fundazol 0.03 g/100 g of green house soil were mixed. After that the required moisture (60% as a whole humidity) and temperature 18-20°C were maintained. *Trichoderma harzianum* G-432 was inoculated in green house substrate in different forms, as conidia adsorbed on barley seeds, spore suspension, chlamydospore suspension and mycelium in liquid molasses medium (Litvinova *et al*., 1991). Then the chaipeka agar medium was prepared with antibiotic streptomycin (100 mg mL^{-1}) and green house substrate samples were taken and inoculated in to the medium. All experiment were repeated three times, results were calculated by formula as $COU \times 10^4$/ g of soil. Where C = colony, O = observation and U = unit.

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RESULT AND DISCUSSION

The present result on growth kinetics of *Trichoderma harzianum* G-432 reveals that it is depended on the form of inoculation. The maximum viability was observed in adsorbed conidia on food substrate (Fig. 1, Table 1). The total count of viable prapagules in substrates increased at first for 20 days and later declined sharply in 40 days and then again increased in 60 days, similar results were observed in spore growth of *Trichoderma harzianum* in soil. it is probably an important character in quick colonization of ecological species and phenomenon plays a significant role in the life of soil microgranisms (Badyi, 1987). Mirshenik (1988) suggested that inoculation increase viability of microorganism possibly because of the hydrolysed organic matter that attract exofermenter microorganisms. It appears that in microecosystem in laboratory with compost or green house soil the organisms with r-strategic life show high level of population (Kojevin, 1989) it seems that *Trichoderma harzianum* is also r-strategic. Our results concur with that of Penekof (1992). Who suggested that *Trichoderma harzianum* is r-strategic. Introducing chlamydospore also manifest highly viable propagules on early and late stage of development. Where as some show a slight inhibition of vital activity at 20th and 30th day of its growth.

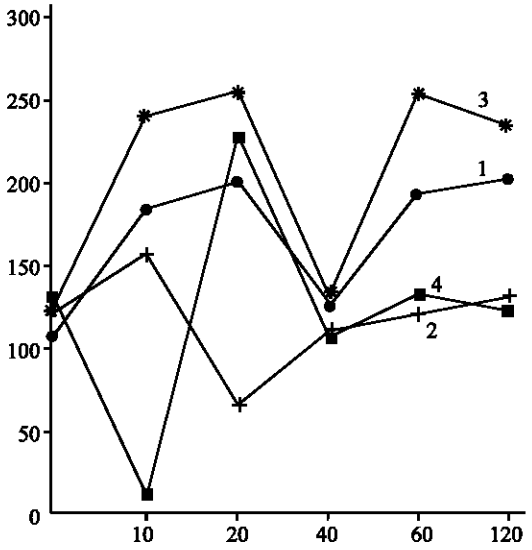

Fig. 1: Effect of mineral fertilization on growth dynamic of G-432 of green house soil
1- Absorbed condia in controlled micro ecosystem. 2- Chalmydospore in controlled micro ecosystem. 3- Absorbed conidia in micro ecosystem with NPK (Nitrogen, Phosphorous, potassium) fertilizer in concentration of 0.25 g/100 g of green house soil. 4- Chlamydospore in micro ecosystem with NPK fertilizer

Table 1: Growth dynamic of G-432 culture of green house soil. Calculated COU (Colony Observing Unit) on chaipaka agar media (COU×10^4 g^{-1} of green house soil)

	Days					
Types of substrate and different forms of *trichoderma harzianum*	0	10	20	40	60	120
1. Adsorbed conidia on barley seeds, shows, No. of colonies	105	180	200	120	180	200
2. Chlamydospore suspension in molasses yeast media, No. of colonies	120	150	55	102	105	120
3. Spore suspension in water, No. of colonies	56	145	55	120	45	21
4. Fungal myscelium, No. of colonies	55	153	105	55	57	20

Table 2: Effect of fundazol on growth dynamic of G-432 culture of green house soil

	Days					
Effect of Fundazol on different forms of culture of *trichoderma harzianum*	0	10	20	40	60	120
1. Adsorbed conidia in control micro ecosystem. No. of colonies	110	175	200	120	195	200
2. Chlamydospore in control micro ecosystem, No. of colonies	120	150	56	110	115	120
3. Adsorbed conidia in mciro ecosystem with fundazol (0.03 g/100 g of green house soil, No. of colonies	60	90	225	75	80	110
4. Chlamydospore in micro ecosystem with fundazol, No. of colonies	20	240	45	120	20	50

Table 3: Dynamic result of G-432 culture depends on integral effect of ecotopic factor (60 and 80 % moisture) and anthropogenic factor (Fundazol in concentration of 0.03 g /100 g of green house soil

	Days					
Integral effect of ecotopic and anthropogenic factor on different forms of culture of *trichoderma harzianum*	0	10	20	40	60	120
1. Adsorbed conidia in micro ecosystem after 60% moisture with fundazol, No. of colonies	40	88	224	80	90	130
2. Chlamydospore in micro ecosystem after 60% moisture with fundazol, No. of colonies	24	240	240	124	32	40
3. Adsorbed conidia in mciro ecosystem after 80% moisture with fundazol, No. of colonies	200	394	316	124	105	200
4. Chlamydospore in micro ecosystem after 80% moisture with fundazol, No. of colonies	200	424	100	200	340	300

Table 4: Dynamic result of G-432 culture depends on integral effect of ecotopic (60, 80% moisture) and anthropogenic factor (NPK fertilizer 0.25 g/100) of green house soil

Integral effect of ecotopic and anthropogenic factor on different forms of culture of *trichoderma harzianum*	Days					
	0	10	20	40	60	120
1. Adsorbed conidia in micro ecosystem after 60% moisture with fertilizer, No. of colonies	124	240	264	124	248	240
2. Chlamydospore in micro ecosystem after 60% moisture with fertilizer, No. of colonies	132	24	224	108	133	116
3. Adsorbed conidia in mciro ecosystem after 80% moisture with fertilizer, No. of colonies	140	340	480	280	456	457
4. Chlamydospore in micro ecosystem after 80% moisture with fertilizer, No. of colonies	124	300	148	200	180	380

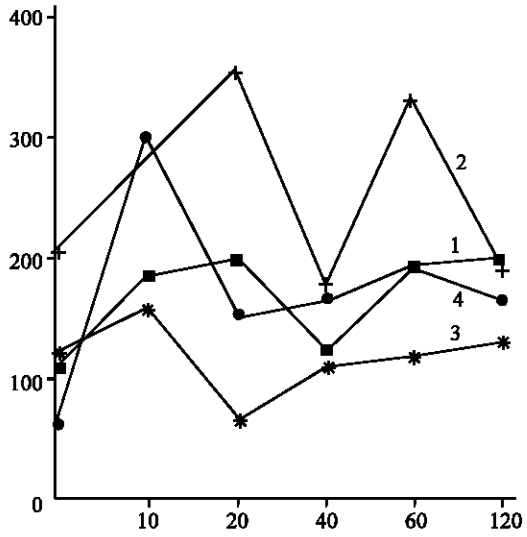

Fig. 2: Dynamic result of G-432 culture of green house upon the ecotopic factor (60-80% moisture) 1- Adsorbed conidia in micro ecosystem after 60% moisture. 2- Adsorbed conidia in micro ecosystem after 80% moisture. 3- Chlamydospore in micro ecosystem after 60% moisture. 4- Chlamydospore in micro ecosystem after 80% moisture

Suspention of spore and mycelium in water showed less viability in green house soil condition. The reduction in viability possibly occurred, owing to the fungal sensitiveness that may also cause inhibition in sporulation as a result overall growth appears impeded. The present finding is in agreement with the results mentioned by Papavizas (1985). Who mentioned different sensitivity of chlamydospore, conidia and mycelium of *Trichoderma harzianum*. Further the anthropogenic activity and ecotopic factors of growth kinetic of microorganism were more viable on adsorbed form of conidia and chalmydospore. It is known that the species of Trichoderma posses high tolerance with pesticide in comparing with many other population of microorganisms, (Siketof, 1982; Velikan of and Cidorova, 1988). We studied the sensitivity of different vital forms of this fungi those are the main producers of Trichodermin (Fig. 2; Table 2). The vegetative propagation of plant in Agrophytocenosis require inoculation of mineral fertilizers in order to give additional support to the introduced population. However, there are contradictive results about the effect of different types of mineral fertilizer on the viability of Trichoderma species, (Salina, 1981; Seiketof, 1982). In our experiment, vital activity of adsorbed conidia, was stimulating but the chalmydospores' population reduced in the beginning stage of growth and restore in latter stage (Fig. 1). According to the result 80% humidity seems to be optimal for vital activity of the culture of *Trichoderma harzianum* G-432 (Fig. 2). The positive correlation ® between population of adsorbed conidia and chlamydospore is 0,8 and 0.7. Hence, it appears that the stimulation of vital fungicide. Since *Trichoderma harzianum* G-432 is highly tolerant to pesticides (Seiketof, 1982; Velikonof *et al*., 1988). The combine research study of growth dynamic of micromycetes showed that the growth stimulation take place in moist condition (Table 3). The result of mineral base fertilizer (Table 4) was that the adsorbed conidia population increase in early stage and remained stable in later stage. The maximum viability of *Trichoderma harzianum* suggested the possibility of its cultivation for economic growth and production of sufficient Trichodermin.

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