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Bio-efficacy of different Botanicals against Leaf Blotch Disease of Turmeric caused by *Taphrina maculans* under *In-vitro* condition

Mukul Kumar* and A.K. Mishra
Department of Plant Pathology, Tirhut College of Agriculture,
Dholi, (R.P.C.A.U) Pusa, Samastipur (Bihar), India.

(Corresponding author: Mukul Kumar)*
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ABSTRACT: Turmeric (*Curcuma longa* L.) is known as the "golden spice" and the "spice of life". It is also called the precious gift of nature and is often referred to as the 'National Heritage'. Turmeric is highly susceptible to many fungal diseases namely, Colletotrichum leaf spot (*Colletotrichum capsici*), leaf blotch (*Taphrina maculans*) and Rhizome rot (*Pythium* spp.) are the most serious diseases leading to crop losses in various parts of the country. Among these diseases the leaf blotch caused by *Taphrina maculans* is a very important disease of the leaves of turmeric that causes a significant decrease in yield due to loss of photosynthetic properties. Turmeric yield losses due to disease have been recorded up to 37.6-52.9 per cent and becoming so colossal in some areas that turmeric cultivation has become uneconomical, especially where susceptible varieties were grown. Pathogen Taphrina is hemi-biotrophic naturally so, it is difficult to distinguish. The culture of the *Taphrina* species is inseparable from the yeast culture. *Taphrina maculans* were isolated from PDA media supplemented with amino acids and yeast extract and extracts from five botanical plants were tested under laboratory conditions to prevent their activity to prevent their radiation growth. All botanicals taken for investigation have significantly inhibited fungal growth compared to control, Neem leaf extraction showed less radial growth of the pathogen, thus providing a greater inhibition effect on the pathogen. Based on a recent study, a decrease in the toxicity of botanicals in *T. maculans* were Neem leaf extract>Pipli leaf extract>Brahmi leaf extract>Tulsi leaf extract>Surpgandha leaf extract.

Keywords: Golden spice, spice of life, Botanicaslec tract, *Taphrina maculans*, Hemi-biotropic

INTRODUCTION

Turmeric (*Curcuma longa* L.) is one of the most important spice plants grown in India. It is also an indigenous Indian plant. It is a well-known remedy often referred to as the 'national heritage' and also called the precious gift of nature. The rhizomatous herbaceous perennial plant in the family Zingiberaceae. It has various uses in flavouring, dyeing, drug preparation, cosmetics and medicine (Dixit *et al.*, 2002). Turmeric is officially included in the Ayurvedic Pharmacopoeia of India, the Pharmacopoeia of the People's Republic of China and the Japanese standards for herbal medicines. The annual production of turmeric in India is approximately 59.86 lakh tons in an area of 892,213 hectares. In Maharashtra, the area cultivated by turmeric is 6146 ha with a production of 8,503 tons (Anonymous, 2021a). Leaf blotch disease caused by *Taphrina maculans* is a serious disease of the turmeric leaves causing a significant decrease in yield due to loss of photosynthetic properties. Poor crop production in the province is due to leaf blight caused by *Taphrina maculans* among other factors that impede its production. Disease loss of turmeric yields due to disease was recorded at 37.6-52.9 percent and increased so much in some areas that the cultivation of turmeric was not economical, especially where endangered species were cultivated. Leaf blotch disease caused by *Taphrina maculans* was reported for the first time from Rangapur, East Pakistan (Butler, 1911). Later, it was observed from all turmeric growing regions of the country (Upadhyay & Pavgi 1967). The individual spots are small, 1-2 mm in diameter and coalesce freely. In severe cases of attack, hundreds of spots appear on both the sides of leaves. The spots are discrete brownish black and mostly confined to lower leaves (Joshi & Sharma, 1982). Due to the proliferation of favourable environmental factors almost year-round and the growth of endangered commercial species in Bihar, the disease poses a serious threat to the cultivation of turmeric.

MATERIALS AND METHODS

Collection of Plant samples: Infected turmeric leaves that show common symptoms of turmeric leaf blight collected from various growing areas of turmeric in northern Bihar in 2017-18 were brought to the Plant Pathology laboratory and first detected under a microscope and back of that packaged under pressure eraser with a herbarium printing press and stored for re-testing at Tirhut College of Agriculture, Dholi, Dr.

Rajendra Prasad Central Agricultural University, Pusa, Samastipur, (Bihar). Both signs and symptoms are closely monitored in the genetically infected areas and are recorded.

Isolation of Pathogen. The culture of *Taphrina maculans* used during this study was divided into different categories of infected turmeric plant leaves collected from various fields of the farmer's farm and from the T.C.A. farm. Compass near the field of meteorology. To separate the pathogen from the leaf showing the obvious signs of infected parts and a thin line of healthy leaves are cut into small pieces 3 mm and the outer surface is sterilized with 1% sodium hypochlorite solution for one minute and washed three times with distilled clear water. Pieces showing yellow-brown blisters were placed in a damp room to produce ascus, which appeared to protrude from the cuticle within 2-4 days. Such pieces were attached to the lid of the Petri plates containing the P.D.A. the middle is composed of amino acids and 2% yeast extracted from Pertiplates allowing ascospores to be extracted from the existing area below. Plates are placed at 20 ± 2°C for 2-3 days. Spores were found to grow in small pink colonies at the end of 48 to 96 hours. This then followed sub-culture in P.D.A. plates through streaking. Pure cultures were obtained by continuing the sub-culturing expansion of isolated colonies performed 3-4 times.

Identification of the pathogen: The pathogen that was isolated from the cultural media has been identified with the help of an explanation developed by Pagvi and Upadhyay (1964) and Kulkarni and Ahmed (1968). The pathogen *Taphrina maculans* produced easily mutilated colonies such as yeast or bacterial colonies. As with all other studies studied to date, *T. maculans* have produced conidia only in cultures without mycelial growth and represent the asexual phase. In sub-culturing growth the growth was limited to the injection line but soon very small pink colonies appeared in the medium part untouched by the injection needle. These colonies remain isolated for a long time. This specialty was observed during sub-culturing, fungi produce bud conidia or blastospores which are sometimes disposed of outside the growing colony of individual colonies. The inoculum was prepared and sprayed on turmeric plants that had been planted for testing. The infected leaf of turmeric was used for recycling according to the normal procedure. The acquired culture was confirmed to grow in a real culture in which segregation was performed to prove pathogenicity.

***In vitro* evaluation of botanicals extract**

Current research is being done about the potential for plant varieties to show the effect of inhibiting the mycelial growth of the affected fungus under in-vitro conditions. Antifungal properties of various plant species, botanicals in three different concentrations were tested against *Taphrina maculans* under in vitro conditions using a toxic dietary process (Nene and Thapliyal, 1993). A total of 5 different plant species namely, Tulsi (*Ocimum sanctum*), Brahmi (*Bacopa monieri*), Neem (*Azadirachta indica*), Pipli (*Piper longum*), Surpgandha (*Rauwolfia serpentina*) without any disease were selected on their basis. Antifungal properties that have been previously reported and are readily available throughout the year. The leaves as parts of the plant are used for research in all cases of botanicals.

PREPARATION OF AQUEOUS EXTRACTS OF BOTANICALS

A sufficient number of new and healthy leaves for selected plant species were collected at the hi-tech horticulture nursery at Dr. Rajendra Prasad Central Agricultural University Pusa, Samastipur, Bihar was also brought to the Plant Pathology laboratory in T.C.A. Dholi, Muzaffarpur, Bihar for further studies. The leaves were first washed thoroughly under running tap water to remove any impurities that had stuck to the surface of the leaf. A sufficient amount of the selected plant leaves are immersed in a solution of sodium hypochlorite (1%) for 30 seconds and then thoroughly washed with clear pure water by washing three times. One ml of pure water used for each gram of fresh maceration ingredients One hundred grams of leaves per sample are then distilled in plain water (100 ml) in a grinding machine for 5-10 minutes. After careful grinding, the extract was first filtered through a three-fold muslin cloth and then by Whatman filter paper number 1. Later, the prepared extract was re-applied with a zeitz filter to free it from germs. This filtrate was taken as stock stock of aqueous aqueous extract and stored at 4OC for further studies. Streptocycline (200 ppm) was mixed as per the requirement to eliminate bacterial growth during fungus mycelium testing. Extraction was then used as a standard solution to extract the plant at 100% concentration or 1: 1 ratio. The inhibitory effects of aqueous extraction of plant extracts were tested against pathogens under investigation of food poisoning.

Poisoned food technique against *Taphrina maculans.*

The principle involved in this experimental method was to make the food a fungus toxicity with a botanicals drink with antifungal properties and to allow the test fungus to grow in the center and record mycelial laboratory inhibition. 6, 8, 10 ml of extracted plant was mixed in 94, 92, 90 ml of PDA medium, to prepare 6, 8 and 10% of plant extracts containing medium respectively. Approximately 15-20 ml of dissolved toxic PDA was poured into sterile Petriplates and allowed to harden. These Petriplates are then injected aseptically into the center of the Petriplates with a 5 mm wide mycelial disc cut with the help of a 5 mm cork borer inserted sterile in a space that lasts for 10-15 days separately. The surface of the inoculum disc was kept in an agar-shaped surface on the plates. Control plates are also grown under the same conditions in the PDA without the aqueous extraction of plant material. All these operations are performed under an aseptic state in the laminar air flow chamber. Three plates are injected into each fungus for all botanicals submerged in water. Plates were placed in the BOD incubator at a temperature of 20°C ± 2°C and detection was recorded. Following botanicals extract were evaluated for their relative efficacy against the pathogen *in vitro*.

Table 1: Common name, scientific name, plant part used and concentrations used of the plant extracts.

Common name	Scientific name	Plant part used	Concentration used
Tulsi	*Ocimum sanctum*	Leaf	6%, 8%, 10%
Brahmi	*Bacopa monieri*	Leaf	6%, 8%, 10%
Neem	*Azadirachta indica*	Leaf	6%, 8%, 10%
Pipli	*Piper longum*	Leaf	6%, 8%, 10%
Surpgandha	*Rauwolfia serpentina*	Leaf	6%, 8%, 10%

The colony diameter were recorded and compared the control with treatment after 10 days of incubation. Formula given by Vincent (1927) was used to calculate the percentage inhibition of growth.

Per cent growth inhibition $I = \left(\frac{C - T}{C}\right) \times 100$

Where,
I = Per cent growth inhibition
C = Colony diameter (mm) in check plate
T = Colony diameter (mm) in the treated plate

RESULTS AND DISCUSSION

The data on radial growth of pathogen culture (*T. maculans*) with respect to different botanical aqueous extract (6%) at time intervals of 72 hrs. starting from 72 to 360 hrs. is presented in table 2. The data indicated that all the botanicals significantly inhibited the fungus growth compared to control in all observations made at different time intervals. Neem leaf extract exhibited minimum radial growth of pathogen in all the days of observation thus giving maximum inhibitory effect on pathogen. Effect of neem leaf extract was found statistically *at par* with tulsi at 72 hrs., brahmi at 216 and 218 hrs. and finally with pipli at 360 hrs. of observation. The next best botanical in terms of conferring inhibitory effect were found sarpgandha, pipli, tulsi and brahmi at 72, 144, 236, 288 and 360 hrs of observation respectively. Based on the last (final) observation, the descending order of fungitoxicity to *T. maculans*. The descending order of fungitoxicity to *T. maculans* by plant extract was as follows:

Neem leaf extract >Pipli leaf extract >Brahmi leaf extract >Tulsi leaf extract >Surpgandha leaf extract.

Aqueous leaf extract of all the botanicals at 8 and 10 per cent strength showed complete suppression of radial growth and pathogen during the period of observation *i.e.*, upto 360 hrs. of inoculation.

Table 2: Effect of Neem, Tulsi, Brahmi, Pipli and Sarpgandha extract (6%) on radial growth (mm) of *Taphrina maculans* at different time intervals.

Treatments	72 hrs (3rd day)	144 hrs (6th day)	216 hrs (9th Day)	288 hrs (12th day)	360 hrs (15th Day)
T_1 Tulsi	9.00	13.67	23.33	31.33	37.50
T_2Brahmi	11.80	13.50	21.17	29.67	37.00
T_3 Neem	8.30	12.50	20.00	28.33	33.33
T_4 Pipli	10.0	14.83	24.33	30.33	35.33
T_5 Sarpgandha	9.70	14.17	24.00	32.00	38.67
T_6 control	29.40	44.67	60.00	71.67	88.33
SEm (±)	0.33	0.26	0.46	0.92	0.93
CD (*p*=0.01)	1.05	0.82	1.43	2.87	2.90
CV (%)	4.46	2.42	2.76	4.24	3.53

*Data is average of 3 replications.

Table 3: Effect of Neem, Tulsi, Brahmi, Pipli and Sarpgandha extract (6%) on inhibition of mycelial growth (%) of *Taphrina maculans* at different time intervals.

Treatments	Per cent inhibition of mycelial growth				
	72 hrs 3rd day	144 hrs 6th day	216 hrs 9th day	288 hrs 12th day	360 hrs 15th day
T1	71.68	72.01	66.65	60.40	59.28
T2	59.77	69.77	64.73	57.58	55.08
T3	69.40	69.40	61.10	56.37	54.54
T4	66.01	66.79	59.43	53.42	51.43
T5	67.11	68.28	59.99	55.36	54.24
T6	0.00	0.00	0.00	0.00	0.00

Table 3 and Fig. 1 depicts the inhibition of mycelial growth (%) of pathogen by botanicals at different time intervals. The data revealed that aqueous extract of tulsi followed by brahmi gave maximum inhibition of mycelial growth (%) across all the days of observation registering 59.28 and 55.08 per cent mycelial growth inhibition on final day of observation (15 day after inoculation).

The result revealed that all the botanicals significantly inhibited the fungus growth compared to control. Initially the minimum (8.30mm) growth of the test-fungus was found in case of neem (6% extract) after 72

hours (3rd day) which further developed slowly and reached to 33.33 mm after 360 hours (15th day) while, in case of Tulsi (6% extract) radial growth observed after 72 are 9.00 mm and reached to 37.50 mm after 360 hours followed by sarpgandha 6% extract in which 9.70 mm radial growth observed which were reached to 38.67mm after 15th day. Neem leaf extract exhibited minimum radial growth of pathogen in all the days of observation thus giving maximum inhibitory effect on pathogen. Effect of neem leaf extract was found statistically *at par* with tulsi at 72 hours, brahmi at 216 and 218 hours and finally with pipli at 360 hours of observation. The next best botanical in terms of conferring inhibitory effect were found sarpgandha, pipli, tulsi and brahmi at 72, 144, 236, 288 and 360 hours of observation respectively depicted in plate 1. Based on the last (final) observation, the descending order of fungitoxicity to *T. maculans*. Aqueous leaf extract of all the botanicals at 8 and 10 per cent strength showed complete suppression of radial growth and pathogen during the period of observation *i.e.*, upto 360 hours of inoculation. Singh *et al*. (2009) revealed that Maximum inhibition of fungal growth was recorded with extracts (25%) of *Allium sativum* (82.01%), followed by *Azadirachta indica* (79.90%), *Curcuma longa* (79.88%) and *Zingiber afficinales* (79.82%) in ethanol solvent. The least inhibition was recorded at 10% concentration of *A. cepa* (36.87%), followed by *Ocimum sanctum* (37.00%) in distilled water. Kothikhar and Koche (2017) earlier conducted a similar experiment on turmeric leaf spot caused by *Colletotrichum dematium* and evaluated three botanical extracts through poisoned food technique and found *Azadirachta indica* seed extract 5% as a superior botanical fungicides which inhibited 74.69% of mycelial growth. Jagtap *et al*. (2013) evaluated total eleven plant extracts under *in vitro* condition against *C. capsici* through poisoned food technique and recorded percent inhibition of mycelial growth of the fungus. *Polyalthia longifolia* exhibited maximum inhibition of 76.15% at 15% concentration followed by rhizome extract *Curcuma longa* with 66.88% and *Allium cepa* 63.96%. Whereas, *Aegale marmelos* and *Parthenium hysterophorus* showed minimum mean inhibition of 20.86 and 28.66%, respectively.

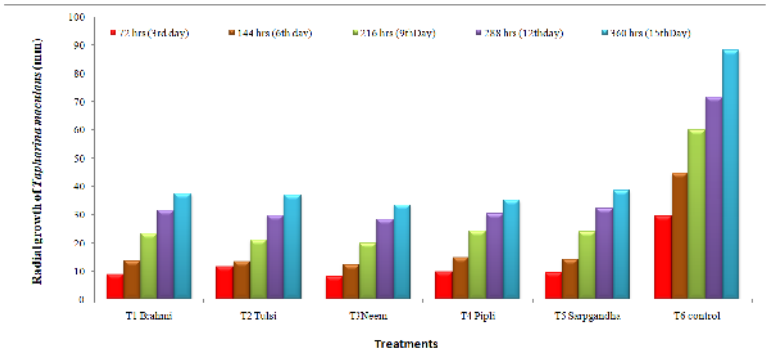
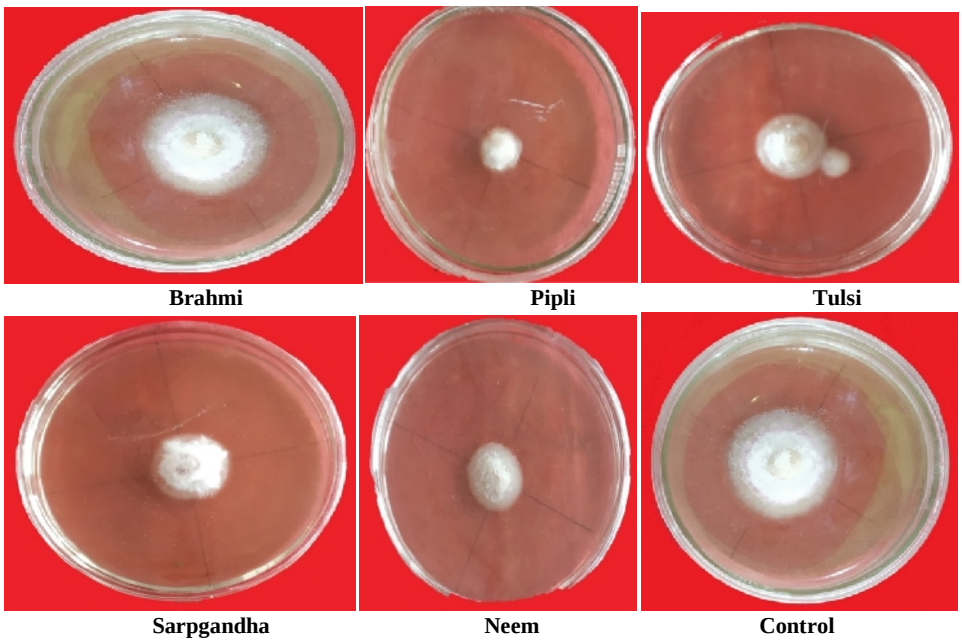


Fig. 1. Effect of Neem, Tulsi, Brahmi, Pipli and Sarpgandha extract (6%) on radial growth (mm) of *Taphrina maculans* at different time intervals.



Kadam *et al*. (2022) reported after an extensive study that per cent germination of blastospore of *Taphrina deformans* were increased upto 20°C beyond which decline intheir germination were recorded. Kangjam *et al*. (2017) reported that garlic clove extract completely checked the growth of *Colletotrichum capsici* under in vitro condition.

CONCLUSION

Based on the last (final) observation, it may be concluded that neem leaf extract, pipli leaf extract and brahmi leaf extract may be used in the reducing the incidence of leaf blotch disease caused by *Taphrina maculans* in turmeric as well as in reducing the toxic chemical residue level in its rhizome. The descending order of fungitoxicity to *T. maculans*. The descending order of fungitoxicity to *T. maculans* by plant extract was as follows:

Neem leaf extract >Pipli leaf extract >Brahmi leaf extract >Tulsi leaf extract >Surpgandha leaf extract.

FUTURE SCOPE

Continuous evaluation of IDM based management practices *i.e.* Compatiblity of newly developed safer fungicides with different Bioagents and botanicals need to be done to save our environment thereby future generation. More cultural and morphological studies of *Taphrina maculans* and its other species need to be further studies as its cultural and morphological characteristics vary with their respective division.

To find out eco-friendly management practices viz. bioagents, botanicals and medicinal plant extracts for organic farming and lastly spray of effective fungicides under IDM pragramme are to be investigated and economics are be studied.

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Conflict of Interest. None.

REFERENCES

Annonymous (2018a). *Annual Report* on All India Coordinated Research Project on spices, Rajendra Agricultural University, Pusa, Samastipur, Bihar.p169.

Bambode, R. S. & Shukla, V. N. (1973). Antifungal properties of certain plant extracts against some fungi. *PKV Res. J*, *2*(1), 1-8.

Butler, E. J. (1911). The leaf sopt of turmeric (*Taphrina maculans* sp. nov.). *Ann. Mycol.*, *9*: 36-39.

Dixit, D., Srivastava, N. K., Kumar, R. & Sharma, S. (2002). Cultivar Variation in Yield, Metabolite Translocation and Partitioning of $14CO_2$ Assimilated Photosynthate into Essential Oil and Curcumin of Turmeric (*Curcuma longa* L.). *Journal of Plant Biology-New Delhi*, *29*(1), 65-70.

Jagtap, G. P., Mali, A. K. and Dey, U. (2013). Bioefficacy of fungicides, bio-control agents and botanicals against leaf spot of turmeric incited by *Colletortricum capsici* African journal of Microbiology Research Vol. & (18), pp. 1865-1873.

Joshi, L. K. and Sharma, N. D. 1982. Disease of ginger and turmeric. *Proc. of the Nat. Seminar on Ginger and Turmeric*, Calicut. April, 8-9, Pp.104-119.

Kothikar, R. B. and Koche M. (2006). Efficacy of botanicals and fungicides for the management of major foliar disease of turmeric (*Curcuma longa* L). M.Sc. Agric. Thesis. Dr. P.D.K.V., Akola.

Kadam, V. K. (2018). Studies on peach leaf curl (*Taphrina deformans* [Berk.] Tul.) and its management. M.Sc. Thesis. Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan, HP, India. p 44.

Nene. Y. L. and Thapliyal, P. N. (1993). Evaluation of fungicides. In: Fungicides in plant disease control (3rd ed.) Oxford, IBM Publishing Co., New Delhi, 331 pp.

Kadam, V. K., Gupta, A. K., Shukla, A. and Sharma, S. K. (2022). Effect of Different Temperature and Relative humidity regimes on the Germination of Blastospore of *Taphrina deformans* (Berk.) Tul. Biological Forum- An international Journal, 14(2): 607-610.

Kangjam, B., Thangaswamy, R., Majumder, D. and Devi, K. J. (2017). Evaluation of Plant Extracts, Biocontrol Agents and Fungicides against the Growth of Turmeric Leaf Spot Pathogen, *Colletotrichum capsici* under in vitro condition. *Environment and Ecology*, *35*(2B): 1173-1178.

Pavgi, M. S. and Upadhyay, R. 1964. Artificial culture and pathogenicity of *Taphrina maculans* Butler. *Sci. Cult*. 30: 558- 559.

Vincent, J. M. (1947). Distortion of fungal hyphae in the presence of certain inhibitors. *Nature*, *159*(4051), 850-850.

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