In vitro evaluation of chemical fungicides against *Corynespora cassiicola* causing Leaf Spots in Tomato

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Department of Botany,
University of Calcutta,
Kolkata 700 019, India

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G.H. SOWMYA[1]*, N. RAJESHWARI [1], H.N. RAMESH BABU[1] AND B.R. SHALINI[2]
[1]Department of Botany and Seed Technology, Kuvempu University, Sahyadri Science College, Shivamogga, Karnataka 577203.
[2]Department of Botany, Tumkur University, Tumkur, Karnataka

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Pathogenic variability and fungicidal sensitivity of the *Corynespora cassiicola* isolates collected from the major Tomato growing region of Chitradurga district of Karnataka were studied for their pathogenic variability by inoculating to susceptible Tomato variety by root dip inoculation method. Three fungicides viz., Mancozeb, Bavistin and Captan at 0.05, 0.1, 0.15 and 0.2 percent concentrations respectivelywere used against isolated pathogen. The*Corynespora cassiicola* isolates showed complete inhibition in Mancozeb at 0.15% &0.2% concentration. However, Bavistin and Captan showed 80% and 77.64% inhibition respectively in 0.2% concentration.

Key words: *Corynespora cassiicola,* Leaf Spot, pathogenicity, tomato, fungicides

INTRODUCTION

Tomato, scientifically called as *Lycopersicon esculentum* Mill. is a most common vegetable crop grown all over the world. It is an important source of minerals, vitamins, essential amino acids, sugars and dietary fibres. During the cultivation, tomato crop is susceptible to various kinds of diseases and disorders (Mary and Giri- 2017). Among all the diseases fungal diseases are the most severe disease and they reduces maximum crop yield.

It is a cosmopolitan vegetable and widely cultivated in almost all the countries of the world including India. Irreversible investment – production ratio for tomato cultivation in recent Indian agricultural systems arise the question, is there any biotic backlogs responsible for such a production loss.Our present investigation is based on the fact that Target leaf spot disease of tomato which is caused by *Corynespora cassiicola*, one of the serious and emerging diseases in India. This pathogen is the natural barrier for tomato production with a disease severity ranging from 35% to 58% which ultimately causes tremendous loss of tomato foliage and fruits. (Adam*et al* -2018). Studies reveal that there is a major toll in the tomato production due to *C. cassiicola.* From the emerging scenario is the real threat for indigenous cultivars and it have been recorded as emerging disease in the tomato crop under change in climatic condition. Hence the attempt was done to control the *C. cassiicola* at different concentrations of chemical fungicides *in vitro* condition.

*Correrspondence: sowmyagyaralli95@gmail.com

MATERIALS AND METHODS

Study site and sample collection

Field survey was done in major vegetable growing region of Chitradurga District during 2019-2020 to estimate the leaf spot disease of Tomato. A randomized sampling method was used for survey and collection of samples (Zainab-2016). Collection of infected material with symptoms like circular, target board or irregular shaped leaf spots, and are dark brown in colour with light brown centers delimited by dark brown rings and surrounded by a yellow halo symptoms on the leaf (Fig 1) were collected with the pre sterilized knife, forceps and cutter. The collected materials are carried in a pre-sterilized zip-lock cover to the laboratory for the microscopic observation and identification.

Isolation and identification of the fungal pathogen

The freshly collected infected materials exhibiting symptoms were brought to the laboratory for isolation. The infected leaves were dissected and

observed under stereo-binocular microscope showed dark and dense clustered sporulation encircled at the main vein or small veinlet adjacent to the spots. Infected leaf tissues were washed with running tap water and leaf bits of 5 to 10 mm size were surface sterilized in 1% Sodium hypochlorite solution for one minute followed by rinsing with sterile distilled water and dried on sterile filter paper. Sterilized tissue bits were placed on Potato Dextrose Agar (PDA) plates added with ampicillin to check the bacterial contamination. The plates were incubated at room temperature for 7–10 days for the growth of mycelium and pure cultures were maintained on PDA slants (Norhito *et al.* 2004; Nirmaladevi *et al*. 2016). Morphological identi-fication was carried based on characteristics of the macroconidia, microconidia and chlamydospores.

Molecular Identification of the pathogen

DNA extraction, PCR amplification and sequencing

The genomic DNA was isolated from fungal mycelium using CTAB DNA extraction method (Wu *et al.* 2001). Seven days old freshly cultured mycelial mats (grown in potato dextrose broth at the temperature of 24±1C) were taken for isolation of genomic DNA.

The nuclear ribosomal internal transcribed spacer (nrITS) was amplified using the primer pairs ITS1 – ITS4 (White *et al*. 1990).The modified protocols of Kantharaja and Krishnappa (2020) were followed for PCR amplification and sequencing. The newly generated sequences were aligned and consensus sequences were generated using BioEdit v.7.2.5 (Hall, 1999). The consensus sequences were used for BLAST search on the NCBI GenBank nucleotide database (https:// www.ncbi.nlm.nih.gov/) to know the sequence similarity and distance tree results and the identified sequences are deposited in the GenBank. (Accession No: OM004059)

Pathogenicity test

Pathogenicity test was performed by Isolated *Corynespora cassiicola* which was inoculated to susceptible tomato seedlings (25 days old) by root cut and dip method at the concentration of 106 spore / ml for 30 min by using Haemocytometer (Nirmaladevi *et al*. 2016). Inoculated seedlings were transferred to pots containing sterilized soil and maintained in poly house. Set of seedlings without inoculation was maintained as control. Symptom expression was recorded regularly from third day of inoculation upto 3 weeks. Disease symptoms like chlorosis, wilting or stunting of the plant, death of the plant were recorded.

In vitro evaluation of fungicides against leaf spots disease of tomato

A total of 3 fungicides were evaluated under in vitro conditions against *C. cassiicola* through food poisoned technique and using PDA as basal medium (Banu and Sharada,2018; Vijay Kumar *et al.,* 2017). The replication of treatments was done thrice and untreated suitable control was maintained. In vitro evaluation of chemical fungicide was carried at four different concentrations viz.,0.05, 0.1, 0.15 and 0.2%. The radial growth of the fungal mycelium was recorded on 10th day when untreated control plates were observed to have maximum growth. The percent inhibition was calculated using the formula.

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

I = percent inhibition of mycelial growth
C = radial growth of fungus in control
T = radial growth of fungus in treatment.

RESULTS

Pathogenicity test

Approximately after 20 Days of inoculation small, dark brown lesions were observedon leaves of all the inoculated tomato plants, which was exactly similar to field condition (Fig 2b). There were no symptoms on water treated control plants. (Fig 2a). Koch's postulate was established by conducting the pathogenicity test with three replications, with the re-isolation of the same pathogen in culture media with characteristic morphometric and cultural characteristics (Fig 3a, b).

In vitro evaluation of fungicides against leaf spots disease of tomato

All three fungicides (Mancozeb, Bavistin and Captan) significantly inhibited mycelial growth compared to the control (Table 1). Among the fungicides, Mancozeb in all concentrations was found to be significantly superior and showing

100% growth inhibition in 0.15% and 0.2% concentration. While inhibitory effect of Bavistin and Captan increased with increase in the concentrations. In 10th day of experimentation, highest inhibition i.e. 100% was shown in the concentration (0.15 & 0.2%) of Mancozeb followed by 0.2% of Bavistin which showed 80% and Captan showed 77.64% inhibition in radial growth of fungal colony. Lowest inhibition was observed in 0.05% Bavistin (42.35%) and Captan (47.05%).

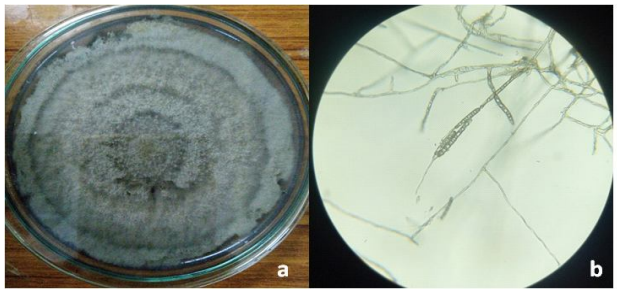

Fig. 3 :(a) *Corynespora cassiicola* culture on PDA, (b) Matured conidia of *Corynespora cassiicola*

Table 1: *In vitro* evaluation of fungicides against leaf spots disease of tomato

Fungicide	Percent (%) growth inhibition of *C. cassiicola* at different concentration (%) of fungicides			
	0.05%	0.1%	0.15%	0.2%
Control			00	
Mancozeb	56.47	81.17	100	100
Bavistin	42.35	50.58	65.88	80
Captan	47.05	70.58	76.47	77.64

Fig. 1 : Natural infection of leaf spot on tomato in field condition.

Fig. 2 : (A)No symptoms on control plants, (B)Leaf spot symptoms expressed on tomato seedlings.

DISCUSSION

Corynespora cassiicola causing leaf spot on tomato is the major disease of tomato in field condition in all over the Karnataka. From emerging scenario, leaf spot has been identified as one of the major threats to tomato production in India as evidence by the severe disease occurrence in farmers plot as well as in the experimental plot through natural infection. Similarly, Adam *et al*. (2018) first reported *C. cassiicola* causing target leaf spot on tomato in West Bengal, India, and Wagner and Louise (2019) first reported *C. cassiicola* causing leaf spot on *Solanum americanum* in Brazil.

In the present study, laboratory testing of three fungicides at four different concentrations (0.05, 0.1, 0.15 and 0.2%) by food poison technique revealed that all three fungicides showed effectiveness in decreasing the fungal growth at increased concentrations. Mancozeb was proved to be the best among the tested fungicides which completely inhibited the fungal growth in two concentrations (0.15% and 0.2%). Bavistin was moderately effective while Capton ranked last among these fungicides. According to Pernezny *et al.* (2002) strobilurin fungicide azoxystrobin and a combination of Mancozeb and fumoxate provided excellent control of target spot. This study can be helpful for generalizing the concept of chemicals against the pathogen and for further research on this area. There is the need of further green-house and field trials for screening these chemicals against the pathogen for additional conformation.

REFERENCES

Kamei,A., Dutta,S., Sarker, K.,Das,S.,Datta,G., Goldar, S. 2018. Target leaf spot of tomato incited by *Corynespora cassiicola*, an emerging disease in tomato production under Gangetic alluvial region of West Bengal, India, *Arch.Phytopath. Plant Protect*.**51**:1039-1048.

Naziya, B.,Sharada, M. S. 2018. Inhibitory effect of Mancozeb on growth and stimulation of resistance against Fusarium wilt of Brinjal, *The Bioscan*. **13**:285-290.

Kantharaja, R., Krishnappa, M. 2020. Morphological and molecular phylogenetic studies on *Battarrea phalloides* (Agaricales): a new report to Indian mycobiota, *J. Threatened Taxa*. **12**:15881-15888.

Nirmaladevi, D., Venkataramana, M., Srivastava, R.K., Uppalapati, S.R., Gupta, V.K., Yli-Mattila, T., Clement Tsui, K.M., Srinivas, C., Niranjana, S.R., Chandra, N.S. 2016. Molecular phylogeny, pathogenicity and toxigenicity of *Fusarium oxysporum* f. sp. *lycopersici*.*Sci. Rep*.**6**: 21367.

Norhito, Y., Jyuichi, S., Mamoru, S., Seizo,S.,Takashi, S. 2004. Physiological races and vegetative compatibility groups of butterhead lettuce isolates of *Fusarium oxysporum* f. sp. *lactucae* in Japan, *J. Gen. Plant Pathol.* **70**:308–313.

Paulina, F.M.A., Giri, R.S. 2017. Screening of biocontrol agent against tomato wilt disease,*Inter. J.Bot.Stud*.**2**:170-175.

Pernezny, K., Stoffella, P., Collins, J., Carroll, A., Beaney, A. 2002. Control of Target Spot Tomato with fungicides, systematic acquired resistance activators and a biocontrol agent, *Plant Protection Science*. **38**: 81-88.

Kumar,V., Singh, G.Tyagi, A. 2017. Evaluation of Different Fungicides against Alternaria Leaf Blight of Tomato (*Alternaria solani*), *Inter. J.Curr.Microbiol. Appl. Sci*. **6**:2343-2350.

Pereira, W.V., May-De-Mio, L.L. 2019. First report of *Corynesporacassiicola* causing leaf spot on *Solanum americanum* in Brazil. *J. Plant Pathol.* **101**: 755.

Wu. Z.H., Wang, T.H., Huang, W., Qu, Y.B. 2001. A simplified method for chromosome DNA preparation from filamentous fungi. *Mycosystema*. **20**:575–577.

Zainab, M. B.,Shinkafi, S.A. 2016.Isolation and identification of fungi responsible for leaf spots disease of Mango (*Mangifera indica* L) in Sokoto State, Nigeria, *Bayero J.Pure Appl. Sci.***9**:166-173.