EFFECT OF DIFFERENT TEMPERATURE ON THE ANTAGONISTIC ACTIVITY OF FUNGAL AND BACTERIAL BIO AGENTS

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Abstract: Antagonistic potential of fungal (*Trichoderma harzianum, Trichoderma viride, Aspergillus niger* & *Penicillium oxalicum*) and bacterial bioagents (*Pseudomonas aeruginosa, Pseudomonas putida* & *Pseudomonas florescens*) was studied against three pathogens *i.e. Fusarium oxysporum, Rhizoctonia solani* and *Pythium ultimum* at four different temperature (20°C, 25°C, 30°C and 35°C). Antagonistic potential of all fungal and bacterial bioagents was found to be significantly influenced by different temperature. With regards to effect of different temperature, among all fungal bioagents, *Trichoderma harzianum* resulted maximum percent inhibition of the pathogens followed by *Trichoderma viride, Aspergillus niger* and *Penicillium oxalicum* at 25°C to 30°C. While as bacterial bioagents, *Pseudomonas fluorescens* exhibited their higher antagonistic potential followed by *Pseudomonas putida* and *Pseudomonas aeruginosa* against all three pathogens at highest temperature *i.e.* 35°C.

Keywords: Biological control, Temperature, Fungal & Bacterial bioagents

INTRODUCTION

Soil borne disease caused by *Pythium, Fusarium Sclerotium, Sclerotium, Verticillium, Thielaviopsis* and *Rhizoctonia* inflict serious damage in agriculture crops. Since indiscriminate use of pesticide has done great harm to human, animal, vegetation and environment so use of chemical against these disease is not an effective measure of control, even though , there is also concern about its ill effect on the environment and food quality. Limitation of the use of chemicals have stimulated interest in alternative means of disease suppression such as biological control which could bring about a reasonably good degree of reduction to crop damage by plant pathogens, ensure sustainablity of production, cost effectiveness and healthy ecosystem (Jensen *et al*., 2000). Though biocontrol agents include all the classes or groups of organism existing in an ecosystem, yet, maximum emphasis for developing biocontrol programmes has invariably gone to fungal and bacterial bioagents. The important genera of fungi studied as biocontrol agents are *Trichoderma, Gliocladium, Aspergillus, Chaeotimum, Penicillium, Neurospora, Fusarium (saprophytic), Rhizoctonia, Dactyella, Arthrobotrys, Catenaria, Paecilomyces, Glomus,* etc. Apart from these, a number of bacterial species/strains have been studies for their plant growth promoting activity and biocontrol potential. These include *Agrobacterium, Actinoplanes, Bacillus, Enterobacter, Erwinia, Pseudomonas, Streptomyces,* etc. Among them currently plant growth promoting rhizobacteria fluorescent Pseudomonads are under intensive research because of their natural occurrence, biocontrol potential as well as plant growth promoting activites. (Mishra, *et al.*, 2001). Having realized the concept of harmful effect of pesticide and exploitation of naturally occurring rhizospheric microbiota against soil borne pathogens the present investigation was aimed to study the effect of different temperature on the antagonistic activity of fungal and bacterial bioagents.

MATERIAL AND METHOD

The present investigation was conducted at Department of Plant Pathology, Janta Vedic College, Baraut, Baghpat U.P.

Source of culture

(a) Causal Pathogens: Isolation of Pathogens (*Fusarium oxysporum, Rhizoctonia solani* and *Pythium ultimum)* done in culture media from diseased plant part collected from field.

(b) Antagonistic fungal and bacterial bioagents: Antagonistic fungal bioagents (*T. harzianum T. viride*, *A. niger* & *Penicillium oxalicum*) and bacterial bioagents (*Pseudomonas aeruginosa*, *Pseudomonas putida* & *Pseudomonas fluorescens*) procured from I.G.F.R.I Jhansi.

The efficacy of fungal and bacterial bioagents on radial growth inhibition of test pathogens i.e. *Fusarium oxysporum, Rhizoctonia solani* and *Pythium ultimum* was studied *in vitro*, through dual culture technique at four different temperatures (20°C, 25°C, 30°C and 35°C)

Dual culture technique

The efficacy of fungal and bacterial bioagents on radial growth inhibition of test pathogens *i.e. Fusarium oxysporum, Rhizoctonia solani* and *Pythium ultimum* at different temperature *i.e.* 20°C, 25°C, 30°C and 35°C was studied *in vitro* by dual

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culture technique. Twenty ml sterlized melted PDA was poured in 90 mm diameter petriplate. After solidification mycelial discs having diameter of 5mm were cut from the young culture of fungal bioagents and test fungus with the help of sterilized cork borer. These discs were placed in the Petri plate containing PDA, maintaining the distance of 4cm between the discs of the test fungus. All the Petri plates were incubated for five days at different temperature *i.e.* 20°C, 25°C, 30°C and 35°C. Each treatment had three replications. Radial growth inhibition of test pathogens was measured at an interval of 24h for five days to record different stages of antagonism. The observations on radial growth inhibition of test pathogens *i.e. Fusarium oxysporum*, *Rhizoctonia solani* and *Pythium ultimum* were recorded after 120 hrs. The percent inhibition over check, noted after 5 days of incubation.

The antagonistic potential of *Pseudomonas* on test pathogens was determined by dual culture technique in a different way. Four discs of the test fungus were placed in the periphery of petriplate at equal distance thereafter the blotting paper discs having the diameter of 10mm dipped in bacterial suspension and placed in the center of petriplates. In check, no blotting paper was placed. These petriplate were incubated at different temperature *i.e.* 20°C, 25°C, 30°C and 35°C for 5days. Each treatment was replicated thrice and the percent inhibition over check, noted after 5 days of incubation the observations on radial growth inhibition of test pathogens *i.e. Fusarium oxysporum*, *Rhizoctonia solani* and *Pythium ultimum* were recorded after 120 hrs. The percent inhibition over check, noted after 5 days of incubation was calculated by the following formula (Vincet, 1947).

$$I = \frac{C - T}{C} X\,100$$

Where,
I = Percent Inhibition
C = Colony diameter in check
T =Colony diameter in treated petriplate.

RESULT AND DISCUSSION

The radial growth inhibition of test pathogens was studied on Potato Dextrose Medium at four different temperature *i.e.* 20°C, 25°C, 30°C and 35°C. The observations thus obtained, are presented in Table 1. It is evident from Table 1 that all fungal and bacterial bio agents varied significantly in their antagonistic potential against *Fusarium oxysporum* at different temperatures. Among the fungal isolates, significantly maximum reduction (1.5 to 2.0) in the growth of the test pathogens was resulted due to *Trichoderma harzianum* followed by *Trichoderma viride* at various temperatures. Though, these fungal biocontrol agents acted more effectively at 25°C to 30°C, exhibiting radial growth minimum 1.5 to 1.7 cm of pathogen and maximum 77.56 to 79.5 percent inhibition over control. It was worthy to note that these bioagents did not visualize any significant difference in their efficacy at different temperature when compared from each other. Remaining other fungal isolates *Aspergillus niger* and *Penecillium oxalicum* grew well at 25°C temperature, thereby resulting in maximum reduction 1.8 and 2.0 cm in growth and 69.5 and 72.0 percent inhibition, respectively over control (Table 1).

Of all the species of *Pseudomonas*, *Pseudomonas fluorescens* showed significantly maximum antagonistic effect against *Fusarium oxysporum* at 35°C followed by 30°C temperature, reducing up to 1.5 and 1.8 cm radial growth and 74.8 and 73.6 percent inhibition respectively over control. The effect of other species of *Pseudomonas viz. Pseudomonas aeruginosa* and *Pseudomonas putida* was also found to be statistically at par with *Pseudomonas florescens* in inhibiting the growth at these temperatures 30°C to 35°C. At the lower ranges of temperatures *i.e.* 20°C and 25°C, these isolates exhibited comparatively least efficacy against the pathogens.

The observations presented in Table 2 show the significant effect of temperature on radial growth inhibition of *Rhizoctonia solani* due to all fungal and bacterial bioagents. Of all the fungal bioagents, maximum reduction (1.6 to 1.7) in growth of pathogens was recorded due to *Trichoderma harzianum* at 25°C to 30°C thereby giving 73.2 to 76.6 percent inhibition respectively over control followed by *Trichoderma viride*. While as the *Aspergillus niger* and *Penecillium oxalicum* exhibited their maximum antagonistic activity at 25°C being resulted 69.6 and 66.1 percent inhibition, respectively. As far as bacterial bioagents is concerned, the highest reduction (1.5) in radial growth was resulted due to *Pseudomonas fluorescens* followed by *Pseudomonas putida* and *Pseudomonas aeruginosa* at 35°C. It was interestingly to note that the efficacy of all the bacterial bioagents at this temperature did not show any significant difference when compared with each other.

It is evident from Table 3 that the growth inhibition of *Pythium ultimum* by all fungal and bacterial bioagents was significantly influenced by fluctuating temperature (20°C to 35°C). Among the fungal bioagents *Trichoderma harzianum* showed significantly maximum reduction (1.4) in the growth of the pathogens at 30°C visualising 78.5 percent inhibition over control. Both bioagents of *Trichoderma i.e. Trichoderma harzianum* and *Trichoderma viride* did not differ statistically in their efficacy at varying temperature when compared to one and another. On the other hand *Aspergillus niger* and *Penecillium oxalicum* inhibited maximum growth (1.9 and 2.0) at 25°C and found to be insignificant in their efficacy from one and another. All bacterial bioagents i.e. *Pseudomonas aeruginosa*,

Pseudomonas putida & *Pseudomonas florescens* resulted in their maximum antagonistic potential against *Pythium ultimum* at 35°C exhibiting 1.3 to 1.5 cm radial growth and 69.6 to 73.5 percent inhibition over control Table 3. Though, efficacy of all these bioagents was found to be statistically at par at this temperature. While at other temperatures, these bio agents reflected comparatively less effectiveness in growth inhibition.

The overall effect of temperature on the antagonistic potential of fungal and bacterial bioagents against the pathogens revealed that all fungal bioagents grew well at 25°C to 30°C and bacterial bioagents grew well at 30°C to 35°C. The results clearly indicate that growth inhibition of *Fusarium oxysporum, Rhizoctonia solani* and *Pythium ultimum* due to fungal and bacterial bioagents is significantly influenced by temperature, *T. harzianum* proved to be most effective against these three pathogens. Besides this there are several instances where the efficacy of fungal and bacterial bioagents has been studied for their antagonistic potential against these soilborne pathogens (Tronsomo and Dennis, 1978; Elad *et al.*, 1983 a, b; Upadhyay and Mukhopadhyay 1983;. Suh *et al.,* 1988; Upadhyay and Rai, 1988.; Sindhu *et al.,* 1997; Saikia *et al*.,1998; Kredics *et al*., 2000)

Grodona *et al.* (1997) and Srivastava and Mall (2008) reported the antifungal activity of *T. harzianum* against soil borne pathogens with 50 per cent inhibition of all pathogens. The fast growing *Trichoderma* sp. caused more inhibition of the pathogens probably due to mycoparasitism and competition for nutrients. Harman *et al.* (1980, 1989) had suggested that mycoparasitism was the principal mechanism involved in controlling *Pythium* damping off of pea seeds. Hyphal parasitism by *Trichoderma* sp. was also observed *in vitro* by many workers (Dennis and Webster 1971 a, b; Chet and Baker *et al.*, 1981; Lifshitz *et al.,* 1986; Waghmare, 2005).

Trichoderma species are known to produce a number of antibiotics, such as trichodermin, trichodermol, harzianum A and harzianolide (Simon and Sivasithamparam 1988; Dennis and Webster 1971 a). In another study, Gupta *et al*., (1999) successfully used *Pseudomonas florescens in vitro* against *Macrophomina phaseolina* and *Fusarium oxysporum* and found antifungal activity of the strain. The production of hydrogen cyanic acid and indole acetic acid was also recorded under normal growth condition. In another experiment, Velzhahan *et al*.,(1999) isolated several strain of *Pseudomonas florescence* from the rhizosphere of rice plants and tested against *Rhizoctonia solani* causing sheath blight in rice and were found to be effective in inhibiting the mycelial growth of the pathogens. Therefore, it is concluded from the study that *Trichoderma harzianum* can be exploited for the management of soil borne pathogens in place of fungicide without disturbing the ecological balance.

Table 1: *In vitro* effect of temperatures on radial growth inhibition of *Fusarium oxysporum* due to Fungal and Bacterial bioagents

Fungal and Bacterial bioagents	TEMPERATURES							
	20°C		25°C		30°C		35°C	
	*RG (cm)	Inhibition (%)	*RG (cm)	Inhibition (%)	*RG (cm)	Inhibition (%)	*RG (cm)	Inhibition (%)
T. harzianum	1.9	52.4	1.7	79.5	1.5	77.5	2.0	65.3
T. viride	2.0	51.6	1.7	73.5	1.6	75.6	2.1	63.6
A. niger	2.1	47.5	1.8	72.0	2.0	70.2	2.3	60.8
P. oxalicum	2.5	39.5	2.0	69.5	2.2	67.3	2.5	58.1
P. aeruginosa	3.0	25.8	2.4	63.0	1.9	71.2	1.6	72.0
P. putida	3.1	24.1	2.3	65.0	1.8	72.6	1.6	72.6
P. florescens	2.7	34.6	2.2	66.0	1.8	73.6	1.5	74.8
Control	4.1	00.0	6.6	00.0	5.6	00.0	5.9	00.0
C.D. at p = 0.05	0.26		0.25		0.20		0.27	

Table 2: *In vitro* effect of temperatures on radial growth inhibition of *Rhizoctonia solani* due to Fungal and Bacterial bioagents

Fungal and Bacterial bioagents	TEMPERATURES							
	20°C		25°C		30°C		35°C	
	*RG (cm)	Inhibition (%)	*RG (cm)	Inhibition (%)	*RG (cm)	Inhibition (%)	*RG (cm)	Inhibition (%)
T. harzianum	1.9	56.3	1.7	73.2	1.6	76.6	2.0	64.7
T. viride	1.9	56.3	1.7	73.2	1.7	75.2	2.2	61.1
A. niger	2.1	51.1	2.0	69.6	2.2	66.9	2.2	60.5
P. oxalicum	2.4	45.1	2.2	66.1	2.3	65.5	2.4	57.6
P. aeruginosa	2.6	39.8	3.0	53.5	2.1	68.4	1.7	69.4
P. putida	2.5	42.8	2.9	56.0	2.0	69.9	1.5	72.3
P. florescens	2.3	47.3	2.7	59.0	2.0	70.8	1.5	73.5
Control	4.4	00.0	6.6	00.0	6.8	00.0	5.6	00.0
C.D. at p = 0.05	0.18		0.62		0.22		0.22	

Table 3: *In vitro* effect of temperatures on radial growth inhibition of *Pythium ultimum* due to Fungal and Bacterial bioagents

Fungal and Bacterial bioagents	TEMPERATURES							
	20°C		25°C		30°C		35°C	
	*RG (cm)	Inhibition (%)	*RG (cm)	Inhibition (%)	*RG (cm)	Inhibition (%)	*RG (cm)	Inhibition (%)
T. harzianum	1.9	49.5	1.6	72.1	1.4	78.5	1.9	61.9
T. viride	2.0	46.9	1.7	71.1	1.6	76.5	2.0	60.0
A. niger	2.1	45.2	1.9	68.3	2.2	66.8	2.2	57.4
P. oxalicum	2.3	39.1	2.0	65.5	2.3	65.3	2.6	48.3
P. aeruginosa	2.8	26.9	2.7	59.4	2.1	68.2	1.5	69.6

P. putida	2.6	30.4	2.1	64.4	2.0	70.2	1.4	71.6
P. florescens	2.4	37.3	2.0	66.1	1.9	72.1	1.3	73.5
Control	3.8	00.0	6.0	00.0	6.8	00.0	5.1	00.0
C.D. at p = 0.05	0.24		0.59		0.21		0.28	

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