

Original Research Article

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**Antifungal Potential of *Trichoderma virens* Secondary Metabolites,
Botanicals and Fungicides against *Exserohilum turcicum*
causing Turcicum Leaf Blight of Maize**

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Maize is considered to be the third most important cereal after rice and wheat in India. It is being cultivated worldwide in a wider range of environmental conditions, because of its greater adaptability. It is known to be affected by more than 62 diseases. Among them, turcicum leaf blight caused by *Exserohilum turcicum* (Pass.) Leonard and Suggs is of significant importance as it drastically reduces the yield by reducing photosynthetic activity of the plant. Effect of secondary metabolites obtained from *Trichoderma virens*, was utilized in the study to understand and overcome many of the problems associated with the application of chemical pesticides in comparison to commercially available various *Trichoderma* formulations. *In vitro* studies have demonstrated that, among the different treatments tested, *Trichoderma* secondary metabolites (TSM), Neem seed kernel extract, combination of TSM with NSKE at 20 per cent concentration has showed highest per cent inhibition of mycelial growth of 61.28, 72.59 and 68.63 per cent respectively. Among the fungicides tested, maximum inhibition of mycelial growth of *E. turcicum* (100 %) was observed in both Tebuconazole (25.9 % EC) and Hexaconazole (5 % EC) at all the different concentrations tested.

Introduction

Maize (*Zea mays* L.) is considered to be the most important cereal crop which ranks third after wheat and rice in India. Maize is cultivated widely around the world because of its greater adaptability to varied environmental condition as well as its ability to produce higher yield. From last few

decades there has been a rapid increase in the area, production and productivity of this crop due to the introduction of high yielding indigenous varieties, exotic hybrids and also the use of improved farm machineries, chemical fertilizers and chemical pesticides. However, these agricultural inputs also lead the maize crop vulnerable to pests and diseases in the farmer's field.

Maize is known to be affected by more than 62 diseases (Singh *et al.*, 2019). Among them, turcicum leaf blight also called as Northern corn leaf blight (NCLB) caused by *Exserohilum turcicum* is one of the most important foliar disease which causes blightening and wilting of the leaves thereby reducing the photosynthetic area which results in severe reduction of grain yield to the extent of 28 to 91 per cent depending upon the intensity of infection (Chenulu and Hora, 1962). This disease not only causes necrosis and premature death of foliage, it also reduces the fodder and grain value of the crop.

The losses occurred due to turcicum leaf blight can be minimized by the foliar application of fungicides and plant extracts (Singh *et al.*, 2019). There is no doubt that, the application of chemical pesticides protects the crop from diseases and increases the crop yield, but at the same time their continuous and overuse has led to some serious ecological problems, *viz.*, hazardous effects on soil microflora and fauna, increased air pollution which directly shows its impact on human and animal health. It also results in the development of resistant strain by the pathogen. The use of plant extracts and bio pesticides in disease management is considered to be safe for the beneficial microorganisms, eco-friendly and an useful alternative for disease management.

Today, enormous number of agricultural products based on the biocontrol agent *Trichoderma* is commercially available worldwide. One of the major factors effectively contributing towards the beneficial biological activity of some *Trichoderma* species is related to their ability to secrete wide variety of secondary metabolites (Reino *et al.*, 2008; Sivasithamparam and Ghisalberti, 1998; Vinale *et al.*, 2014). Considering these factors, an attempt was made to explore the feasibility of utilizing the naturally produced compounds such as

secondary metabolites by *Trichoderma virens*, four plants extract and three chemical fungicides against *E. turcicum* under *in vitro* condition.

Materials and Methods

In vitro* evaluation of *Trichoderma virens* secondary metabolites (TSM) against *Exserohilum turcicum

The methodology described by Dennis and Webster (1971) was followed to extract Non-volatile metabolites (crude extract) produced by *Trichoderma virens*. Pure culture of *Trichoderma virens* was collected from the department of Plant Pathology, UAHS, Navile, Shivamogga for further *in vitro* studies.

Procedure for extraction of TSM

To obtain the secondary metabolites under laboratory condition, the edge of seven days old *Trichoderma virens* culture was cut using sterilized cork borer and inoculated into 100 ml sterilized potato dextrose broth in 250 ml conical flask and incubated at 30 °C in an orbital shaker at 150 rpm. After that, the culture filtrate was filtered through muslin cloth followed by Whatman paper no 40 filter paper for removing mycelial mat and further passed through membrane filter assembly and then centrifuged at 3000 rpm for 10 min. The supernatant thus obtained was stored and utilized for further studies.

These *Trichoderma virens* secondary metabolites were evaluated under laboratory conditions by following the "Poison food technique" (Grover and Moore, 1962).

Poison food technique

The secondary metabolites of required quantity was added to molten PDA medium to

obtain a final concentration of 2.5, 5, 10, 15 and 20 % v/v basis. Later, 20 ml of the poisoned medium was poured into sterilized Petri plates. A sterile cork borer was used to make a mycelial disc from seven days old culture of *E. turcicum* and one such disc was placed at the centre of each agar plate. The plate without secondary metabolites served as control. Four replications were maintained for each concentration. Such plates were incubated at room temperature (27 ± 1 °C) and the radial growth was measured when fungus attained maximum growth in control plates. The efficacy of the secondary metabolites was expressed as per cent inhibition of mycelial growth over control, which was calculated by using the formula given by Vincent (1947).

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

Where,

I= Per cent inhibition

C= Radial growth of the pathogen in control plate

T= Radial growth of the pathogen in treated plate

In vitro* evaluation of botanicals against *Exserohilum turcicum

The efficacy of four botanical extracts *viz.*, NSKE, Tulsi, Parthenium, Pongamia were tested against *Exserohilum turcicum*.

Preparation of NSKE

Neem seed kernel extract was prepared by following the procedure as described by Matharu and Chahil (2013).

Preparation of other botanicals

Fresh leaf sample of different botanicals collected and identified based on their

taxonomical characters. These samples were washed thoroughly using tap water followed by sterile distilled water.

Surface sterilization was done using one per cent sodium hypochlorite solution and finally rinsed thrice in sterile distilled water. 100 g of the sample was taken and crushed in a surface-sterilized pestle and mortar by adding 100 ml sterile distilled water (1:1 w/v). A ground stock solution of all the leaf extracts was collected by filtering through double layered muslin cloth followed by membrane filter assembly (Mohana and Raveesha, 2007).

Poison food technique

The botanicals of required quantity was added to molten PDA medium to obtain a final concentration of 2.5, 5, 10, 15 and 20 % v/v basis with three replications each and remaining procedures was same as explained under evaluation of TSM.

In vitro* evaluation of TSM in combination with botanicals against *Exserohilum turcicum

To check the compatibility and efficacy of combination of TSM and botanicals, the required equal proportion of TSM and botanicals were added separately into sterilized molten and cooled potato dextrose agar to get the desired (2.5, 5, 10, 15 and 20 %) concentration with three replications each and remaining procedure was same as explained under evaluation of TSM.

In vitro* evaluation of fungicides against *Exserohilum turcicum

To check the efficacy of three fungicides *viz.*, Tebuconazole, Hexaconazole and Carboxin (37.5 %) + Thiram (37.5 %) at 50, 100, 150, 250 and 500 ppm concentration against

Exserohilum turcicum, *in vitro* evaluation was carried out by following “Poison food technique” with three replications each and further per cent inhibition of mycelial growth over control was calculated by using the formula given by Vincent (1947).

Results and Discussion

In vitro evaluation of TSM against *Exserohilum turcicum*

Per cent inhibition of *Exserohilum turcicum* by *Trichoderma virens* secondary metabolites ranged between 16 to 62 per cent. However, among different concentrations tested, highest mean per cent inhibition of mycelial growth (61.28) was found at 20 per cent concentration followed by 53.83, 27.50 and 20.52 per cent inhibition at 15, 10 and 5 per cent, respectively. The least per cent mycelial inhibition (16.75) was observed at 2.5 per cent concentration (Table 1).

The reduction of mycelial growth was gradually increased as the concentration of secondary metabolites increased. Similar results were also obtained by Rahman *et al.*, (2011); Bae *et al.*, (2016); Joshi *et al.*, (2016) and Meena *et al.*, (2017) who reported higher inhibition at higher concentrations. This could be attributed to the ability of *T. virens* to produce various biologically active compounds.

As reported, *T. virens* secretes (both volatile and non-volatile) compounds like, alkylpyrones, peptaibols, polyketides, sesquiterpenes, siderophores and terpenoids which play an important role in reducing the mycelial growth through antibiosis (Zeilinger *et al.*, 2016). Whereas, some secondary metabolites Gliotoxin and Gliovirin secreted by *T. virens* belongs to chemical category Diketopiperazine which exhibit fungistatic

activity. Apart from that, Trichovirin II belongs to the chemical category of Peptaibols, involved in inhibition of glucan synthase activity, which in turn leads to the prevention of the reconstruction of the pathogen cell walls, thus facilitating the disruptive action of β -glucanases (Vinale *et al.*, 2014).

In vitro evaluation of selected botanicals against *Exserohilum turcicum*

The findings revealed that, plant extracts tested was found more effective in inhibiting mycelial growth of *E. turcicum* with increasing concentration. Among them, at all the concentration tested, neem seed kernel extract was highly efficient in inhibiting mycelial growth.

However, highest mycelial inhibition (72.59 %) was observed at 20 per cent concentration of NSKE followed by leaf extract of *Ocimum sanctum* (65.96 %) and *Parthenium hysterophorus* (54.52 %). Whereas, least mycelial inhibition (25.77 %) was recorded at 2.5 per cent concentration of *Pongamia pinnata* (Table 2).

The present findings are in agreement with the results obtained by Harlapur *et al.*, (2007), Vishwanath *et al.*, (2018) and Kiran and Patil (2019) who reported that neem seed kernel extract @ 5 per cent concentration showed maximum inhibition of mycelial growth of *E. turcicum* (56.64 %).

The efficacy of NSKE against *E. turcicum* mainly attributed to the presence of active compounds like Azadirachtin, Nimbolin, Nimbin, Salannin, Nimbidin, Nimbidol and Quercetin which possess the ability to disturb the cell wall synthesis as well as it causes damage in the cell wall and retards activation of the pathogen.

Table.1 *In vitro* evaluation of *Trichoderma virens* secondary metabolites against *Exserohilum turcicum*

Concentration (%)	Inhibition (%)
2.5	16.75 (24.14) *
5	20.52 (26.92)
10	27.50 (31.61)
15	53.83 (47.18)
20	61.28 (51.50)
S.Em ±	0.35
C.D. at 1 %	1.47
*Figures in parenthesis are arc sine transformed values	

Table.2 *In vitro* evaluation of selected botanicals against *Exserohilum turcicum*

Treatment	Inhibition (%)				
	2.5 %	5 %	10 %	15 %	20 %
NSKE	50.11 (45.05) *	58.37 (49.80)	62.04 (51.95)	65.70 (54.14)	72.59 (58.41)
<i>Ocimum sanctum</i>	38.52 (38.35)	47.44 (43.52)	54.15 (47.36)	60.37 (50.97)	65.96 (54.29)
<i>Parthenium hysterophorus</i>	27.11 (31.35)	38.67 (38.43)	45.78 (42.56)	51.52 (45.85)	54.52 (47.57)
<i>Pongamia pinnata</i>	25.77 (30.49)	28.62 (32.33)	30.85 (33.73)	36.25 (37.01)	44.00 (41.54)
	S.Em ±			C.D. at 1 %	
Botanicals (B)	0.237			0.91	
Concentration (C)	0.265			1.01	
F X C	0.530			2.03	
*Figures in parenthesis are arc sine transformed values, NSKE - Neem seed kernel extract					

Table.3 *In vitro* evaluation of TSM in combination with botanicals against *Exserohilum turcicum*

Treatment	Inhibition (%)				
	2.5 %	5 %	10 %	15 %	20 %
TSM +NSKE	57.19 (49.11)*	65.07 (53.75)	66.07 (54.35)	66.93 (54.87)	68.63 (55.92)
TSM + <i>Ocimum sanctum</i>	45.74 (42.54)	56.30 (48.60)	58.55 (49.91)	61.63 (51.70)	63.74 (52.96)
TSM + <i>Parthenium hysterophorus</i>	30.07 (33.24)	34.63 (36.03)	45.41 (42.35)	50.00 (44.98)	61.78 (51.79)
TSM + <i>Pongamia pinnata</i>	27.74 (31.77)	32.93 (35.00)	34.18 (35.76)	40.85 (39.71)	58.59 (49.93)
	S.Em ±			C.D. at 1 %	
TSM + Botanicals (T)	0.144			0.55	
Concentration (C)	0.161			0.61	
T X C	0.321			1.23	
*Figures in parenthesis are arc sine transformed values, TSM-Trichoderma secondary metabolites					

Table.4 *In vitro* evaluation of different fungicides against *Exserohilum turcicum*

Treatment	Inhibition (%)				
	50 ppm	100 ppm	150 ppm	250 ppm	500 ppm
Tebuconazole (25.9 % EC)	100 (89.96)*	100 (89.96)	100 (89.96)	100 (89.96)	100 (89.96)
Hexaconazole (5 % EC)	100 (89.96)	100 (89.96)	100 (89.96)	100 (89.96)	100 (89.96)
Carboxin (37.5 %) + Thiram (37.5 %)	50.96 (45.53)	61.67 (51.73)	80.55 (63.81)	89.92 (71.47)	100 (89.96)
	S.Em ±			C.D. at 1 %	
Fungicides (F)	0.107			0.42	
Concentration (C)	0.138			0.54	
F X C	0.239			0.93	
*Figures in parenthesis are arc sine transformed values					

In vitro* evaluation of TSM in combination with botanicals against *Exserohilum turcicum

Study with respect to TSM compatibility with other botanicals and their efficiency against *E. turcicum* under *in vitro* condition revealed that TSM was compatible with all the botanicals tested. However, treatment involving TSM + NSKE was found to be highly effective in inhibiting mycelial growth (68.63 %) at 20 per cent concentration. Least per cent inhibition (27.74) was noticed in the treatment involving TSM + *Pongamia pinnata* at 2.5 per cent concentration (Table 3).

When two components were added together, the inhibition of *E. turcicum* was enhanced than their individual treatments. It indicates that, TSM and botanicals can work together without affecting each other properties, thereby increasing the inhibition of the mycelial growth compared to individual treatment.

In vitro* evaluation of different fungicides against *Exserohilum turcicum

Among the three fungicides tested under *in vitro* conditions, maximum inhibition of mycelial growth of *E. turcicum* (100 %) was

recorded in both Tebuconazole (25.9 % EC) and Hexaconazole (5 % EC) at all the different concentrations tested *viz.*, 50, 100, 150, 250 and 500 ppm tested. Whereas, Carboxin (37.5 %) + Thiram (37.5 %) showed cent per cent inhibition at the concentration of 500 ppm. However, it exhibited 89.92, 80.55 and 61.67 per cent inhibition of pathogen growth at 250, 150 and 100 ppm concentrations, respectively. The least mycelial inhibition of 50.96 per cent was observed at a concentration of 50 ppm (Table 4). These results were in accordance with the results obtained by Manu *et al.*, (2017) and Bhatt and Kumar (2019) who evaluated different fungicides against *Exserohilum turcicum* and indicated that Propiconazole (25 % EC) and Tebuconazole (25.9 % EC) have toxic effect on the growth and development of the pathogen under *in vitro*. It is because both the fungicides possess the ability to inhibit sterol biosynthesis and demethylation process in the pathogen cell and thereby altering cell membrane structure and function.

In conclusion the TSM can be effectively utilized for the management of turcicum leaf blight of maize through an ecofriendly approach. Looking at the consumer preference in the modern world, it is quite understood that, consumer needs a food product which is free from any pesticide

residues. Under such situation we can explore different ways and means to utilize secondary metabolites of biocontrol agents like *Trichoderma virens* in the management of plant disease thereby providing the safe, residue free food to the consumer.

References

- Bae, S. J., Mohanta, T. K., Chung, J. Y., RYU, M., Park, G., Shim, S., Hong, S. B., SEO, H., BAE, D. W., BAE, I., KIM, J. J. and BAE, H., 2016, *Trichoderma* metabolites as biological control agents against *Phytophthora* pathogens. *Biol. Control*, 92: 128–138.
- Bhatt, B. and Kumar, P., 2019, Bioefficacy of fungicides, plant extracts and essential oils against *Exserohilum turcicum* (Pass.) Leonard and Suggs causing northern leaf blight of maize. *J. Appl. Nat. Sci.*, 11(2): 257-262.
- Chenulu, V. V. and Hora, T. S., 1962, Studies on losses due to *Helminthosporium* blight of maize. *Indian Phytopathol.*, 15: 235-237.
- Dennis, L. and Webster. J., 1971, Antagonistic properties of species-groups of *Trichoderma* I production of non-volatile antibiotics. *Trans. Br. Mycol. Soc.*, 57 (1): 25-39.
- Grover, R. K. and Moore, J. D., 1962, Toxicometric studies of fungicides against brown rot organisms *Sclerotinia fructicola* and *S. laxa*. *Phytopathol.*, 52: 876- 880.
- Harlapur, S. I., Kulkarni, M. S., Wali, M. C. and Kulkarni, S., 2007, Evaluation of plant extracts, bio-agents and fungicides against *Exserohilum turcicum* (Pass.) Leonard and Suggs. Causing turcicum leaf blight of Maize. *Karnataka J. Agric. Sci.*, 20(3): 541-544.
- Joshi, D., Singh, P., Singh, A. K., Lal, R. J. and Tripathi, N., 2016, Antifungal potential of metabolites from *Trichoderma* spp. against *Colletotrichum falcatum* Went causing red rot of Sugarcane. *Sugar Tech*, 18(5): 529–536.
- Kiran, B. M. and Patil, P. V., 2019, Integrated management of leaf blight of sweet sorghum caused by *Exserohilum turcicum* (Pass.) Leonard and Suggs. *Indian Phytopathol.*, 72: 63-69.
- Manu, T.G., Naik, G. B., Sayipratap, B. R. and Balagar, M. S., 2017, Efficacy of fungicides, botanicals and bio-agents against *Exserohilum turcicum*. *Chem. Sci. Rev. Lett.*, 6(24): 2100-2107.
- Mathuru, K. S. and Chahil, G. S., 2013, Role of neem based botanical pesticide in organic agriculture. *Popular kheti*, 1(4): 178-179.
- Meena, M., Swapnil, P., Zehra, A., Dubey, M. K. and Upadhyay, R. S., 2017, Antagonistic assessment of *Trichoderma* spp. by producing volatile and non-volatile compounds against different fungal pathogens. *Arch. Phytopathol. Plant Prot.*, 50(14): 629-648.
- Mohana, D. C. and Raveesha, K. A., 2007, Antifungal evaluation of some plant extracts against some plant pathogenic field and storage fungi. *J. Agric. Technol.*, 4(1): 119-137.
- Rahman, M. A., Rahman, M. M., Azad, A. K. and Alam, M. F., 2011, Inhibitory effect of different plant extracts and antifungal metabolites of *Trichoderma* strains on the conidial germination and germ tube growth of *Colletotrichum capsici* causing chilli anthracnose. *Int. J. Agron. Agric. Res.*, 1(1): 20-28.
- Reino, J. L., Guerrero, R. F., Galan, H. R. and Collado, I.G., 2008, Secondary metabolites from species of the biocontrol agent *Trichoderma*. *Phytochem. Rev.*, 7: 89-123.
- Singh, R., Jakhar, D. S., Kumari, R., Kumar, P. and Lal, I., 2019, Management of

- turcicum leaf blight in maize (*Zea mays* L.). *J. Biotech. Crop Sci.*, 8(12): 115-117.
- Sivasithamparam, K. and Ghisalberti, E. L., 1998, Secondary metabolism in *Trichoderma* and *Gliocladium*. In: Taylor and Francis London (Ed.), *Trichoderma and Gliocladium.*, pp: 139-191.
- Vinale, F., Sivasithamparam, K., Ghisalberti, E. L., Woo, S. L., Nigro, M., Marra, R., Lombardi, N., Pascale, A., Ruocco, M., Lanzuise, S., Manganiello, G. and Lorito, M., 2014, *Trichoderma* secondary metabolites active on plants and fungal pathogens. *Open Mycol. J.*, 8: 127-139.
- Vincent, J. M., 1947, Distortion of fungal hyphae in the presence of certain inhibitors. *Nature*, 150: 850.
- Vishwanath, V., Abhilasha, A. L., Sunil, Z. and Sobita, S., 2018, Efficiency of plant extracts and *Trichoderma* spp. against turcicum leaf blight of Maize caused by *Exserohilum turcicum* (Pass.) Leonord and Suggs. *Annals of Plant Prot. Sci.*, 25(2): 338-341
- Zeilinger, S., Gruber, S., Bansal, R. and Mukherjee, P. K., 2016, Secondary metabolism in *Trichoderma*-chemistry meets genomics. *Fungal Biol. Rev.*, 30(2): 74-90.

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