Environment & Ecology 35 (3A) : 1975—1978, July—September 2017
Website: environmentandecology.com ISSN 0970-0420

Antagonistic Activity of Some Selected Strains of Fungus and Bacteria Against the Causal Agents of Charcoal Rot (*Macrophomina phaseolina*) in Fenugreek and Their Application in Green House Conditions

Priyanka Meena, V. K. Gaur, M. K. Meena, Ashok Meena, Veer Singh

Received 21 October 2016; Accepted 22 November 2016; Published online 13 December 2016

Abstract *Trichoderma* and other species isolated from infested soil were tested for their antagonism to *Macrophomina phaseolina* on Czaper's dox agar medium in petri dishes. The antagonistic potential of each antagonist was studied. A 5 mm diameter disc of antagonist was placed individually at one end of the petri dish containing Czapek's dox agar medium and just opposite to that a 5 mm diameter disc of the pathogen was placed. Three replications were maintained for each antagonist. In control, the pathogen alone was inoculated. The petri dishes were incubated at 28 ± 10C for seven days in a BOD incubator and observations were recorded. Microorganisms which inhibited growth of *M. phaseolina* were termed as antagonistic microorganisms. The antagonistic reaction of isolated fungi against pathogen was classified as mild, intermediate and antagonist. Four fungi *Aspergillus niger, Trichoderma atroviride, T. harzianum, T. viride* gave distinct antagonistic reactions, showing stunting of *Macrophomina phaseolina* colony and a clear zone of inhibition between colonies of antagonist and the pathogen. *Trichoderma harzianum* was most effective among all antagonists in reducing charcol rot of fenugreek followed by *Trichoderma viride, Trichoderma atroviride* and *Aspergillus niger.*

Keywords Antagonist, Agar medium, *Macrophomina phaseolina.*

P. Meena, V. K. Gaur, A. Meena
Department of Plant Pathology,
College of Agriculture,
Swami Keshwanand Rajasthan Agricultural University,
Bikaner, India

V. Singh
Department of Entomology,
College of Agriculture,
Swami Keshwanand Rajasthan Agricultural University,
Bikaner, India

M. K. Meena*
AICRP on Niger,
Regional Research and Technology
Transfer Station (OUAT), Semiliguda,
Sunabeda, Koraput, Odisha 763002, India
e-mail : manojmeena22@gmail.com
*Correspondence

Introduction

The pathogen being soil-borne and its propagules distributed randomly in soil is difficult to be controlled by fungicide. Moreover, the fungicides are effective only on the active metabolic stage of the propagules and not on resting structure. Soil application of fungicides is an expansive and deleterious to non target microflora. Biological agents could be an important

Table 1. Effect of microorganisms on the growth of *Macrophomina phaseolina* on Czapek's dox agar medium.

Sl. No.	Microorganisms	Microorganism inhibiting growth of *Macrophomina phaseolina* (%)
1	*Alternaria alternata*	47.65 (43.65)
2	*Aspergillus clavatus*	42.89 (40.91)
3	*Aspergillus flavus*	43.71 (41.38)
4	*Aspergillus japonicus*	39.49 (38.93)
5	*Aspergillus nidulans*	37.64 (37.83)
6	*Aspergillus niger*	58.33 (49.79)
7	*Chaetomium globosum*	45.5 (42.41)
8	*Cochliobolus lunatus*	33.17 (35.16)
9	*Curvularia lunata*	44.36 (41.76)
10	*Drechslera halodes*	41.94 (40.36)
11	*Fusarium oxysporum*	40.55 (39.55)
12	*Penicillium aurantiogriseum*	24.47 (29.60)
13	*Penicillium javanicum*	20.16 (26.67)
14	*Rhizoctonia solani*	38.35 (38.25)
15	*Rhizopus stolonifer*	44.72 (41.96)
16	*Trichoderma atroviride*	58.03 (49.62)
17	*Trichoderma harzianum*	69.25 (56.32)
18	*Trichoderma viride*	63.60 (52.89)

component in the control of *M. phaseolina* if effective and reliable formulations were redily available and could be integrated with chemical fungicides [1]. Biological control has been considered as an alternative to control this disease since it is highly selective method lasting over time [2]. Numerous researches have been focused on searching and selecting antagonist microorganisms on diverse soil pathogens. Among the most used are bacterias like *Bacillus, Pseudomonas*, and *Streptomyces*, fungi of the *Trichoderma, Penicillium, Gliocladium, Aspergillus, Rhizopus* genera. These microorganisms, natural inhabitants of diverse substrates, in laboratory tests (*in vitro*) as well as in the greenhouse and field, have demonstrated antagonistic activity on a wide ranging group of pathogens such as *Sclerotium rolfsii, S. cepivorum, Rhizoctonia solani, Pythium ultimum, Phytophthora parasitica,* and *M. phaseolina* [3]. Biological control has become a critical component of plant disease management and it is a practical and safe approach in various crops. Bioprotectants provide unique opportunity for crop production, since they grow, proliferate, colonize and protect the newly-formed plant parts to which they were not initially applied. Microorganisms indicating such properties were utilized for possible biological control of charcoal rot by addition of the cultures of antagonists to infected soil.

The disease is of wide occurrence where climatic conditions are dry and temperature remains high. Therefore, it was very important to undertake the studies to find out the cause for disease development and its suitable management measures. Thus it was obvious to study rhizosphere mycoflora and their antagonistic reactions to pathogen *Macrophomina phoseolina* (Tassi) Goid. ultimately leading to biological management of charcol rot disease.

(The author is very much grateful to Swami Keshwanand Rajasthan Agricultural University, Bikaner for providing necessary funds and resources for carrying out this research work).

Materials dmd Methods

Trichoderma and other species isolated from infested soil were tested for their antagonism to *Macrophomina phaseolina* on Czapek's dox agar medium (Agar-agar 15.0 g, sodium nitrate 2.0 g, di potassium hydrogen phosfate 1.0 g, potassium chloride 0.50 g, magnesium sulfate 0.50 g, ferrous sulfate 0.01 g, sucrose 30.0 g, distilled water 1000 ml, in petri dishes [4].

The antagonistic potential of each antagonist was studied. A 5 mm diameter disc of antagonist was placed individually at one end of the petri dish containing Czapek's dox agar medium and just opposite to that a 5 mm diameter disc of the pathogen was placed. Three replications were maintained for each antagonist. In control, the pathogen alone was inoculated. The petri dishes were incubated at 28 ± 10C for seven days in a BOD incubator and observations wre recorded. Microorganisms which inhibited growth of *M. phaseolina* in above method were termed as antagonistic microorganisms [5].

Radial growth of *M. phaseolina* was recorded and inhibition percent was calculated using formula

Table 2. Effect of antagonistic microorganisms to minimize charcol rot of fenugreek in green house conditions. Sem 2.50, CD 5% 7.70, CD 1% 10.79, CV (%) 9.0, General mean 47.01.

Antagonist	Mean population/g soil (pre-sowing) *M. phaseolina*	Mean population/g soil (pre-sowing) Antagonist	Mean population/g soil (after 100% mortality in control) *M. phaseolina*	Mean population/g soil (after 100% mortality in control) Antagonist	Disease incidence (%)	Disease control (%)
Control (Soil infected with *M. phaseolina* alone)	1685.65	–	6852.36	–	100.00 (90.00)	
Aspergillus niger (Soil infected with *M. phaseolina + Aspergillus niger*)	1220.87	2865.18	5125.87	14854	63.33 (52.78)	36.67
T. atroviride (Soil infected with *M. phaseolina + T. atroviride*)	1454.65	3557.36	4165.8	11554.25	33.33 (35.22)	66.67
T. harzianum (Soil infected with *M. phaseolina + T. harzinum*)	1690.87	440.20	3029.41	3987.14	20.00 (26.07)	80.00
T. viride (Soil infected with *M. phaseolina + T. viride*)	1550.38	610.78	3617.85	3841.25	26.67 (31.00)	73.33

using by different researchers.

$$\text{Percent growth inhibition} = \frac{C - T}{C} \times 100$$

C=Radial growth of *M. phaseolina* in control (mm), T=Radial growth of *M. phaseolina* in presence of antagonist (mm).

Antagonist in green house conditions

The highly pathogenic isolate of *Macrophomina phaseolina* isolated from fenugreek was multiplied on sand maize medium for 15 days. The antagonistic microorganism *Trichoderma harzianum, Trichoderma viride, Trichoderma atroviride* and *Aspergillus niger* were also multiplied on sterilized sorghum grains side by side. The separate media for pathogen and antagonists were taken with the view that they will not utilize the medium of each other for growth and multiplication in pots. After multiplication of both organisms on their respective media, *Macrophomina phaseolina* and antagonist were added in equal quantity in pots filled with sterilized soil. Seeds of highly susceptible variety Khushboo (selection- 55) of fenugreek after surface sterilization with 0.1% mercuric chloride were sown in each pot and ten plants in each pot were maintained after germination. A control with only pathogen was maintained. The disease was recorded till there was 100% mortality in control pots. Soil sample for isolation of antagonists and pathogen were also taken at two stages i.e. pre sowing in unsterilized soil and after 100% mortality in control in sterilized soil. Population of pathogen (*Macrophomina phaseolina*) and antagonists was calculated on the basis of per 'g' soil [4].

Disease control in various experiments were calculated by using the following formula,

$$\text{Disease control (\%)} = \frac{\text{Root rot incidence inoculated control (\%)} - \text{Root rot incidence in treatments (\%)}}{\text{Root rot incidence in inoculated control (\%)}} \times 100$$

Results and Discussion

Eighteen fungi were tested for their antagonistic activity against Bikaner isolate of *Macrophomina* *phaseolina* on Czapek's dox agar medium *in vitro* (Table 1). The antagonistic reaction of isolated fungi against pathogen was classified as mild, intermediate and antagonist. All the microorganisms showed reaction to *Macrophomina phaseolina*. Two microbes viz. *Penicillium aurantiogriseum* and *Penicillium javanicum* which showed reactions up to 30% inhibition of growth were termed as mild antagonist. Twelve microbes which formed inhibition zone between 31 to 50% viz. *A. alternate, A. clavatus, A. flavus, A. japonicus, A. nidulans, Curvularia lunata, Chaetomium globosum, Cochliobolus lunatus, Drechslera halodes, Rhizoctonia solani, Fusarium oxysporum* and *Rhizopus stolonifer* were termed as intermediate antagonist. Similar results with other fungi have previously been reported [6, 7]. Four fungi viz. *Trichoderma viride, T. harzianum, T. atroviride, Aspergillus niger* which developed a zone of inhibition more than 50% were termed as antagonist. Mutual antagonism and mutual intermingling of the pathogen and antagonist in few cases were also observed.

Four fungi viz. *Trichoderma harzianum, Trichoderma viride, Trichoderma atroviride* and *Aspergillus niger* in susceptible Khsusboo)Selection-55) variety of fenugreek were able to reduce the charcol rot incidence in sterilized soil filled in earthen pots. Highly virulent Bikaner isolate was taken in this experiment. An apparent correlation between the decrease in disease incidence of charcol rot of fenugreek and the magnitude of population soil of *Macrophomina phaseolina* and antagonists have been illustrated (Table 1). The mean population of *Macrophomina phaseolina* and antagonists was optimum in naturally infested unsterilized soil of fenugreek (pre-sowing). Population of each antagonist assayed revealed its increase over *Macrophomina phaseolina*. The mean population of *Macrophomina phaseolina* observed with treatment of *Trichoderma harzianum, Trichoderma viride, Trichoderma atroviride* and *Aspergillus niger* was 3029.41, 3617.85, 4165.80 and 5125.87 respectively, in sterilized soil. In control *Macrophomina phaseolina* population was 6852.36. A significant decrease in incidence of disease by antagonists was found as compared to control. *Trichoderma harzianum, Trichoderma viride, Trichoderma atroviride, Aspergillus niger* minimized charcoal rot incidence (%) in fenugreek upto 80.00, 73.33, 66.67, 43.34 and 36.67, respectively as compared to control where 100% mortality was observed. *Trichoderma harzianum* was most effective among the antagonists trial in minimizing the disease followed by *Trichoderma viride, Trichoderma atroviride* and *Aspergillus niger*. These results are comparable with Valiente et al. [8] and Etebarian et al. [1].

In present studies, a direct correlation was found between the population of pathogen and percent mortality of plants which was inversely proportionate to the population of antagonists. *Trichoderma harzianum* was most effective among the antagonists in checking the disease followed by *Trichoderma viride, Trichoderma atroviride* and *Aspergillus niger.*

References

1. Etabarian HR (2006) Evaluation of *Streptomyces* strains for biological control of charcol stem rot of melon caused by *Macrophomina phaseolina*. Pl Pathol J 5 : 83—87.
2. Nico AI, Monaco C, Dal Bello G, Alippi YH (2005) Efectos de al adición de enmiendas orgánicas al suelo sobre la capacidad patogenica de *Rhizoctonia solana* II. Microflora asociada y antagonismo *in vitro* de los aislados más frecuentes. RIA 34 : 29—44.
3. Singh N, Pandey P, Dubey R, Maheshwari D (2008) Biolological control of root rot fungus *Macrophomina phaseolina* and growth enhancement of *Pinus roxburghii* (Sarg.) by rhizosphere competent *Bacillus subtilis* BNI. World J Microbiol Biotechnol 24 : 1669—1679.
4. Barari H, Foroutan A (2016) Biocontrol of soybean charcoal root rot disease by using *Trichoderma* spp. Cercetari agronomics in Moldova 69 : 41—51.
5. Paul NC, Kim WK, Woo SK, Park MS, Yu SH (2007) Fungal endophytes in roots of *Aralia* species and their antifungal activity. J Pl Pathol 23 : 287—294.
6. Benitez T, Rincon AM, Limon MC, Codon AC (2004) Biocontrol mechanisms of *Trichoderma* strains. Int Microbiol 7 : 249—260.
7. Harman GE, Howell CR, Viterbo A, Chet I, Lorito M (2004) *Trichoderma* spesies-opportunictic, avirulant plant symbionts. Nature Reviews Microbiol 2 : 43—56.
8. Valiente C, Diaz K, Gacitua S, Martinez M (2008) Control of charcoal root rot in *Pinus radiate* nurseries with antagonistic bacteria. World J Microbiol and Biotechnol 24 : 557—568.