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PA Chaudhary
M.Sc. Scholar, Department of Plant Pathology, C. P. College of Agriculture, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar, Gujarat, India

Manisha S Shinde
Assistant Professor, Department of Polytechnic Agriculture, Ardarkrushinagar Dantiwada Agricultural University, Deesa, Gujarat, Inida

JK Patel
Assistant Research Scientist, Department of Plant Pathology, Potato Research Station, SDAU, Deesa, Gujarat, India

MK Chandaragi
Assistant Research Scientist, Cotton Research Station, SDAU, Talod, Gujarat, India

Corresponding Author:
PA Chaudhary
M.Sc. Scholar, Department of Plant Pathology, C. P. College of Agriculture, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar, Gujarat, India

Survey, isolation, identification and pathogenicity of cumin root rot pathogen (*Macrophomina phaseolina* (Tassi) Goid)

PA Chaudhary, Manisha S Shinde, JK Patel and MK Chandaragi

Abstract
Cumin (*Cuminum cyminum* L.), locally known as "Jeera" is an important and extensively cultivated spices crop. Diseases are the major constrain in economic crop production as they inflict heavy losses. Cumin is attacked by many diseases during seed germination to seed production and maturity. The present investigation was carried out on various pathological aspects to generate scientific information on this important pathological problem and to develop suitable management strategy to prevent losses due to root rot of cumin in *in vitro.* The root rot disease was found more severe proportion on popular varieties of cumin *viz.,* GC-4, GC-2 and local in three district of northern Gujarat *viz.* Banaskantha, Patan and Mehsana. The highest mean per cent disease incidence was observed in the Patan district (35.55%). The tissue isolation from stem and root of infected plant revealed the association of *Macrophomina* sp. which was identified after purification and cultural and morphological studies and found that it was *Macrophomina phaseolina*.

Keywords: Cumin, root rot, survey, identification, pathogenicity and *Macrophomina phaseolina*

Introduction
India is the largest consumer of spices. The major part of the spices total production is consumed indigenously and only about 10% is spent or export. The seed spices play a vital role in the export of spices from India. Cumin crop is generally grown in sandy to loam soil. It matures in about 100 to 115 days. The market value of cumin depends much on its quality and therefore, the farmers are very much concerned with the quality of the produce, besides the yield. Cumin seed contain 2.5 to 4.5 per cent volatile oil and 10 percent fatty oil. Indian cumin seeds are found to contain about 3.5 per cent volatile oil by their weight. The seeds also contain cumin aldehyde or cuminol, which attributes for the aroma and special medicinal properties. In addition to this, seeds also contain 6.2 per cent moisture, 17.7 per cent protein, 23.8 per cent fat, 9.1 per cent crude fiber, 35.5 per cent carbohydrate, 7.7 per cent mineral matter, 0.09 per cent calcium, 0.45 per cent phosphorus, 0.048 per cent iron, 1.6 per cent sodium, 2.1 per cent potassium and also vitamin B_1, B_2, niacin, vitamin-C and vitamin-A etc. (Sankaracharya and Natrajan, 1971) [16]. Production and quality of cumin crop is mainly affected by attack of diseases and pest. There are three major diseases and two minor diseases of cumin *viz*., wilt (*Fusarium oxysporium* f. sp. *cumini*, P and P), alternaria blight (*Alternaria burnsii*, Uppal *et al.,* 1938) [20] and powdery mildew (*Erysiphe polygony*), and two minor diseases *viz*., root rot and leaf spot disease (*Chaetomium sulphureum*). Besides them the mycoflora *viz*., *Alternaria*, *Curvularia*, *Aspergillus*, *Helminthosporium* and *Fusarium* adversely affected seed germination and cumin growth (Swarup and Mathur 1972; and Singh, 1977) [17, 14]. In Gujarat, cumin has been cultivated on substantial area every year especially under stress conditions where biotic stresses are major constrain in attainable productivity. Among various biotic stresses, Root rot has been recorded in some cumin growing areas of Gujarat state. Therefore, an attempt has been made to generate scientific information on root rot disease on cumin incited by *Macrophomina phaseolina* (Tassi) Goid which have become a serious problem in hampering the production in all cumin growing areas of India in general and Gujarat in particular in North Gujarat cultivation area of cumin is larger as compared to rest part of Gujarat. Therefore, the present investigation has been undertaken with the following aspects to the study of root rot disease of cumin.
The wilt is common in cumin, however past four year root rot disease is recorded in cumin. It is prevalent in all the cumin growing countries. *M. phaseolina* was recovered from some

diseased samples, showing blackening of the stem and root rot symptoms, and these isolates were confirmed to be pathogenic to cumin seedlings, Similarly, this pathogen was determined on cumin plant. Hence survey were conducted during 2019-20 in different districts of North Gujarat to know the cause of disease.

Materials and Methods

Survey of cumin root rot disease

An intensive roving survey was conducted in main cumin growing regions of North Gujarat such as Banaskanth, Mehsana and Patan district. From each district three talukas were selected, and minimum three samples from different villages were collected and per cent root rot disease incidence was recorded.

$$\text{Per Cent Disease Incidence} = \frac{\text{Number of infected plants observed}}{\text{Total No. of plants assessed}} \text{X } 100$$

Isolation, identification and pathogenicity of cumin root rot pathogen

Sample collection

Cumin plants with typical root rot symptoms were collected from different cumin growing fields and brought to the laboratory for examination and isolation. The roots of such infected plants were wash with running tap water to remove all adhered soil particles and they were subject for tissue isolation.

Isolation of root rot pathogen

Isolation of the pathogen from diseased specimens was made by tissue isolation technique. The typically infected root and stem portion near collar region was cut into small pieces with the help of sterilized knife and again wash with sterilized water. These pieces were disinfect for one minute in 4% sodium hypochlorite solution. To remove residues of sodium hypochlorite, the pieces was wash thrice in sterilized distilled water. Such pieces then transfer aseptically on to potato dextrose agar medium in Petri dishes. The Petri dishes were incubate for five days at 28±2 ℃ temperature. A typical growth of the fungus was transfer aseptically on PDA slants. Periodical sub-culturing and multipliication was done on the same medium to keep the culture fresh for further study.

Identification: To identify the pathogen, morphological and cultural characters of the fungus isolated from cumin infected plants was studied in laboratory under microscope and compared with those given in literature.

Result and Discussion

Survey of cumin root rot disease

To find out the status of root rot disease in Banaskantha, Patan and Mehsana districts, a roving survey was conducted to assess the severity of root rot of cumin during *Rabi* 2019-20 in major cumin growing areas. The observations on per cent disease incidence were recorded and presented in Table 1. It is very clear from the results that root rot was present in all the surveyed area in Banaskantha, Mehsana and Patan. Varieties *viz*., GC-2, GC-4 and local were growing extensively in the area showed root rot incidence of cumin. The root rot incidence was recorded ranging from 0 to 79 per cent in Banaskantha, Mehsana and Patan during the year 2019. The root rot incidence of cumin was recorded ranging from 11 to 45 per cent (29.33%) during the year 2019 in Banaskantha district. The highest disease incidence was observed at Kumbhalgadh (45%) in local variety of cumin while, the lowest in Jegol (11%) in GC-2. In Patan district the root rot incidence of cumin was recorded ranging from 0 to 79 per cent (35.55%) during the year 2019 in Patan district. The highest disease incidence was observed at Hajipur (79%) in local variety of cumin while, the no root rot incidence were recorded in Thakrasan, Nandotri and Tavadiya in Patan district. In Mehsana, the root rot incidence of cumin was recorded ranging from 8 to 24 per cent (29.33%) during the year 2019 in Mehsana district. The highest disease incidence was observed at Jilosan (24%) in local variety of cumin while, the lowest in Maktupur (8%) in GC-2. Overall, the root rot incidence was more in Patan district (35.55%) followed by Banaskantha district (29.33%) and Mehsana district (14.66%). The root rot incidence was found highest in Hajipur (79%) followed by Vijaynagar (65%) and Runi (63%) village of Patan district. The data presented in Table 1 clearly showed.

The result of present investigation are more or less similar with finding of Mohanpriya *et al.*, they recorded maximum dry root rot incidence (25.84%) in cow pea during 2013-14 due to *Macrophomina phaseolina* in different location of Cuddalore, Thiruvannamalai and Vellore districts of Tamilnadu. Rani *et al.*, (2017) [18] recorded root rot (*R. solani*) incidence in range of 1.00 to 59.19 per cent in different fenugreek fields of Karnataka. The maximum root rot incidence was recorded in Yattinagudda village of Dharwad district. Deepa *et al.*, (2018) [5] noted root rot (*R. bataticola*) in rainfed chickpea field in range of 3.27 to 35.73 per cent during 2015-2016 in eight district of Northern Karnataka.

The root rot pathogen of cumin (*Macrophomina phaseolina*) was identified and reported by Sharma (2011) [22] with ITCC number 6906 (Anonymous, 2011) [1]. This is first time where the information on the status of root rot of cumin in North Gujarat is created.

At disease was present in all the villages in three districts except Siddhpur taluka during surveyed. The popular variety GC-2, GC-4 and local were found susceptible and hence proved as a major constraint in profitable cultivation of cumin in North Gujarat. This suggests for urgent need to develop management strategies including resistant varieties.

Isolation, identification and pathogenicity of cumin root rot pathogen

Collection of sample

Cumin plants (GC-4) showing the typical root rot symptoms were collected from farmer's field and brought to the laboratory and subjected to tissue isolation for further investigation.

Table 1: Root rot disease incidence of cumin in different district

District	Taluka	Village	Variety	Disease incidence (%)	Average disease incidence (%)
Banaskantha	Dantiwada	Dantiwada	GC-4	21	22.33
		Dhaneri	GC-4	35	
		Jegol	GC-2	11	
	Tharad	Lodhnor	Local	28	30.66
		Bhapi	GC-2	35	
		Budhanpur	Local	29	
	Palanpur	Kumbhasana	GC-4	29	35.00
		Kumbhalgadh	Local	45	
		Gadh	GC-4	31	
Mean					29.33
Patan	Siddhpur	Thakrasan	GC-4	0	0.0
		Nandotri	Local	0	
		Tavadiya	Local	0	
	Radhanpur	Vijaynagar	GC-2	65	52.33
		Bandhval	Local	43	
		Kamalpur	GC-4	49	
	Patan	Runi	GC-4	63	54.33
		Kamlivada	GC-2	21	
		Hajipur	Local	79	
Mean					35.55
Mehsana	Unjha	Maktupur	GC-2	8	10.66
		Varvala	GC-2	11	
		Bhramanvada	GC-4	13	
	Mehsana	Jilosan	Local	24	16.33
		Dediyasan	GC-4	16	
		Udaipur	Local	9	
	Visnagar	Valam	Local	11	17.00
		Kansi	GC-2	18	
		Ganeshpura	GC-4	22	
Mean					14.66

Isolation and purification of pathogen

The fungus was isolated on PDA from infected roots of cumin plants under aseptic conditions. The fungus started emerging on PDA after 48 hrs of incubation at 28 ±2 °C temperature. The fungus initially started to grew as dirty white mycelium, then turn to fluffy with blackish mycelium growth on (PDA) (Plate II). After seven days, minute black sclerotial bodies formed on PDA. The culture was further purified by single hyphal tip method and the purified culture was maintained on PDA slants for further studies. The periodical sub-culturing and multiplication were made on PDA plates to keep the culture fresh and to use throughout the investigations.

The isolation and purification of *M. phaseolina* has been made by several research worker from castor (Nakrani, 1991) [13], mungbean (Wyllie, 1993; Kale, 1999; Tandel, 2004) [21, 8, 19]; urdbean (Suryawanshi *et al.,* 2008) [15], soybean (Ahmed *et al.,* 2010; Cummings and Bergstrom, 2013) [2, 3] and chickpea (Lakhran *et al.,* 2018) [9]. Thus the result obtained from the study as similar with the earlier research worker reported.

Identification of causal organism

Cultural and morphological characters

The mycelium of the fungus was initially white, gradually turned brown to black in colour due to formation of numerous small black sclerotia after 7 days. The sclerotia formed in culture were black, hard and measured 24.4 × 19 µm in diameter (Table 2). The results of present investigation are more or less similar with the Tandel (2004) [19], Suryavanshi *et al.,* (2008) [15], Ashraf *et al.,* (2015) [23], and Nakrani (1991) [13].

Proving pathogenicity of associated causal organism

To confirm Koch's postulates, the pathogenic nature of the fungus *Macrophomina phaseolina* isolated from infected cumin plant was established by employing soil inoculation method in GC-4 variety of cumin. The results presented in Table 3 and showed that the pathogenicity was proved positively in the methods of inoculation. The pathogenicity of *Macrophomina phaseolina* isolated from infected cumin plant was successfully proved by soil inoculation method which confirmed it's pathogenic nature producing root rot disease in susceptible cumin cultivar GC-4. These methods successfully produced root rot symptoms similar to those under natural conditions as well as described in the literature, confirmed the pathogenic nature of the fungus. The causal agent of cumin root rot was identified and confirmed as *M. phaseolina* (Tassi.) Goid.

On standing plants, rotting symptoms were observed after 18 and 22 days of inoculation in soil inoculation techniques. In soil inoculation method 86.66 per cent plant developed diseased symptoms. The re-isolation from the artificially inoculated and infected cumin plants yielded the culture of *Macrophomina phaseolina* identical and similar to original in all the respects. The pathogenicity of cumin root rot pathogen was proved quickest and most efficiently by soil inoculation method. The pathogenic nature of *Macrophomina phaseolina* has been proved in various crops by Deshkar *et al.,* (1973) [4] and Hooda and Grover (1988) [7] in mungbean, Dhingra and Sinclair (1978) [6] in soybean, Mayee and Guard (1981) [10] in sorghum, Garcia *et al.,* (2003) [24] in common bean and Chaudhary (2013) [25] in pigeon pea.

Table 2: Measurement of sclerotia

Sr. No.	Length (µm)	Width (µm)
1	45	20
2	22	18
3	20	22
4	20	20
5	15	15
Average	24.4	19

Table 3: Pathogenicity test of *Macrophomina phaseolina* on cumin in pots by soil inoculation methods

Sr. No.	Inoculation method	Total number of plant	Days required to produce root rot symptoms (DAS)	Disease incidence (%)
1	Soil inoculation	15	18	86.66
2	Control	15	-	0.00

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