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Biological control of Maydis leaf blight pathogen (race 'O' of *Drechslera maydis*) of maize with *Drechslera oryzae* and *Helminthosporium sativum*

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The feasibility of controlling Maydis leaf bight (race 'O' of *Drechslera maydis*) of maize with *Drechslera oryzae* and *Helminthosporium sativum* was studied. The protection of maize plants against *D. maydis* on sterilized soil could be achieved by prior inoculation of the non pathogen followed by inoculation of the pathogen as well as by mixed inoculation with both. However, a better result was obtained with mixed inoculation as compared to preinoculation. The non pathogens were antagonistic to *D. maydis* by inhibiting linear growth in the culture. Culture filtrate of the non pathogens also resulted in a high inhibition of conidial germination of the pathogen. Similar results were also recorded with the extract of maize leaves inoculated with the non pathogens.

Key Words : Biological control, leaf blight, maize, pathogen

INTRODUCTION

Maydis leaf blight of maize (*Zea mays* Linn.) caused by *Drechslera maydis* Nisikado race 'O' is one of the major foliar diseases of this crop in India as it is widely prevalent over different parts of the country including West Bengal and it may cause appreciable damage to the standing crop in terms of both yield and crop value under the favourable conditions (Payak and Sharma, 1978; 1985). The disease appears in the *kharif* season (July to September) and it attacks maize at various stages of plant growth. Characteristic symptoms are produced on leaves in the form of small lesions with parallel sides and buff to brown border with little chlorosis. In case of severe infection the lesions may coalesce exhibiting a blight like appearance. Although the disease occurs every year in different parts of the country 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 control of this disease in practice.

MATERIALS AND METHODS

The study was undertaken during *kharif* season (July to September) in the Department of Plant Pathology, Bidhan Chandra Krishi Viswavidyalaya, Kalyani (22.5 ^{0}N & 89.0 ^{0}E) located on the Gangetic plains of West Bengal. The pathogen race 'O' of *D. maydis* was isolated from the infected leaves of maize while *Drechslera oryzae* (Breda de Hann) Subram. & Jain and *Helminthosporium sativum* P. K. & B. known nonpathogenic organisms to maize were isolated from the infected leaves of rice and wheat respectively. Seeds of a susceptible maize variety NEH (North Eastern Himalayan) composite were sown during the first week of July in large (31 cm) earthen pots filled up with sterilized garden soil and compost (5:1 ratio). Before sowing N, P and K fertilizers in the form of ammonium sulphate, super phosphate and muriate potash were added to the soil in pots @ 400 kg, 250 kg and 120 kg ha^{-1} respectively. After germination a single plant was raised in each pot using near optimum irrigation with sterile tap water.

Protection experiment

Protection experiment was conducted in two ways : (a) by prior inoculation with the nonpathogens followed by inoculation with the pathogen (x) and (b). by mixed inoculation with both pathogen and non pathogens (+). The pathogen and the nonpathogens from the stock cultures were separately grown on potato dextrose broth (PDB) in 250 ml Erlenmeyer flasks at 28 ± 1°C for 10 days. Mycelial mat from each of the flasks was then taken out separately and the conidial suspension was prepared following the method described by Pal (1998). The conidial suspension so prepared contained approximately 5×10^5 conidia ml^{-1} which was found optimum for invasion of maize leaves.

In case of preinoculation, the test plants were first inoculated with the conidial suspension of the nonpathogens about 7 days before inoculation with the conidial suspension of the pathogen when the test plants were 45 to 50 cm high. The test plants were inoculated by pouring approximately 10 ml of the conidial suspension of each of the pathogen and the non-pathogens following the 'whorl inoculation' method (Sharma, 1982) while in case of mixed inoculation the test plants (45 to 50 cm high) were inoculated similarly with the mixture of an equal volume (10 ml) of both pathogen and the nonpathogen. Each treatment contained 5 plants and was replicated 5 times. In one set the test plants were inoculated with the pathogen only and the other set was kept uninoculated as check. Disease symptom was recorded 21 days after inoculation following 1 (very slight to slight infection) to 5 (very heavy infection) disease rating scale (Sharma, 1982). Percentage of reduction to infection on maize plants was calculated using a similar formula described by Kaiser and Sengupta (1977).

Tests for antagonism against the pathogen by non-pathogen *in vitro*

Antagonism *in vitro* was studied by placing simultaneous 6 mm fungal discs of the pathogen and the nonpathogen on potato dextrose agar (PDA) plates at a distance 4 cm apart from each other. Such plates replicated 5 times were incubated at 28 ± 1°C and those were periodically examined for the inhibition zone, and the final data were recorded after 7 days.

Effects of culture filtrates of *D. oryzae* and *H. sativum* on germination of conidia of the pathogen (*D. maydis*)

Culture filtrates of the nonpathogens grown in 250 ml Erlenmeyer flasks on PDB at 28 ± 1°C was collected after 10 days. The culture filtrates thus collected were centrifuged at 5000 rpm for 15 minutes to eliminate spore, and were then decanted into 10 ml vials for immediate use. Conidia of the pathogen were collected from the mycelial mat grown in 250 ml Erlenmeyer flasks on PDB at 28 ± 1°C for 10 days following the method described by Pal (1998). Using different concentrations of the culture filtrate the percentage of conidial germination of the pathogen was studied in cavity slides at 28 ± 1°C.

Effects of extracts from the maize leaves inoculated with the nonpathogens (*D. oryzae* and *H. sativum*) on conidial germination of the pathogen (*D. maydis*)

Maize plants (45 cm high) of the variety NEH composite raised in 31 cm earthen pots containing sterilized soil were inoculated separately with the nonpathogens following the 'whorl inoculation' method. The inoculated leaves were collected after 7 days. Using 100 g (fresh weight) of the leaves the extract was collected in sterile distilled water following the method described by Singh (1991). Using different concentrations of the extracts as test solutions the germination of conidia of the pathogen was studied similarly as before.

RESULTS AND DISCUSSION

The possibility of biological control of race 'O' of *D. maydis* infecting maize by inoculation of the host with *D. oryzae* and *H. sativum* pathogenic to rice and wheat respectively but nonpathogenic to maize has been demonstrated in the present study. The data presented in Table 1 showed that a significant reduction in the disease incidence was achieved by prior inoculation of the non-pathogens followed by inoculation of pathogen as well as by mixed inoculation with the both. However, a better result was obtained with the mixed inoculation as compared to pre-inoculation. Knowledge on this aspect in respect of *D. maydis* of maize, was

however, lacking. Some workers, however, reported the biocontrol of some pathogenic species of *Helminthosporium in vivo* using a nonpathogenic fungal species. Sinha and Trivedi (1969), for example, reported that the inoculation of a susceptible variety of rice with an avirulent strain of *D. oryzae* reduced the disease when subsequently inoculated with a virulent strain, while Islam and Nandi (1985) in the pot experiment observed that a cell suspension of *Bacillus megaterium* before inoculation of *D. oryzae* prevented the disease incidence in rice.

Table 1 : Inhibition of race 'O' of *D. maydis* of maze by *D. oryzae* and *H. sativum* nonpathogen to maize

Plants inoculated with[1]	Disease index			%Reduction of infection
	Max.	Min.	Average	
D. oryzae x D. maydis	3.0	2.0	2.4	40.0
H. sativum x D. maydis	2.5	2.0	2.2	45.0
D. oryzae + D. maydis	2.5	1.5	2.0	50.0
H. sativum + D. maydis	2.0	1.5	1.8	55.0
Control				
D. maydis	4.5	3.5	4.0	
Uninoculated	0.3	0.0	0.0	
SE(mean)			±0.38	
CD(at P=0.05)			0.79	

[1]x-preinoculation ; +-mixed inoculation

The antagonistic effects of some fungi nonpathogenic to different cereal crops were also demonstrated against their respective pathogenic species of *Helminthosporium* in the culture. Mangiarotti *et al.* (1987) for example, reported that *Penicillium chrysogenum* and *P. charlesii* were markedly active against *D. maydis* and *D. oryzae.*

Table 2 : *D. oryzae* and *H. sativum* showing antagonism to race 'O' of *D. maydis* on PDA plates

Plate seeded with	Inhibition zone		
	Maximum	Minimum	Average
D. oryzae + D. maydis	2.5	2.0	2.1
H. sativum + D. maydis	2.8	2.3	2.4

Table 3 : Germination of conidia of race 'O' of *D. maydis* at different concentrations of culture filtrate of *D. oryzae* and *H. sativum*

Cultural filtrate of	% Germination of conidia at different concentrations (%) of the cultural filtrate					
	10	20	40	60	80	100
D. oryzae	74.0	66.0	55.0	46.0	39.0	36.0
H. sativum	71.0	65.0	50.0	45.0	38.0	34.0
Control (sterile distilled water)						82.0

Table 4 : Germination of conidia of race 'O' of *D. maydis* at different concentrations of extract of maize leaves inoculated with *D. oryzae* and *H. sativum*

Leaf inoculated with	% Germination of conidia at different concentrations (%) of the leaf extract					
	10	20	40	60	80	100
D. oryzae	48.0	40.0	35.0	28.0	17.0	14.0
H. sativum	45.0	36.0	31.0	27.0	16.0	11.0
Control (sterile distilled water)						83.0

Mostafa *et al.* (1992) also observed that *Trichoderma* spp. were most effective against mycelial growth of *D. teres*. In the present study the two nonpathogens *D. oryzae* and *H. sativum* also inhibited the mycelial growth of the pathogen *D. maydis* in the culture (Table 2). Using different concentrations of the culture filtrates of the nonpathogens it was further observed that the conidial germination of the pathogen gradually decreased with the increase in the concentration of the culture filtrate (Table 3). Similar results on conidial germination was also recorded by using different concentrations of the extracts from maize leaves inoculated with the nonpathogens (Table 4). This kind of antagonism might be attributed due to the production of some antifungal toxic substance(s) on maize leaves by the nonpathogens *D. oryzae* and *H. sativum* which probably inhibited germination, growth and sporulation of the pathogen *D. maydis* and its subsequent spread. Bilis and Hill (1988) also reported that sporulation capacity of *H. sativum* was greatly reduced in the leaf lesions of inoculated wheat plants which was subsequently treated with the conidial suspension of *T. harzianum* as compared to untreated control. However, the present study is an exploratory one. Further study is necessary to know the mechanism of such resistance and its application in the field for the confirmation of such findings.

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REFERENCES

Bilis, C. L. and Hill, J. P. 1988. Effect of *Trichoderma harzianum* on sporulation of *Cochliobolus sativum* on excised wheat seedling leaves. *Phytopathology*, **78** : 656-659

Islaman, K. Z. and Nandi, B. 1985. Control of brown spot of rice by *Bacillus megaterium Zeit pflan krankheiten pflanzen* **92** : 241-246.

Kaiser, S. A. K. M. and Sengupta, P.K. 1977. Inhibition of wilt symptoms by *Fusarium oxysporum f.* sp. *udum* in pigeon pea (*Cajanus cajan*) induced by other formae speciales of *Fusarium oxysporum. Phytopath Medit,* **16** : 1-4.

Mangiarotti, A., Frate, G. G. Del., Picco, A.M. and Caretta, G. 1987. Antagonistic activity *in vitro* of some saprophytic fungi occurring on the phytoplane of rice, wheat and maize. *Boletin Micologico.,* **3** : 183-189.

Mostafa, M., Barrault G., Albertini, L. 1992. Biological control of *Drechslera teres, in vitro* activity of antagonistic microorganisms on mycelial growth and conidial germination. *Crypt Mycologie,* **13** : 125-133.

Pal, Debasis 1988. *Studies on some aspects of Drechslera maydis Nisikado race 'O' causing leaf blight of maize in India.* Ph. D. Thesis submitted to the Bidhan Chandra Krishi Vizwavidyalaya, West Bengal, p. 30.

Payak, M. M. and Sharma, R. C. 1978. Researches on diseases of maize. PL-480 Project, Final Technical Report (April 1969-March 1975). *Indian Council of Agri. Res.* New Delhi, pp. 228.

Payak, M. M. and Sharma, R. C. 1985. Maize diseases and approaches to their management in India. *Trop. Pest. Manag.,* **31** : 302-310.

Sharma, R. C. 1982. Maydis leaf blight of maize. Techniques of scoring for resistance to diseases of maize in India. Notes used for the training course held at Andhra Pradesh Agricultural University, Hyderabad, AICMIP, IARI, New Delhi, pp. 6-9.

Singh, R. D. N. 1991. *Studies on some aspects of charcoal rot of maize caused by Macrophomina phaseolina* (Tassi) Goid. Ph. D. Thesis submitted to the Bidhan Chandra Krishi Viswavidyalaya, West Bengal, pp. 230.

Sinha, A. K. and Trivedi, N. 1969. Immunization of rice plants against *Helminthosporium* infection. *Nature. London.* **223** : 9.

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