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RESEARCH PAPER

Evaluation of botanicals, bioagents and ITK's against *Exserohilum turcicum* (Pass.) Leonard and Suggs., causal agent of *Turcicum* leaf blight of maize

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Abstract: Maize (*Zea mays* L.) is one of the important cereal crops in the world standing next to wheat and rice. Maize is prone to several diseases among which *Turcicum* leaf blight (TLB) caused by *Exserohilum turcicum* is one of the most devastating foliar fungal disease causing grain yield losses ranging from 28-91 per cent. TLB is widely distributed in all maize growing areas of our country. Among the evaluated botanicals, Margoneem (Azadirachtin 0.15%) and Perfekt (Herbal mixture) were significantly superior to other botanicals with 100 per cent mycelial growth inhibition of *E. turcicum* at all concentrations. Among the bio-agents, *Trichoderma harzianum* and *Pseudomonas fluorescens* showed the maximum per cent mycelial growth inhibition of 100 and 68.90 per cent, respectively. Of the various Indigenous Technical Knowledge evaluated, panchagavya @ 10 per cent and cow urine @ 10 per cent proved to be most effective with 70.97 and 62.35 percent of inhibition of conidial germination.

Key words: Bioagents, Botanicals, *Exserohilum turcicum*, ITK's, Maize

Introduction

Maize (*Zea mays* L.) is the world's third leading cereal crop, after wheat and rice. It has got immense potential and hence called as 'Miracle crop' and also called as 'Queen of cereals' (Dey *et al.,* 2013). Among the major foliar diseases of maize, Turcicum leaf blight (TLB) of maize caused by *Exserohilum turcicum* reported to cause significant loss in grain yield from 24-91 per cent (Pant *et al.,* 2000; Nwanosike *et al*., 2015). The disease was first reported by Passerini in 1876 from Italy. In India, the disease was first reported by Butler *et al*. (1907). TLB is caused by the ascomycete fungus *Exserohilum turcicum*. The nomenclature of *Exserohilum turcicum* was given by Leonard *et al*. (1989). The perfect state of *Exserohilum turcicum*, *Setosphaeria turcica* is rarely found in nature (Leonard and Suggs, 2009). TLB is considered to be one of the most devastating foliar diseases in Karnataka resulting in grain yield loss of 28 to 91 per cent (Pandurangegowda *et al*., 1992). NCLB or TLB has long elliptical, grey-green lesions and as the lesions mature, they become tan with darkish zones of fungal sporulation. The lesions are 3-15 cm in length and lesions look like cigar shaped. This infection starts inhibiting photosynthetic activities on leaf surface. The lesions appear dark grey, olive or black and the grey black mold layer is the pathogen's conidiophores and conidia (Hooda *et al*.,2017).

In recent years, the need for integrated approach in managing the diseases of corn is augmented and crucial. Therefore, there is a great demand for new methods to supplement the existing disease management strategies to achieve better blight control. Managing TLB with resistant cultivars is not practical. Fungicides dominate as the most common method for management of TLB disease (Jakhar *et al.,* 2017). Conversely, increasing use of chemicals is a growing concern for both environment and public health. Use of plant products in disease management is a contemporary eco-friendly approach and gaining popularity considering that of its benefits over chemical compounds. These plant extracts are easily biodegradable, without any residue, non-phytotoxic and are easily absorbed by the plants and cost effective. The presence of naturally occurring substances in plants with antifungal properties had been stated and tested towards wide range of fungi infecting many commercially important crops. In the recent past, the philosophy of crop protection has shifted from the use of environmentally unsafe chemical pesticides to the eco-friendly approaches (Pathania *et al.,* 2021). This study primarily aimed to evaluate the efficacy of commercially available botanicals, bioagents and ITK's against *E.turcicum* under *invitro* conditions.

Material and methods

Isolation of the pathogen

The leaves of maize plants severely infected by turcicum leaf blight showing typical leaf blight necrotic lesions were collected from experimental fields of AICRP on Maize, Main Agricultural Research Station, University of Agricultural Sciences, Dharwad. The standard tissue isolation procedure was followed to isolate the pathogen. The leaf bits showing typical symptoms of *turcicum* leaf blight were surface sterilized in 0.1 per cent sodium hypochlorite solution for 30 seconds and repeatedly washed separately in sterilized distilled water to remove the traces of sodium hypochlorite. Later the sterilized bits were aseptically transferred to sterilized Petri plates containing Potato Dextrose Agar (PDA) in aseptic condition in laminar air flow. The inoculated Petri plates were incubated at room temperature (27±1ºC) for 15 days for fungal growth. The growth of the fungus was conspicuous after 24 h of incubation. The pure colonies developed from the bits were transferred to

PDA slants and incubated at room temperature for 15 days. Abundant sporulation was observed after 15 days of incubation. The pathogen was purified following hyphal tip isolation technique. The hyphal tip culture of the fungus was sub-cultured on PDA slants and kept in the laboratory at 27±1 ºC for 15 days and was used for further studies.

Evaluation of commercial botanicals

The various commercial botanicals were tested *in vitro* against *E. turcicum* by poisoned food technique. (Table 1) Potato dextrose agar (PDA) medium was prepared in flasks and sterilized. Desired concentration of botanicals (0.25%, 0.5% and 1%) were prepared by mixing with medium under constant stirring. The medium was poured into sterilized Petriplate and allowed to solidify. A disc of test fungus grown on solid medium was cut with the help of the sterilized cork borer and placed aseptically in the middle of the Petri plate and incubated at room temperature for 7 days. The culture discs grown without the botanical on PDA medium served as control and the diameter of the fungal colony was measured after incubation. Experiment was conducted in three replications. Per cent inhibition of growth of the pathogen was calculated using the formula given by Vincent (1947).

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

Where; I=Per cent in hibition, C = Growth of mycelium in control, T= Growth of mycelium in treatment

The following commercial botanicals were evaluated at three concentrations *viz*., 0.25%, 0.5% and 1%.

Evaluation of bio-agents

The efficacy of bio-agents was tested against *E. turcicum* under *in vitro* conditions using dual culture method (Table 2). The bioagents were evaluated by inoculating the pathogen obtained from actively growing cultures at one side of the Petri plates and antagonist at exactly opposite side of the same Petri plate. In case of evaluation of fungal antagonist, mycelial disc of test fungus was placed at one end of the Petri plate and antagonist fungus was placed opposite to it on the other end. Incase of evaluation of bacterial antagonists, bacterium was streaked at one end and mycelia disc of fungus was placed at other end of Petri plate. Four replications were maintained for each treatment. Per cent inhibition of mycelial growth was calculated using the formula given by Vincent (1947).

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

Evaluation of Indigenous technology knowledge (ITK's)

Various ITK's such as Panchagavya, Jeevamrutha, Cow urine, Brahmastra and Neemastra were evaluated under *in vitro* conditions by using cavity slide method at three concentrations *viz.,* 2.5%, 5% and 10%. Experiment was conducted with four replications. Percent spore germination over the control was calculated by using the formula given by Vincent (1947).

$$\text{Percent spore germination (I)} = \frac{C-T}{C} X\ 100$$

Where C= Germination of conidia in control, T = Germination of conidia in treatment

Table1. Details of *in vitro* evaluated commercially available botanicals

Botanical
Margo Neem (Azadirachtin 0.15%)
Nimbicidine (Azadirachtin 0.03%)
Perfekt (Herbal mixture)
Crude Pongamia oil (Karanjin)
Jojoba oil
Cinnamon oil
Crude Neem oil

Table 2. Details of *in vitro* evaluated bioagents

Bioagent	Accession No.
Trichoderma harzianum Rifai	MH027645.1(IOF strain)
Pseudomonas fluorescens (Flugge) Migula	NAIMCC-B-01981 (IOF strain)
Bacillus subtilis (Ehrenberg) Cohn	MT383652.1(IOF strain)
Neofusicoccum parvum (Penny cook and Samuels) Crous, Slippers and Phillips	Endophyte (IOF strain)
Control	

Table 3. Efficacy of commercially available botanicals on inhibition of mycelia growth of *E.turcicum*

Botanicals	Percent mycelia growth inhibition Concentration(%)			Mean
	0.25	0.5	1.0	
Margoneem (Azadirachtin 0.15%)	100(90.00)*	100(90.00)	100(90.00)	100(90.00)
Nimbicidine (Azadirachtin 0.03%)	64.90(53.66)	73.60(59.08)	100(90.00)	79.50(67.58)
Perfekt (Herbal mixture)	100(90.00)	100(90.00)	100(90.00)	100(90.00)
Crude pongamiaoil (Karanjin)	76.03(60.68)	72.36(58.28)	72.36(58.28)	73.58(59.08)
Jojoba oil	65.00(53.72)	67.30(55.12)	86.23(68.22)	72.84(59.02)
Cinnamon oil	64.90(53.66)	72.13(58.13)	75.50(60.33)	70.84(57.37)
Crude neem oil	91.93(73.49)	96.60(79.37)	98.56(83.14)	95.69(78.66)
Mean	80.39(67.88)	83.14(69.99)	90.37(77.13)	84.63(71.67)
S.Em. ±	C.D.at 1%			
Botanicals (B)	0.09	0.36		
Concentration (C)	0.06	0.24		
B x C	0.16	0.63		

*Figures in paranthesis indicate arcsine transformed values

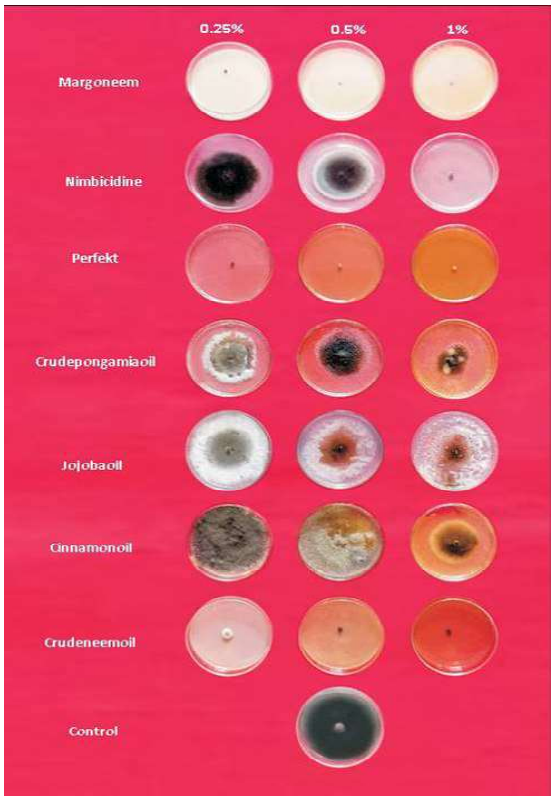


Fig1.Efficacy of commercially available botanicals on inhibition of mycelial growth of *turcicum*

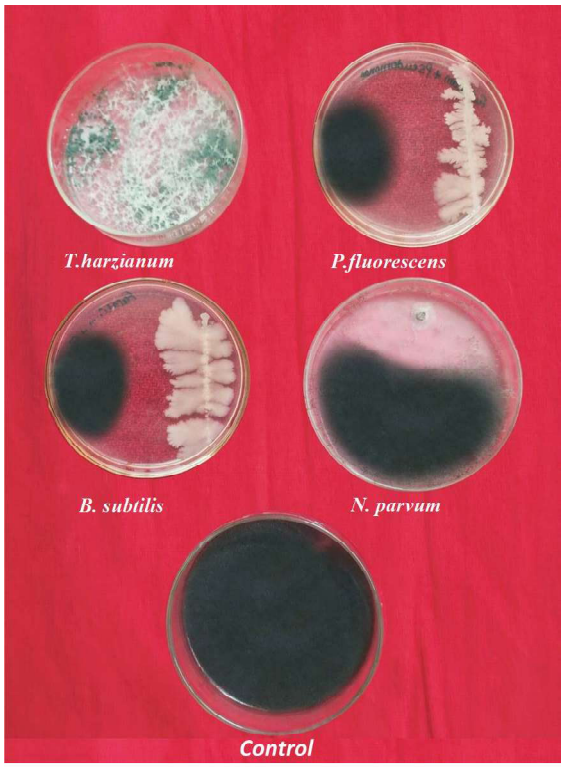


Fig 2. Efficacy of bioagents on inhibition of mycelial growth of *E.turcicum*

Procedure for preparation of ITK's

Panchagavya:7 kg cowdung and 1kg of cow ghee were mixed thoroughly for 2 days. Then 3 litres of cow urine and 10 litres of water were added to this mixture and kept it for 15 days. Daily the mixture was stirred 2-3 times. After 15 days, 3 litres of sugarcane juice or 250 g jaggery, 2 litres cow milk, 2 litres cow milk curd, 2 litres coconut water, 100 g yeast and 12 over ripened bananas were added and the mixture was allowed to ferment for 15 days. Then the contents were stirred 2-3 times daily in clock wise direction.

Jeevamrutha: A 200-litre capacity drum was taken and half of it was filled with water. Cowdung was mixed into it. For this mixture, 10 litres of cow urine and 2 kg of powdered jaggery were added, ensuring that they dissolved properly. Then 2 kg pulse flour was added and stirred. Five hundred gram of bund soil was added to the mixture. It was stirred in clockwise direction. The remaining half of the water was added to the drum. The mixture was thoroughly stirred two to three times daily for up to one week.

Table 4. Efficacy of bioagents on inhibition of mycelia growth of *E.turcicum*

Treatments	Bioagents	Percent mycelial growth inhibition
T1	*Trichoderma harzianum*	100.00 (90.00)*
T2	*Pseudomonas fluorescens*	68.90 (56.10)
T3	*Bacillus subtilis*	64.10 (53.19)
T4	*Neofusicoccum parvum*	63.17 (52.63)
Mean	74.04 (62.98)	
S.Em.±	0.13	
C.D.at1%	0.55	

Cow urine: Cow urine diluted with water in a ratio of 1:20.

Brahmastra:3 kg neem leaves were crushed in 10 litres of cow urine. 2 kg custard apple leaf, 2 kg papaya leaf, 2 kg pomegranate leaves, 2 kg guava leaves were crushed with water. Later, these two were mixed and were boiled for 5 min until it became half. This was kept for 24 hrs and the filtrate was squeezed.

Neemastra: 5 kg neem leaves were crushed with water, 5 litres cow urine and 2 kg cowdung were added to it and was allowed to ferment for 24 hours with intermittent stirring, later the filter was squeezed and the extract was diluted to100 litres.

Results and discussion

At all the concentrations (0.25,0.5 and1%), Margo neem (Azadirachtin 0.15%) and Perfekt (Herbal mixture) were most effective botanicals with 100 per cent mycelial inhibition. Crude neem oil was effective at higher concentration (10%) with overall mycelial inhibition of 95.69 per cent. Crude pongamia oil (Karanjin) and Jojoba oil were statistically on par with the mycelia inhibition of 73.58 and 72.84 per cent, respectively. Cinnamon oil showed least mycelia inhibition (70.84) in all the different concentrations tested (Table 3 and Fig 1).

Botanicals offer a promising alternative to the use of the fungicides. They are easily accessible, non-phytotoxic, renewable, inexhaustible and readily biodegradable, making them suitable for plant protection in integrated disease management. Wani *et al* (2017) reported that neem oil inhibited the maximum mycelial growth of the pathogen. Antifungal activity of neem is due to different types of terpenoids and phenolics. Pooja *et al*. (2020) has also proved antifungal potential

Table 5. Efficacy of ITK's on inhibition of conidial germination of *E.turcicum*

ITK's	Percent conidial germination inhibition Concentration (%)			Mean
	2.5	5	10	
Brahmastra	26.26 (30.82)*	26.56 (31.02)	28.96 (32.55)	27.26 (30.95)
Neemastra	40.46 (39.50)	48.53 (44.15)	64.55 (53.95)	51.18 (48.97)
Cow urine	54.20 (47.41)	65.36 (53.94)	67.50 (55.24)	62.35 (52.2)
Panchagavya	66.26 (54.49)	72.50 (58.37)	74.16 (59.45)	70.97 (57.44)
Jeevamrutha	25.83 (30.53)	27.33 (31.51)	27.40 (31.56)	26.85 (31.20)
Control	1.34 (6.64)	1.34 (6.64)	1.34 (6.64)	1.34 (6.64)
Mean	35.72 (36.70)	40.27 (39.38)	43.98 (41.54)	39.99 (39.22)
S. Em.±	C.D.at1%			
ITK's(I)	0.51	1.98		
Concentration(C)	0.39	1.53		
Ix C	0.88	3.44		

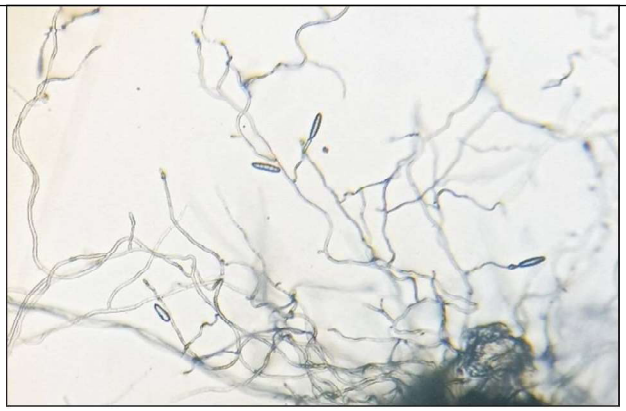
of neem against *E. turcicum* and confirmed that ethyl acetate fraction of *Azadirachta indica* found to be most effective in retarding the mycelia growth.

In the *in vitro* evaluation of bioagents using the dual culture technique, it was observed that there was significant difference in the per cent mycelial inhibition of *E. turcicum* among the various bioagents tested. *Trichoderma harzianum* exhibited the maximum mycelia inhibition of 100 per cent followed by *Pseudomonas fluorescens* which showed 68.90 per cent of mycelia inhibition. On the other hand, the least mycelia inhibition of 63.17 per cent was recorded in *Neofusicoccum parvum*. *Bacillus subtilis* showed 64.10 per cent of mycelial inhibition which was on par with *Neofusicoccum parvum* (Table 4 and Fig 2).

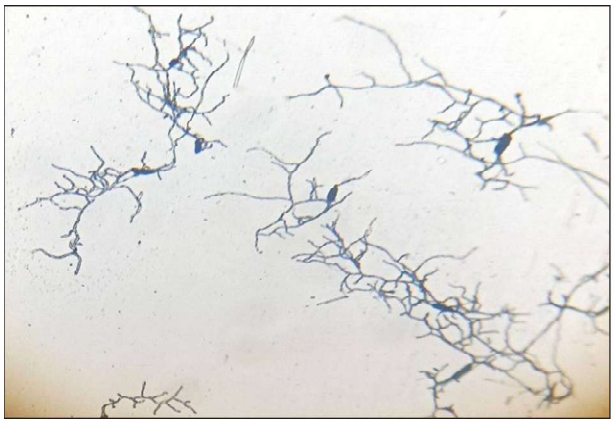
Bioagents contain the living antagonistic microorganisms which offer a promising nonchemical approach to manage plant diseases by reducing pathogen inoculums levels. This method helps to prevent pollution and health hazards associated with fungicide usage. The observed results are in line with the findings of Manu *et al*. (2017). Previous studies by Harlapur *et al*.(2007) and Khedekar *et al*. (2012) reported that *Trichoderma harzianum* was effective in inhibiting the mycelia growth of *E. turcicum.*

Panchagavya was most effective in inhibiting the conidial germination of *E.turcicum*, with an overall inhibition of 70.97 per cent followed by cow urine with 62.35 per cent of inhibition. Neemastra was found to be effective at higher concentration (10%) with over all inhibition of 51.18 per cent. Jeevamrutha and Brahmastra were least effective in all the concentration with inhibition of 26.85 and 27.26 per cent, respectively (Table 5 and Fig 3).

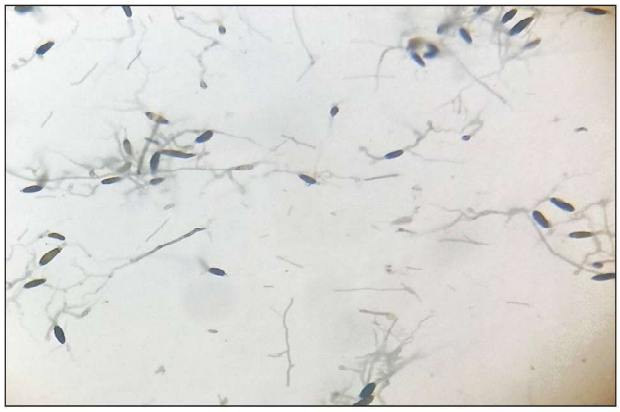
Panchagavya is a mixture of five cow-derived substances: milk, urine, dung, curd and ghee. While its effectiveness against *E. turcicum* is not fully studied but there is emerging evidence suggesting its potential as a sustainable and ecofriendly alternative. Several mechanisms could contribute to Panchagavya's potential against *E.turcicum*. Panchagavya contains various micro organisms that may have antagonistic effects on fungal pathogens. Panchagavya contains bioactive compounds with anti microbial properties. Studies have shown that cow urine contains phenolic compounds, alkaloids and volatile constituents that exhibit antimicrobial effects. These



Panchagavya at 10 per cent



Cow urine at 10 per cent



Control (Sterile distilled water)

Fig 3. Spore germination inhibition of *Exserohilum turcicum* in Panchagavya and Cow urine

compounds could potentially hinder the development and spread of *E. turcicum*. The observed results are in line with the findings of Wani *et al*. (2017).

Conclusion

Among the tested ITK's against *E. turcicum,* Panchagavya showed the maximum per cent of conidial inhibition at all three different concentrations (2.5%, 5% and 10%) with mean as 70.97 per cent. Cow urine was the second best effective ITK with 62.35 per cent of conidial inhibition followed by Neemastra with 51.18 per cent of conidial inhibition. Brahmastra and Jeevamrutha showed least conidial inhibition of 27.26 and 26.85 per cent, respectively at all the concentrations. At all the tested concentrations (0.25, 0.5 and 1%), Margoneem (Azadirachtin 0.15%) and Perfekt showed 100 per cent mycelial inhibition against *E. turcicum* followed by crude neem oil with per cent mycelial inhibition of 91.93, 96.60 and 98.56 at 0.25, 0.5 and 1 per cent, respectively. Nimbicidine (Azadirachtin 0.03%) proved to be effective at higher concentration. Among the tested bioagents, *Trichoderma harzianum* demonstrated the 100 per cent mycelia inhibition followed by *Pseudomonas fluorescens* with 68.90 per cent of mycelia inhibition. The lowest mycelial inhibition of 63.17 per cent was observed in *Neofusicoccum parvum* which was on par with *Bacillus subtilis* with 64.10 per cent of mycelia inhibition. The effective botanicals (Margoneem @ 0.25%, Perfekt @ 0.25%), bioagents (*T. harzianum* @10g/l*, P. fluorescens* @ 10g/l) and ITK's (Panchagavya @10%, Cow urine @ 10%) can be used for the further field evaluation in the integrated disease management of TLB of maize.

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