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Research Note

Potential of *Trichoderma* spp. as biocontrol agents against pathogens causing maydis leaf blight of maize

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ABSTRACT: Efficacy of bioagents against *Helminthosporium maydis* was studied *in vitro* and under field condition at Trihut college of Agriculture, Dholi (Muzaffarpur) in 2005 and 2006. Among the three native antagonist isolates of *Trichoderma viride*. *Trichoderma harzianum* and *T. virens* screened, *T. viride* inhibited the radial growth of *H. maydis* to an extent of 60.7% followed by *T. harzianum* (55.1%) and *T. virens* (52.6%). Studies on hyphal interaction between antagonists and test fungus revealed disorganization of protoplasmic content and lysis of host hyphae. Soil application of bioagents was more effective in protecting the crop than foliar spray. Soil application of *T. viride* resulted in minimum disease intensity (2.6) followed *by T. harzianum* (2.8) and *T. virens* (3.0). Soil application of *T viride* also gave maximum yield (18.6q ha^{-1}) followed by *T. harzianum* (17.7q ha^{-1}) and *T. virens* (17.3q ha^{-1}), respectively. These results suggest the need to augment soil application of *Trichoderma* for obtaining effective management of maydis leaf blight.

KEY WORDS: Antagonism, *Helminthosporium maydis, Trichoderma, Zea mays.*

Maize (*Zea mays*) is an important cereal crop cultivated in India and other parts of the world. The crop is often affected by maydis leaf blight caused *by Helminthosporium maydis* Nishikado and Mianke.This disease is very common and invariably prevalent in northern region of Bihar and causes considerable loss every year (Jha *et al.*, 2004). In recent years, an increasing consciousness about environmental pollution due to pesticides and development of fungicide resistant strains in plant pathogens have challenged plant pathologists to search for eco-friendly tools for disease management. The possible use of fungal antagonists of maize pathogen has been viewed as an alternative disease management strategy. *Trichoderma* spp. are extensively exploited for control of soil borne pathogens (Rudresh *et al.*, 2005; Srinivasulu *et al.*, 2005). Although bioagents have been found effective in inhibiting the growth of *H. maydis in vitro*, they fail to control the disease in the field when applied as foliar sprays in most of the cases (Jha *et al.*, 2004). Soil application of antagonists has been reported for the management of diseases in other crops (Sharma, 1999), but there is no information on biological management of maydis leaf blight in maize by soil application of antagonists. With this in view, the present investigation was carried out to examine the efficacy of *Trichoderma* spp. against maydis leaf blight of maize under laboratory and field conditions.

The studies were conducted in Department of Plant Pathology and research farm of Tirhut College of Agriculture, Dholi, Muzaffarpur (RAU Pusa), Bihar, during 2005 and 2006. Three antagonistic fungi, *viz*., *T. viride, T. harzianum* and *T. virens* were evaluated *in vitro* to test the antagonism against *H. maydis* by dual culture technique using potato dextrose agar (PDA) medium (Mortan and Straube, 1955). All the plates were incubated in a BOD incubator at 28 ± 1ºC. Observation on colony diameter of *H. maydis* was recorded at 96 and 144h of incubation and the per cent inhibition of pathogen was calculated as described by Vincent (1947). Hyphal interaction between the antagonists and the test fungus was studied when the colonies of the two fungi came in contact with each other. Small mycelial fragments from the zone of interaction of the pathogen and antagonists were taken out with the help of a needle and mounted in lactophenol and examined under a microscope for hyphal interaction. Slides were also prepared from the areas where the test fungus was overgrown by the antagonists to study the hyphal interaction between the test fungus and the antagonists. Re-isolation was done by taking a 6mm disc from the area

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where pathogen colony had already overgrown by the antagonist and placing centrally on PDA.

A field trial was laid out in randomized block design (RBD) using a susceptible variety Kiran of maize with 3 replications in 5 x 4.5m² plots .The bioagents, *viz.,T. viride, , T. harzianum* and *T. virens* as soil application and spray were evaluated in sick plot condition to find out the most efficacious one against maydis leaf blight. For inoculation of bioagent in soil, the antagonists were multiplied on farm yard manure (FYM) as described by Kuruvilla and Sivaprakasam (1994). Bioagent enriched in talc formulation FYM was spread over the plots @ 100g / m² one week before sowing as pre-reatment and upper layer of soil was mixed. Bioagents were used as foliar spray @ 1.0gL^{-1} of water during evening hours (between 4 and 5 PM), when the first symptom of disease was noticed. Two consecutive sprays at an interval of 10 days were given. Observation on disease intensity was recorded at weekly interval using 1–5 scale proposed by Payak and Sharma (1987). The yield data were recorded after harvest of the crop.

The observations recorded on the inhibition of test fungus in dual culture test (Table 1) revealed that all the three antagonists tested inhibited the growth of *H. maydis in vitro*. Highest inhibition was recorded in case of *T. viride* (60.7%) followed by *T. harzianum* (55.1%) and *T. virens* (52.6%). Inhibition zone was observed in case of *T. viride* and *T. virens* followed by mycoparasitism whereas only mycoparasitism was noticed in case of *T. harzianum*. In all the three cases, the *H. maydis* mycelium did not grow when transferred on to fresh media indicating its death. Microscopic observations made from the mycelial interaction between antagonists and *H. maydis* revealed lysis and protoplasmic disintegration of hyphae of the test fungus at many locations. Mycoparasitism through physical contact by coiling and pathogen cell lysis in case of *Sclerotium rolfsii* by *T. harzianum* has been reported by Upadhyay and Mukhopadhyay (1986). The disintegration of mycelia of test fungus may be due to action of enzymes produced by *Trichoderma* spp. (Elad *et al,* 1982) and production of volatile and non-volatile chemical compounds (Srinivasulu *et al*., 2005). The pathogen cell lysis and protoplasmic disintegration of the mycelium by *T. harzianum* and *T. virens* have been reported earlier (Chet *e t al*., 1981).

The result revealed that soil application of bioagents was more effective in protecting the crop than foliar spray. Soil application of *T. viride* resulted in minimum disease intensity (2.6) followed by *T. harzianum* (2.8) and *T. virens* (3.0) (Table 2). Spraying of bioagents, *viz., T. viride, T. harzianum* and *T. virens* was less effective than soil application and showed 3.8, 3.9 and 4.1 of disease intensity, respectively. Soil application of *T. viride* also gave maximum yield (18.6q ha^{-1}) followed by *T. harzianum* (17.7q ha^{-1}) and *T. virens* (17.3q ha^{-1}), respectively. All the treatments reduced the disease intensity and increased the grain yield significantly when compared to check. The per cent increase in yield varied between 24 and 48% depending upon disease intensity. Similar results were recorded against pigeon pea wilt caused by *Fusarium udum* under field conditions (Prasad *et al.,* 2002). Sharma (1999) also reported that soil application of mycelial preparation of *T. harzianum* a week before sowing reduced the stem rot of chickpea caused by *S. sclerotiorum* considerably.

The control of maydis leaf blight disease by *Thichoderma* spp. might be attributed to the pronounced colonization of rhizosphere by antagonists in advance to the pathogen and also by mycoparasitism. The possible mechanisms involved in antagonism of soil borne pathogens are antibiosis, enzyme production as well as mycoparasitism (Harmon, 2000; Chet, 1987). In the present investigation, all the three *Trichoderma* spp., *viz,. T. viride, T. harzianum and T. virens* were very effective against *H. maydis in vitro*. Further, under field conditions, soil application of bioagent enriched FYM was more effective in protecting the crop than foliar spray.

Table 1. Antagonism of *Trichoderma* against *H. maydis in vitro*

Treatment	Colony diameter of antagonists (mm)	Colony diameter of *H. maydis* (mm)	Inhibition of growth of *H. maydis* (%)	Mode of action
T. harzianum	70.0	20.0	55.1(47.8)	Mycoparasitism
T. virens	69.0	21.0	52.6(46.2)	Antibiosis followed by mycoparasitism
T. viride	72.5	17.5	60.7(51.4)	Antibiosis followed by mycoparasitism
H. maydis		44.6	–	–
SEM (±)		0.377	0.521	
CD (P=0.05)		1.14	1.51	

Table 2. Biological control of maydis leaf blight of maize in field

Treatment	Dose	Disease intensity			Yield (q ha^{-1})			Increase in yield over control (%)		
		2005	2006	Pooled mean	2005	2006	Pooled mean	2005	2006	Pooled mean
T. viride (SA)	100g / m²	2.6	2.7	2.6	19.3	18.0	18.6	89.2	85.5	87.3
T. harzianum (SA)	100g /m²	2.8	2.9	2.8	18.3	17.2	17.7	79.4	77.3	78.3
T. virens (SA)	100g /m²	3.1	3.0	3.0	17.0	16.2	17.3	66.6	67.0	66.8
T. viride spray	1.0 g / l	3.8	3.9	3.8	14.6	13.8	14.3	43.1	42.2	42.6
T. harzianum spray	1.0 g / l	3.9	4.0	3.9	13.4	12.8	13.4	31.3	29.8	30.5
T. virens spray	1.0g / l	4.1	4.2	4.1	12.4	12.0	12.2	25.4	23.7	24.5
Control	–	4.6	4.7	4.6	10.2	9.7	9.9	–	–	–
CD at 5%		0.42	0.44	0.22	0.52	0.63	0.33			

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