Published with Open Access at Journal BiNET
Vol. 16, Issue 01: 1330-1336
Journal of Bioscience and Agriculture Research
Journal Home: www.journalbinet.com/jbar-journal.html

Cultural and morphological characterization of *Ceratocystis fambriata* causing mango sudden death disease in district Muzaffargarh, Punjab, Pakistan and its chemical control

Shanam Naz, Abdul Rehman, Muhammad Zeshan Ahmed, Luqman Amrao and Salman Ghuffar

Dept. of plant pathology, University of Agriculture Faisalabad, Pakistan

✉ For any information: ask.author@journalbinet.com

Article received: 17.11.17, Revised: 15.02.18 and First available online: 25 February 2018.

ABSTRACT

Mango (Mangifera indica L.) is an important fruit of Pakistan which is widely cultivated throughout the world. Fungal diseases of mango are the most important biological constraint to productivity. Out of which sudden death disease causes significant losses. The present investigation is carried out to characterize Ceratocystis fambriata causing mango sudden death disease and its management by application of different fungicides. During the survey 14 isolates of Ceratocystis fambriata causing sudden death disease was collected from Muzaffargarh district. The isolates were identified on the basis of cultural and microscopic features. After 6 days during cultural studies maximum growth was observed on Czepk and Malt extract medium (85 mm) and minimum growth was on PDA (74.1 mm). The colonies colors range from grayish, slightly white to light brown having smooth, circular and regular or irregular margins on the surface. Perithecia was black to brown, smooth, circular and regular or irregular, Aascospore was hat shaped ranging 4.18- 6.34 µmx 3.15 - 4.88 µm, Aleurioconidia ranging 17.1- 18.5 µmx 9.04-10.09 µm, Endoconidia ranging 22.5-24.2 µmx 3.90-5.30 µm. During pathogenicity test, result showed that infection started after fifteen days of inoculation. The pathogen covered 1.3 cm after fifteen days. Final reading was taken on the forty five day, when the pathogen colonized up to 3.25 cm. Among different fungicides the results after 2, 4 and 6 days revealed that Topsin- M was found most effective for controlling the growth of Pathogen that showed colony diameter 0.20, 0.50 and 1.3 cm at 200 ppm followed by Score, Nativo, Cabriotop and Copper oxychloride respectively.

Key Words: *Mango, Ceratocystis fambriata, Chemical control and Muzaffargarh*

Cite Article: Naz, S., Rehman, A., Ahmed, M. Z., Amrao, L. and Ghuffar, S. (2018). Cultural and morphological characterization of *Ceratocystis fambriata* causing mango sudden death disease in district Muzaffargarh, Punjab, Pakistan and its chemical control. Journal of Bioscience and Agriculture Research,16(01), 1330-1336. **Crossref:** https://doi.org/10.18801/jbar.160118.165

I. Introduction

Mangifera indica L. commonly known as Mango, is an important fruit crop mostly cultivated in regions with tropical and subtropical environment. It is favorite fruit in sub-continent, also called as the King

Published with open access at journalbinet.com
EISSN: 2312-7945, © 2018 The Authors, Research paper

of fruits (Purseglove, 1972). Among the entire world, Pakistan is sixth largest mango producer country (FAO, 2009). Mango is infected due to number of a biotic and biotic factors which are responsible for pre and post-harvest losses such as anthracnose, fruit rot, leaf blight and malformation etc. Among all, Mango sudden death disease is recently introduced which is destroying the fruit crop and economically damaging the Pakistan. Symptoms of this disease are reported as wilting, vascular (xylem and phloem tissues) discoloration and gum exudation (Talpur and Khuhro, 2003). Asif et al. (2011) examined that *Ceratocystis fambriata* is a well known vascular shrivel pathogen highly infected the mango trees. Its incidence is reported in Punjab, Pakistan is 10-28%. Fateh et al. (2006) isolated the pathogen from xylem and phloem tissues from declining plants of mango in Sindh, Pakistan. Morphological characteristics of pathogen are studied by (Ferrari and Pichenot, 1974) as colonies were initially brown then becoming black on PDA medium. Perithecia brown to black, necks almost 800-900 µm long with ostiolar hyphae. Ascospores elliptical 4-8 x 2-5 µm, colorless, non-septate, give hat shaped appearance. In Oman *ceratocystis fimbriata* was also confirmed as the primary causal organism of mango sudden death by pathogenicity test (Al-Adawi et al., 2006). Vijaya et al. (2007) conducted an experiment of three systemic and five non-systemic fungicides with two concentrations in vitro condition against the Ceratocystis spp. Two systemic fungicides carbendazim and propiconazole were show best result to control the growth of pathogen at both the concentrations (0.05 and 0.1%). Keeping in the view, the aim of the study is conducting survey of Mango sudden death disease caused by *ceratocystis fimbriata* from mango growing areas located in Muzaffargarh region of Punjab, Pakistan as well as morphological characterization by using different parameters, pathogenicity for conformation and finally their chemical control.

II. Materials and Methods

Survey was conducted in the four Tehsils of district Muzafargarh (Muzafargarh, Jatoi, KotAddu and Ali pur) of Punjab Province to collect the disease samples from declined mango trees having the typical symptoms of Mango sudden death disease. Samples were brought to Mycology lab of department of plant pathology, University of agriculture Faisalabad for morphological characterization of causal agent. Colonies were purified by using single spore method on different nutrient media such as Potato Dextrose Agar (PDA), Czapek Dox Agar (CDA), and Malt Extract Agar (MEA), for growth comparison. After 6 days the diameter of each colony was measured and identified on the basis of cultural and morphological characteristics by using taxonomic key (Ellis, 1971). After obtaining the pure pathogens, Pathogenicity test was conducted for the confirmation of pathogens. For this purposePathogenicity test was conducted on young mango seedling (24 month old) growing in 25 cm diameter pots containing a mixture of peat moss and loamy soil. The seedlings were inoculated with isolate of Ceratocystis spp. Two seedlings per treatment were inoculated on the bark of mango seedling after making I- shaped incision (10 mm long) made with sterile knife. Seedling inoculated with only CzapekDox Agar (CDA) medium was served as controls. Then inoculated stem portion were covered with moist cotton pads and wrapped with parafilm to maintain humidity for ten days. After removing the parafilm the inoculated seedlings were assessed weekly for symptoms development. To fulfill the Koch's postulates, the re-isolation was done from the artificially inoculated stem portion. Different fungicides like Topsin M, Copper oxychloride, Nativo, Cabriotop, and Score at different concentrations 50, 100, 150, and 200 ppm were used for in vitro management of *Ceratocystis fimbriata* by using poison food technique. Data was recorded after 2, 4, and 6 days and subjected to statistical analysis.

III. Results and Discussion

During survey 14 isolates were found causing sudden death disease on Mango as shown in Table 01. Most of the isolates were found to have similar cultural and morphological characteristics and identified as *Ceratocystis fimbriata* (Table 02 and Table 03). Different types of fungal colonies were isolated and sub-cultured on different media for purification purpose. After 2, 4 and 6 days, total number of isolates sporulated on all the three media but on Czepk-agar and PDA medium maximum (85 mm) percent of sporulation was recorded as compared to Malt extract minimum growth was recorded (72.6 mm) as shown in Table 03. The colonies were flat, raised with different colors ranging from grayish, slightly white to light brown having smooth, circular and regular or irregular margins on the surface. Perithecia was black to brown, smooth, circular and regular or irregular, Aascospore was

hat shaped ranging 4.18- 6.34µmx 3.15 -4.88 µm, Aleurioconidia ranging 17.1- 18.5 µmx 9.04-10.09 µm, Endoconidia ranging 22.5-24.2 µmx 3.90-5.30 µm. During Pathogenicity test, result showed that infection started after fifteen days of inoculation. The pathogen covered 1.3 cm after fifteen days. Final reading was taken on the forty five day, when the pathogen colonized up to 3.25 cm.Among different fungicides the results after 2, 4 and 6 days shown in figure 01, figure 02 and figure 03 revealed that Topsin- M was found most effective for controlling the growth of Pathogen that showed colony diameter 0.20, 0.50 and 1.3 cm at 200 ppm followed by Score, Nativo, Cabriotop and Copper oxychloride respectively.

Table 01. List of isolates collected from different regions of Muzafargarh district

S. No	Locations	Date(s) of sampling	No of Isolates	Names of Isolates
1	Muzafargarh	June , 2012	3	SDMCf1 SDMCf2 SDMCf3
2	KotAddu	June , 2012	4	SDKCf1 SDKCf2 SDKCf3 SDKCf4
3	Ali pur	July , 2012	5	SDACf1 SDACf2 SDACf3 SDACf4 SDACf5
4	Jatoi	July , 2012	2	SDKCf1 SDKCf2
Total	**4**		**14**	

Table 02. Cultural characteristics of *Ceratocystis fambriata*

S. No.	Isolates	Colony Color	Growth type	Average Colony Diameter in 6 days (mm)	Types of margins
1	SDMCf1	Grayish	Flat	82	Smooth, circular, regular
2	SDMCf2	Grayish	Flat	80	Smooth, circular, regular
3	SDMCf3	Light brown	Flat	72	Smooth, circular, irregular
4	SDKCf1	Slightly Whitish	Flat	76	Regular
5	SDKCf2	Greyish white	Flat	74	Smooth, regular
6	SDKCf3	Slightly white	Flat	80	Regular
7	SDKCf4	Greyish	Flat	85	Smooth, circular, regular
8	SDACf1	whitish	Flat	75	Smooth regular
9	SDACf2	Greyish	Flat	85	Smooth, circular, regular
10	SDACf3	Greyish	Flat	80	Smooth, circular, regular
11	SDACf4	Light brown	Raised	72	Smooth, circular, irregular
12	SDACf5	Greyish	Flat	82	Smooth, circular, regular
13	SDJCf1	Greyish	Flat	85	Smooth, circular, regular
14	SDJCf2	Light brown	Raised	72	Smooth, circular, irregular

Published with open access at journalbinet.com
EISSN: 2312-7945, © 2018 The Authors, Research paper

Table 03. Microscopic study of *Ceratocystis fambriata*

S. No.	Isolates	Aleurioconidia (µm) (L x B)	Endoconidia (µm) (Lx B)	Ascospore (µm) (L x B) (Hat shaped)	Perithecia (Black to brown)
1	SDMCf1	18.1 x 10.04	22.5 x 4.50	5.13 x 4.27	Smooth, circular, regular
2	SDMCf2	18.2 x 10.05	23.6 x 4.90	5.24 x 4.38	Smooth, circular, regular
3	SDMCf3	17.1 x 09.04	23.2 x 4.12	5.19 x 4.18	Smooth, circular, irregular
4	SDKCf1	18.5 x 10.09	23.8 x 4.70	4.24 x 3.38	Regular
5	SDKCf2	18.4 x 10.08	23.1 x 4.10	4.18 x 3.21	Smooth, regular
6	SDKCf3	18.1 x 10.04	23.6 x 4.90	5.28 x 4.29	Regular
7	SDKCf4	18.5 x 10.10	22.6 x 3.90	5.46 x 4.68	Smooth, circular, regular
8	SDACf1	18.7 x 10.14	24.2 x 5.10	5.13 x 4.27	Smooth regular
9	SDACf2	18.25 x 10.44	23.6 x 4.90	6.24 x 4.38	Smooth, circular, regular
10	SDACf3	17.31 x 10.02	22.6 x 3.90	4.26 x 3.15	Smooth, circular, regular
11	SDACf4	17.1 x 9.04	23.4 x 4.30	5.29 x 4.18	Smooth, circular, irregular
12	SDACf5	18.5 x 10.06	24.2 x 5.30	5.37 x 3.84	Smooth, circular, regular
13	SDJCf1	18.5 x 10.04	23.4 x 4.30	5.24 x 4.38	Smooth, circular, regular
14	SDJCf2	17.1 x 09.06	23.6 x 4.70	6.34 x 4.88	Smooth, circular, irregular

Table 04. Mean diameter (mm) of the colony of on *Ceratocystis fambriata* different cultural media

S. No	Day 2	Day 4	Day 6
	Czepk		
01	22.5	75.3	85.00
02	15.2	62.8	85.00
03	20.5	70.0	85.00
04	16.6	60.9	85.00
05	19.0	78.0	85.00
	MEA		
06	12.5	51.3	75.6
07	12.2	55.4	72.6
08	15.8	58.1	74.6
09	12.6	55.3	69.9
10	20.8	69.4	77.9
	PDA		
11	25.2	64.8	85.0
12	29.4	72.6	85.0
13	24.6	69.8	85.0
14	16.4	65.0	78.0
15	22.4	64.4	82.8

Table 05. Mean lesion length of inoculated plants

Sr. No.	Plant No.	11-6-2013	26-6-2013	11-7-2013
1	P1	1.3	1.025	1.325
2	P2	2.25	2.425	3.0
3	P3	2.625	2.625	3.175
4	P4	1.55	2.275	2.7
5	P5	2.275	1.7	2.175
6	P6	1.25	1.725	2.1
7	P7	0.55	1.025	1.575
8	P8	2	2.2	3.25
9	P09	1.175	1.375	1.7
10	P10	1.75	2.5	3.15
11	P11	0.6	1.625	2.4
12	P12	0.975	1.325	2.125
13	P13	1.725	1.775	2.725
14	P14	1.625	1.95	2.625
15	P15	1.15	2.175	2.925

Published with open access at journalbinet.com
EISSN: 2312-7945, © 2018 The Authors, Research paper

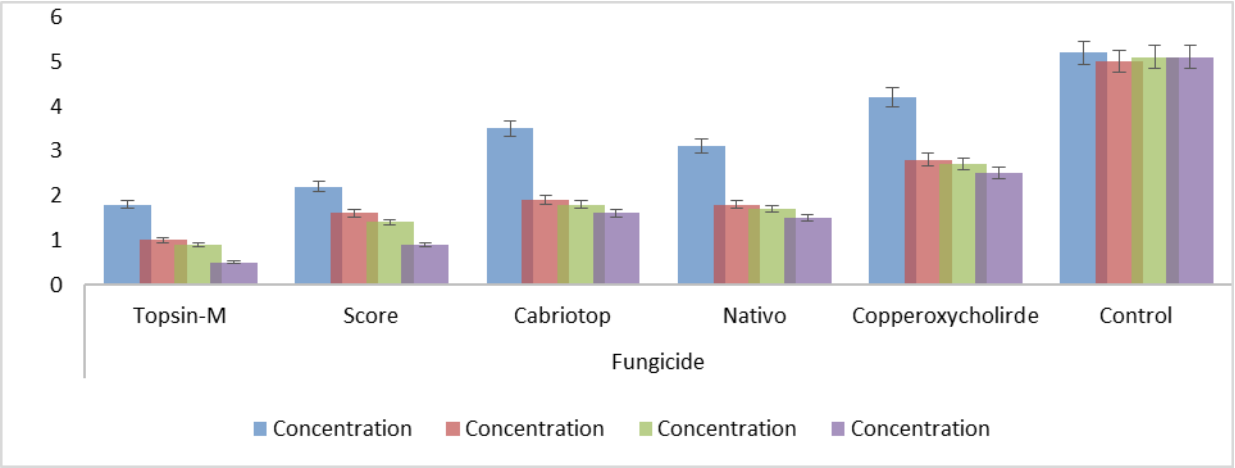

Figure 01. *In vitro* evaluation of fungicides after 2 days mycelial growth of *Ceratocystis fimbriata*

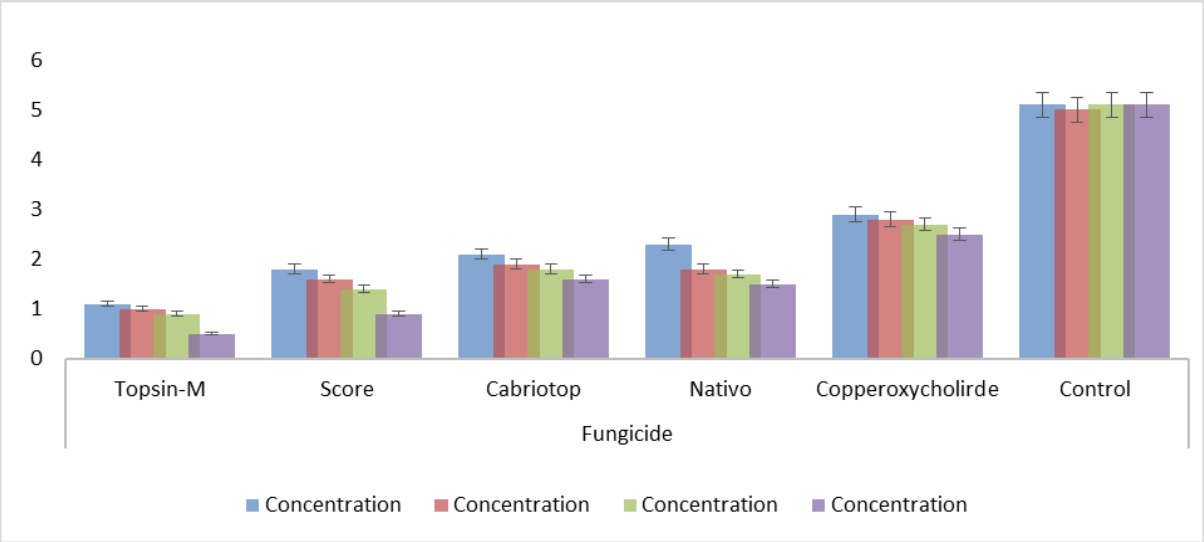

Figure 02. *In vitro* evaluation of fungicides after 4 days mycelial growth of *Ceratocystis fimbriata*

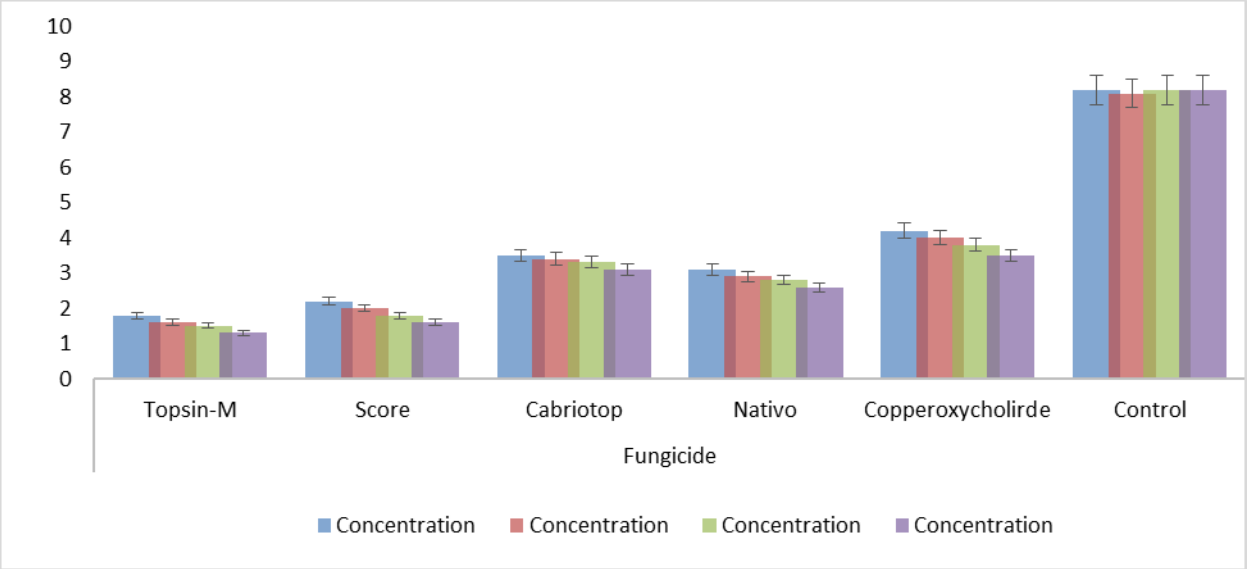

Figure 03. *In vitro* evaluation of fungicides after 6 days mycelial growth of *Ceratocystis fimbriata*

IV. Conclusion

Fourteeen isolates were collected causing sudden death disease of mango from different region of Muzaffargarh district. Isolates were identified as *Ceratocystis fambriata* on the basis of cultural and morphological and characterization. Pathogenicity test was conducted for confirmation of pathogen. Topsin-M was found most effective for controlling the growth of Pathogen that showed colony diameter 0.20, 0.50 and 1.3 cm at 200 ppm after 6 days.

V. References

[1]. Al-Adawi, A. O., Deadman, M. L., Al- Rawahi, A. K., Al-Maqbali, Y. M., Al-Jahwari, A. A., Al-Saadi, B. A., Al-Amri, I. S. and Wingsfield, M. J. (2006). Etiology and causal agents of mango sudden decline disease in the Sultanate of Oman. European J. Pl. Pathol. 116(4), 247- 254. https://doi.org/10.1007/s10658-006-9056-x

[2]. Asif, F., Faisal, S., Munawar, K. R.,Chrys, A. N.,Gulbhar, P. and Nazim, L. H. (2011). Trend of Mango Sudden Death syndrome (MSDS) in relation to fungal microflora and nematodes fauna in Punjab, Pakistan. Pak. J. Nematol. pp. 45-51.

[3]. Ellis, M. B. (1971). Dematiacioushyphomycetes. CABI, Kew, England. p. 608.

[4]. FAO Statistics. (2009). Statistics database on World Wide Web. http://apps.fao.org/page/colections?subset=agriculture

[5]. Fateh, F. S., Kazmi, M. R., Ahmad, I. and Ashraf, M. (2006). Ceratocystis fambriata isolated from vascular bundles of declining mango trees in Sindh, Pakistan. Pak. J. Bot. pp. 1257-1259.

[6]. Ferrari, J. P. and Pichenot, M. (1974). *Ceratocystis fimbriata* responsabled'une grave maladie du plataneen France: la tachechancreuse. Compte Rendu des Séances del'Académie des Sci. 2787-2789.

[7]. Purseglove, J. W. (1972). Mangoes West of Ind. Act Hort. pp. 107-174. https://doi.org/10.17660/ActaHortic.1972.24.30

[8]. Talpur, M. A. and Khuhro, R. D. (2003). Relatives population of mango hopper species on different mango varieties. J. Asia- Pascific Entomol. 6, 183-186. https://doi.org/10.1016/S1226-8615(08)60184-3

[9]. Vijaya, H. K., Kulkarni, S. and Hedge, Y. (2007). Chemical control of sett rots of Sugarcane caused by *Ceratocystis paradoxae*. Karnatka J. of Agri. Sci. pp. 62-64

HOW TO CITE THIS ARTICLE?

Crossref: https://doi.org/10.18801/jbar.160118.165

APA (American Psychological Association)

Naz, S., Rehman, A., Ahmed, M. Z., Amrao, L. and Ghuffar, S. (2018). Cultural and morphological characterization of *Ceratocystis fambriata* causing mango sudden death disease in district Muzaffargarh, Punjab, Pakistan and its chemical control. Journal of Bioscience and Agriculture Research, 16(01), 1330-1336.

MLA (Modern Language Association)

Naz, S., Rehman, A., Ahmed, M. Z., Amrao, L. and Ghuffar, S. "Cultural and morphological characterization of *Ceratocystis fambriata* causing mango sudden death disease in district Muzaffargarh, Punjab, Pakistan and its chemical control". Journal of Bioscience and Agriculture Research, 16.01(2018): 1330-1336.

Chicago and or Turabian

Naz, S., Rehman, A., Ahmed, M. Z., Amrao, L. and Ghuffar, S. "Cultural and morphological characterization of *Ceratocystis fambriata* causing mango sudden death disease in district Muzaffargarh, Punjab, Pakistan and its chemical control". Journal of Bioscience and Agriculture Research,16 no.01(2018):1330-1336.

Published with open access at journalbinet.com
EISSN: 2312-7945, © 2018 The Authors, Research paper