Characterization and Chemical Management of Cumin wilt Disease Caused by *Fusarium oxysporum*

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ABSTRACT

Cumin is the current leading currency earning crop in India, as per Agriwatch production estimate, Jeera production for 2021-22 marketing period is estimated at 391,291 MT, generating 8,600 crore rupees. Despite its importance, cumin is threatened by a serious, destructive disease called Cumin Wilt, which is caused by *Fusarium oxysporum*. The disease's impact has reduced income at both the household and national levels. The current study was carried out to look into chemical control strategies for the disease. The research was carried out from November to March (Rabi season) in Ravipura Kampa, Gujarat, India. Field observation of infected cumin wilt was followed by laboratory isolation, characterization of the causative agent, and assessment of the effects of various fungicides. In chemical management different fungicides were used. In laboratory tests, *F. oxysporum*, which is characterized by whitish mycelia growth and chlamydospores, was found to be the causative agent of the cumin wilting symptoms. There are two types of conidia: macro conidia and micro conidia. All the fungicides showed varying effects against *F. oxysporum*. This resulted in a complete stoppage of mycelial growth, followed by 5% EC formulation of Hexaconazole 2800 ppm, 50% WP CAPTAN3500ppm, Azoxystrobin 1600ppm, Azoxystrobin 11% + Tebuconazole 18.3% w/w SC 1000 ppm, Epoxyconazole + Flyxapyroxald 3500 ppm, Isopropanol azole 4000 ppm, Mancozeb 5000 ppm, Pyarclostrobin 13.3%+ Epoxyconazole 5% EC 4000 ppm, and Tebuconazole 25.9% EC 2000 ppm.

KEY WORDS: CHEMICAL FUNGICIDES, CUMIN, *FUSARIUM* WILT, *IN VITRO*.

INTRODUCTION

India has been a land of spices and the largest producer, consumer, and exporter of spices. Rajasthan and Gujarat are the important states to produce cumin. The crop suffers from several diseases. Among the major threats to the cumin is wilt. When stem was cut longitudinally, brownish discoloration is observed. Mathur and Mathura (1956) reported wilt of cumin from Rajasthan and identified the casual organism to be *Fusarium oxysporum*. Based on host specificity, Patel and Prasad finally named it as *Fusarium oxysporum* f. sp. *cumini*. *Fusarium* is largest genus of filamentous fungi widely distributed in agricultural soils. It contains large number of destructive plant pathogens such as *F. avenaceum, F. eumartii, F.oxysporum* and particularly *F. solani,* which is potential cause of vascular wilt, root rot and fruit rot as well as influence seed germination in different host plants. The fungus is soil borne in nature and commonly found in all crop growing areas in world. The fungus belongs to ascomycetes and cause wilt disease of several important crops. Fungus produces micro conidia, macro conidia and chlamydospores, which persist in soil for several years. The pathogen is seed borne in nature too and transmits from one place to another through seed material (Watt, 2006, Akter *et al.,* 2021).

Crop plants are highly susceptible to attack of *Fusarium* spp. during the pre- and post-emergence stages (Nawar, 2007). Pathogens can quickly develop in light sandy soils, triggering root rot disease in a variety of crops in different parts of the world (Celar, 2000). The fungus produces toxic substances that are responsible for inducing wilt symptoms in susceptible cultivars. Brown blotches form on the wilted area. The disease causes withering signs in the seedling stage. Plants are damaged during every phase of its development. The infected plants turn yellowish and show characteristic dropping of leaves leading to mortality of plant. Brown

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discolorations can also be seen on the vascular bundle, and the plants are easily pulled from the soil. Although there are a lot of approaches and procedures for controlling plant diseases, only a handful have been proven effective. The pathogen is managed using a combination of biological, cultural, and chemical methods. Varieties of systemic and non-systemic fungicides have been tested against the pathogen, having varied results. Several scientists have concluded that systemic fungicides are superior for disease management (Rafique *et al.,* 2016).

MATERIAL AND METHODS

Isolation of pathogen: The Pathogen of cumin wilt was isolated from wilted part of cumin plant (Ravipura kampa-Gujarat India). Small (5 by 5 mm) sections were excised from margins of browning stem bases and surface-sterilized in 75% ethanol for 10 sec, then sterilized in 0.5 % sodium hypochlorite for 1 min, followed by three rinses with sterile water. The fragments were then placed on to potato dextrose agar (PDA) and incubated at 27°C. Fungal growth was examined daily for up to7 days. Isolates were transferred to fresh PDA and purified by single-spore culturing. All fungal isolates were placed on fresh PDA slants and stored at 4°C Akter *et al,.*(2021).

Effect of temperature, carbon sources, different nutrient media, *pH and salt* on linear growth of pathogen: There were four temperatures selected for study 4°C, 28°C, 35°C and 40°C. Twelve sterile Petri-dishes with PDA medium were used (three plates for each treatment). With the help of 5 mm-diameter sterile cup borer a disc of inoculum of a 7- day old cultures, and then transferred to the center of each plate in close contact with the medium. The plates were incubated for 7 days at the following temperature range: 4°C, 28°C, 35°C and 40°C. The fungal growth was estimated daily by measuring the colony size and results were recorded for each degree of temperature, (Khilare and Ahmed, 2012).

Effect of carbon sources on linear growth of pathogen was investigated by using four different sources. Those carbon sources were dextrose, sucrose, maltose and starch. The amount of 2.0 gm of each sugar was added to the potato agar medium. The Petri-dishes were inoculated with 2 mm disc taken from 7 days old culture of the isolate and incubated in complete darkness at 28°C for 7 days. Readings were taken daily by measuring the growth diameters and the average was recorded.

Effect of different nutrient medium on linear growth of pathogen was assessed by taken five types of media those were potato dextrose agar (PDA), malt extract agar (MEA) and Czepek's Dox agar, Rose bangle agar, white bran medium. Four plates (9 cm diameter) were prepared from each medium and then the plates were inoculated with 5 mm disc from 7 days old culture of isolate. The plates were incubated in complete darkness for 7 days at 28°C and then the rate of fungal growth was estimated daily by measuring the colony size (Chittem and Srikant Kulkarni, 2008)

Effect of pH and salinity on linear growth of pathogen: 5.0, 6.0, 7.0, 8.0 and 9.0 pH were taken. The pH was adjusted in PD Amedium. Salinity was adjusted with NaCl in medium. Four concentrations of NaCl were taken for study 0.10,0.25,0.50and0.75percent were maintained. All Petri plates were incubated at 28°C for a week. Radial growth observations were taken at 24 hour intervals up to one week, radial growth was measured by scale Gordon *et al,* (2019).

Effect of different fungicides on pathogen at laboratory level: 5% EC formulation of Hexaconazolek, 50% WP CAPTAN, Azoxystrobin, Azoxystrobin 11% + Tebuconazole 18.3% w/w SC, Epoxyconazole + Flyxapyroxald, Isopropanol azole, Mancozeb, Pyarclostrobin 13.3%+ Epoxyconazole 5% EC, and Tebuconazole 25.9% EC Fungicide tested to analyze their effect on growth of pathogen . Each fungicides was dissolved in water to obtain a final concentration 100 ppm, 500 ppm, 1000 ppm, 1500 ppm, 2000 ppm, 2500 ppm, 3000 ppm, 3500 ppm, 4000 ppm, then inoculated into sterile PDA with three replicate.

These five dilutions for each chemical were made based on their recommended dose. Three plates poured with PDA medium served as control. Then 5 mm disc was cut from the edge of 7 days old culture of the fungus and disc was placed in the center of Petri dish and kept in BOD incubator for 8 days for the full growth of fungus was observed, in case of control where no chemicals were applied in medium. The inhibition in the radial growth of pathogen mycelium was recorded and percent inhibition in the mycelium of pathogen was recorded by comparing the mycelial growth of fungus in the treated plates with control. The effect of each chemical was evaluated as percentage of reduction (Sultana & Ghaffar 2010).

Figure 1: Wilted cumin sample

Figure 2: Growth of pathogen on PDA

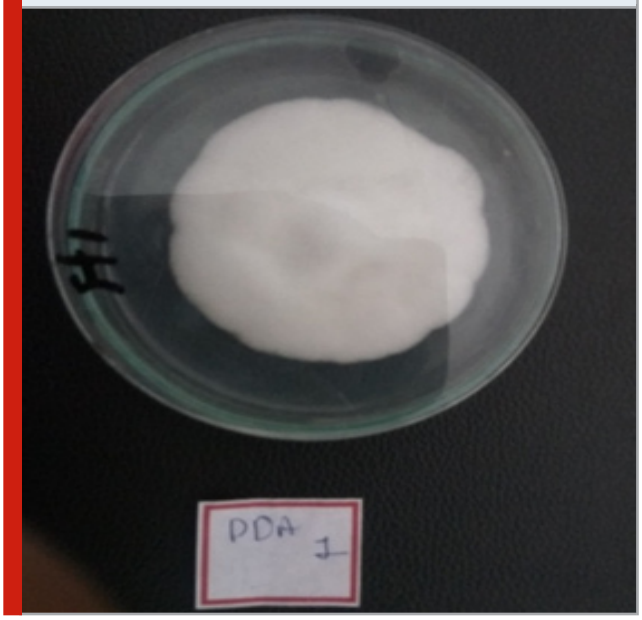


Figure 3: Microscopic observation of macro and micro conidia

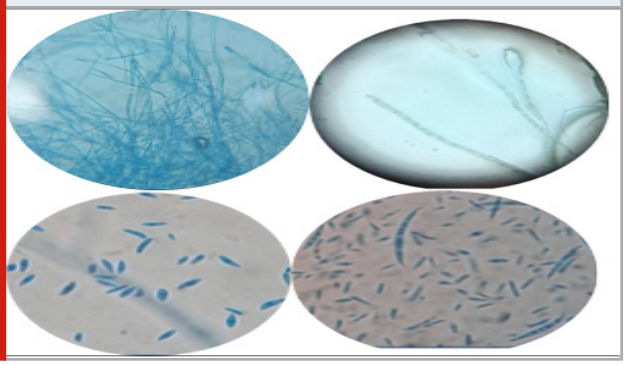


Figure 4

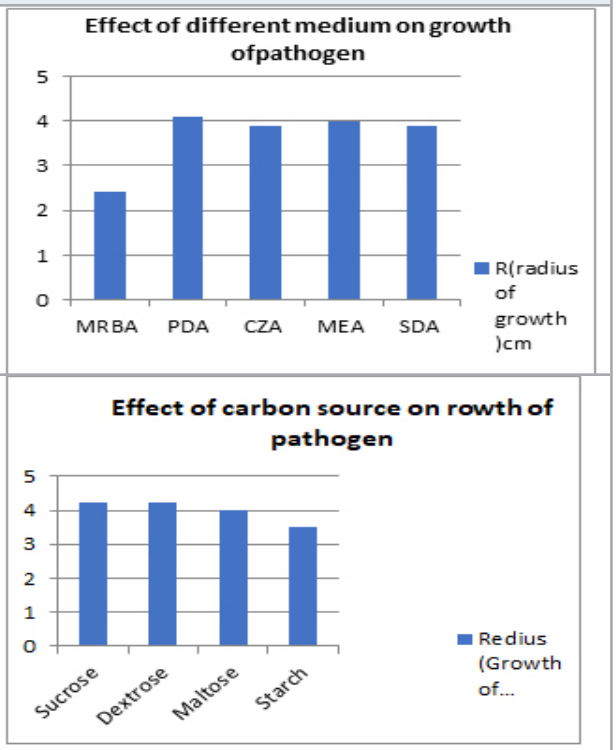
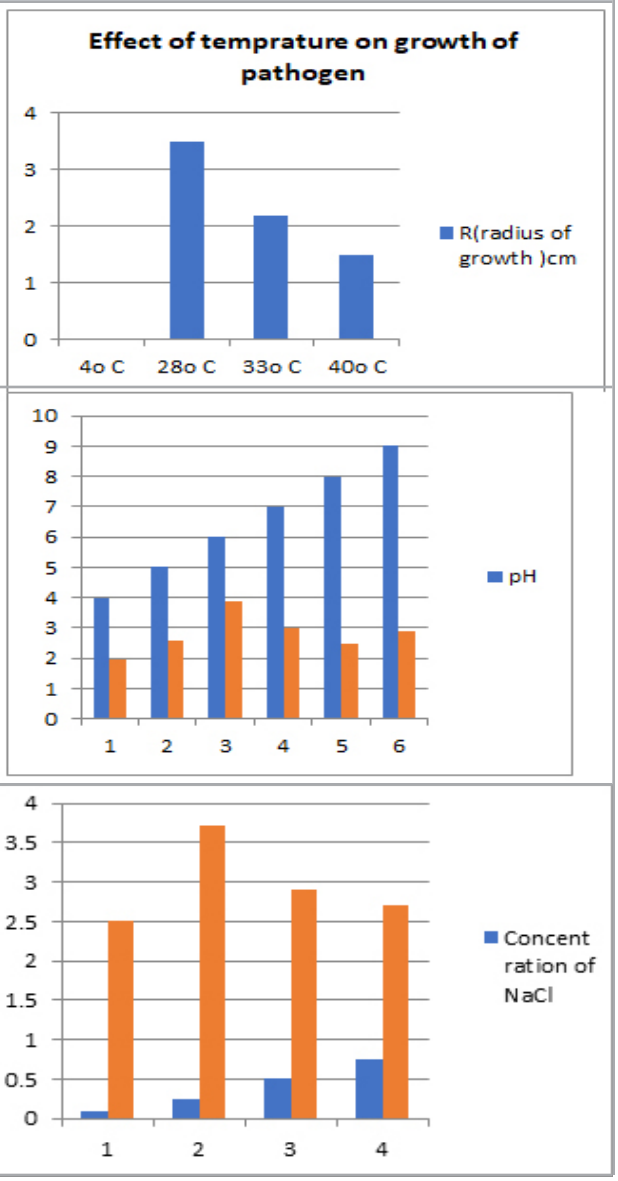


Figure 5



Where: R = dc – dt x 100/dc 25 Whereas:
R = Percentage of reduction
dc = diameter of control colony
dt = diameter of treatment colony

RESULTS AND DISCUSSION

In the present study, the experiments were carried out under laboratory conditions including all the available management practices and further their efficacy was tested in farm conditions. Standard tissue isolation technique was followed to get culture of causal organism from the wilted

plants (figure 1), showing the symptoms. *Fusarium* was isolated consistently from wilted cumin sample. Repeated sub-culturing was done to obtain pure culture on basal medium PDA (figure 2).

Hyphal tip method was used to obtain pure culture as detailed in material and methods. The pathogen was identified as based on their morphological and cultural characters as *F.oxysporum* by referring to Booth (1971)

Cultural studies: Cultural characters were studied on five different solid media and four carbon sources were studied to analyze effect of carbon source on linear growth of pathogen, maximum growth was observe in Potato dextrose agar medium, dextrose and sucrose carbon source. For the majority of the isolates, the 27°C temperature, pH of 6, and salt content of 0.25 percent resulted in the fastest *in vitro* radial growth.

The symptoms of *Fusarium* wilt caused by *Fusarium oxysporum* appeared as leaf chlorosis, yellowing of foliage followed by wilting and dropping of leaves. The xylem became brown in colour of the stem and finally death of the above ground parts. Similar symptoms were also reported by Altinok (2005) and Mac Hardy and Beckman (1981). Isolated fungal pathogens were identified based on cultural and microscopic characteristic and using available literatures. Altinok (2005) and Mwaniki *et al.* (2011) isolated *F. oxysporum* from the eggplant vascular tissues in Tanzania. Sahar *et al.* (2013) also isolated *Fusarium oxysporum* from infected roots of egg plant in Pakistan. *F. oxysporum* was isolated from *Fusarium* wilt of tomato plant by Amini and Sidovich (2010). *F.oxysporum* was also isolated from roots and stem of wilt infected tomato plant (Nirmaladevi and Srinivas, 2012 and Joshi *et al*., 2013).

Chemical agents used were found to be effective for the inhibition of mycelial growth of *Fusarium oxysporum* under *in vitro* conditions (Table 1). Khan *et al.* (1997) and Sharma (2006) found that Bavistin completely inhibited the mycelial growth of *F. oxysporum.* Hossain and Bashar (2011) also reported that complete inhibition of mycelial growth was observed in *Fusarium oxysporum* and *F. pallidoroseum* with Dithane M-45 and Cupravit at 50 and 800 ppm concentrations, respectively. The effectiveness of these fungicides against other fungi has been reported (Daradhiyar, 1980; Sommer, 1982; Kalra and Sokhi, 1985; Singh *et al*., 1997; Patel *et al*., 2005; Banyal *et al*., 2008).

Table 1. Effect of chemical fungicides against *F. oxysporum* f. sp. *cumini*

No.	Trade name of fungicides	Name of active compound	Concentration of fungicides	Radial growth (mm)
1	Folicur	Tebuconazole	2000ppm	0.00
2	Amistar Top	Azoxystrobin	1500ppm	0.00
3	Opera	Pyarclostrobin + Epoxyconazole	4000 ppm	0.00
4	Adexar	Epoxyconazole + Flyxapyroxald	3500 ppm	0.00
5	Custodia	Azoxystrobin + Tebuconazole	1000 ppm	0.00
6	Curzate M8	Cynoacetamide	3500 ppm	0.00
7	Dithane M45	Mancozeb	5000 ppm	0.00
8	Captaf	WP Captan	3500 ppm	0.00
9	Contaf	Hexaconazole, Triazol	2800 ppm	0.00
10	Azaka	Azoxystrobin	1600 ppm	0.00

The pathogen was identified as based on their morphological and cultural characters as *F. oxysporum* by referring to Booth (1971). Generated Sequence from the Microbial Sample (CWP-1). DNA sequence was submitted to NCBI; accession number of DNA sequences is OR178141.1. Furthermore, *Fusarium oxysporum* f. sp. *cumini* was confirmed to be the causative agent of cumin wilt disease.

Among the fungicides tested in this study Folicur treatment reduced mycelium development at concentrations ranging from 500 ppm to 2000 ppm, similar to our founding Shcherbakova et al., 2020, reported Folicur at 400 ppm completely suppressed the germination of the pathogen.

Our findings of Custodia fungicide demonstrated that the fungicide was successful in stopping all mycelia development between 500 ppm and 1000 ppm, similarly, 500 ppm concentration of Custodia exhibits a fungistatic action, Poussio *et al.,* 2021. Opera was successful in inhibition of all mycelial development between 2500 ppm and 4000 ppm, 500 to 1500 parts per million concentrations of Amistar top fully inhibited growth of mycelia; similar to our study Amistar top 500 and 1000 ppm and opera 2500, 3000 ppm were concluded to be fungistatic and strongly suppressed the mycelial development of *F. oxysporum* f. sp. *lycopersici*, according to Chavan *et al.,* 2021. Curzate M8 successfully impeded mycelial growth from 1500

ppm to 3500 ppm. At doses between 1600 and 2800 ppm, Contaf fungicide proved to be effective. Similar to our investigation, Khamari and Patra's 2018 reported Curzate M8 - 1000 ppm has 99.60 % mycelial inhibitions and 1000 ppm of contaf was effective on *Fusarium spp*.

Mycelia had been significantly reduced by Dithen M45 between 3500 ppm. At doses between 1600 and 2800 ppm, Contaf fungicide proved to be effective. Similar to our investigation, Khamari and Patra's 2018 reported Curzate M8 - 1000 ppm has 99.60 % mycelial inhibitions and 1000 ppm of contaf was effective on *Fusarium spp.* Mycelia had been significantly reduced by Dithen M45 between 3500 and 5000 ppm. Similarly, Hossain and Bashar 2011 reported complete inhibition of mycelial growth with Dithane M-45.

Azaka concluded to be of an effective dose range of 400 ppm to 1600 ppm, similarly using 0.030% (300 ppm) Azaka (Azoxystrobin 18.2% + Difenoconazole 11.4 %), Undhad et al., 2022 achieved 98.80 % mycelial inhibition. Wieczyński, et al., 2016 found similar results Captaf, 2000 ppm and 3500 ppm was the efficacious dosage shown in our results, similar results were obtained by Garkoti *et al.,* 2013. Song *et al.* (2022) also reported that Tebuconazole 25.9% EC was found to be the most effective fungicide in inhibiting the mycelial growth of *F. oxysporum* in solidified poisoned PDA observed.

Similarly, among non-systemic fungicides, Mancozeb has the best performance and this result is supported by the findings of Singh *et al.,* 2000. They recorded Mancozeb to be best for growth inhibition of *F. solani* and *F. oxysporum*. Allen *et al.,* 2004 also recorded percent inhibition of all four *Fusarium* species by Mancozeb. The findings of the present study are in agreement with those of Pandey *et al.,* 2005). They reported that *Fusarium* is best controlled by the use of Mancozeb.

CONCLUSION

The observed wilt syndromes on cumin fields are the fungal disease symptoms caused by a soil borne pathogen known as *Fusarium oxysporum*. Salinity, pH, carbon source and temperature have a significant effect on fungal growth. These characteristics can aid in disease monitoring and raising farmer awareness.

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Availability of data and materials: All data and material used can be availed from corresponding author upon request.

Ethics approval and consent to participate: Not applicable.

Competing interests: Authors declare that they have no competing interest.

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