

**Current Journal of Applied Science and Technology** 

23(3): 1-6, 2017; Article no.CJAST.35899 Previously known as British Journal of Applied Science & Technology ISSN: 2231-0843, NLM ID: 101664541

## Evaluation of Fungicides and Botanicals against Mango (*Mangifera indica*) Anthracnose

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#### Authors' contributions

This work was carried out in collaboration between all authors. Author PK designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors RS and RP managed the analyses of the study. All authors read and approved the final manuscript.

#### Article Information

DOI: 10.9734/CJAST/2017/35899 <u>Editor(s):</u> (1) Ndongo Din, Department of Botany, Faculty of Science, University of Douala, Cameroon. <u>Reviewers:</u> (1) Carlos Alberto Oliveira de Matos, São Paulo State University (Unesp), Campus of Itapeva, Brazil. (2) Godwin Michael Ubi, University of Calabar, Calabar – Nigeria. Complete Peer review History: <u>http://www.sciencedomain.org/review-history/20803</u>

**Original Research Article** 

Received 1<sup>st</sup> August 2017 Accepted 28<sup>th</sup> August 2017 Published 4<sup>th</sup> September 2017

### ABSTRACT

Mango anthracnose disease caused severe damage to mango yield so its control is required. For this purpose the current investigation was carried out under *in vitro* and under field conditions during, 2016 at CCS, HAU, Hisar. The experiment was carried out through poison food technique under *in vitro* and through foliar spray under field conditions. Five fungicides along with three botanicals were evaluated *in vitro* against *Colletotrichum gloeosporioides*, the causal agent of anthracnose disease of mango. Carbendazim completely inhibited mycelial growth up to 100 per cent. Copper oxychloride was found least effective among all five fungicides. Among botanicals eucalyptus leaf extract was found most effective in inhibition of fungal growth up to 58.5 and 70.4 per cent at 5 and 10 per cent concentration, respectively. Neem leaf extract at 10 per cent concentration inhibited mycelial growth up to 57.0 percent. The best performing *in vitro* two fungicides and two botanicals when tested under field condition, carbendazim (0.1%) twice at 15 and 30 DAI (days after initiation) of disease was found most effective in controlling the disease up to 71.43 per cent and 65.22 per cent in Langra and Dashehari cultivars, respectively.

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#### 1. INTRODUCTION

Mango (Mangifera indica L.) is one of the world's most important fruit of the tropical and subtropical countries. It is cultivated extensively as a commercial fruit crop in India. China, Indonesia, Thailand and Mexico, India is the world's largest producer of mango followed by China and Thailand. In India, it is cultivated in an area over 2,163,470 hectares with a production of 1,852,980 metric tonnes of fruit. However, in Haryana, mango is cultivated in area over 9,288 hectares with a production of 8,872 metric tones [1]. Various biotic and abiotic stresses cause immense loss to mango crop throughout the world and destructive disease of mango are those caused by fungi, bacteria, viruses and phytoplasma. Among biotic stresses, mango anthracnose is the most serious fungal disease that causes maximum damage in mango. Singh et al. [2] reported that fungicides viz., Bavistin (carbendazim), Contaf (hexaconazole) and Score (difenconazole) had completely inhibited the growth of pathogen at 100 µg/ml concentrations tested in vitro, while copper oxychloride was least effective as it did not cause substantial reduction in growth of pathogen. Sharma and Verma [3] reported that the systemic carbendazim completely (100%) fungicide inhibited mycelia growth of C. gloeosporioides at all the concentrations in vitro conditions. Kolase et al. [4] reported that the systemic fungicides, carbendazim was found to be most effective, while non-systemic fungicide, mancozeb showed the best inhibition of C. gloeosporioides, causing anthracnose of mango. Neem leaf extract was found effective in mycelial growth inhibition (35.21%) at 5 per cent concentration [4]. In several other studies neem, garlic, eucalyptus and akk extract were used for the management of C. gloeosporioides causal agent of mango anthracnose under in vitro and in vivo conditions. Eucalvptus also showed the highest mycelial growth inhibition at all concentrations among all the selected plant extracts [5,6]. Since, the pathogen is very difficult to manage due to its long viability in infected plant parts and wide host range, therefore the present study was carried out with the objective to evaluation of fungicides and botanicals for the control of mango anthracnose under in vitro and field conditions.

#### 2. MATERIALS AND METHODS

#### 2.1 In vivo Evaluation of Fungicides

The efficacy of five fungicides viz., carbendazim, propiconazole, copper oxychloride, captan and mancozeb on the growth of C. gloeosporioides was tested in vitro using the standard procedure of poison food technique as given by Mayer [7]. Stock solution of each fungicide was prepared in double strength i.e. 100, 250, 500 and 1000 ppm by dissolving weighed or measured quantity of funaicide in а measured volume of sterilized water. The double strength potato dextrose agar medium was also prepared and sterilized at 15 lbs pressure for 20 minutes. An equal volume of each test chemical solution and PDA was mixed in a sterilized conical flask and poured aseptically in the Petri plates. After solidification of medium, each Petri plate was centrally inoculated with 5 mm disc of fungus taken from 10 days old culture of C. gloeosporioides with the help of sterilized cork borer and incubated at  $25\pm1$  °C. Suitable controls were maintained for each chemical. Four replications of each fungicide were maintained and completely randomized design (CRD) was experimental lay out. Colony diameter of the fungus of each treatment along with control was recorded with metric scale (mm) till the fungus of controlled treatment occupied the full area of Petri plate within which it was growing. The per cent inhibition of mycelial growth over control was calculated by following formula given by Vincent [8].

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

Where,

- C= Radial growth of *C. gloeosporioides* mycelium in control.
- T= Radial growth of *C. gloeosporioides* mycelium in treatment.

#### 2.2 In vitro Evaluation of Botanicals

The efficacy of three plants extracts *viz.* neem leaf extract, neem seed kernel extract and eucalyptus leaf extract on the growth of *C. gloeosporioides* were tested *in vitro* (Table 1) using the standard procedure of poison food technique as given by Mayer [7].

Name of plants	Scientific name	Family	Plant part used
Eucalyptus	Eucalyptus camaldulensis	Myrtaceae	Leaves
Neem	Azadirachta indica	Meliaceae	Leaves
			Seed kernel

Table 1. Different plant extract used for botanical assay

Fresh leaves of neem and eucalyptus, and seed kernel of neem were collected and washed thoroughly with distilled water. The respective plant parts were grinded in appropriate volume of water separately in a grinder (1:1 w/v). The grinded material was passed through double fold muslin cloth and the filtrate thus obtained was filtered through Whatman filter paper no. 1. The aqueous extracts collected were subjected to filter sterilization. Stock solution of each plant was further diluted to desired extract concentrations of 5% and 10%. The results were expressed in terms of per cent inhibition of mycelial growth over control.

# 2.3 *In vivo* Evaluation of Fungicides and Botanicals

field experiment conducted А was at of Department experimental orchard of Horticulture, CCS HAU, Hisar during, 2016 to test the efficacy of two fungicides and two botanicals which were found best under in vitro. Field experiment was conducted on cv. Dashehari and Langra cultivars by using randomized block design (RBD) with three replications. For this study, eighteen mango plants were selected then two best fungicides T<sub>1</sub>: 1<sup>st</sup> most effective fungicide-carbendazim (0.1%) and T<sub>2</sub>: propiconazole (0.05%) and two best botanicals ( $T_3$ : 1<sup>st</sup> most effective botanical-neem leaf extract (10%),  $T_4$ :2<sup>nd</sup> most effective botanical-eucalyptus leaf extract (10%) and  $T_5$ : alternate spray of  $T_1$  and  $T_3$ ) were sprayed twice at 15 days interval starting from initiation of disease in the March end. Mango leaves were sprayed with plain water and served as control. The disease severity was recorded on randomly selected 20 mango leaves/treatment/replication and disease severity was assessed in the July by using 0-5 scale *i.e.* 0 = no infection, 1 = 20%area infected, 2 = 40% area infected, 3 = 60%area infected, 4 = 80% area infected, 5 = 100%area infected. Per cent disease severity (PDS= Sum of all disease rating/total no. of plant examined  $\times$  highest grade  $\times$  100) was calculated by using formula given by Prabakar et al. [9] and per cent disease control was calculated by using formula of Das and Raj [10].

Per cent disease control =  $[{(\% \text{ Disease severity in control} - \% \text{ Disease severity in treatment})/\% \text{ Disease severity in control} x 100]$ 

## 3. RESULTS AND DICUSSION

### 3.1 In vitro Evaluation of Fungicides

Evaluation of five fungicides was tested in vitro conditions; carbendazim completely inhibited mycelial growth up to 100 per cent at 100 ppm concentration (Table 2), while propiconazole at 500 ppm concentration completely inhibited up to 100 per cent. Mancozeb provided complete inhibition up to 100 per cent at 1000 ppm concentration as compare to 24.5 per cent inhibition at 100 ppm concentration. Captan inhibited fungal growth up to 80.0 per cent at 1000 ppm concentration, while it inhibited 52.2 per cent at 100 ppm concentration. Copper oxychloride was found least effective among all five fungicides, as this fungicide inhibited 55.0 per cent of mycelial growth even at 1000 ppm concentration. Kolase et al. [4] also reported that systemic fungicide, carbendazim was found to be most effective in inhibition of mycelial growth of gloeosporioides. These results are in С. agreement with that of Sudhakar [11] and Prashanth [12]. The systemic fungicide carbendazim had completely inhibited mycelial growth up to 100 per cent at all the concentrations under in vitro conditions [3,13]. Singh et al. [2] reported that fungicides carbendazim, hexaconazole and difenconazole had completely inhibited the growth of pathogen at 100 µg/ml concentrations tested in vitro. Copper oxychloride was found least effective in reducing the growth of pathogen. Effectiveness of mancozeb at higher concentrations only was also observed even up to 1000 ppm in present studies.

#### 3.2 In vitro Evaluation of Botanicals

Efficacy of three plant extract was tested *in vitro*; eucalyptus leaf extract was found most effective in inhibition of fungal growth up to 58.5 and 70.4 per cent at 5 and 10 per cent concentration, respectively (Table 3).

Treatments	Per cent growth inhibition at different concentration (ppm)				Mean
	100*	250*	500*	1000*	-
Mancozeb 75%WP	24.5 (29.7)	42.3 (40.5)	56.4 (48.6)	100.0 (89.1)	55.8 (52.0)
Carbendazim 50%WP	100.0 (89.1)	100.0 (89.1)	100.0 (89.1)	100.0 (89.1)	100.0(89.1)
Copper oxychloride 50%WP	18.3 (25.3)	26.0 (30.6)	43.7 (41.3)	55.0 (47.8)	35.8 (36.2)
Propiconazole 25%EC	75.1 (60.0)	82.3 (65.0)	100.0 (89.1)	100.0 (89.1)	89.4 (75.8)
Captan 50%WP	50.3 (45.1)	65.6 (54.0)	73.3 (58.9)	79.2 (62.8)	67.1 (55.2)
Mean	53.6 (49.9)	63.2 (55.9)	74.7 (65.4)	86.8 (75.6)	
	Treatment Concentration		Treatment ×		
		Concentratio		on	
SEm±	0.3	0.3		0.7	
C.D. (p=0.05)	(0.9)	(0.8)		(1.9)	

Table 2. Evaluation	of different f	funaicides a	against C.	gloeosporioides in vitro

Note: SEm- Standard error of mean; C.D.- Critical difference

\*Mean of four replications

Figures in parenthesis indicate angular transformed values

#### Table 3. Evaluation of different botanicals against C. gloeosporioides in vitro

Treatments	Per cent	growth inhibition	Mean	
	5 (%)*	10 (%)*		
Eucalyptus leaf extract	58.5 (49.8)	70.4 (56.9)	64.5 (53.4)	
Neem leaf extract	45.9 (42.6)	57.0 (49.0)	51.5 (45.8)	
Neem seed kernel extract	42.2 (40.4)	53.3 (46.8)	47.8 (43.6)	
Mean	48.9 (44.3)	60.2 (50.9)		
	Treatment	Concentration	Treatment × Concentration	
SEm±	0.2	0.2	0.3	
C.D. (p=0.05)	(0.7)	(0.6)	NS	

Note: SEm- Standard error of mean; C.D.- Critical difference

\*Mean of three replications

Figures in parenthesis indicate angular transformed values

Neem leaf extract at 10 per cent concentration inhibited mycelial growth up to 57.0 per cent, while it was 45.9 per cent at 5 per cent concentration. Neem seed kernel extract was found the least effective among all three botanicals as it inhibited 53.3 per cent mycelial growth at 10 per cent concentration. Eucalyptus leaf extract at 5 and 10 per cent has showed the highest mycelial growth inhibition among all the selected plant extracts [5,6]. Effectiveness of eucalyptus leaf extract against C. gloeosporioides is supported by the finding of Prasanth [12]. Kolase et al. [4] also reported that neem leaf extract was found effective in mycelial growth inhibition (35.21%) at 5 per cent concentration. Neem seed kernel extract was found least effective in present studies among all three botanicals as it inhibited 53.3 per cent mycelial growth at 10 per cent concentration.

## 3.3 *In vivo* Evaluation of Fungicides and Botanicals

All the five treatment viz. carbendazim (0.1%), propiconazole (0.05%), eucalyptus leaf extract (10%), neem leaf extract (10%) and carbendazim (0.1%) + eucalyptus leaf extract (10%) tested under field conditions, significantly controlled mango anthracnose (Table 4). Foliar spray with carbendazim (0.1%) twice at 15 and 30 DAI (days after initiation) of disease was found most effective in controlling disease up to 71.43 per cent and 65.22 per cent in Langra and Dashehari Foliar spray with cultivars, respectively. carbendazim (0.1%) followed by eucalyptus leaf extract (10%) was found effective in controlling disease up to 61.90 per cent and 56.52 percent in in Langra and Dashehari cultivars, respectively. Results with foliar spray of

Treatments	Name of chemicals	cv. Langra		Cv. Dashehari	
		Per cent disease severity*	Control (%)	Per cent disease severity*	Control (%)
	Carbendazim @0.1%	10.00 (18.38)	71.43	13.33 (21.38)	65.22
$T_2$	Propiconazole @0.05%	13.89 (21.82)	60.31	17.78 (24.92)	53.63
$T_3^2$	Eucalyptus leaf extract @10%	18.33 (25.32)	47.62	22.78 (28.49)	40.58
$T_4$	Neem leaf extract @10%	23.33 (28.86)	33.33	28.33 (32.14)	26.09
T <sub>5</sub>	Carbendazim @0.1% followed by Eucalyptus leaf extract@10%	13.33 (21.38)	61.90	16.67 (24.06)	56.52
$T_6$	Control	35.00 (36.25)	-	38.33 (38.23)	-
C.D. (p=0.05)		(2.72)	-	(1.91)	-
SEm±		0.85	-	0.59	-
C.V. (%)		5.82	-	3.67	-

Table 4. Evaluation of different fungicides and botanicals against mango anthracnose in vivo

Note: SEm- Standard error of mean; C.D.- Critical difference; C.V. – Coefficient of variation \* Mean of three replications

Figures in parenthesis indicate angular transformed values

propiconazole (0.05%) at same DAI were found at par with the above treatment, as it controlled the disease up to 60.31 per cent and 53.63 per cent in Langra and Dashehari cultivars, respectively over untreated control. Eucalyptus leaf extract (10%) was also found effective in reducing the disease severity. Effectiveness of carbendazim (0.1%) against *C. gloeosporioides* is supported by the work of Raghuwanshi et al. [14] and Prashanth et al. [15]. Results are in agreement with that of Faiz et al. [6], where they reported that eucalyptus leaf extract showed significant disease severity reduction under natural field conditions.

#### 4. CONCLUSION

In conclusion, carbendazim was found to be the most effective *in vitro* and *in vivo*, for the control of *C. gloeosporioides* in mango. Among the botanical, eucalyptus leaf extract proved to have the highest antifungal potency in controlling the pathogen under *in vitro* and *in vivo* conditions. So, it can be used as an eco-friendly approach for the control of mango anthracnose disease.

#### **COMPETING INTERESTS**

Authors have declared that no competing interests exist.

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Peer-review history: The peer review history for this paper can be accessed here: http://sciencedomain.org/review-history/20803