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The Ability of *Trichoderma harzianum* Isolates in Controlling Rapeseed Sclerotinia Rot in Comparison with the Effects of *Bacillus subtilis* and Benomyl Fungicide

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Abstract: In this investigation, the ability of nine *Trichoderma harzianum* isolates which had been isolated from soil and root rapeseed field were compared to one isolate of *Bacillus subtilis* and 2 per thousand benomyl solution to control rapeseed sclorotinia rot. These isolates had shown good control of *Sclerotinia sclerotiorum* in *in vitro* condition. In this experiment seed of Talaye variety were planted in pots of 15 cm diameter and offer seedling emergence they were thined to one seedling. The plants were maintained at 20±2°C and 90% R.H until flowerbuds appeared. After flowerbud appearance the plants were inoculated with 7mm diameter mycelium disk put on low, middle and uppear leaf of each plant. The controls were treated with 10^7 mL^{-1} of spores of the antagonist fungus, a 10^8 mL^{-1} bacterial suspension and with a 2 per thousand benomyl solution separated at 2 different times. One set of control plants were treated 3 days ahead of inoculation and another set were treated on the same day. Assessment of the inhibitory effect of treatment on fungal pathogen were done 3 days after inoculation of the leaves by measuring the diameter of the lesions produced and comparing with sterile water control. The results obtained show that: there is a significant difference between the biological treatment agents. The shortest diameter of the lesions on inoculated plants belonged to T_1 and T_3 isolates of *T. harzianum* and also to *B. subtilis*. There was no significant difference between T_4 and *B. subtilis* in other word, use of any of these three would have same effect. The two isolates T_1 and T_9 of *T. harzianum* and *B. subtilis* when used at the same time that plants are inoculated with the pathogen would have the most effect. Comparing the bonomyl effect with that of biological agents, it had significantly superior effect. The position of the leaf on the plant had not significant effect.

Key words: Agents, antibiosis, application time, biocontrol

INTRODUCTION

Many diseases, depending on the environmental conditions, have been identified in different parts of the world that threaten rapeseed production every year and sometimes lead to the high economic losses (Azizi *et al*., 1990; Saadat, 1981). Among all the characterized diseases of rapeseed, the rapeseed *Sclerotinia* stem rot is particularly important. This disease is caused by the fungus *Sclerotinia sclerotiorum,* known as a polyphagous pathogen. In Canada, the fungus can infect over one hundred plant species (Bardin *et al*., 2001). In the recent years, the damaging nature of S. *sclerotiorum* has encouraged some researchers to employ the fungus as a biological agent to control weeds in the fields. Infection of plants by the fungus takes place in 2 different ways. In his research on sunflower crown rot, attributed the primary infection to the myceliogenic germination of sclerotic (Purdy, 1979). With an emphasis on ascospor's role in the infection of cruciferaceous plants, stated that the existence of dead and withered petals were necessary for initiation of the infection (Williams *et al*., 1996). Accordingly, the ascospores use the dead parts of the flower as a primary source to feed on. The high damage caused by S. *sclerotic* (Purdy, 1979) *orum* on various plants in different areas, is the reason for using different methods to control this fungus. Swtated that flooding a field for 26-31 days could be effective in controlling the disease (Moor, 1949). Steadman *et al*. (1974) showed that decreasing the plant population intensity (thinning) was also effective in the disease control. Saur (1983) Showed effectiveness of the fungicide 'Vincozolin' on the disease control if used simultaneously with the first release of the ascospores. In another case different biological agents

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have been used to biologically control the disease. Noticed that some antagonistic fungi such as *Conithyrium minitants* and isolates of *Trichoderma* sp. caused the death of S. *sclerotiorum* sclerotia (Campbell, 1947; Jones *et al*., 1969). Studies (Hoes *et al*., 1975) showed that the species C. *minatans, T viride*, *Talaromyces jlavus*, *Sporidesmium sclerotiorum* and *Gliocladium catenulatturs*, introduced into soil in sunflower farm, parasitized S. *sclerotiorum* and reduced its population by 90%.

The fungal species *C. minitans*, *T harzianum*, *T virid*, *G. roseum*, *G. virens* and *paecilomyces lilacinus* had an antagonistic effect on the sclerotia of *S. sclerotiorum* and among these species, *G. virens* and *C. mintans* were the most effective ones in parasitizing the sclerotia and reducing their viability (Whipps and Budge, 1990). A few studies have so far been done on the biological control of *S. sclerotiorum* using bacteria. Examined the effects of 10 bacterial isolates from bean flowering buds on the sclerotinia rot disease and showed that the treatment of plants with *Erwinia herbicola* strain Bland *Bacillus polymyxa* strain B8 caused 40% reduction in Preparation of antagonistic fungi (Yuen *et al*., 1991).

MATERIALS AND METHODS

Isolation of 9 fungi isolates from roots of rapeseed plant and soils of rapeseed fields. The antagonistic ability of these isolates and of 5 other isolates, prepared from the sclerotia of S. *sclerotiorum* during culturing on medium, was studied on PDA medium through dual culture. Eight isolates with more antagonistic activity (Table 1) were chosen and under greenhouse condition their antagonistic activities, to control rapeseed sclerotinia rot, were compared to that of a *Bacillus subtilis* isolate was been gettin from Evin Plant Pests and Diseases Research Institute, Tehran and of 0.2% Benomyl solution.

Ascospores' germination of S. *sclerotiorum*. Using four strains of *Bacillus* sp. in soil decreased production of the apotesium about 36% and increased the rapeseed yield by 11-15% (Lyth *et al*., 1993) reported that spraying pea leaves with the suspension, prepared from Alfa-87 A isolate of *Bacillus ceraus* significantly reduced the damage caused by the contaminant S. *sclerotiorum* ascospores (Huang *et al*., 1993). Besides; further researches, carried out by them, made clear that the metabolites produced by *B. cereus* inhibited the ascospores' germination and hyphal growth of S. *sclerotiorum.* Teo (cited in 2), studying on the bean *Sclerotinia* rot, showed that spraying bean leaves with the *Bacillus subtilis* suspension for 2 consecutive years considerably reduced the damage caused by the disease.

Table 1: Fungi isolates used in biologicale control of Sclerotinia stem rot

Isolates codes	T_1	T_3	T_5	T_7	T_8	T_{10}	T_{11}	T_{13}
Isolation from Seed source	Soil	Roots	Soil	Soil	Roots	Soil	Soil	Soil

Table 2: Isolated sclerotia from different host plants

Isolates codes	SR_1	SR_3	SS_1	SS_4
Host plant	Rapeseed	Rapeseed	Sunflower	Sunflower

In this study the effectiveness of different bacterial isolates, specially isolates of *B. subtilis* in controlling rapeseed *Sclerotinia* rot, in comparison with *Trichoderam harzianum* and the fungicide, Benomyl was assessed.

The rapeseed variety 'Talayeh' was used in greenhouse experiments in 2002. Five disinfected seeds were sown in 25 em-diameter pots containing sterile soil (pH = 6.8, Ec = 1.4). The pots were placed and grown in greenhouse at 22±2 DC with 90% humidity. Then one of the seedlings per pot, showing a more healthy appearance was selected for incubation with *S. sclerotiorum* and the remaining 2 plants were eliminated. The selected plants were inoculated with SR_1 isolate of *Sclerotiorum* after flower bud appearance.

Inoculum preparation and host plant inoculation: Among the 4 different isolates of S. *sclerotiorum*, from 2 plant species (Table 2), SR_1 which had shown more virulence in pathogenicity test on the variety 'Talayeh' was chosen for inoculation.

The method, established by Inglis and Boland (1992) was used for inoculation. Accordingly, the plants were inoculated with a 7 mm-diameter mycelium disk by placing on lower, middle and upper leaves of each plant. Control plants were inoculated with a disk, prepared from the culture medium without mycelium. The medium contained 0.5% petal extract of rapeseed and 4% agar. The inoculated plants were incubated at 22±2 DC and 90% humidity.

Control treatment of inoculated plants with *S. sclerotiorum* was done with 10^8 per mL bacterial suspension, 10^7 spores per ml antagonistic fungus and 2 per thousand (0.2%) Benomyl solution at two different stages. One group of plants was treated 3 days ahead of inoculation and other group on the same day as inoculation was done. Both groups of plants were incubated at 22±2 DC and 90% humidity and the inhibitory effect on fungal pathogen was assessed 3 days after inoculating the leaves. The assessment was done through measuring the diameter of the resultant lesions and comparing with the control, sterile water-inoculated plants. The experiment was done, as a factorial, in a completely random blocks design with 12 treatments and 3 blocks. The statistical analysis of this biological control experiment was performed using Spss 9.0, Exell and MSTAT-C software.

RESULTS AND DISCUSSION

Comparison of the lesion diameters on the leaves of *S. sclerotiorum*-inoculated rape-seed plants (cultivar 'Talaye') with that of the biological agents-Benomyl-and non-treated (control) plants revealed significant differences among the plants (Table 3). In terms of the lesion diameters, significant differences were also observed depending on what time, simultaneously as inoculation with *S. sclerotiorum* or three days pre-inoculation, the biocontrol agents were applied. Based on the comparison of the lesion diameter mean values,

The simultaneous application of the biological control agents with the inoculation was more effective in controlling the *Sclerotinia* rot disease on rapeseed. Besides, there appeared to be a significant correlation between the type of the biological control agents and time of their application. In other words, the isolates of biological control agents, depending when they are applied, may be either curative (if used at the time of inocultaion) or preventive. The isolates T_3 and T_{13} of *Trichoderma harzianum* and *Bacillus subtilis* also were most effective if applied at the time of inoculation with *S. sclerotiorum*, whereas the isolates T_5 and T_7 appeared to be more effective when applied three days before the inoculation. Compared to the biological control agents, the fungicide Benomyl was the most effective treatment, regardless of being applied at the time of inoculation or three days before that.

As can be seen on Table 3, the position of leaf on the plant did not show either a significant lesion diameter effect or any significant interaction with the other variants (type of the control agents or the application time). However, the infection rates on the lower leaves were generally less than those on the middle and upper leaves. This could be due to the thickness of the lower leaves or their cuticles.

The non-treated control plants with 23.33, as the mean value of lesion diameters, were significantly different from the ones treated by the biological control agents which had 14.23 as the mean value of lesion diameter. This meant that any treatment, by the fungicide or any of the biological control agents (bacterial or fungal), compared to non-treated control, was effective in the disease control.

The fungicide Benomyl with a related mean lesion size of 0.22 was more effective in the disease control in comparison with the biological control treatments, with 14.23 as the mean lesion size.

In terms the disease control ability, there was no significant difference between *T. harzianum* isolates T_3 and T_{13} and the isolate of *B. subtilis*.

Table 3: Analysis of variance for data obtained for S seclerostiorum control by different treatments

Treatments	Degrees of freedom	Mean of squares
Replication	2	803.125**
Control agents	10	731.084ns
Time versus control agents	1	50.29**
Control agents versus check	1	41.56**
Benomyl versus biocontrol agents	1	107.32**
Bacterial isolate versus fungus isolates	1	0.59
Among fungi isolates	7	895.5412
Time of application	1	721.237
Leaf positions	2	399.257
Leaf positions × control agents	20	30.25**
Time × control agents	10	753.342**
Time ×Leaf position	2	45.899ns
Time× control agents × Leaf position	20	44.034ns
Error	130	41.265

Furthermore, microscopic studies on mechanism of the destructive effects of the bacterial isolates on the *S. sclerotiorum* mycelia indicated that the isolates T_3 and T_{13} were able to control the disease via vaculization and lysis of the pathogen hyphal cells. In other words, these isolates produce various metabolites, which have an antibiosis effect on the pathogenic fungus. These results are in accordance with and supported by, the other reports (Lyth *et al*., 1993; Line *et al*., 1993). Further studies on the dual culture of the fungi isolates with *S. sclerotiorum* on nutrient agar (PDA) and Czapek-Dox Agar (CZDA) showed that the bacterial isolates could also inhibit the production of hyphal branch as well as its development on the media.

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