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Biological Control of Charcoal Rot Pathogen (*Macrophomina phaseolina*) which Infects Maize by Nonpathogenic *Fusarium solani* f. sp. *psidii*

R. D. N. Singh and S. A. K. M. Kaiser*

Abstract

An exploratory study was undertaken of the possibility of controlling charcoal rot (*Macophomina phaseolina*) of maize by inoculation of the host with *Fusarium solani* f.sp. *psidii* the incitant of wilt of guava but nonpathogenic to maize. It was observed that the protection of maize plants on sterilized soil could be achieved against *M. phaseolina* by inoculation of the host with the nonpathogen before inoculation with the pathogen (77.3%) and also by mixed inoculation with the pathogen and the nonpathogen (86.7%). *F. solani* f.sp. *psidii* was antagonistic to *M. phaseolina by* reducing growth arid spread of the pathogen in the inoculated taaize stalks. The antagonist was also able to inhibit linear growth and reduced sclerotia production of *M. phaseolina* in culture. Culture filtrate of the nonpathogen also resulted in a high inhibiti6n of germination of sclerotia of the pathogen. Similar phenomenon on sclerotial germination was also observed in the extract of maize stalks inoculated with the nonpathogen.

1 Introduction

Concept on the biological control of plant diseases first came from the report of Weindling (1934) who observed that a saprophyte *Trichoderma lignorum* (*T. viride*) reduced the pathogenicity of *Rhizoctonia solani*. A few years later it was demonstrated by Muller et al. (1939) who observed that the avirulent strains of *Phytophora infestans* prevented potato tuber rots by subsequent inoculation with sporangia of the virulent strains of the late blight pathogen. Following the classical work of Muller et al (1939) many workers were successful to induce resistance to diseases in plaint by simultaneous or successive inoculation with two pathogens (one pathogenic and the other nonpathogenic) where the interactions were among different races of pathogens (McCLURE 1951), among different species of a genus (MATTA 1966) wand among different genera belonging to different types of disease inciting agents (PHILLIPS et al. 1967).

Although charcoal rot (*Macrophomina phaseolina* (Tassi) Goid) is a major stalk rot disease of maize (*Zea mavs* Linn.) in India capable of causing appreciable damage to

* Department of Plant Pathology, Agricultural Research Complex Bidhan Chandra Krishi Viswavidyalaya Kalyani-741235, West Bengal/India

standing crop in comparatively dry and low rainfall areas of the COUNTRY (RENFRO AND ULLSTRUP 1976, PAYAK AND SHARMA 1978), the information is scanty on its management using biological methods. The present paper reports on the possibility of some antagonist(s) to be used in the biological management of this disease in future.

2 Materials and methods

The study was undertaken during *rabi* season (December to April) in the Department of Plant Pathology, Bidhan Chandra Krishi Viawavidywalaywa, Kalyani, West Bengal. From ecological point of view the existing *weather* condition at Kalyani during rabi season are conducive for the development of charcoal rot of maize (KAISER AND DAS 1988). The pathogen *M. phaseolina* was isolated from the sclerotia occuring in the diseased maize stalk while *Fusarium solani* (Mart.) Sacc. f.sp. *psidii* Sengupta observed nonpathogenic to maize, was isolated from wilted guava plant. A susceptible maize inbred line, *CM-2O6,* was planted at the end of December in large (31 cm diameter) earthen pots filled with sterilized garden soil and compost (5:1 ratio). Before planting 12 g of ammonium sulphate (AS), 7.5 g of superrphosphate (SP) and 3.6 g of muriate of potash (MP) were mixed in the soil of each pot folbawing the normal agronomic practices with N, P and *K* fertilizer doses @ 400 kg of AS, 250 kg of SP and 120 kg of MP/ha. After germination one plant was maintained in each pot using near optimum irrigation with sterile tap water at regular intervals.

Protection experiment

Protection experiments with nonpathogenic organism was conducted in two ways: (a) by prior inoculation with the nonpathogen followed by inoculation with the pathogen (x) and (b) by mixed inoculation with both (+). The pathogen and the nonpathogen were separately grown in potato dextrose broth (PDB) in 250 ml Erlenmeyer flasks at 29 + 1°C for 14 days. Fungal suspesion was then prepared separately by adding 150 ml sterile tap water to the mycelial mat of each flask. 750 ml of the pathogen suspension was found optimum for inducing infection to an individual plant. For nonpathogen also, same volume of suspension prepared as above was found optimum for invasion of maize roots. In prior inoculation, the plants were first inoculated with nonpathogen suspension about 10 days before flowering. This was followed by inoculation with pathogen suspension when 50% of the preinoculated plants had flowered. In mixed inoculation, the individual plants at 50% flowering stage were inoculated with the mixture of equal volume (750 ml each) of both the organisms. Each treatment contained 10 plants and was replicated 5 times. In one set of check, the plants were inoculated with the pathogen only and in another set they were left uninoculated. Disease symptoms were recorded 25 days after inoculation by splitting open the internodes longitudinally following 1 to 10 (1= very slight to slight infection and ion very heavy infection leading to the premature wilting) scale (PAYAK AND SHARMA, 1978). Percentage reduction on charcoal rot incidence in those maize plants was calculated using a formula described earlier (KAI-

SER AND SENGUPTA, 1977).

Symptoms on roots and stalk produced by the pathogen or the nonpathogen alone and their combinations were also observed by recording average length of roots, colour of invaded roots and stalk, and percentage of roots infected .

*Antagonism to the pathogen (***M. phaseolina***) by the nonpathogen (***F. solani *f.sp.* psidii***)*

Antagonism to the pathogen by the nonpathogen in maize stalk (*in vivo* was directly studied by the toothpick method of inoculation (YOUNG, 1943). The pathogen and the nonpathogen were separately multiplied on round bamboo toothpicks at 29 ± 1°C for 14 days and were used for inoculation. In one Set, the plants were first inoculated with the nonpathogen at a spot on the basal internode at flowering followed by inoculation with the pathogen after 7 days at another spot 2.5 cm apart on opposite side. In another set, the plants were simultaneously inoculated with the pathogen and the nonpathogein similarly as before. For comparison, one set of plants were inoculated with the pathogen alone. Each treatment contained 6 plants and Was replicated 5 times. Disease severity was studied 21 days after inoculation following the 1 to 10 scale as before.

Antagonism *in vitro* was observed by placing mycelial discs (6 mm diam.) of the pathogen and nonpathogen simultaneously on PDA plates at a distance 4 cm apart from each other. Such plates, replicated 5 times were incubated at 29 ± 1 C and data on the inhibition zone were recorded after 7 days.

For studying the sclerotia population 5 discs (10 mm diam.) were cut after 9 days from the portion of the colony of the pathogen facing the nonpathogen and these were placed in 50 ml sterile distilled water in 100 ml Erlenmayer flasks. The flasks were thoroughly shaken in a shaker for 30 minutes and the suspension was passed through two screens of 170 μm and 300 μm to eliminate mycelium and the substratum on which the fungus was grown. The number of sclerotia per field under the low power of a microscope was then counted.

Effect of culture filtrate of the nonpathogen on germination of sclerotia of the pathogen

The pathogen was grown on PDB in 250 ml Erlenmey flasks at 29 ± 1°C for 10 days. The culture of each flask was passed through muslin cloth and the culture filtrate was then centrifuged at 3,300 g for 20 minutes at 40°C after which the clear supernatant was obtained and it was immediately used as test solution. Sclerotia of the pathogen were harvested from the mycelial mat (grown on PDB in 250 ml Erlenmeyer flasks at 29 ± 1 C for 14 days) through repeated blending, then suspending in sterile water followed by decantation and finally passing the scierotial mass through two screens of 170 μm and 300 μm followed by washing with running tap water. Harvested mass was filtered by

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adding few drops of 2% sodium hypochlorite solution, then washed in sterile water and air dried. Viability of sclerotia was then tested on moist filter paper and nearly 100% of them were found viable through germination. Germination of sclerotia was studied by adding one drop of sclerotial suspension (approximately 20 sclerotia/field under the low power of a microscope) of different eccentric aliens (25%, 50%,75%, and one drop of the test solution double groove slides incubated in moist chamber at 29 + 1°C. The slides, replicated 5 times, were then observed under microscope at different time intervals (6 hr, 12 hr, 18 hr and 24 hr).

Effect of extract from maize stalk innoculated with the nonpathogen on sclerotial germination of the pathogen

The test plants were inoculated with the nonpathogen at the basal inter-modes at flowering using toothpicks (YOUNG, 1943) as before. The inoculated internodes were then collected after 12 days when the pith turned into pink in colour. Using 250 g of fresh weight of those internodes the extract was collected in sterile glass distilled water in a cold room at 40.C through repeated crushing followed by blending and finally straining through muslin cloth Using different concentrations (25%, 50%, 75% and 100%) of this extract (initially treated as 100% after extraction) as test solutions germination of sclerotia was studied in groove slides similarly as before.

3 Results

Protection against charcoal rot incidence

Fig. 1 shows that a high significant reduction in charcoal rot incidence in maize was achieved by prior inoculation with the nonpathogen followed by inoculation with the pathogen (77.3%) as well as by mixed inoculation with both (86.7%). However, none of these test plants were wilted while in case of inoculation with the pathogen alone a few plants died.

*Symptoms produced by the pathogen (***M. phaseolina***) or nonpathogen (***F. solani f. sp. psidii***) alone and their combinations*

Table 1 shows the characteri5tic symptoms on maize plants inoculated with the pathogen 88 evidenced by dark brown discolouration of internodes and presence of sclerotia in the di8integrated pith and disorganised tissues of *roots.* similar type of symptoms, except formation of sclerotia, extending up to basal internode were noticed in plants inoculated with the pathogen and nonpathogen in combinations. when inoculated with the non-pathogen alone, the invaded roots became light brown to pink in colour and the pith of the basal internode also became pink but in such case the root length remained normal, while in others they were shorter.

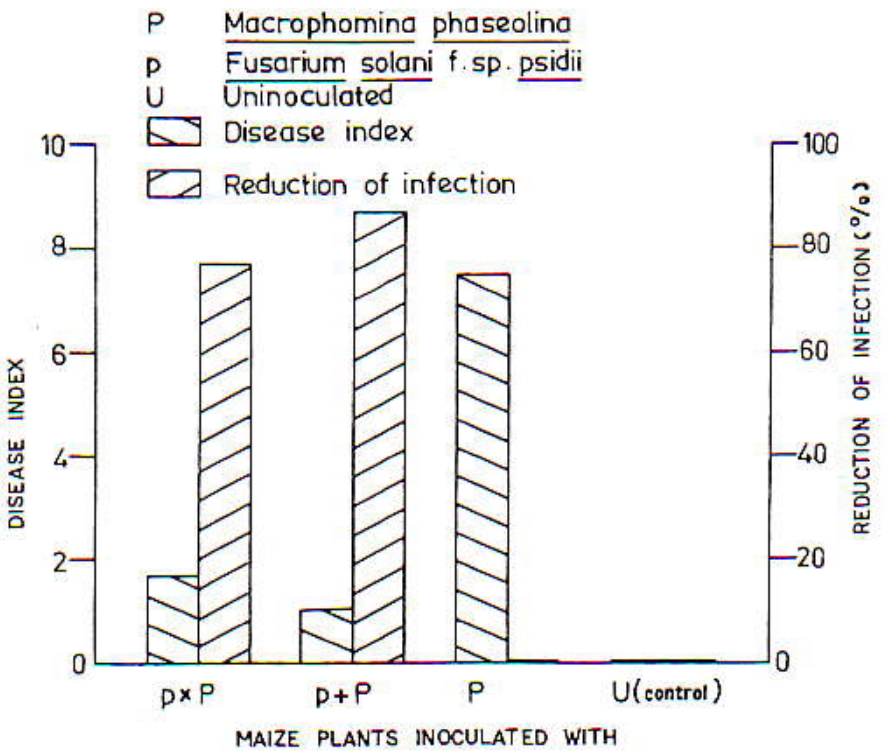

Fig. 1: Effect of preinoculation of maize plant with the nonpathogen *F. solani* f. sp. *psidii* followed by inoculation with the pathogen M. phaseolina (pxP) and also by mixed inoculation with both (p+P). C.D. (at P = 0.05) for the comparison of disease index = 1.26

Table 1: Symptoms on roots and stalk of maize plant inoculated with the pathogen *M. phaseolina* (P), the nonpathogen *F. solani* f. sp. *psidii* (p) and their combinations

Plant inoculated with	Symptom
P	Early symptoms were drying of the lowermost leaves from the tip upward and dark browning of root tips. In advance stage, the lower internode became straw colour and disintegration of pith occurred up to fifth internode. small, black clerotia (47 to 125 μm in diam.) were noticed in the pith of affected stalks and in the disorganised tissues of roots. About 90% roots were invaded and they became dark brown. Roots were shorter (19 to 22.5 cm) compared to uninoculated ones (23 to 30 cm).
p	About 70 to 75% roots were invaded and they became light brown. Secondary roots were reduced in number but the root length remained normal. Pith of the first internode only become pink in colour.
p x P	About 40 to 50% roots were invaded and they became almost dark brown, the root length became shorter (16.5 to 20.5 cm) than the previous cases. Black brown discoloration of the pith up to 25 to 40,' of the first internode was noticed.
p + P	Similar type of symptoms were noticed as in the previous cases but less number of roots (35 to 40%) were invaded and the root length was less shorter.

Antagonism to M. phaseolina *by* F. solani *f. sp.* psidii

Table 2 shows that *F. solani* f. sp. psidii was antagonistic to *M. phaseolina* in maize stalk where it significantly reduced growth of the pathogen, while table 3 shows that *F. solani* f.sp. *psidii* inhibited linear growth and reduced sclerotial population of M. phaseolina but the reduction in sclerotial population was not significant.

Effects of culture filtrate of *F. solani* f. sp. *psidii* and the extract of maize stalks inoculated with the same on sclerotial germination of *M. phaseolina* are presented by fig.2 and Fig.3 respectively. Fig.2 shows that percentage of sclerotial germination decreased in an almost linear fashion with increase in the concentration of culture filtrate. while fig .3 shows that sclerotial germination also followed similar pattern with increase in the concentration of plant extract.

4 Discussion

The possibility of biological control of charcoal rot pathogen *M. phaseolina* infecting maize by inoculation of the host with *F. solani* f. sp. *psidii* the incitant of wilt of guava but nonpathogenic to maize, has been demonstrated in the present study. There are few reports on the biological control of *M. phaseolina* inciting diseases on different crop plants. Singh and Mehrotra (1980) reported control of wilt of gram by coating the seed with species of *Bacillus* and Strreptomyces while Pande (1985) reported control of preemergence death of horsegram by adding culture of *T. viride* to the seed as well as by mixing with soil. Elad et al. (1986) also reported that wheat bran preparation of *T. harzianum* to soil reduced incidence of dry root rot of beans. The above two species of *Trichoderma* were also found to be antagonistic *to M. phaseolina in vitro* resulting in the inhibition of linear growtii and microsclerotia production. In the present study tlie nonpathogen *F. solani* f. sp. *psidii* also inhibited linear growth and reduced sclerotial production of the pathogen M. *in vitro* study further showed inhibition of sclerotial germination in different concentrations of the culture filtrate of the nonpathogen. The nonpathogen was also antagonistic to the pathogen *in vivo* by reducing growth and spread of tile pathogen in the inoculated maize stalk. Extract from the maize stalks innoculated with the nonpathogen was also highly effective against sclerotial germination of the pathogen. This antagonism observed in the present study might be attributed to the production of some antifungal or toxic substance(s) by *F. solani* f. sp. *psidii* primarily in the rhizosphere and later in the tissues of roots and stalk of maize plants resulting in the inhibition of growth and spread of *M. phaseolina*. Such phenomenon of biocontrol in plant diseases has been reported by different workers. kalyansundaram (1958), for example, reported that *F. lycopersici* was able to produce fusaric acid in the rhizosphere of tomato plants that inhibited growth of many soil fungi while Kaiser and Sengupta (1977) demonstrated that the extract of pigeon pea seedlings inoculated with *F. oxysporum* f. sp. *vasinfectum* and f. sp. *ciceri* pathogenic to cotton and gram respectively showed the antifungal properties on conidial germination and mycelial growth of *F. oxysporum* f. sp. *udum* pathogenic to pigeon pea. Howell and Stipanovic (1979)

further reported that the improved emergence of cotton seedlings by treating the seed with *Pseudomnas fluorescens* against *R. solani* was as a result of antagonism due to production of antibiotic "pyrolnitrin" by the nonpathogen. However, the present study is an exploratory one. Further study is necessary to know the mechanism of such biocontrol and its application in the field for the confirmation of such findings.

Table 2: *F. solani* f. sp. *psidii* (p) showing antagonism to *M. phaseolina* (P) in maize stalk

Stalk inoculated with	Disease index		
	Maximum	Minimum	Average
p x P	2.50	1.50	2.00
p + P	2.00	1.00	1.60
P (control)	8.00	6.00	7.50
C.D. (at P = 0.05)			0.99

Table 3: *F. solani* f. sp. *psidii* (p) showing antagonism to *M. phaseolina* (P) in PDA plates

Plate inoculated with	Inhibition zone	Number of sclerotia (x 10)
p+P	2.30	2.10
P	0.00	3.20
C.D. (at P = 0.05)		1.60

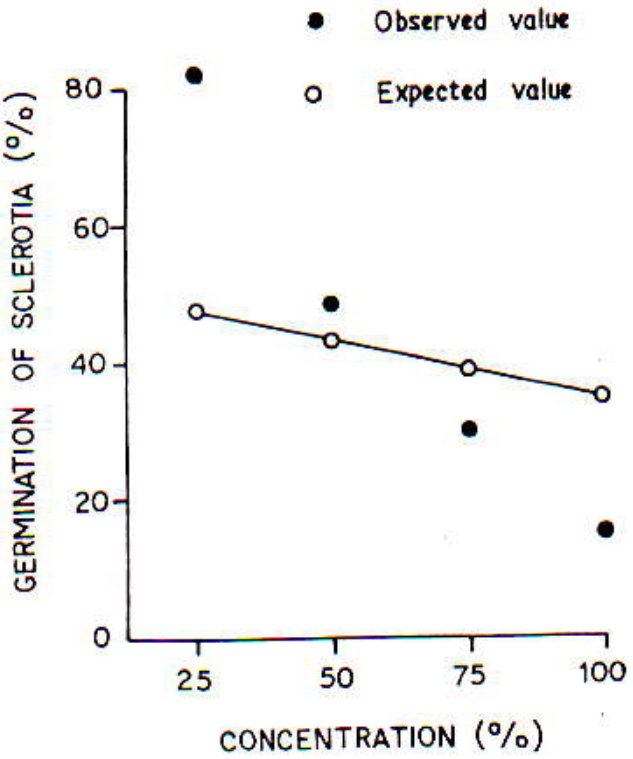

Fig. 2: Effect of culture filtrate of *F. solani* f. sp. *psidii* on the germination of sclerotia of *M. phaseolina*

Regression equation % Germination of sclerotia: Y = 58.12 - 0.17 X (r - 0.99**)

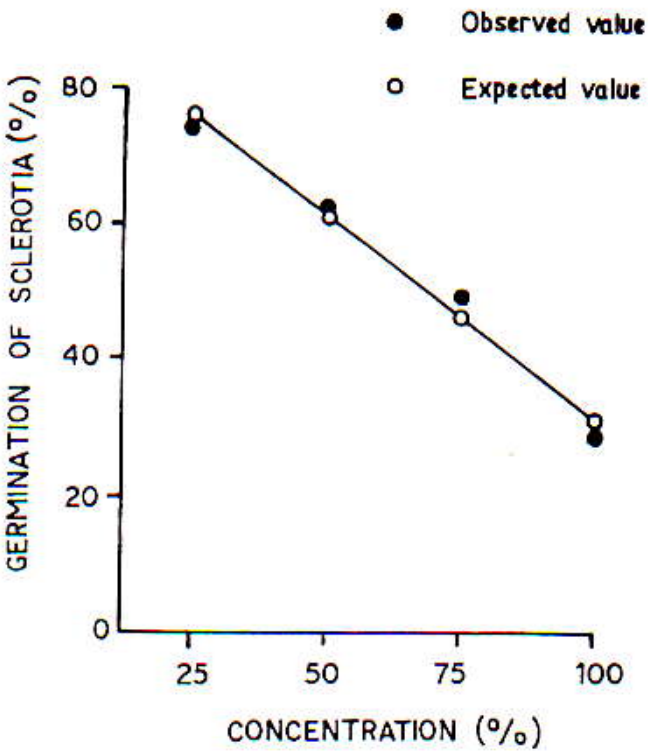

Fig. 3: Effect of extract of maize stalk inoculated with *F. solani* f. sp. *psidii* on the germination of solerotia of *M. phaseolina*
Regression equation % Germination of sclerotia: Y = 91.00 - 0.60 X (r = 0.99**)

Zusammenfassung

Biologische Bekämpfung der Schwarzfäule (*Macrophomina phaseolina*) am Mais durch das Nichtpathogen *Fusarium solani* f. sp. *psidii*

In vorliegenden Untersuchungen wurde die Interaktion zwischen dem Erreger der Guava-Welke (*Fusarjum solani* f. sp. *psidii*) und jenem der Schwarzfäule am Mais (*Macrophomina phaseolina*) untersucht.

Während die Inokulation mit *F. solani* f. sp. *psidii* vor der Infektion mit *M. phaseoline* die Schwarzfäule am Mais um 77,3% unterdrückte, hatte die gemeinsame Inokulation einen Bekämpfungseffekt von 86,7%. In beiden Fällen wurden keine welken Pflanzen festgestellt.

Die antagonistische Wirkung von *F. solani* f. sp. *psidii* gegenüber dem Erreger der Schwarzfäule drückte sich im signifikant reduzierten Wachstum und der Anzahl der Sclerotien des Pathogens aus.

Kulturfiltrate von *F. solani* f. sp. *psidii* und Extrakte des inokulierten Maisstengels führten bei *in vitro* Versuchen mit zunehmender Konzentration im Kulturfiltrat zur Verminderung der Keimung der Sclerotien des Pathogens.

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