Research Article

Evaluation of Plant Growth-Promoting Fungus *Aspergillus terreus* Isolates for Improving Physiological Parameters and Management of Charcoal Rot of Soybean

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Abstract

The nine isolates of *Aspergillus terreus* were isolated from rhizosphere of soybean crop and their antagonistic potential against *Macrophomina phaseolina* (Tassi) Goidanich was established under laboratory and field conditions. However, the highest suppression was recorded with isolate 3 of *A. terreus* except at 48 hours and 72 hours. The isolates 1 and 4 were highly but equally suppressive. All the isolates significantly inhibited the mycelia growth of pathogen under poison food technique with maximum inhibition recorded in isolate 1, isolate 3 and isolate 2. All the nine isolates were more suppressive under poison food technique than dual culture technique. The highest (96.56%) relative water content (RWC) was recorded in *A. terreus* isolate 1 treated plant under *in-vivo* condition. Similarly, the lowest (18.18%) disease incidence was also recorded with the same isolate (*A. terreus* isolate 1). Chlorophyll content of the plant was not affected with or without inoculation of test pathogen as the highest chlorophyll content (31.4) was recorded in isolate 7 in both the conditions. It can be concluded from findings that the *A. terreus* native isolate can be used as bioagent for suppression of charcoal rot of soybean.

Keywords: Plant-growth promoting fungi, *Macrophomina phaseolina*, antagonism and disease suppression

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Introduction

The roles of fungi in maintaining the soil health, vegetations, crop improvement and ground carbon sequestration, are of interest to bioenergy and agricultural researches [1]. Plant-growth promoting fungi (PGPF), resident to rhizosphere of any crop, are capable of enhancing plant growth through numerous mechanisms including the protection of roots against infection by minor and major pathogens [2], [3], enhancing the availability of nutrients to the host plant, lowering of the ethylene level within the plant or by the enhanced production of stimulatory compounds, such as plant growth regulators [4]. These PGPF belongs to several genera viz., *Fusarium*, *Penicillium*, *Aspergillus*, *Phoma*, *Trichoderma* and various sterile fungi that can suppress several soil borne diseases by inducing systemic resistance besides promoting growth [5], [6]. In recent years, *Aspergillus* species have been the subject of intensive research as they produce a large number of active specialized metabolites. The profiles of biosynthetic families of metabolites are species specific, some well-know bioactive metabolites, such as penicillin, viridivatin, mevinolin, pseurotin A and cyclopiazonic acid are even secreted by different sections of *Aspergillus* [7] species too.

Rhizosphere soil of soybean cultivars have been explored to obtain the potential bioagents that can suppress the menace of *Macrophomina phaseolina* (Tassi) Goidanich causing charcoal rot. Out of 157 fungal species isolated from plant rhizosphere, many isolates belonging to *Aspergillus terreus* has been isolated owing to their dominant occurrence in soil. Many researchers have reported the complete inhibition of *Ophiobolus graminis* and *Fusarium udum* by *Aspergillus terreus* that produces antifungal substances [8, 9]. Charcoal rot caused by the fungus, *M. phaseolina*, have emerged as serious concern for cultivation of soybean under climate change scenario worldwide as it can survive as microsclerotia (masses of fungal tissue) for two or more years in dry soil. In India, epiphytotics occur in areas where temperature ranges from 35-40ºC during the crop season and the disease can cause up to 80% yield losses. Hence, present study was undertaken with objective to know the probable mode of parasitism offered by root resident mycofloral against pathogenic fungi and their impact on physiological parameters and plant health, on artificial inoculation.

Materials and Methods

Isolation of pathogens from diseased specimens and rhizosphere

Diseased soybean plants exhibiting typical symptoms of dry root rot were collected from the sick plants of AICRP on Soybean experimental field of Jawaharlal Nehru Krishi Vishwa Vidyalaya (22º49'- 22º80'N; 78º21'- 80º58'E), Jabalpur in the Central India during 2015-16. The pathogen was isolated and further purified through hyphal tip method and sub-cultured on PDA slants at 4ºC for further use. Dilution plate method was used to isolate the *Aspergillus terreus* isolates from soil samples of chickpea plant showing different level of wilt symptom, on Rose Bengal Agar medium (RBA). Plates with RBA medium was added with 0.1 ml ($=10^{-4}$) of suspension and incubated at 22 ± 2ºC for 15 days. The colonies were transferred to test tubes containing Potato Dextrose Agar (PDA) medium. The *Aspergillus terreus* isolates were designated as AT1, AT2, AT3, AT4, AT5, AT6, AT7, AT8 and AT9 throughout the study.

Evaluation of antagonistic potential of beneficial fungi under in-vitro conditions

The antagonistic potentials of *Aspergillus terreus* isolates were evaluated against the *Macrophomina phaseolina* (Tassi) Goidanich through dual culture technique [10]. A five mm disc of different fungal isolates was cut out from the seven days old culture and placed close to one end of the Petri-plate containing 20 ml solidified PDA medium. At the opposite end, a similar disc from the culture of the pathogen *M. phaseolina* was placed simultaneously. The Petri-plates were incubated at 25±2ºC in a BOD incubator and the inhibition of the growth of the pathogen by the antagonistic fungi was measured after 48 hrs, 72 hrs and 96 hrs of incubation till both occupies the entire space of Petri-plate.

Culture filtrate of AT isolates grown in PDA broth grown for 10 days were collected after passing it twice through Whatman filter paper No. 1. These filtrates were used to amend Petri-plates containing PDA at 5 per cent concentration and incubated at 25+2°C and observations were recorded after 48, 72, 96, 120, 144 and 168 hours, respectively; an un-amended Petri-plate served as check (control). Each treatment was replicated thrice and the experiment was repeated twice.

The antagonism was measured on the basis of inhibition of the pathogen by the bio agent by the following formulae

$$\text{Inhibition} = \frac{\text{Radial growth in control(C) - Radial growth in the treatment (T)}}{\text{Radial growth in control(C)}}$$

Assessment of antagonistic potential of A. terreus isolates under in-vivo conditions

The inoculum of test fungus was produced on sand + wheat flour mix (9:1), moistened with water and autoclave twice for 90 minutes on two consecutive days. Thirty days after the sowing of the seeds, the culture filtrate of individual beneficial fungi were added into the pots that were already containing the *M. phaseolina* inoculum (spreaded on sand + wheat flour mix) were added @ 5 gm/kg of potting mix. Two sets of experiments with three replicates for each treatment were maintained. The experiment was done in two sets under two different poly-houses. Ten soybean seeds were sown in each clean pot at the 2-3 cm deep in six pots for each strain of *A. terreus* along with un-inoculated control.

Relative water content (RWC)

Measurements of RWC [11] were performed on leaves collected from chickpea plants. Individual leaves were first removed from the stem with tweezers and were weighed immediately (fresh mass, FM) to obtain minimum 0.5 gram from each sample. In order to obtain the turgid mass (TM), leaves were floated in distilled water inside a closed Petri dish. At the end of the imbibition period, leaf samples were placed in a pre-heated oven at 80ºC for 48 hr to obtain the dry mass (DM). Values of FM, TM and DM were used to calculate RWC, using the following equation:

$$\text{RWC (\%)} = [(\text{FM - DM})/ (\text{TM - DM})] \times 100$$

Chlorophyll content index

Chlorophyll Content Index was estimated through the portable chlorophyll meter [12]. Fully expanded leaf sample from three places of each plant of different treatments has been selected for estimation of chlorophyll content index.

The mean of triplicate readings taken using SPAD-502 (SPAD-502, Minolta, Japan) around the midpoint near the midrib of each sample were recorded for different treatment of chickpea leaf and averaged.

Disease incidence

The percent dry-root rot incidence of each treatment was calculated by using following formulae.

$$\text{Disease incidence (\%)} = \frac{\text{No. of plants exhibiting wilt symptom}}{\text{Total number of plant observed}} \text{ X } 100$$

Results

Evaluation of Aspergillus terreus isolates against M. phaseolina through dual culture technique

Under *in-vitro* condition, nine isolates of *A. terreus* were tested to evaluate their growth suppressing ability against *M. phaselina* but none of them were found significantly effective against the pathogen (**Table 1**). The isolate 2, 4 and 6 were not suppressive at 72 hours but had significantly reduced the growth at 96 hours compared to control. The isolate 3 was suppressive at 48hrs but was not found effective at 72 hrs; however, it again became suppressive towards pathogen at 96 hours.

Table 1 *In –vitro* analysis of *Aspergillus terreus* isolates against *Macrophomina phaseolina* through dual culture technique

***Aspergillus terreus* isolates**	**Mycelia growth (in mm)**			**Mean**
	48 hours	**72 hours**	**96 hours**	
Isolate1	29.30	36.65	52.32	39.42
Isolate2	29.98	37.64	54.31	40.64
Isolate3	27.72	35.04	53.91	38.89
Isolate4	30.20	37.44	52.92	40.19
Isolate5	31.28	36.85	56.15	41.42
Isolate6	31.50	37.44	59.11	42.68
Isolate7	29.53	36.65	55.12	40.43
Isolate8	30.62	36.05	54.53	40.40
Isolate9	28.64	35.44	52.71	38.93
Control	35.04	38.62	57.19	43.62
Mean	30.38	36.78	54.82	
	C.D.	SEm±		
Hours (P≤ 0.05)	1.11	0.39		
Fungus (P≤ 0.05)	0.61	0.21		
Hours x Fungus	1.93	0.68		

Screening of Aspergillus terreus isolates against M. phaseolina through poison food technique

Nine isolates of *A. terreus* were assessed against *M. phaseolina* through poison food technique. It has been recorded that all the isolates had effectively reduced the mycelial growth of pathogen (**Table 2**). Inhibitory effect of *A. terreus* isolates were varying among themselves but isolate 1 (29.33 mm), 3 (30.48 mm), 2 (31.16 mm) 6 and 9 (32.01) and (32.65) were statistically at par with each other in suppressing the growth of pathogen followed by isolate 7, 4, 5 and 8. There was increase in growth of pathogen at all studied time. *A. terreus* isolate 1 was found to be suppressive throughout the studied period. In rest isolates, the suppression was not recorded at different interval of time.

Impact of A. terreus isolates on physiological parameters and disease incidence under in-vivo conditions

The effects of culture filtrate of different isolates of *A. terreus* were tested under *in-vivo* condition on RWC, chlorophyll content and on disease incidence (**Table 3**). The highest (96.56%) RWC was recorded in isolate 1 followed by isolate 3 (96.38%), isolate 4 (95.43%) and isolate 2 (90.56%). Similarly, the lowest (18.18%) disease incidence was recorded in isolate 1 followed by isolate 3 (25.0%), isolate 4 (30.0%) and isolate 2 and 7 (33.33%). The chlorophyll content of leaves ranged from 24.9 to 31.4 in culture filtrate inoculated plants of different isolates prior to *M. phaseolina* inoculation while 24.9 to 31.4 in *M. phaseolina* inoculated plants. The highest chlorophyll content was recorded in isolate 7 (31.4) in without *M. phaseolina* inoculations while the same isolate (i.e. isolate 7) out performed

in *M. phaseolina* inoculated plants.

Table 2 Screening of *Aspergillus terreus* isolates against test pathogen through poison food technique

Aspergillus terreus isolates	Mycelia growth (in mm)			Mean
	48 hours	72 hours	96 hours	
Isolate1	21.68	26.31	39.99	29.33
Isolate2	22.50	28.87	42.11	31.16
Isolate3	22.77	27.72	40.95	30.48
Isolate4	30.64	35.45	47.85	37.98
Isolate5	32.98	36.45	52.71	40.71
Isolate6	23.04	29.54	43.45	32.01
Isolate7	26.79	33.61	46.12	35.51
Isolate8	33.19	36.85	54.51	41.52
Isolate9	23.04	31.07	43.83	32.65
Control	34.84	46.12	55.32	45.43
Mean	27.15	33.20	46.68	
	C.D.	SEm±		
Hours (P≤ 0.05)	0.42	0.15		
Fungus(P≤ 0.05)	0.23	0.08		
Hours x Fungus	0.73	0.26		

Table 3 Impact of *Aspergillus terreus* isolates on physiological parameters and disease incidence of soybean crop

Aspergillus terreus isolates	RWC (%)	Chlorophyll content (SPAD-502)		Disease incidence (%)
		BI	AI	
Isolate1	96.56	29.1	27.8	18.18
Isolate2	90.56	24.9	25.8	33.33
Isolate3	96.38	27.8	27.8	25.00
Isolate4	95.43	29.6	26.4	30.00
Isolate5	85.49	31.2	24.9	50.00
Isolate6	79.38	28.4	25.0	44.44
Isolate7	79.66	31.4	31.4	33.33
Isolate8	69.14	29.8	25.9	75.00
Isolate9	73.60	27.5	26.8	66.66
Control	75.36	28.0	28.9	61.53
SE m±	0.47	0.36	0.40	0.60
CD at (5%)	1.40	1.08	1.21	1.56
CV	0.94	2.18	2.59	2.30

BI: Before inoculation of pathogen: AI; After inoculation of pathogen

Discussion

Disease management with fungicides is uneconomical because of the soil and seed borne nature of the pathogen, besides being hazardous to the environment. The uses of biocontrol agents is gaining momentum as it is environment friendly and are compatible with other models of agriculture in integrated pathogen management [13]. Significant growth suppression of the *M. phaseolina* by *Aspergillus terreus* was observed under dual culture technique. The maximum mycelia growth reduction of test pathogen was recorded with isolate 3. The isolates 1 was found effective against test pathogen at all the recorded time. Such antagonistic effect of isolates could be due to rapid sporulation and toxic metabolite producing capacity of the test pathogen. [14] reported the mycoparasitic interaction between *A. terreus* and sclerotia of *Sclerotinia sclerotiorum* (Lib.) de Bary. Abundant sporulation by mycoparasite on the sclerotial surface followed by host cell wall disruption, as observed under scanning electron microscopy, led to the inhibition of pathogen's growth. Similar observation was also recorded by other scientist [15]; [16] against many plant pathogens. Inhibitory effect of *Aspergillus* species is also in confirmatory with the similar reports of [17] in case of other pathogenic fungi. Significantly higher inhibition of *M. phaseolina* by *A. terreus* was observed under poison food technique to dual culture method. All the nine tested isolates of *A. terreus* inhibited the growth of test pathogen at all the studied time intervals. The maximum inhibition was recorded in culture filtrate of isolate 1 at 48, 72 and 96 hours, respectively to rest of the isolates. The isolate 3 was next best to isolate 1 in inhibiting the growth at 72 and 96

hours. This could be due to the secretion of different extracellular enzymes by different isolates at different time. [18] clearly demonstrated that the mycoparasite characteristics of *Aspergillus* sp. ASP-4 enhanced through its ability to produce a range of extracellular enzymes, such as chitinases and other antifungal extrolites which help in colonization of *Sclerotinia sclerotiorum* sclerotia. The results obtained from pot experiments revealed that the minimum disease incidence and was recorded in plants treated with culture filtrate of isolates AT1 (18.18%) and also recorded the maximum percent of RWC in their leaves. The *A. terreus* isolate 1 was the only isolate that was found suppressive against the test pathogen under both *in-vitro* and *in-vivo* conditions.

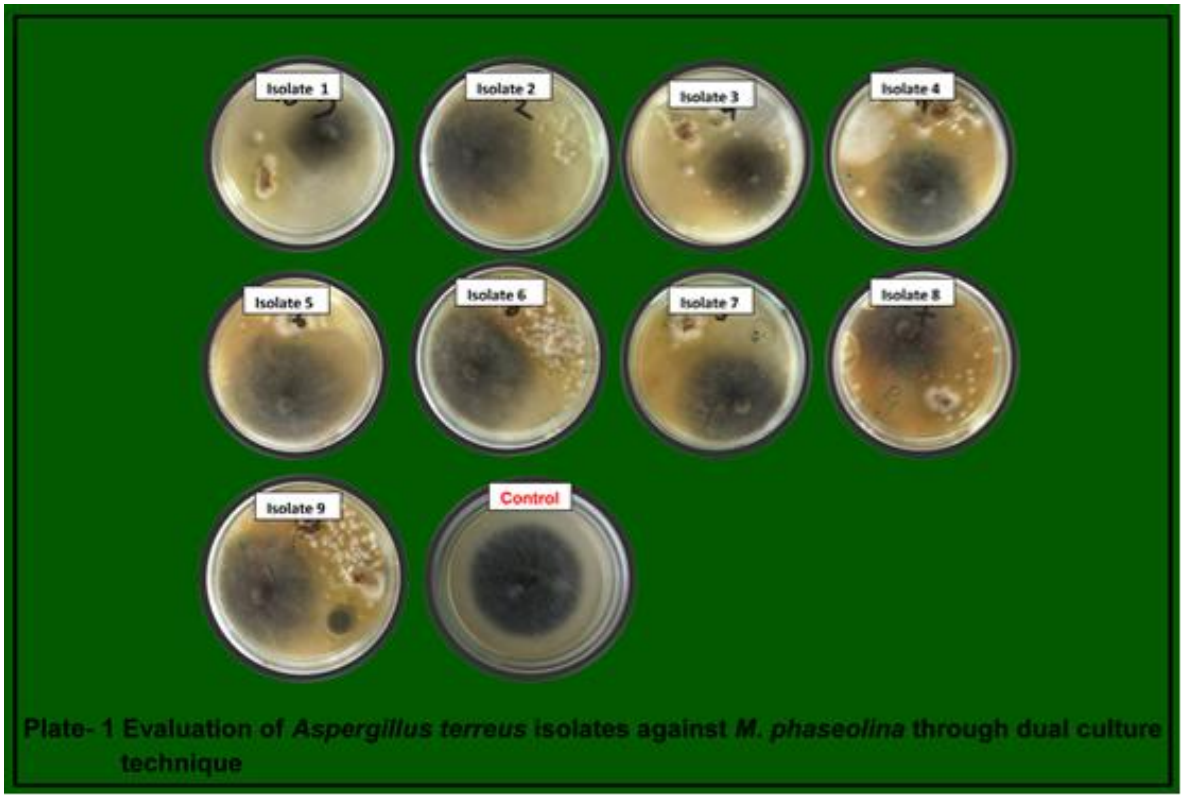

Plate- 1 Evaluation of *Aspergillus terreus* isolates against *M. phaseolina* through dual culture technique

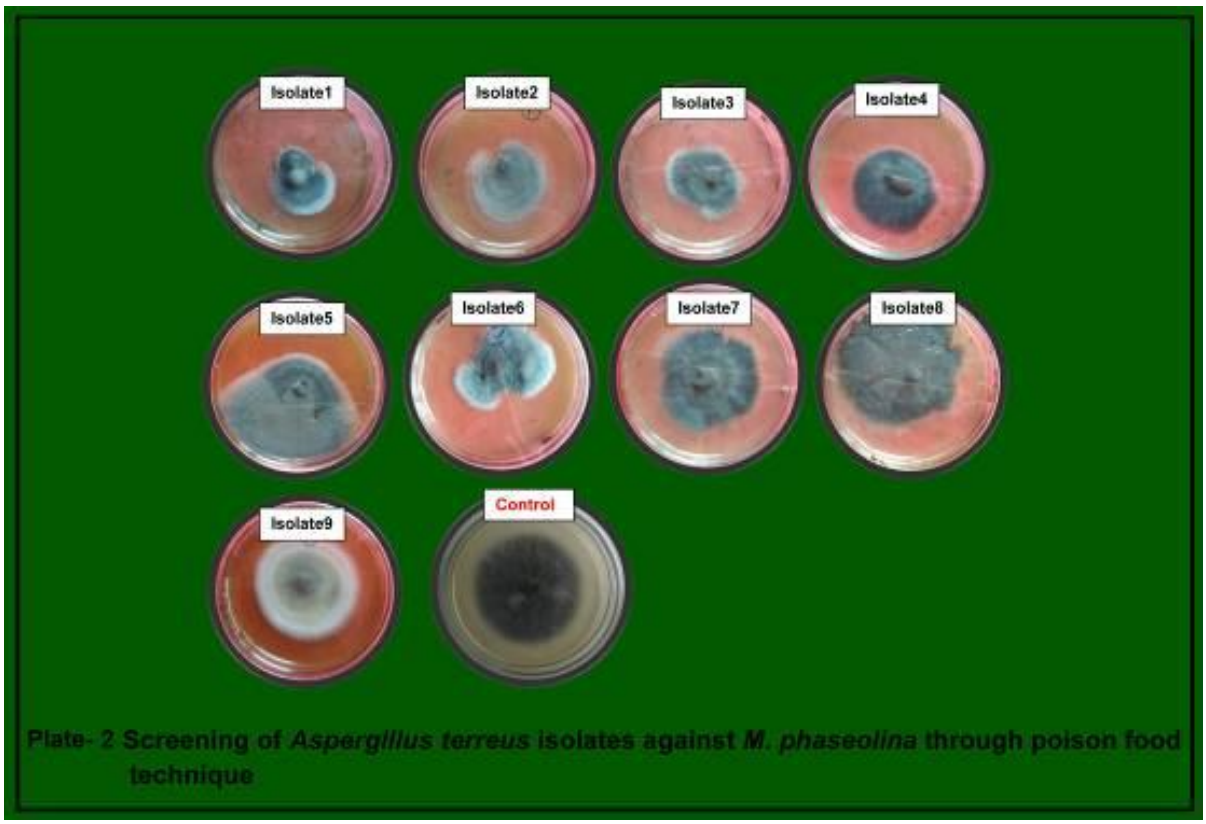

Plate- 2 Screening of *Aspergillus terreus* isolates against *M. phaseolina* through poison food technique

Conclusion

Rhizosphere harbor variety of micro-organisms that have significant effect on crop health management. Dominance of *A. terreus* in soil is well documented in literature but its use as bioagent against soil borne fungi has not been explored much. Nine isolates of *A. terreus* have been evaluated and found effective against *M. phaseolina* under laboratory conditions. Soil application of these isolates also gave promising result under field conditions for managing charcoal rot disease. The result of this research will help in bio-engineering of rhizosphere through including more native bioagents against *M. phaseolina*.

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