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In vitro antifungal activity of plant extracts (Sterilized and unsterilized) against *Macrophomina phaseolina* (Tassi) Goid. cause stem canker of pigeonpea [*Cajanus cajan* (L.) Millsp.]

■ Mulji Jehani **and** D.M. Pathak*

Department of Plant Pathology, College of Agriculture, Navsari Agricultural University,Campus Bharuch, **Maktampur (Gujarat) India**

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***Corresponding author:**
Email : dmpathak@nau.in

ABSTRACT

The present paper reports the antifungal activity of plant extracts (5 % Concentration) from seven plant species *viz*., *Bougainvillea spectabilis, Zingiber officinali, Datura stramonium, Curcuma longa, Ocimum sanctum, Hibiscus bombycideron, Tegetes erecta*. Antifungal activity was tested against *Macrophomina phaseolina*. All plant extracts (sterilized and unsterilized) exhibited considerable distinction in radial mycelial growth of tested pathogen. Overall, *Curcuma longa* appeared significantly the most effective and suppressed the radial mycelial growth (sterilized plant extract, 47.13% and unsterilized plant extract, 48.28 %) of the *Macrophomina phaseolina*. However, *Ocimum sanctum* exhibited minimum inhibition (sterilized plant extract, 25.29 % and unsterilized plant extract, 22.99 %) against *Macrophomina phaseolina*. It may be concluded from the present investigation that *Curcuma longa* can be utilized for the management of *Macrophomina phaseolina.*

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INTRODUCTION

Biological screening of plant extracts is carried out throughout the world for the determination of their antifungal activity. Synthetic chemicals used to control plant diseases not only pollute the environment, but are also harmful to human health. Because of environmental and economic considerations, plant scientists are involved to find the cheaper and more environmental friendly bio-compounds for the control of plant diseases using diffusates from different plants (Gerretsen and Haagsma, 1951; Kumar *et al*., 1979 and Naidu and John, 1981). Many studies have shown that plants are sources of diverse nutrient and non nutrient molecules, many of which showed antioxidant and antimicrobial properties

which can protect the human body against both cellular oxidation reactions and pathogens. Thus it is important to characterize different types of plants for their antimicrobial potential (Mothana and Lindequist, 2005; Bajpai *et al*., 2005 and Wojdylo *et al*., 2007). The trend towards the environmental friendly pesticides with alarming levels of pestresistance to commonly used pesticides has led to search new antimicrobial agents from various sources including extracts of plants. The major characteristics of such biopesticides are that they should have minimal toxic effects to human and other organisms, rapid degradation and often a narrow spectrum of the activity (Loper *et al*., 1991). The stem canker is caused by *Macrophomina phaseolina* Ashby is one of most serious and oldest known disease (Nene *et al.*, 1984). *Macrophomina phaseolina* causing stem canker of pigeonpeais one of most serious and oldest known disease (Nene *et al*., 1984). It is soil inhabiting fungi, an important root pathogen and causes dry root rot / stem canker, stalk rot orcharcoal rot of over 400 plant species (Mahrshi, 1986). It has a wide host range and is responsible for causing losses of more than 500 cultivated and wild plant species (Indera *et al*., 1986). Wide host range of *M. phaseolina* suggested that it is non-host specific fungus. The most common method for controlling the pathogen is the use of fungicides but the development of resistance in pathogenic fungi to common fungicides and increasing residual hazardous effects on human health and environmental pollution has given a thrust to search for new plant derivatives that can obstruct the fungal pathogenicity. Use of natural products for the management of fungal diseases in the plants is considered as a good alternate to synthetic fungicides, due to their less negative impact on the environment. Plant extracts are not only easy to prepare but are also non-polluting and low priced as compare to commercial fungicides. The objective of present study was to determine the *in vitro* antifungal activity of plant extracts against *Macrophomina phaseolina*.

MATERIAL AND METHODS

This work was conducted in Department of Plant Pathology, N.M. College of Agriculture, Navsari agricultural University. Navsari during 2014 to determine the antifungal activity of *Bougainvillea spectabilis, Zingiber officinali, Datura stramonium, Curcuma longa, Ocimum sanctum, Hibiscus bombycideron* and *Tegetes erecta* against fungal *Macrophomina phasiolina* by using food poisoning technique (Naz *et al*., 2006).

Isolation of pathogens:

Pigeonpea plants (GT-1) showing the typical stem canker symptoms were collected from N.A.R.P. Research Station, NAU, Bharuch as well as from the farmers' field and brought to the laboratory and subjected to tissue isolation. After 48 hrs of incubation the isolated fungus initially started to grow as dirty white mycelial growth, then turn to fluffy, blackish mycelial growth on sterilized potato dextrose agar (PDA) medium (potato starch: 20 g, dextrose: 20 g, agar: 20 g and distilled water to make the volume 1 litre. After eight 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. After purification of the pathogen as described cultural and morphological characters of the fungus on PDA, were studied for identification and compared with those described in the literature. The pure culture was also sent to Indian Type Culture Collection (I.T.C.C), Division of Plant Pathology, I.A.R.I., New Delhi-110 012 and was identified as *Macrophomina phaseolina* (Tassi.) Goid (I.T.C.C. No. 9572.14). The studies on the cultural and morphological characters of isolated *Macrophomina* sp. showed its close identity with *Macrophomina phaseolina* (Tassi.) Goid as described by Nakrani (1991) and Agrawal (1993) were also similar with our present finding. Thus, the *M. phaseolina* causing pigeonpea stem canker.

Preparation of extracts:

Sterilized plant extracts:

Healthy fresh plant parts *i.e.*, leaves, bulbs or rhizomes were washed thoroughly with fresh water and finally rinsed with sterilized distilled water. Fifty gram of plant parts were cut into small pieces and minced with the help of a grinder by adding 50 ml sterilized distilled water. The phyto-extracts were filtered through double layered muslin cloth in 150 ml conical flasks and plugged with non-absorbent cotton. These filtered extracts were autoclaved at 15 lbs pressure for 20 minutes. Autoclaved extract were individually added into previously sterilized

PDA plates @ 10 per cent and mixed thoroughly at the time of pouring in the previously sterilized Petri plates. The Petri plates were inoculated aseptically after solidification by placing 5 mm diameter mycelial disc at the center, cut aseptically with cork borer from 10 days old pure culture of *M. phaseolina*. Three replications of each treatment were maintained. The plate without plant extracts served as control. The Petri plates were incubated at 27 ± 2^0C temperature till the complete coverage in control plate.

Unsterilized plant extracts:

Healthy fresh plant parts *i.e.*, leaves, bulbs or rhizomes were washed thoroughly with fresh water. Fifty gram of plant parts were cut into small pieces and minced with the help of a grinder by adding 50 ml water. The plant extracts were filtered through double layered muslin cloth in 150 ml conical flasks and plugged with non-absorbent cotton. These unsterilized filtered extracts were individually added into PDA plates @ 10 per cent and mixed thoroughly at the time of pouring in the Petri plates. The Petri plates were inoculated aseptically after solidification by placing 5 mm diameter mycelial disc at the center, cut aseptically with cork borer from 10 days old pure culture of *M. phaseolina*. Three replications of each treatment were maintained. The plate without plant extracts served as control. The Petri plates were incubated at 27 ± 2^0 C.

Observations on colony diameter were recorded up to the complete coverage of control plates, which were inoculated with only pathogen. Radial growth of the pathogen was recorded and per cent growth inhibition was calculated by following formula (Vincent, 1947):

$$\text{Growth inhibition (\%)} = \frac{C - T}{C} \times 100$$

where,

C = Growth of pathogen in control after incubation

T = Growth of pathogen in treatment after incubation.

RESULTS AND DISCUSSION

The aqueous extracts of commonly available seven plant species were evaluated *in vitro* sterilized as well as un-sterilized solution for their inhibitory effect on the mycelial growth and sclerotial formation by *M. phaseolina*. The results presented in Table 1 and depicted graphically in Fig. 1 sterilized and in Fig. 2 un-sterilized revealed that all the plant extracts inhibited the growth of the fungus as compared to control. The turmeric extract (*Curcuma longa* L., 46mm) allowed minimum growth of the pathogen followed by marigold (*Tegetes erecta* L., 49mm), ginger (*Zingiber officinalis* Rosa., 53mm), Jasud (*Hibiscus bombycideron* L., 59mm), Bougainvillea (*Bougainvellea spectabilis* L., 60mm), Dhatura (*Dhatura stramonium* L., 65mm) and *Tulsi* (*Ocimum sanctum* L., 65mm) in sterilized plant extracts. Whereas, turmeric extract (*Curcuma longa* L., 45mm), allowed minimum growth of the pathogen followed by marigold (*Tegetes erecta* L., 50mm), ginger (*Zingiber officinalis* Rosa., 55mm), Jasud (*Hibiscus bombycideron* L., 57mm), Bougainvillea (*Bougainvellea spectabilis* L., 59mm), Dhatura (*Dhatura stramonium* L., 65mm) and *Tulsi* (*Ocimum sanctum* L., 67mm) in un-sterilized plant extracts.

Table 1 : Efficacy of different plant extracts (botanicals) against *Macrophomina phaseolina in vitro*

Sr. No.	Name of plant	Botanical name	Sterilized extracts solution		Un-sterilized extracts solution	
			Average Colony diameter of pathogen (mm)	Growth inhibition over control (%)	Average Colony diameter of pathogen (mm)	Growth inhibition over control (%)
1.	Bougainvillea	*Bougainvillea spectabilis* L.	60.00	31.03	59.00	32.18
2.	Ginger	*Zingiber officinalis* Rosa.	53.00	39.08	55.00	36.78
3.	Datura	*Datura stramonium* L.	65.00	25.29	65.00	25.29
4.	Turmeric	*Curcuma longa* L.	46.00	47.13	45.00	48.28
5.	*Tulsi*	*Ocimum sanctum* L.	65.00	25.29	67.00	22.99
6.	Jasud	*Hibiscus bombycideron*	59.00	32.18	57.00	34.48
7.	Marigold	*Tegetes erecta*	49.00	43.68	50.00	42.53
8.	Control	---	87.00	---	87.00	--
	S.E.±		0.62		1.29	
	C.D. (P=0.05)		1.85		3.86	
	C.V. %		3.28		6.73	

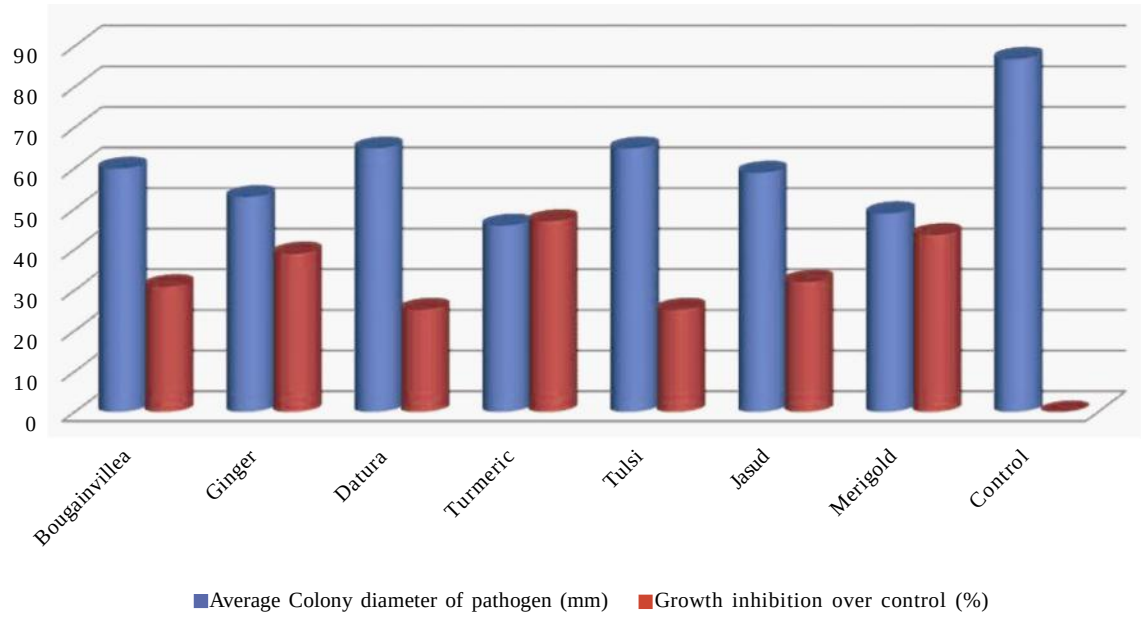

Fig. 1: Efficacy of different plant extracts (botanicals) against *Macrophomina phaseolina in vitro* (Sterilized)

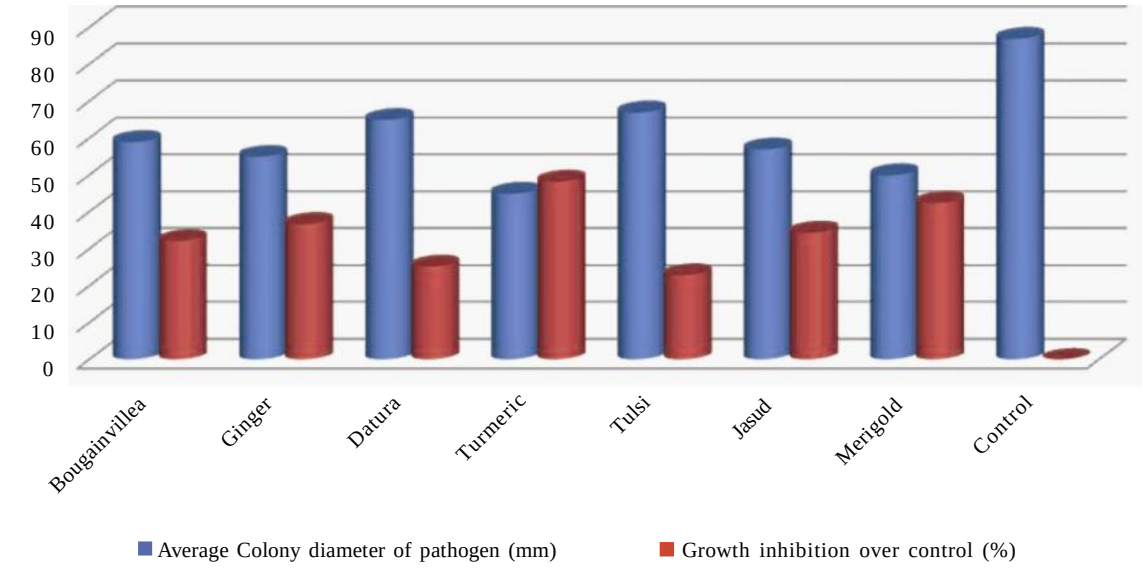

Fig. 2 : Efficacy of different plant extracts (botanicals) against *Macrophomina phaseolinain vitro* (Un-sterilized)

The turmeric rhizomes extract (*Curcuma longa* L., 47.13%) showed maximum growth inhibition of the pathogen followed by marigold leaves (*Tegetes erecta* L., 43.68%), ginger rhizomes (*Zingiber officinalis* Rosa., 39.08%), jasud leaves (*Hibiscus bombycideron* L., 32.18%), bougainvillea leaves (*Bougainvellea spectabilis* L., 31.03%), dhatura leaves (*Dhatura stramonium* L., 25.29%) and tulsi leaves (*Ocimum sanctum* L., 25.29%) in sterilized plant extracts. Whereas, turmeric rhizomes extract (*Curcuma longa* L., 48.28%) showed maximum growth inhibition of the pathogen followed by marigold leaves (*Tegetes erecta* L., 42.53%), ginger rhizomes (*Zingiber officinalis* Rosa., 36.78%), jasud leaves (*Hibiscus bombycideron* L.,

34.48%), Bougainvillea leaves (*Bougainvellea spectabilis* L., 32.18%), dhatura leaves (*Dhatura stramonium* L., 25.29%) and tulsi leaves (*Ocimum sanctum* L., 22.99%) in un-sterilized plant extracts.

The present studies are in confirmation with those described by earlier workers Datar (1999) studied the effect of botanicals on *M. phaseolina* and reported that out of four rhizomes and bulbs extracts tested, ginger (*Zingiber officinalis* Rosa.) extract was found most inhibitory to *R. bataticola*. Dubey and Dwivedi (1991) reported fungitoxic properties of *Acacia arabica* L., *Allium cepa* L. and *A. sativum* against vegetative growth and sclerotial viability of *M. phaseolina*.

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