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Post-Harvest Rhizome Rot and Reduction in Curcumin Content in Turmeric (*Curcuma longa* L.)

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Post harvest rhizome rots of turmeric are caused due to many fungal pathogens. The association of different *Fusarium spp.* and fungi belonging to hyphomycetes group were found with infected turmeric rhizomes. The pathogenesity of these fungi was proved under laboratory condition. The mean percent colonization and mean percent infection of turmeric rhizomes by incision method and pinprick method were studied and both were higher in incision method as compared to pinprick method.Highest percent infection was found in case of *Fusarium oxysporum* followed by *Fusarium solani*. Curcumin was extracted by solvent extraction method from both healthy and diseased rhizomes and the percent reduction in curcumin content of diseased sample over healthy sample was studied under laboratory condition and highest reduction in curcumin content was found to be 25.19% in the diseased sample affected by *Fusarium solani*.

Key words: Curcumin; Incision Method; Pin Prick; Post Harvest; Rhizome Rot; and Turmeric.

Turmeric (*Curcuma longa* L.) is an annual spice crop grown as kharif crop in India. It is the third important spice crop in India next to chilli and black pepper and important for both domestic and industrial use. A heavy damage is occurring due to post-harvest rhizome rot of turmeric at storage and transit condition. Rhizome rot of turmeric due to *Fusarium solani* was reported by Dohroo (1988). Kumar and Roy (1990) found many fungi associated with post-harvest roting of turmeric like *Aspergillus flavus, Aspergillus niger, Cladosporium cladosporioides, Drechslera (Setosphaeria rostrata, Fusarium moniliforme, Fusarium oxysporum, Macrophomina phaseolina, Pythium aphanidermatum, Rhizoctonia solani* and *Sclerotium* (*Satarium*) rolfsii. They observed that injured and uninjured rhizomes of *Curcuma longa*, the turmeric of commerce harbour several rhizome rot fungi which become prominent in monsoon and reach at minimum in summer months in samples collected from different markets from West Bengal. Mandal(1980) reported that rhizome rot by *Fusarium moniliforme* and *Fusarium solani* in turmeric starts from stem end of rhizome and some wound. Percent colonization and percent infection can be studied by incision and pin- prick method in in vitro condition as given by Kuffman et al. (1973) and Baayen et al. (1990). Curcumin is the antioxidant chemical found in turmeric and responsible for yellow colour of turmeric (Rakhunde et al., 1998). Here in this study attempts have been made to study the effect of infection of fungal pathogens on curcumin content of turmeric rhizome after harvest. In this regard curcumin was isolated by solvent extraction method as was given by Milan Suhaj in 2006. Curcumin content from healthy rhizome was estimated by solvent extraction method given by Sadasivan and

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Manickam (1992). Percent reduction in cucumin content was calculated for diseased sample and compaired with healthy sample and was found to be highest in case of *Fusarium solani*.

MATERIALS AND METHODS

Isolation of pathogens

Sample of rotten rhizomes, asserted according to the visual symptoms, were fumigated in formalin chamber for 15 minutes, then the rotten rhizomes were transferred (4-5 pieces) to the PDA-plates aseptically inside the culture room, which was previously disinfected by spraying HCHO and with UV light. The plates were incubated at room temperature for 5 days and the growths of the fungi were recorded on the 5th day of incubation. Further purification was done in PDA whenever necessary.

Pathogenecity Test

Pathogenecity test was done by using healthy rhizome with the help of pin prick and incision method under aseptic condition.

Effect of different inoculation method on symptoms development by four fungi

Surface sterilization of healthy rhizomes

The two methods such as incision method to study the infection by different fungi. Turmeric rhizomes were surface sterilized in 1% mercuric chloride for two minutes and subsequently washed thoroughly with four changes of sterile water arranged in different Petridishes. The surface sterilized rhizomes were emerged in sterile water for 10 minutes to soften the tissues.

Preparation of inoculation plates

Ten cm diameter Petridishes were wrapped in paper and sterilized in hot air oven at 160°C for 2 hours. Blotter paper were cut at 12 cm dia and stacked together and sterilized at 60°C for 24 hours. The sterilized Petridishes were lined with the sterilized blotters. In incision method, a dip cut of about 5 mm was made on the side of the individual rhizome with the help of a flame sterilized spear head needle In case of pin prick method, sterilized rhizomes were pricked individually 3-4 times with the help of a flamed needle. Three numbers of sterilized rhizomes were placed on the blotting paper in each replication and for each fungus. Thirty two such Petridishes were prepared for inoculation of four fungi with four replications, of which 16 plates were used for colonization study and 16 for infection study.

Inoculation

Prior to inoculation all fungi were separately grown on PDA for 7 days. For inoculation, fungal mycelium was transferred from the actively grown edges of the colony and inoculated on the incision given on the rhizomes and on the pin pricked area. The plates were incubated at room temperature for 20 days. Observations on colonization were recorded on 1, 5, 10, 15 and 20 days after incubation at room tempe rature. Percent colonization was calculated by the formula:

$$\% \text{ colonization} = \frac{\text{Area colonized in mm}^2}{\text{Total area in mm}^2} \text{x100}$$

For studying percent infection of turmeric rhizomes, inoculated rhizomes were washed thoroughly after removing the mycelial growth carefully. The washed rhizomes were then dried in shed in the laboratory and the percent infection was calculated by the formula:

$$\% \text{ infection} = \frac{\text{Area infected in mm}^2}{\text{Total area in mm}^2} \text{x100}$$

Extraction and Estimation of Curcumin

The turmeric rhizome contains a variety of pigments. It is used as a natural dye in food industries, cosmetics and pharmaceutical products as an antimicrobial principle. The powder contains a large number of aromatic compounds; curcumin is the major compound responsible for the characteristic colour. In pure form, it is an orange yellow crystalline powder, soluble in alcohol and glacial acetic acid. Curcumin content is used as an index for the quality of the produce. Extraction of curcumin was done following the method given below (Sadasivam and Manickam, 1992).

$$\text{Curcumin content} = \frac{0.0025 \text{ x } A_{425} \text{ x volume made up x dilution factor x } 100}{0.42 \text{ x weight of the sample (g) x } 1000}$$

Since 0.42 absorbance at 425 nm = 0.0025 g curcumin. (Sadasivan and Manickam, 1992)

Experimental Results

Effect of Different inoculation Methods on Symptom Development: Percent colonization

of turmeric rhizome and % infection by *Fusarium oxysporum, F. solani, Fusarium sp.* and unidentified fungi was tested in 2 methods namely incision method (IM) and pin-prick method (pm). Observations were recorded in, day, 5th day, 10th day, 15th day and 20th day after inoculation. The results are presented in Table 1 and 2.

The data presented in Table 1 indicated that no evident growth of any inoculated fungi was noticed in 1 day after inoculation in either incision method or pin prick method. Scanty growth was observed in 5th day after inoculation, maximum colonization being 3.57 by unidentified fungi in incision method and minimum being negligible growth, which was not measurable by *F.solani* in pin-prick method and by *Fusarium sp.* In both incisions as well as in pin prick method.

Considerable colonization of turmeric rhizome was observed on 10 day after inoculation. Colonization was as high as 22.29% by unidentified fungi in incision method and the minimum colonization was 5.94% by *Furasium sp.* in pin prick method of inoculation. Colonization was moderate (12.5% - 16.88%) by *Fusarium sp, F. solani* and *F.oxysporum* in incision method. On 15 day after inoculation, colonization of turmeric rhizome was still higher in comparision to colonization 10 day after inoculation. In incision method, the colonization varied from a maximum 39.23% by unidentified fungi to a minimum of 25.45% by *Fusarium sp*. In pin-prick method, colonization was maximum 24.67% by unidentified fungi to a minimum of 10.73% by unidentified fungi to a minimum of 10.73% by *Fusarium sp*. Colonization, in pin-prick method was 12.55% and 11.24% respectively by *F. oxysporum* and *F. solani*. Highest % colonization of turmeric rhizome was observed on 20 day after inoculation. On 20 days after inoculation, a maximum colonization of 100% was observed in incision method by unidentified fungi. This fungus was also able to colonize as high as 78.35% of turmeric rhizome in pin-prick method. *Fusarium oxysporum* colonize 67.24% in incision method and 29.48% in pin-prick method, compared to *F. solani* 65.52% in incision method and 27.64% in pin-prick method and by *Fusarium sp.*, 58.92% in incision method and 25.28% in pin-prick method. Since the colonization was 100% by the unidentified fungi in incision method, the period of observation was restricted to 20 days. In general, the incision method was observed to be superior over the pin prick method in colonizing turmeric rhizomes over a period of time (Table 1).

Percent infection of turmeric rhizome by four fungi in incision method and pin prick method are presented in table 2. A perusal of data indicated that no infection was evident 1 day after inoculation. Scanty infection (1.25%) was recorded by *F. oxysporum* in incision method. *F. solani* and *Fusarium sp* recorded 1.1% and 0.75% infection in incision method only. No visual infection could be observed by *F. oxysporum, F. solani, Fusariuim sp.* and unidentified fungi in pin-prick method

On 10 days after inoculation, % infection was maximum 5.72% by *F. oxysporum* 5.05% by *F. solani*, 4.32% by *Fusarium sp.* and 2.75% by unidentified fungi in incision method. In pin-prick method, the infection was maximum 1.95% by *F. oxysporum*, 1.73% by *F. solani*, 1.30% by *Fusarium sp.* and 0.84% by unidentified fungi. On 15 days after inoculation, the infection was maximum 10.53% by *F. oxysporum* followed by 9.87% by *F. solani*, 9.33% by *Fusarium sp*. and 5.24% by unidentified fungi in incision method. In pin-prick method however, in infection was maximum 3.25% by *F. oxysporum*, followed by 2.95% by *F. solani*, 2.76% by *Fusarium sp.* and 1.89% by unidentified fungi.

On 20 days after inoculation, a maximum 15.66% infection was observed in rhizomes inoculated with *F. oxysporum* followed by 14.47% by *F. solani*, 13.75% by *Fusarium sp.* and 10.25% by unidentified fungi in incision method. In pin-prick method, the infection was maximum 7.49% by *F. oxysporum*, 6.92% by *F. solani*, 6.40% by *Fusarium sp*. and 3.62% by unidentified fungi (Table 2). Percent colonization and percent infection by two fungi was faster in incision method than in pin-prick method. (Table 1 and 2)

Estimation of Curcumin Content

Solvent extraction method (Sadasivam and Manickam, 1992) was employed to estimate the curcumin content in diseased and healthy rhizomes. For each sample, 100 mg of turmeric powder thoroughly mixed in 200 ml of absolute alcohol were taken in conical flask. Optical density was measured at 425 nm. The OD value, curcumin content (%) and percent reduction in curcumin content over healthy sample were estimated and presented in table no 4 and plate 6. From the data

presented in table 3, it was observed that curcumin content of turmeric rhizome was differently reduced due to infection by different fungi. Curcumin content was maximum 2.167% in healthy rhizomes followed by 1.955% in rhizomes with unidentified Hyphomycetes, 1.690% in rhizomes with *Fusarium oxysporum* and the least 1.621% in rhizomes with *Fusarium solani*.

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Table 1. Mean % colonization of turmeric rhizome by 4 fungi in Incision method and pink prick method

Fungi	Method	Mean % colonization of turmeric rhizomes Days after inoculation (DAI)				
		1 day	5days	10 days	15 days	20 days
Fusarium	Incision method	0	2.78	16.88	31.46	67.24
oxysporum	Pin-prick method	0	1.25	7.76	12.55	29.48
F. solani	Incision method	0	2.71	14.23	26.59	65.52
	Pin-prick method	0	*	6.15	11.24	27.64
Fusarium sp.	Incision method	0	*	12.05	25.45	58.92
	Pin prick method	0	*	5.94	10.73	25.28
Unidentified	Incision method	0	3.57	22.29	39.23	100
Fungi	Pin prick method	0	1.82	10.82	24.67	78.35

*Growth of the fungi was negligible

Table 2. Mean % infection of turmeric rhizome by four fungi in incision and pin prick method

Fungi	Method	Mean % colonization of turmeric rhizomes Days after inoculation (DAI)				
		1 day	5days	10 days	15 days	20 days
Fusarium	Incision method	0	1.25	5.72	10.53	15.66
oxysporum	Pin-prick method	0	*	1.95	3.25	7.49
F. solani	Incision method	0	1.1	5.05	9.87	14.47
	Pin-prick method	0	*	1.73	2.95	6.92
Fusarium sp.	Incision method	0	0.75	4.32	9.33	13.75
	Pin prick method	0	*	1.30	2.76	6.40
Unidentified	Incision method	0	*	2.75	5.24	10.25
Fungi	Pin prick method	0	*	0.84	1.89	3.62

*Growth of the fungi was negligible

Table 3. Estimation of curcumin content of turmeric rhizomes

Sample taken	Causal pathogen	OD value	Curcumin content (%)	% reduction in curcumin Over healthy sample
Healthy	No pathogen	1.821	2.167	0.00
Diseased	*F. oxysporum*	1.420	1.690	22.01
Diseased	*F. solani*	1.362	1.621	25.19
Diseased	Unidentified	1.643	1.955	18.16

percent reduction of curcumin content in different diseased rhizomes varied from 18.16% to 25.19%. A maximum reduction in curcumin content of 25.19% was observed in sample with *F. solani*, followed by 22.01% with *F. oxysporum* and 18.16% with the unidentified fungi.

DISCUSSION

A number of workers have reported losses due to rhizome rots of turmeric differently. The involvement of the isolated fungal pathogens in turmeric may cause concern in future particularly in traditional turmeric growing areas. Since these species of *Fusarium* are facultative parasites and are also soil dwellers, there is every possibility that the turmeric soil may become sick, as turmeric is continuously cultivated in the same soil for years together as monoculture. The diseased rhizomes exhibited visual symptoms of white rot, pink rot and black rot. Mandal (1981) reported rhizome rot of turmeric caused by *F. moniliforme* and *F. solani*. Symptoms described by Mandal (1981) matches with the symptoms of pink rot observed in the present study. Dohroo (1988) has reported *F.solani* as pathogenic causing rhizome rot of turmeric.

Kumar and Roy (1990) found many fungi associated with post-harvest rotting of turmeric. Among these, notable fungi are *Aspergillus flavus, A. niger, Cladosporium, cladosporioides, Drechslera rostrata, Fusarium moniliforme, F. oxysporum, Macrophomina phaseolina, Pythium aphanidermatum, Rhizoctonia solani* and *Sclerotium rolfsii*.

Effects of different inoculation methods on symptoms development by different fungi indicated that incision method was superior over the pin-prick method. This was probably due to higher wound area which greatly facilitated for colonization, establishment and subsequent infection in comparison to pin-prick method where in the wound area was less. Colonization of turmeric rhizomes by different fungi was faster than infection throughout the period of study, and colonization was not a measure of infection. This view is corroborated from the observation that even if the colonization was 100%, the infection was 10.25%. In general, the percentage colonization after 20 days of inoculation in incision method varied from 58.92% up to as high as 100% but the percent infection remained restricted from 10.25% to 15.66% only.

In the present study, Curcumin was estimated from apparently healthy and diseased rhizomes by employing solvent extraction method given by Sadasivan and Manickam(1992) as per Suhaj, 2006. Curcumin content of turmeric rhizome was differently reduced due to infection by different fungi. Curcumin content in healthy rhizomes was found to be 2.167% while, it was 1.690% in diseased rhizomes associated with *F. oxysporum*, 1.621% in rhizomes associated with *F. solani*, and 1.955% in diseased rhizomes associated with unidentified fungi. Percent reduction in curcumin content due to these pathogens was in the range of 18.16 to 25.19%.

REFERENCES

1. Baayen, R P and Schrama, R M. Comparision of five stem inoculation methods with respect to phytoalexin accumulation and *Fusarium* wilt development in carnation. *Netherland Journal of Plant Pathology*. 1990; **96**: 315-320.
2. Dohroo, N P. Fusarium solani on Curcuma longa. *Indian Phytopathology.* 1988; **40**: 275.
3. Sadasivan, S and Manickam, A. *Biochemical Methods for Agricultural Sciences.* 1992; Wiley Eastern Limited. P 185.
4. Harish, K, Roy, A N and Kumar, H. Occurrence of fungal rot turmeric (*Curcuma longa*) rhizomes in Delhi market. *Indian Journal of Agricultural Sciences.* 1990; **60**(3), 189-191.
5. Kuffman, H E, Reddy, A P K, Hsieh, S P and Merca, S D. An improved technique for evaluating resistance of rice varieties to *Xanthomonas oryzae. Plant Disease Reporter.* 1973; **57**(6): 537-541.
6. Mandal, N C and Dasgupta, M K. Post harvest diseases of perishables in West Bengal. III. Further addition to new diseases in India. *Indian Journal of Mycology and Plant Pathology,* 1980; **10**: 31.
7. Rakhunde, S D, Munjal, S V and Patil, S R. Curcumin and essential oil contents of some commonly grown turmeric (*Curcuma longa* L.) cultivars in Maharashtra. *Journal Food Science and Technology* 1998; **35**: 352-354.
8. Suhaj, M. Spice antioxidant isolation and their antiradical activity: a review. *Journal of Food Composition and Analysis.* 2006; **19**: 531-537.