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IN-VITRO EFFICACY OF NATIVE BIO-CONTROL AGENTS FROM MAIZE REGIME AGAINST *EXSEROHILUM TURCICUM* IN RESPONSE TO BOTANICALS AND FUNGICIDES

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ABSTRACT
Three native *Trichoderma* spp. *viz*. *T. koningii* (phylloplane), *T. virens* (soil) and *T. asperellum (*soil) were collected and identified from maize regime including rhizosphere and phylloplane based on their morphological characters using Trichoderma home illustrations. *In-vitro* efficacy three native and two *Trichoderma* spp. from ICAR laboratory were tested against *E. turcicum* in response to plant extracts and fungicides. *T. harzianum* showed 54.14 % mycelial inhibition followed by *T. viride* (53.88%). Among plant extracts, lantana 10% showed maximum inhibition of 43.87% mycelia inhibition of *E. turcicum*. While Datura 10% and 20% concentration was found less effective in comparision. Mancozeb 75% WP at both the doses (@1.25 and 2.5 g/l) was found same effect inhibiting 94.44% mycelia inhibition of *E. turcicum*. Whereas, Amistar 25 SC inhibited 33.33% in half dose and a least 31.85% inhibition was observed in full dose.

INTRODUCTION

Maize (*Zea mays* L.) is one of the important cereal crops and it is 3rd major crop in India after rice and wheat. It is grown throughout the year and predominantly as *kharif* crop with 85 per cent of the area under cultivation in the season. It accounts for 9 per cent of total food grain production in the country.

In spite of being important crop, it is affected by several foliar diseases. Among them, turcicum leaf blight is most important worldwide *per se* and Meghalaya in particular. It is caused by *Exserohilum turcicum* (Pass.) Leonard and Suggs. (syn.*Helminthosporium turcicum* Pass.) with the teleomorph of Setosphaeria turcica (Lutterell) Leonard & Suggs.) affecting photosynthesis with severe reduction in grain yield losses up to 16 - 98% (Kachapur and Hedge, 1988). Disease epidemics at an early stage cause premature death of blighted leaves which lose their nutritive value even as fodder (Payak and Sharma, 1985; Kachapur and Hegde, 1988; Patil *et al*., 2000).

In India, the disease is prevalent in all the maize growing areas. During rainy season, the disease is prevalent in the Himalayan region; in peninsular India it is prevalent during both rainy and post rainy seasons and in the plains of eastern India during post rainy season (Dutta *et al*., 2008). It causes a serious problem in the states of Karnataka, Himachal Pradesh, Uttar Pradesh, Uttarakhand, Orissa, Andhra Pradesh and North Eastern Hill states. The disease appears regularly in the mid hills of Meghalaya in the *kharif* season. Barapani, Meghalaya also considered as a hot spot for turcicum leaf blight, where during cropping season a sizeable amount of disease is observed every year (Dutta *et al*., 2012).

Although the yield losses due to turcicum leaf blight can be reduce by certain application of fungicides, botanical and biological control methods. Use of native bio-control agent in disease management is considered as eco-friendly and sustainable approach for disease management. In Meghalaya research work on important aspect of the disease and pathogen has not been done systematically. Information on native bio-control agents to be used as bio control agents was also lacking. Therefore, the present study was carried out to explore the feasibility of several native bio control agent present in maize regime and their management strategy with the combination of plant extracts and fungicides as efficient and effective components against turcicum leaf blight of maize *in vitro* condition.

MATERIALS AND METHODS

The present investigation was carried out at Division of Crop Health, ICAR Research Complex for NEH Region, Umiam, Meghalaya.

Sample collection

Samples were collected from the field of ICAR NEH region, Umiam. The sample, for phylloplane, collected were those characterized by deep green and disease free from upper part of the maize plants. Soil samples were collected from 5-6 cm

depth from rhizosphere soil of healthy maize plant using soil augur.

Isolation of native bio-control agents from Phylloplane

The isolation of *Trichoderma spp*. from the phylloplane of maize leaf was done by the method described by Joshi (2008). The collected leaf samples were cut into small pieces aseptically in a laminar flow chamber and mixed thoroughly. One gram (w/v) sample was taken in a 250 ml sterilized conical flask containing 100 ml of sterilized deionised water and stirred vigorously for 30 minutes to detach the surface propagules. A suspension of 1:100 was then obtained and 10 ml of this suspension was transferred aseptically to a 250 ml conical flask containing 90 ml of sterilized deionised water to get a dilution of 1:1000. The suspension (500 μl) was transferred aseptically into sterilized Petriplates. The media was poured as stated above Rose Bengal Agar (Martin, 1950) and incubated at 26ºC for 5 days.

Isolation of native bio- control agents from soil

The collected soil samples were composited and representative samples were used for isolation. The soil samples were homogenized and then spread on a clean paper for shade drying for a week. All unwanted materials were removed then ground to fine powder. The samples were then sieved through 2 mm sieves. The powdered sample (10g) was transferred to 100 ml sterilized deionised water to make solution samples. This solution sample was diluted to 10^{-6}.The solution samples were mixed properly by shaking for 5 minutes and 10 ml of aliquot was taken and transferred to 90 ml water blank containing sterile deionised water. The suspension was stirred for one minute, before it was further diluted to 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} for isolation of *Trichoderma* spp. 1- ml of suspension from respective dilutions was transferred aseptically into Petriplates. Then 15 ml of melted Trichoderma Semi-selective Medium (TSM) was poured. The plates were swirled manually for uniform distribution of the suspension in medium and were allowed to solidify. The plates were incubated at 26ºC for 5 days in order to develop fungal colonies. The colonies with characteristic growth of particular organism were observed under microscope. The growth of bio-agents was further purified by hyphal-tip culture method. Cultures were identified based on the shape and size of the conidia, conidiophores, branching and phialides arrangements with the help of keys available on Trichoderma Home(http://nt.ars-grin.gov/taxadescriptions/keys/trichodermaindex .cfm). The identified cultures were maintained by sub-culturing on agar slants.

Identification of native bio-control agents

For identification of native isolates, three different culture media namely Potato Dextrose Agar (PDA), Corn Meal Agar (CMA) and Malt Extract Agar (MEA) were used under different conditions. The isolates were grown in different media and incubated at 26°C under complete darkness, complete light and alternate day and night with three replication of each isolates. Then, the Identification was made through visual as well as microscopic observation. Visual observations were made on the basis of colony character, mode of mycelial growth, colour, odour and changes of medium colour posses by isolates on different media. Microscopic observation studies were carried out on examination of shape, size, arrangement and development of phialides. Then sample was compared to a taxonomic key for the genus using Trichoderma Home (Samuels *et al*., 2002) and Manual book (Rahaman *et al*., 2009).

Maintenance of culture

The isolated and purified *Trichoderma* spp. were sub-cultured on PDA slant and allowed to grow at 26ºC for 3 days. The pure cultures so obtained were maintained by storing in refrigerator at 4ºC and revived monthly for further studies.

In vitro* efficacy of bio-control agents against *E. trucicum

Three *Trichoderma spp*.(*Trichoderma koiningii, T. virens* and *T. asperellum*) isolated from phylloplane, soil and two *Trichoderma spp*. (*T. viride* and *T. harzianum*) received from ICAR plant pathology lab were evaluated against *E. turcicum* by using dual culture technique (Adebola and Amadi, 2010). Petriplates was inoculated with 5-mm diameter mycelia disc of 5 days old cultures of *E. turcicum* and bio-agent were inoculated at equal distance from the periphery. The plates were incubated at 25ºC.The experiment was laid in 3 replications and control consisted individual plates for bio-agents and the pathogen. Observations on radial growth were recorded every 24 hrs after inoculation until the growth was full in control plate of the pathogen (90 mm diameter). The radial growth of the pathogen as well the bio-agent was measured and the per cent growth inhibition of the test pathogen over control was calculated according to the formula given by Vincent (1927) as described below.

In-vitro* efficacy of botanicals against *E. turcicum

Two botanicals namely Lantana (*Lantana camara*) and Datura (*Datura innoxia*) were evaluated for their bio-efficacy against *E. turcicum* using poisoned food technique (Nene and Thapliyal, 1979). Three concentrations (5%, 10% and 20%) of each plant extract were evaluated. Clean and dried fresh plant materials with equal (w/v) and sterilised distilled water were mixed using mortar and pestle. Each plant species extract, first filtered through two fold muslin cloth and then through 0.2 μm syringe filter (Whatman®). The required quantity sterile filtrate plant extract was incorporated separately in 100 ml melted sterilized PDA. Then, the poisoned medium was poured into Petriplates under aseptic conditions. The mycelial disc of 5mm diameter taken from 5 days old culture of *E. turcicum* was inoculated at the centre of the plates in three replications and incubated at 25ºC. The radial growth (cm) of *E. turcicum* was recorded every 24 hrs after inoculation until the control plate attained full growth. The per cent inhibition (%) on growth of the mycelium was calculated by using the formula of Vincent (1927) as describe below.

In-vitro* efficacy of fungicides against *E. turcicum

Two fungicides Mancozeb 75% WP (2.5 g/l and 1.25 g/l) and Azoxystrobin 23% SC (2.0 ml/l and 1.0 ml/l) at different concentrations were used for efficacy against radial growth of the *E. turcicum* using poisoned food technique (Nene and Thapliyal, 1979). Two concentrations of each fungicide were incorporated into 100 ml melted PDA. The food poisoned media were poured into Petri plates for each treatment under aseptic conditions and pathogen was inoculated. A 5-mm disc of mycelium taken from 5 days old culture of *E. turcicum*

was inoculated at the centre of the plates in three replications and incubated at 25^{0}C. Medium without fungicides served as control. The observations were recorded every 24 hr after inoculation in term of radial growth (cm) until the control plate attained full growth. The per cent inhibition on growth of the mycelium was calculated by using the formula of Vincent (1927).

$$I = \frac{C - T}{T} \times 100$$

Where,

I = Per cent inhibition of mycelial growth

C = Colony diameter in control

T = Colony diameter treatment

RESULTS AND DISCUSSION

Isolation and identification of native bio-control agents from maize regime

Three *Trichoderma* spp were isolated from phylloplane and rhizosphere using serial dilution method. *Trichoderma* spp. posses light green colour colony surrounded by white mycelium on Trichoderma Selective Medium (TSM). The *Trichoderma koningii* was isolated from the collected leaves sample from maize phylloplane region on TSM. The standard methodology was followed as observed by McFadden and Sutton, 1975. Different *Trichoderma* spp. were isolated from the collected soil sample from maize rhizosphere region on Trichoderma Selective Medium. Two species of Trichoderma namely *T. asperellum, and T. virens* were identified. The standard methodology was followed as used by Rahman *et al*., 2009.

Identification of native bio-control agents

The *Trichoderma spp*. isolated from maize regime were identified as *T. koningii* (phylloplane), *T. viren* (soil) and *T. asperellum (*soil). According to their morphological characters such as colony, phialide and spore size produced in different media such as corn meal agar (CMA), Potato Dextrose Agar (PDA) and Malt Extracts Agar (MEA) on different conditions *i.e*. Dark, light and alternate day and light respectively. According to their respective characters possessed, the isolates were identified using Trichoderma home.

In-vitro efficacy of bio-control agents against *E. turcicum*

Five *Trichoderma* spp. viz., *T. koningii* from phylloplane, *T. virens and T. asperellum* from soil & *T. viride* and *T. harzianum* from ICAR Plant Pathology lab were screened under *in vitro* conditions against *Exserohilum turcicum* for their antagonistic activity. It is apparent from data presented in table 1 that all the antagonistic effects showed significantly inhibited the growth of *E. turcicum* ranging from 48.75 to 54.14 per cent over control. Among them, *T. harzianum* (54.16%) showed highest mycelial inhibition followed by *T. viride* (53%) and *T. asperellum* (51%), these differences in inhibition might be attributed to microbial interactions such as competitive ability, production of toxic metabolites leading to antibiosis by the antagonist over pathogen. The antagonistic effects of *T. harzianum* and *T. viride* observed in the present study were in tune with the findings of Mahamood *et al*., 1995, Ramachandra, 2000 and Kumar *et al*., 2010.

In-vitro efficacy of botanicals against *E. turcicum*:

The antimicrobial effect of two plant extracts on radial growth and per cent inhibition of *E. turcicum* were tested at three different concentrations *i.e*. 5%, 10% and 20% by poisoned food technique and data on inhibition radial growth of *E. turcicum* are presented in table 2. It revealed that lantana 10% and 20% was found effective in inhibiting radial growth of *E. turcicum* by per cent inhibition of 43.87% and 42.36%, respectively. While Datura at 10% and 20% was found less effective as compare to *Lantan camara i.e*. 29.87% and 30.46%, inhibition respectively. The present study also revealed that both concentration of *Lantan camara* showed comparable effect without significance differences. The inhibitory effect of *L. camara* might be due to the presence of antimicrobial compounds such as phenolic compounds, flavonoids, saponins, tannins, phlobatanins, glycosides and alkaloids, which retarded the growth and activation of the pathogen. Similar finding on antimicrobial effects of

different aqueous plant extracts have been reported by Harlapur *et al*., 2007 and Meena *et al*. (2003). However, the present investigations are found contrary with the result received by Harlalpur *et al*. (2007) who reported that Neem Seed Kernel Extract (NSKE) @ 5.0 per cent showed maximum inhibition, whereas lantana 10% showed 43.87 % inhibition over control. The present findings are in tune with the work of Singh and Singh, 2014, Yoon *et al*., 2010, Sanjeev *et al*., 2009. Patil and Kulkarni (2002) reported, among the extracts of 35 plants evaluated *in vitro* against *Exserohilum hawaiiensis*, *Eucalyptus globus* inhibited the maximum mean spore germination (88.48%), followed by *Lantana camara* (87.54%) and *Flacourtiara montchii* (85.43%). Similar results on

Table 1: *In-vitro* bio-efficacy of bio- control agents against *E. turcicum*

Bio control agents	Radial growth(cm*) 1st DAI#	2nd DAI#	3 DAI#	% inhibition over control
Trichoderma koningii (Phylloplane)	1.77	2.59	2.67	49.87
T. virens (Soil)	1.81	2.59	2.73	48.75
T. asperellum (Soil)	1.75	2.55	2.61	51.00
T. viride (ICAR)	1.76	2.39	2.45	53.88
T. harzianum (ICAR)	1.73	2.37	2.44	54.14
Control	2.13	3.47	5.32	
SE(d) ±	-	-	0.06	
CD(p = 0.05)	-	-	0.16	

* Mean of five replications. # DAI = days after inoculation

Table 2: *In- vitro* bio- efficacy of plant extracts against *E. turcicum*

Plant extracts	Radial growth (cm*) at 5 DAI#	% inhibition over control
Lantana 5%	8.54	0.35
Lantana 10%	4.81	43.87
Lantana 20%	4.94	42.36
Datura 5%	8.56	0.12
Datura10%	6.01	29.87
Datura 20%	5.96	30.46
Control	8.57	-
SE(d) ±	0.09	
CD($p = 0.05$)	0.23	

* Mean of three replication; #DAI = days after inoculation

Table 3: *In-vitro* efficacy of fungicides on radial growth of *E. turcicum*

Fungicides	Doses	Radial growth (cm*) at 6 DAI#	% inhibition
Mancozeb 75% WP	1.25g/l	0.50	94.44
Mancozeb 75% WP	2.5g/l	0.50	94.44
Amistar 23% SC	1.0ml/l	6.13	31.85
Amistar 23% SC	2.0ml/l	6.00	33.33
Control		9.00	-
SE(d) ±		0.07	
CD($p = 0.05$)		0.19	

* Mean of five replications. #DAI = days after inoculation

antifungal activity of aqueous extracts of different plants has been reported by various workers (Rahman *et al*., 2007, Meena *et al*., 2003, Hegde *et al*., 2014).

In-vitro* efficacy of Efficacy of fungicides against *E. turcicum

Two fungicides were evaluated against *E. turcicum* at two doses by poisoned food technique and the data are presented in table 3. The fungicides tested effectively inhibited the mycelial growth of *Exserohilum turcicum* over control. Among the two fungicides, Mancozeb 75% WP at both the doses (1.25 and 2.5 g/l) showed 94.44% inhibition. as compared to Amistar 25 SC. The inhibitory effect of Mancozeb 75% WP inhibited the mycelial growth and development of the pathogen .The present investigations are found in agreement with the finding of Sanjeev kumar and A.K. Mauriya, 2015; Kumar *et al*., 2010; Harlalpur *et al*., 2007 and (Singh and Gupta, 2000). But the present study showed 94.44% inhibition whereas the earlier worker reported 100% inhibition. The effectiveness of fungicides carboxin, mancozeb and propiconazole against *E. turcicum* has been reported by several authors (Bhaliya and Jadeja, 2014, Kumbhar *et al*., 2012, Ramachandra and Kalappanavar, 2006 and Ambhore *et al*., 2003).

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