Effect of botanicals against dieback and gummosis (*Botryodiplodia theobromae* Pat) of mango

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ABSTRACT

Dieback and gummosis incited by *Botryodiplodia theobromae* Pat has become a major constraint in cultivation of mango in India inflicting significant yield losses. A study was undertaken to evaluate the efficacy of nine plant extracts viz Ghrit Kumari (*Aloe vera*) fleshy leaves, lemongrass (*Cymbopogon citrates*) leaves, lantana (*Lantana camara*) leaves, eucalyptus (*Eucalyptus hybrida*) leaves, mint (*Mentha piperata*) leaves, parthenium (*Parthenium hysterophorus*) leaves, garlic (*Allium sativum*) cloves, onion (*Allium cepa*) bulbs and hemp (*Cannabis sativa*) leaves having anti-microbial properties. The plants were collected and evaluated in vitro against *B theobromae* using poisoned food technique. Among these all *Cannabis* sp suppressed the mycelial growth and showed the minimum mean diametric growth of the fungus with 71.55 mm. It was followed by garlic with 74.33 mm of diametric growth. Hence study opened further opportunities for advanced investigations of botanicals against the dieback and gummosis of mango.

Keywords: Mango; dieback; gummosis; botanicals; *Botryodiplodia theobromae*

INTRODUCTION

Mango (*Mangifera indica* L) is one of the most flavoursome and delectable fruits grown on Indian demesne. It has deep roots established into Indian soil way back to 25 to 30 million years where it first appeared in northeast India, Myanmar and Bangladesh and later transcended down to south India. An area of 2.29 million hectares and a production of 20.8 million tonnes has been recorded in India (Anon 2020).

Mango suffers from several infectious diseases caused by many phytopathogens. Among these the dieback and gummosis caused by *Diplodia natalensis* and *Lasiodiplodia theobromae* (Pat) Griff and Maubl (syn *Botryodiplodia theobromae*) is a serious disease. Studies have shown that the most common and famous varieties of mango are highly susceptible to dieback disease caused by *B theobromae*. The dieback is a serious disease of mango causing considerable damage to tree health, fruit yield and even post-harvest losses. *Botryodiplodia theobromae* is a dothidiomycetous fungus having *Botryosphaeria rhodina* as its teleomorph. It causes stem end rotting in mango along with oozing gummy exudations on the wounded bark and cut portions of the tree. The fungus survives as pycnidia on the bark of the diseased wood. These pycnidia form two-celled conidia which have a peculiar characteristic of striations on the surface. These conidia are dissipated with rain splashes and wind on to the freshly cut and wounded areas of the wood. Thakur (2017) reported that the conidia of the fungus germinate on the bark obstructing the vascular pathways of the plant which results in canker formation, necrosis and eventually dieback of the infected portion of the tree. However this disease can be controlled using different fungicides as well as plant extracts that have antifungal properties and characteristics. In the present study investigations were carried out to evaluate the plant extracts for effective management of dieback and gummosis in mango to obtain optimum production and better economic returns.

MATERIAL and METHODS

The present investigations were conducted during March-October 2019 in the Department of Plant Pathology, Dr YS Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh. The detailed methodology is described below:

Isolation of *B theobromae*

The pathogen was isolated from infected twigs and gummy exudates collected from mango trees grown in Himachal Pradesh. Repeated isolations were carried out from the infected twigs at the junction of the diseased and healthy portion. The initial growth observed was picked up aseptically with the help of a sterilized inoculating needle purified by hyphal tip method (Hansen 1926), transferred to PDA slants, and incubated in BOD at temperature 25 ± 1°C for mycelial growth and used for further investigations. The pure culture of the microorganism was examined under the microscope and identified based on morphological characters through documented instandard authentic description (Shah et al 2010). The culture was granted accession number MN453809 by NCBI.

Collection and preparation of plant extracts

Healthy plant parts (500 g each) of different plants viz Ghrit Kumari (*Aloe vera*) fleshy leaves, lemongrass (*Cymbopogon citrates*) leaves, lantana (*Lantana camara*) leaves, eucalyptus (*Eucalyptus hybrida*) leaves, mint (*Mentha piperata*) leaves, parthenium (*Parthenium hysterophorus*) leaves, garlic (*Allium sativum*) cloves, onion (*Allium cepa*) bulbs and hemp (*Cannabis sativa*) leaves having anti-microbial properties were collected and evaluated in vitro against *B theobromae* using poisoned food technique (Falck 1907). The plant parts were washed with distilled water, air-dried and crushed in 500 ml of sterilized distilled water. The crushed product was filtered through a muslin cloth to collect the filtrate. Each solution of the botanicals was considered as 100 per cent which was further diluted to the required concentrations of 10, 20 and 50 per cent. The filtrates were sterilized in an autoclave by tyndallization at 5 psi pressure for 30 minutes for three successive days and kept in the refrigerator for further use.

A double-strength potato dextrose agar medium was prepared in distilled water and sterilized at 121°C and 15 psi pressure for 20 minutes. The required quantities of plant extracts were added into sterilized PDA medium to give required concentration and poured separately into each sterilized Petri plate under aseptic conditions. These were allowed to solidify and then inoculated with 3 mm mycelial discs from seven days old culture of the fungus and incubated at 25 ± 1°C. Simultaneously a control treatment was also maintained without adding plant extracts by growing the fungus only on PDA medium. Each treatment was replicated thrice. The observations on diametric mycelial growth of the fungus were recorded after every 24 hours of inoculation till the control plates were fully covered with the mycelium.

The per cent inhibition of mycelial growth was calculated by the formula given by Vincent (1947):

$$I = \frac{C - T}{C} \times 100$$

where I= Per cent inhibition over control, C= Mycelial growth of the test pathogen in control (mm), T= Mycelial growth of the test pathogen in treatment (mm)

RESULTS and DISCUSSION

The results revealed that among the nine plant extracts screened for their inhibitory effect on the mycelial growth of *B theobromae* at different concentrations, all doses of *Cannabis* sp suppressed the mycelial growth and showed the minimum mean diametric growth of the fungus with 71.55 mm. It was followed by garlic with 74.33 mm of diametric growth. Eucalyptus and *Aloe vera* showed a mean diametric growth of 86.00 and 85.56 mm respectively and were statistically at par with each other which was closely followed by lemongrass with 87.33 mm of mean diametric growth. Maximum mean diametric growth was recorded in cultures mediated with onion bulb extract, lantana leaf extract, mint leaf extract and parthenium leaf extract with 88.89, 88.78, 88.44 and 88.44 mm respectively thus failing to inhibit the mycelial growth of the pathogen remarkably. Similarly highest inhibition of the fungus was recorded in *Cannabis* leaf extract with 20.49 per cent inhibition followed by garlic clove extract with 17.40 per cent. Eucalyptus, *Aloe vera* and lemongrass were found to be comparatively less effective with 4.93, 4.44 and 2.96 per cent inhibition respectively. Ahmed and Sultana (1984) reported that garlic bulb extract inhibited the germination of spores and growth of the mycelia of *B theobromae*. Suresh et al (2016) and Bui et al (2018) showed garlic as the best phyto-extract against mycelial growth of *B*

Table 1. In vitro effect of plant extracts on *B theobromae*

Botanical	Diametric growth (mm) under concentration				Per cent growth inhibition under concentration			
	10%	20%	50%	Mean	10%	20%	50%	Mean
Lantana	88.33	88.66	88.33	88.78^{e}	0.74 (4.03)	1.48 (6.88)	1.85 (7.72)	1.35^{d} (6.21)
Mint	89.00	88.33	88.00	88.44^{e}	1.11 (4.87)	1.85 (6.35)	2.22 (8.37)	1.72^{d} (6.53)
Onion	89.00	88.66	89.00	88.89^{e}	1.11 (4.87)	1.48 (6.88)	1.11 (4.87)	1.23^{d} (5.54)
Garlic	89.00	89.00	45.00	74.33^{b}	1.11 (4.87)	1.11 (4.87)	50.00 (44.98)	17.40^{b} (18.24)
Cannabis	88.00	71.66	55.00	71.55^{a}	2.22 (8.37)	20.37 (26.8)	38.89 (38.56)	20.49^{a} (24.58)
Parthenium	89.00	89.00	87.33	88.44^{e}	1.11 (4.87)	1.11 (4.8)	2.96 (9.86)	1.72^{d} (6.53)
Aloe vera	88.33	84.33	85.33	86.00^{c}	1.85 (7.72)	6.29 (14.37)	5.18 (13.09)	4.44^{c} (11.73)
Eucalyptus	87.33	86.00	83.33	85.56^{c}	2.96 (9.86)	4.43 (12.1)	7.41 (15.78)	4.93^{c} (12.58)
Lemongrass	88.33	87.66	86.00	87.33^{d}	1.85 (7.72)	2.59 (9.02)	4.43 (12.1)	2.96^{c} (9.61)
Mean	88.59	85.93	78.59		1.56 (6.35)	4.53 (10.24)	12.67 (17.26)	

Figures represented by different alphabets differ significantly; Angular transformed values in parentheses

	$CD_{0.05}$	
	Diametric growth	Growth inhibition
Botanicals	0.95	(2.73)
Concentration	0.55	(1.58)
Botanicals × Concentration	1.67	(4.75)

theobromae. Lantana, mint, onion and parthenium showed minimum growth inhibition as 1.35, 1.72, 1.23 and 1.72 per cent respectively. Irrespective of the different botanicals evaluated, the significant inhibitory effect was noticed with increasing dose levels from 10 to 50 per cent. Sabalpara (1983) studied 21 plant botanical extracts against mango dieback and found that only *Eucalyptus* sp caused significant inhibition of *B theobromae* mycelial growth and inhibition. Lantana, mint, onion and parthenium showed minimum growth inhibition of 1.35, 1.72, 1.23 and 1.72 per cent respectively.

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