IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

Evaluation Of Disease Incidence And Severity Of *Tinospora Cordifolia* With Special Reference To Parbhani District

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Abstract

Tinospora cordifolia, also known as "Guduchi" in Sanskrit, is a genetically diverse and a commercial crop with a niche market in Marathwada region. This crop has recently been studied for fungal infections causing a devastating loss in the yield and quality of the medicinal products. Since this plant is potent medicinal and wild crop, not much of attention has been given to study the causative agents in the fungal infections. A survey was done in the Marathwada region to identify the causative agents in diseases of this plant. Seven different districts in the Marathwada region of Maharashtra were selected to find the disease severity index. A five point grading system was implied for the same. The observations were taken in 3 different seasons, from 2019 to 2021. The study found sever yield losses by *Colletotrichum capsici* and *Colletotrichum dematium*. The pathogenicity test confirmed the causative agents, showing similar diseased symptoms in test plants. The Disease severity index was found to be highest in Pathri district in rainy season. The lowest was found in Gangakhed. The study confirms the need of human interventions in controlling yield losses in the plants.

Key words

Disease severity index, *Tinospora cordifolia*, *Colletotrichum*

Introduction

Tinospora cordifolia, also known as "Guduchi" in Sanskrit, is a genetically varied, big, deciduous climbing shrub with greenish yellow characteristic blooms found at higher altitudes (Singh, 2020). It is evolved to sustain in a wide range of environments and is known for withstanding high temperature of tropical regions. *T. cordifolia* leaves are simple, alternating, glabrous exstipulate with a 12–15 cm long petiole, round, heart-shaped, ovate lamina, and 7–9 nerved (Yates et al., 2022). This plant's stem is somewhat succulent, long, filiform, and climbing in nature. Aerial roots grow from branches with tetra to penta-arch main structure. When the external skin is removed, a greenish-colored mucilaginous material is revealed. The bark ranges in hue from pale milky to brownish. Flowers are unisexual and yellow in hue. Male flowers form a group, but female flowers have a single inflorescence. Six sepals (in 2 pair of 3 each) (Rout, 2006). Petals are smaller than sepals, and it has six free and membranous petals. Flowering and fruiting occur from March to June, and they are orange-reddish in colour, meaty, aggregated in groups of 1–3, and ovoid on a stout stalk (Bharathi et al., 2018). Medicinally, the plant has been shown to contain a variety of phytochemicals such as berberine, palmatine, tembetarine, tinosporin, tinosporide, tinosporaside, cordifolide, cordifol, tinocordiside, and tinocordifolioside, among others (Singh et al., 2020). This plant is very important in India because of its usefulness in treating a variety of illnesses, as documented in Ayurveda. Microbes such as *E. coli, S. aureus, S. pyrogens, B. subtilis, and P. vulgaris* are treated with the plant (Mishra & Padhy, 2013). It's also utilised to treat chronic debilitating conditions including dyspepsia, fever, and urinary tract infections. *T. cordifolia* production is subjected to many abiotic and biotic factors that seriously compromise the final yields. Among the menacing biotic factors, leaf blast is a widespread disease capable of devastating the unprotected plant, consequently resulting in reduction of physiological maturity, biomass and yield of the crop (Mishra et al., 2012). The disease is found to affect the plant at all growth stages from seedling stage, causing lesions and premature drying of young leaves, to affecting the mature leaves and generating leaf spots (Shivanna et al., 2014). Several fungi have been identified to be responsible for the blast disease in this plant. The disease severity has seldom been studied in this crop. Since this crop has special importance in India, it is essential to survey the prevalence of this disease and report the disease incidences from local niche . Hence a survey has been reported along with the disease severity index by grading the disease on a 5 point scale and identify the causative microbe.

Materials and methods

Survey of foliar disease

A survey of several foliar diseases of *T. cordifolia* was conducted in Selu, Manwat, Pathri, Sonpeth, Jintur, Parbhani and Gangakhed regions (Savithramma et al., 2007). This included a diverse range of plant varieties and diseases for research in Maharashtra, India. The plants were cultivated on the proprietary fields of farmers in their respective regions. During the survey, the plant parts were collected, and species were deposited as voucher specimens in the herbarium at the Department of Botany, Nanded University (Carter et al., 2007). During the collection, the plant leaves showing disease symptoms were collected in sterile plastic ziplock bags. These leaf samples were used to identify the causative fungus in the leaf spot.

Disease severity index

The incidence of fungal infections was investigated using leaf samples taken from several districts in the Parbhani area. During the years 2018-2021, disease incidence was observed in three seasons: summer, rainy, and winter, in the months of April, August, and December, respectively. The infection was monitored, and the percentage of infection was computed as the Disease Severity Index (DSI) using standard approach as stated by Mayee and Datar (1986). One hundred leaves from *T. cordifolia* plant, both healthy and diseased, were randomly picked and segregated based on disease infection. These leaves were divided into five categories for the purpose of calculating the Disease Severity Index (DSI) using Mayee and Datar (1986) five-point scale 5 point scale, ranging from 0 to IV. The Grade 0 was healthy leaves with no infection, grade I with 1 to 25 percent infection, grade II with 26 to 50%, grade III with 51 to 75% and grade IV with 76 to 100% infection. The disease severity index was calculated with the following formula:

$$\text{Disease Severity Index (DSI)} = \frac{\sum all\ ratings}{no\ of\ observations * (all\ ratings - 1)} * 100$$

Isolation, purification and identification of the fungal pathogens

The infected leaves were properly cleansed with sterile distilled water, and little portions of diseased leaf spot pieces of 3 mm^2 together with some healthy tissues were cut using a scalpel. These leaf portions were then submerged in a 0.1 percent sodium hypochlorite solution for 30 seconds to remove surface impurities before being rinsed three times with sterile distilled water. Excess water was removed using sterile blotting paper (Mishra et al., 2012). These pieces were inoculated aseptically on sterilised petriplates with Potato Dextrose Agar (PDA) and incubated at 27 °C for 10 days (da Silva Santos et al., 2022). The fungal culture was purified using the single spore isolation approach described by Reddy et al (2006). The fungal isolates were kept on PDA slants for further research. To identify fungal pathogens, fungal culture characteristics, mycelium, reproductive structure, and morphological features were observed using the literature of (Alexopoulos et al., 1996) and (Hunter & Barnett, 2019).

Pathogenicity test

The isolated fungal pathogens were tested on live *T. cordifolia* plant grown indoors in triplicate to confirm the pathogenicity of the isolated cultures (Jie et al., 2009). The healthy leaves of the *T. cordifolia* plants were inoculated with the isolated cultures. The inoculated leaves were marked and observed for visible infectious symptoms. The growth of pathogens was recorded in millimeters.

Statistics

The standard error of mean was calculated using IBM SPSS 24.

Results

Disease severity index

The observations of the disease severity found in the selected locations at three different seasons in the districts of Marthwada are shown in Table 1. The observations clearly showed that there was not much of difference In the Disease Severity index at summer or winter seasons Throughout the observation tenure of 2019 to 2021. A distinct variability was found In the disease severity index in the rainy seasons. The Disease Severity Index spanned from the 14 to 18.5% in the summer season in the three years. Similarly, its pain from 13 to 19.5% in the winter season. A larger variability in the severity index was found during the rainy season. The Disease Severity Index was found to be highest of about 43.75 at Pathri District, whereas lowest as 16.7% in Manwat. There is no particular trend of increase or decrease in the disease severity index and might probably be due to the variability in the relative humidity or the extent of rain during the respective seasons.

Table 1 Disease severity index of fungal infections on *T. cordifolia* at different districts of Marathwada region, taken in the year from 2019 to 2021

Year	Districts of Marathwada region																				
	Selu			Manwat			Pathri			Sonpeth			Jintur			Parbhani			Gangakhed		
	S	R	W	S	R	W	S	R	W	S	R	W	S	R	W	S	R	W	S	R	W
2019	17.20	35.00	19.00	18.20	32.25	13.50	18.20	43.75	19.20	18.50	25.70	19.00	15.00	28.20	19.25	14.00	28.20	17.50	18.50	26.70	15.25
2020	17.20	30.00	18.70	17.00	16.70	17.70	17.20	31.70	19.00	17.00	30.00	17.00	16.75	39.00	18.70	16.50	27.70	19.00	16.50	27.00	18.20
2021	17.00	21.80	18.70	18.20	43.35	21.00	18.00	23.70	17.70	17.00	27.20	19.50	18.70	43.50	17.00	18.70	32.25	15.25	14.00	43.00	16.50
S.E	0.01	0.19	0.01	0.04	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.01	0.04	0.01	0.01	0.01	0.01	0.01	0.01	0.01

Where S is summer, R is rainy, and W is winter season. All the observations are in percent, except standard error (S.E).

Identification and isolation of causative pathogenic fungi

The isolated cultures were found to show homology with *Colletotrichum capsici* and *Colletotrichum dematium.* Most of the cultures found in all the 7 observation sites had these fungi in common and hence giving a higher probability to be the causative pathogen.

Pathogenecity test

After 6 days, the disease signs appeared on live plant of *T. cordifolia*. The grading system used in the study is shown in the Figure 1. The disease symptoms were fully established as leaf spot. These symptoms were identical to those seen in naturally infected *T. cordifolia* leaves at the observation sites. *Colletotrichum capsici* and *Colletotrichum dematium* were discovered by microscopic observation of spores and conidia. The trend of infection by the causative fungi is shown in figure 1.

Figure 1 Disease severity grading system and infections found after artificial inoculation in pathogenicity test in *T. cordifolia* used in the research. The gradings of infections of leaves are 0, I. II, II and IV from left to right respectively.

Conclusion

The grading of fungal infection based on disease severity helped in the segregation and assessment of infections in *T. cordifolia*. The grading system reduced bias in observations and increases the mean assessment, rendering an easy identification of severity of the infections. This provided a very low standard error in the reading, hence proving to be a robust method for grading the severity of the disease. The larger number of observations was found to reduce the error in the readings. Though, the method is laborious and time consuming and there is still room to generate a faster and less labor-intensive method. The observations found *Colletotrichum capsici* and *Colletotrichum dematium* to be the causative agent in the plant disease, which was later confirmed by pathogenicity test. These fungal strains are found to be predominantly infecting citrus fruits, though found in selected plants as well.

The disease severity was found to be highest in the Pathri district and went on decreasing during the rainy season. This might probably be due to the human intervention of use of synthetic pesticides to reduce the funal infections. The disease severity index was found to escalate in the Jintur, Parbhani and Gangakhed district and is recommended to use fungal control agents to reduce the disease.

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