STUDIES ON THE EFFECT OF ABIOTIC AGENTS ON FUNGITOXICITY OF THE LEAF EXTRACT OF *CURCUMA MALABARICA* AGAINST *RHIZOCTONIA SOLANI*

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ABSTRACT
India is among the five major potato producing countries of the world and it contributes to over 5% of the world production. The present study carried out to investigate the effect of physical factors on antifungal activity of plant extract *Curcuma malabarica* against the test fungus *Rhizoctonia solani* Kuhn, which causes Black Scurf disease of potato. It was found that the plant extract of *Curcuma malabarica* retain their fungitoxicity to great extent under extremes of physical factors like temperature, autoclaving, storage, increased density of inoculums.
Keywords: Plant extract, Antifungal activity, Black scurf disease & Physical factors.

INTRODUCTION

Curcuma malabarica is a plant of the family Zingiberaceae. It is widely grown as a kind of pepper in countries of South and Southwest Asia, and in these countries, as well as in India. The rhizomes of the plant are used widely in the cure of stomatitis, as a stimulant, anti-flatulent, diuretic, anti-diarrheic, antiemetic, antipyretic, purgative, and also to clean and heal ulcers, wounds and other kinds of skin disorders (Matsuda *et al*., 2001).

Significant antifungal activities have been demonstrated in extracts of members of the Zingiberaceae, particularly *Alpinia galanga, C. zedoaria, Zingiber purpureum* and *Z. officinale* (Ficker *et al*., 2003). Investigation of the antifungal action of the volatile oil of *C. zedoaria* revealed that a concentration of 2000 ppm is sufficient to cause complete inhibition of the mycelium of the fungus *Colletotricum falcatum*. This concentration also brought about partial mortality of other fungi tested, but was inefficient against *Aspergillus niger* (Singh *et al*., 2002).

Potato (*Solanum tuberosum* L.) is an annual, herbaceous and dicotyledonous plant belonging to genus *Solanum* and family Solanaceae, a major vegetable crop of India. India is among the five major potato producing countries of the world and it contributes to over 5% of the world production. It is cultivated on 1.2 million hectares which accounts for approximately 0.67% of the total cropped area of India, Potato belongs to one botanical species *Solanum tuberosum* but it comprises of thousands of varieties that vary in their characteristics.

Among the various fungal diseases of potato crop, black scurf disease of potato caused by, *Rhizoctonia solani*, (Ahmed *et al*., 1995; Khan *et al*., 1995) is the serious and most commonly observed disease with the characteristic symptoms of black scurf (dark brown to black colored hard masses of sclerotia, irregularly shaped and superficial, varying from small, flat, barely detectable blotches to large and raised lumps adhering tightly to the skin) on tubers and stem canker are the result of *Rhizoctonia* disease complex in potato (Tsror, 2010).

Extract of *Curcuma malabarica* having strong antifungal activity against *Rhizoctonia solani* Kuhn. causes Black scurf Disease of Potato (Chaudhary *et al.* 2017). Therefore in the present study the plant extract were tested for some physical factors like temperature, autoclaving, storage, increased density of inoculums

MATERIAL AND METHODS

Preparation of crude extract

20 grams of freshly collected disease-free leaves of *Curcuma malabarica* were surface sterilized with sodium hypochlorite solution (4%) for 2 min followed by washing with sterilized distilled water to remove all the traces of sodium hypochlorite. The sample was then chopped into small pieces and macerated to pulp using a sterilized pestle and mortar. The pulp was squeezed by double layered sterilized muslin cloth and filtered through Wattman's No. 1 filter paper. The crude extract thus obtained was subjected to antifungal testing against the test fungus *Rhizoctonia solani* Kuhn.

Microbial Cultures and Growth Conditions The plant extracts of *Curcuma malabarica* were assayed for antifungal activity against the fungal strain *Rhizoctonia solani*. Kuhn. (MTCC No. 4633) obtained from Microbial Type Culture (MTCC), Chandigarh.. This fungus was grown on PDA plate at 25^0C- 27°C and maintained with periodic sub – culturing at 4^oC. The pure culture of the test fungus was maintained. The assessment of fungitoxicity was done by poisoned food technique (Grover and Moore 1962).

Inoculum disc: Seven days old culture of the test fungus was used for the preparation of inoculum disc of 4 mm in diameter.

Antifungal assay: A volume of 0.5 ml of each concentration was aseptically poured into the petriplate followed by the addition of 9.5 ml of melted PDA and was swirled gently to achieve thorough mixing of the contents. In the control set, no extract was used. After the solidification of the media, one inoculum disc of the test fungus was aseptically inoculated upside down at the centre of the petriplate and incubated at 25-27oC. Fungitoxicity was recorded in terms of the % inhibition of mycelial growth and calculated using the following formula (Vincent 1947).

$$\text{Percent Inhibition} = \frac{dc - dt}{dc} \times 100$$

Where: *dc* – average diameter of fungal colony in control sets.

dt – average diameter of fungal colony in treatment sets.

Extract was tested for various physical factors like temperature, autoclaving, storage and increased inoculums.

Effect of temperature: Conical flask containing the extract was heated on different temperatures from 20 ^{0}c to 120 ^{0}c and mixed with PDA medium in pre sterilized Petriplates and allowed to solidify. Petriplates were inoculated with 4 mm disc of test fungus and incubated at 25-27°C. Observation was recorded on seventh day. (Table 1)

Effect of autoclaving: Conical flask containing the extract was autoclaved (15 lbs/inch2 pressure for 15 min.) and mixed with PDA medium in pre sterilized Petriplates and allowed to solidify. Petriplates were inoculated with 4 mm disc of test fungus and incubated at 25-27 °C. Observation was recorded on seventh day (Table 2).

Effect of storage: The extract was stored for different time periods and mixed with PDA medium in pre sterilized Petriplates and allowed to solidify. Petriplates were inoculated with 4 mm disc of test fungus and incubated at 25-27°C. Observation was recorded on seventh day (Table 3).

Effect of increased inoculum:

By number: PDA medium was autoclaved and poured into pre sterilized Petriplates. Requisite amount of extract was mixed with PDA medium and allowed to solidify. After complete solidification of the medium, petriplates were inoculated with increasing the number of disc and incubated at 25-27°C. Observation was recorded on seventh day (Table 4).

By diameter: PDA medium was autoclaved and poured into pre sterilized Petriplates. Requisite amount of extract was mixed with PDA medium and allowed to solidify. After complete solidification of the medium, petriplates were inoculated with increasing the diameter of disc and incubated at 25 -27°C. Observation was recorded on seventh day (Table 5).

RESULTS AND DISCUSSION

The study reveals that the extract of *Curcuma malabarica* retained their fungitoxicity when they were exposed upto 80^0C temperature, however when they were exposed above than 80^0c temperature there was significant loss in their fungitoxic potential (Table 1). When the extract of *Curcuma malabarica* were autoclaved, the extracts showed reduction in fungitoxic potential (Table 2). When the extract was stored for different periods of time, the extract retained their fungitoxic potential upto 120 days (Table 3).

When the extract was subjected to increased inoculum density by increasing the number of disc, there was no effect on fungitoxicity of extract of *Curcuma malabarica* (Table 4). On increasing the diameter of inoculums disc there was no effect on fungitoxicity of plants extract (Table 5). On the basis of above results it was clear that the plant extract of a *Curcuma malabarica* retained their fungitoxicity even under extreme condition of physical factors. Therefore the plant extract of *Curcuma malabarica* can be used as alternative of chemical fungicides.

Table 1. Effect of temperature on antifungal activity of extract

Name of Plant	Temp. ^{0}c	% inhibition of mycelial growth	
		Control	Treatment
Curcuma malabarica	40	0	100
	60	0	100
	80	0	100
	100	0	85
	120	0	20

Table 2. Effect of Autoclaving on antifungal activity of extract

Name of Plant	% inhibition of mycelial growth of *Rhizoctonia solani* Kuhn.
Curcuma malabarica	68.29

Table 3. Effect of storage on Fungitoxicity of extract

% inhibition of mycelial growth of *Rhizoctonia solani* Kuhn.	
Storage Period	***Curcuma malabarica***
20	100
40	100
60	100
80	100
100	100
120	100

Table 4. Effect of increased inoculums on Fungitoxicity of plant extract (By increasing the number of discs)		
No. of disc inoculated	***Curcuma malabarica***	
(5mm diameters)	Control	Treatment
2	+	-
4	+	-
6	+	-
8	+	-
10	+	-
12	+	-
14	+	-

Table 5. Effect of increased inoculums on Fungitoxicity of plant extract (By increasing the diameter of discs)		
No. of disc inoculated	***Curcuma malabarica***	
(diameter in mm)	Control	Treatment
5	+	-
6	+	-
7	+	-
8	+	-
9	+	-
10	+	-
12	+	-

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