Greener Journal of Agricultural Sciences
Vol. 9(1), pp. 86-89, 2019
ISSN: 2276-7770

DOI Link: http://doi.org/10.15580/GJAS.2019.1.012119024
http://gjournals.org/GJAS

Isolation and Characterization of Mango Shoot Dieback Pathogen

Garuma Nemera Roge

Ethiopian Institute of Agricultural Research (EIAR)
Holetta Agricultural Research Center, P.O BOX 31

ARTICLE INFO

Article No.: *012119024*
Type**:** *Research*
DOI: *10.15580/GJAS.2019.1.012119024*

Submitted: *21/01/2019*
Accepted: *29/01/2019*
Published: *10/03/2019*

Corresponding Author
Garuma Nemera Roge
E-mail: *grmn2007@ gmail. com*
Phone: *+(251) 9-17-88-88-69*

Keywords: *Mango shoot dieback; Colletotrichum spp.; Pestalotiopsis spp.; Isolates*

ABSTRACT

Mango, Mangifera indica, is an economically important tropical fruit, which is produced in many countries around the world, and this plant is affected by more than 140 different plant pathogens during its life cycle. The study was conducted to isolate and characterize causal pathogen (S) of mango shoot dieback. Samples were taken from top, middle and bottom of diseased mango canopy. The samples were cultured using PDA. Two fungal pathogen species: Colletotrichum spp. and Pestalotiopsis spp. were isolated. The isolates were characterized based on their colony growth (cm), colony color, colony shape, and colony growth orientation after their further purification. The mean length of the diameter of mycelia growth for the three representatives of all isolates, i.e. top, middle and bottom showed the isolates from middle of the crown of the mango tree exhibited relatively highest mean length (5.88 cm) diameter whereas the mean length of the diameter for the isolates from top and bottom were 4.63 cm and 5.63 cm respectively. The isolates from top showed the least mean diameter length (4.63 cm). The diameter length of isolated from top was significant to the isolates from middle and bottom. The color of colony at front side of isolates from which Colletotrichum spp. was identified showed whitish color whereas the same isolates had blackish and yellowish color at their reverse side. Pastalotiopsis spp. showed whitish and yellowish colors at their front and reverse sides respectively. Circular and irregular colony shape, and spreading and upward colony growth habit (orientation) was observed on both identified fungal pathogens. The overall results showed that mango shoot dieback can be caused by more than two pathogens with different morphological characteristics.

INTRODUCTION

Mango (*Mangifera indica*) is an economically important tropical fruit, which is produced in at least 90 countries around the world (Evans, 2008). During all stages of their life cycle, mango trees can be attacked by over 140 different plant pathogens inciting diverse diseases some of which have become a limiting factor for mango production (Haggag, 2010). Almost every part of mango trees; stem, branch, twig, root, leaf, petiole, flower and fruit are affected by various diseases. These diseases manifest themselves as several kinds of symptoms such as rots, dieback, mildew, necrosis, scab; stem bleeding, wilt, spots, canker, sooty mould, malformation, unknown etiology and disorders. Some of these diseases have become limiting factor in mango cultivation (Prakash, 2004).

Tip dieback or decline, which is a complex disease, is considered a serious problem in various mango producing countries (Khanzada *et al*., 2004a, 2004b). The etiology of this disease remained unclear for several years due to the different causal agents associated with it (Ploetz *et al*., 2003). Therefore, this work was aimed at identifying and characterizing fungal pathogen(s) associated with mango shoot dieback.

MATERIALS AND METHODS

The study was carried out in Southwestern part of Ethiopia in 2017 located at $7^0$42'N latitude and $36^0$50'E longitude with an altitude of 1710 m.a.s.l. Samples of infected mango shoots were collected from top, middle and bottom of the crown of mango tree from the Horticulture garden of Jimma University College of Agriculture and Veterinary Medicine and the samples were brought to plant pathology laboratory of the University.

Isolations

Isolations were made from symptomatic plant samples showing dieback symptom (dark brown lesions, necrosis and brown discoloration) on shoot tissues. Small pieces between the healthy tissues and infected one were cut into (2-4 $mm^{2)}$**.** Plant materials were surface disinfected by sequential washing in house hold bleach (NaOCl 5 %) for 1 minute, and then rinsed in distilled sterilized water three times and dried in sterile filter paper and placed in 9 cm-diameter Petri dishes containing sterilized Potato Dextrose Agar medium (PDA).Plates were incubated at 25°C in the dark until the fungi growth appeared. Pure cultures were obtained by excising a hyphal tip from colony margins emerging from the tissue pieces onto fresh PDA and incubated at the same conditions following Espinoza *et al*. (2009).

Data Collection

Data were collected starting from the growth of the fungus mycelia four days after purification. Data on colony growth in diameter (cm), colony color, colony shape, and colony growth orientation were recorded at three days interval.

Identification of the Pathogens

The pathogens were identified based on their cultural and morphological characters. A loop full of fungal culture grown on PDA plates were taken on a clean glass slide with a drop of distilled water and observed under compound microscope at 10 X 40 magnifications for the presence of conidia and spores. After observing the spores of each plate, two types of spores were identified. And the spores were compared with previously identified fungal spores of the same type and finally confirmed with (Ismail *et al*., 2012).

RESULTS

Phenotypic characteristics of observed fungal spores indicated that two fungi species were associated with the mango shoot dieback viz., *Colletotrichum* spp. and *Pestalotiopsis* spp. (Figure 1A & 1B). Five isolates (26.32%) of the isolates characterized morphologically were *colletotrichum* spp*.* whereas 73.68% were confirmed to be *pestalotiopsis* spp.(Table 1)*.*The isolates*,* thus grouped into two: those isolates in which *colletotrichum* spp. were found and the other ones whose isolates were confirmed containing *pestalotiopsis* spp*.*

Table 1. Occurrence of *Colletotrichum* spp*.* and *Pestatiopsis* spp. identified from the purified culture.

S. No	Isolates	*Colletotrichum* spp.	*Pestalotiopsis* spp.
1	TR11	-	+
2	TR21	-	+
3	TR22	-	+
4	TR31	-	+
5	TR41	-	+
6	MR11	-	+
7	MR12	-	+
8	MR21	-	+
9	MR31	-	+
10	MR41	-	+
11	MR42	+	-
12	BR11	+	-
13	BR21	-	+
14	BR22	+	-
15	BR23	+	-
16	BR31	-	+
17	BR32	+	-
18	BR41	-	+
19	BR42	-	+

+ = Present, - = absent

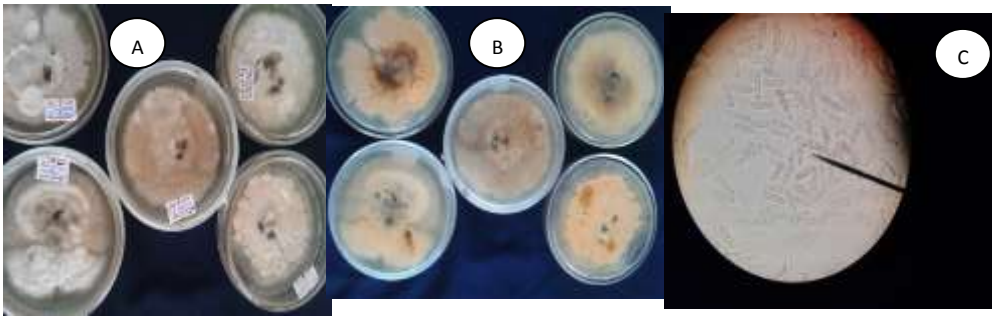

Figure1A. *Colletotrichum* spp. The Upper side of the colonies (A), the Reverse side of the same colony (B), and, (C) typical spore shape in microscopic view

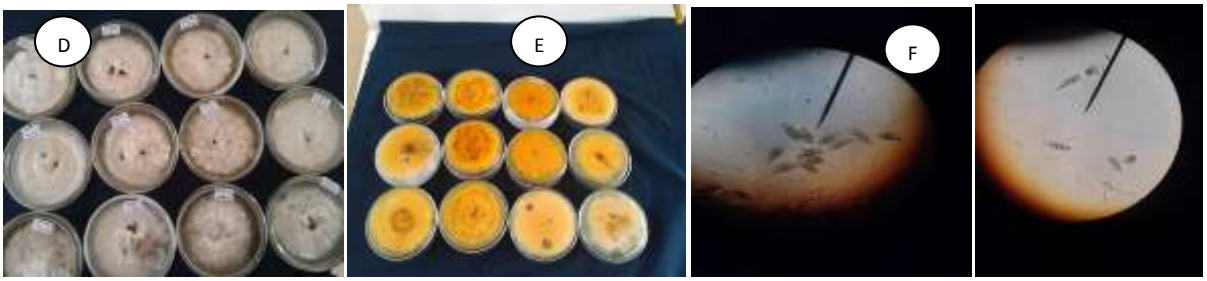

Figure1B. *Pestalotiopsis* spp.The Upper side of the colonies (D), the Reverse side of the same colony (E) and, typical spore shape in microscopic view (F).

Each isolate were measured in terms of colony growth after purification. The result showed that as the incubation day increased, colony growth in top, middle and bottom increased (Table 2).The total mean value of fungal colony from top, middle and bottom were 5.63 cm, 5.13cm and 5.24 cm respectively. The colony color at front side of isolates from which *Colletotrichum* spp. was identified showed whitish color. However, the isolates showed blackish and yellowish color at the reverse side of the isolates (Figure 1A), whereas the isolates from which *Pestalotiopsis* spp. was identified showed whitish color at front side and yellowish color at the reverse side (Figure 1B).

The result showed that fourteen isolates of *Pestalotiopsis* spp. were identified based on conidia characteristics observed under compound microscope at 10x40. The conidia were characterized containing four septated with apical and basal cells hyaline, and three median cells were from olivaceous to light or dark brown (Figure 1B). On the other hand, five isolates of *Colletotrichum* spp. were identified based on microscopic characteristics of conidia at 10x40 magnifications. From the result, the shape of *Colletotrichum* spp. was found to be cylindrical (Figure 1A).

Table 2. Morphological characterization of fungal colony.

S.No.	Isolates	Colony growth (cm) Days 4	Days 7	Days 10	Days 13	Mean	Colony color	Colony Shape(edge)	Colony growth direction/orientation
	Top	7/1/2017	10/1/2017	13/1/2017	16/1/2017				
1	TR11	3.5	5	6.2	7.5	5.55	White	Circular	Spreading
2	TR21	3	5.5	7	9	6.13	White	Irregular	Spreading
3	TR22	2.6	4	6.7	7	5.08	White	Irregular	upward
4	TR31	2.6	4	6.8	7.5	5.23	white	Irregular	upward
5	TR41	2.2	6	7.5	9	6.18	Yellowish	Circular	Spreading
						5.63			
	Middle								
6	MR11	2.6	5	6.3		3.48	White	Circular	Upward
7	MR12	2.2	4	4.2	9	4.85	White	Circular	Spreading
8	MR21	1.5	4.6	6.7		3.20	Black	Circular	Spreading
9	MR31	3	9	9	9	7.50	White	Circular	Upward
10	MR41	2.5	5.8	7.2	9	6.13	White	Circular	Upward
11	MR42	2	4.9	6.5	9	5.60	White	Circular	Spreading
						5.88			
	Bottom								
12	BR11	1.1	3.7	5.5	9	4.83	White	Circular	Upward
13	BR21	0.9	3.7	4.5	9	4.53	Gray	Irregular	Upward
14	BR22	1.1	4.8	6.5	8.5	5.23	Gray	Circular	Spreading
15	BR23	2	5.5	7.9	8.5	5.98	White	Circular	Upward
16	BR31	2.4	4.7	6.3	9	5.60	White	Circular	Upward
17	BR32	1.5	4.2	5.5	9	5.05	Gray	Irregular	Upward
18	BR41	2.1	9	9	7.5	6.90	White	Circular	Upward
19	BR42	1.5	3.5	4.7	5.5	3.80	Gray	Irregular	Spreading
						4.63			

DISCUSSION

The mean length of the diameter of mycelia growth for the three representatives of all isolates showed varying growth. The isolates from middle of the crown of the mango tree exhibited relatively highest mean length (5.88 cm) diameter, whereas the mean length of the diameter for the isolates from top and bottom were 5.63 cm and 4.63 cm respectively, the isolated from bottom showing the least mean diameter length (4.63 cm) (Table 2). The diameter lengths of isolates from bottom were significant to the other two isolates.

This study found two fungi pathogens to be causal of mango shoot dieback; and the finding agrees with Saeed *et al*. (2017) that mango shoot dieback disease can be associated with different fungi. Therefore, from the results of the present study, it can be concluded that mango shoot dieback can be a caused by more than two different pathogens and isolation and characterization of these pathogens should be carefully cultured for isolation starting from sample taking as some pathogens may be associated with different samples taken from different parts of the tree as this is the case in this study (Table 1). After taking representative sample, particular procedures should be carefully followed to isolate the pathogen (s) associated with a particular plant disease symptom. Characterization of the isolated pathogen (S) should be done with the existing facilities to categorize the pathogen (S) for further studies provided all necessary facilities fulfilled to observe all possible characters.

ACKNOWLEDGMENT

I am grateful to Jimma University College of Agriculture and Veterinary Medicine (JUCAVM) and to the Plant Pathology laboratory of the Agriculture campus. I also want to extend my gratitude to the lab technicians who were with me to help me get the necessary lab materials.

REFERENCES

Espinoza JG, Briceño EX, Chávez ER, Úrbez-Torres JR, and Latorre A (2009). *Neofusicoccum* spp. associated with stem canker and dieback of blueberry in Chile. Plant Disease 93: 1187-1194.

Evans EA (2008). Recent trends in world and U. S. mango production trade, and consumption. University of Florida, IFAS Extension.

Haggag WM (2010). Mango diseases in Egypt. Agriculture and Biology Journal of North America 1: 285-289.

Ismail AM (2012). Studies on the fungal diseases of mango with particular reference to diseases Caused by *Botryosphaeria* species. Doctoral Thesis, University of Catania Faculty of Agriculture, Egypt.

Khanzada MA, Lodhi AM and Shahzad S (2004a). Mango dieback and gummosis in Sindh, Pakistan caused by *Lasiodiplodia theobromae*. Plant Health Progress Online:http://www.plantmanagementnetwork.org/pub/php/diagnosticguide/2004/mango/.

Khanzada MA, Lodhi AM and Shahzad S (2004b). Pathogenicity of *Lasiodiplodia theobromae*and *Fusarium solani* on mango. Pakistan Journal of Botany 36: 181-189.

Ploetz RC (2003). Diseases of mango. In: Ploetz R.C. (ed.). Diseases of Tropical Fruit Crops, pp. 327-363. APS Press, St. Paul, MN, USA.

Prakash O (2004). Diseases and disorders of mango and their management. In: Naqvi SAMH (ed.). Diseases of Fruits and Vegetables, pp. 511-619. Kluwer Academic Publishers, Dordrecht, the Netherlands.

Saeed EE, Sham A, AbuZarqa A, A Al Shurafa K, S Al Naqbi T, Iratni R, El-Tarabily K, F AbuQamar S (2017). Detection and Management of Mango Dieback Disease in the United Arab Emirates. *International journal of molecular sciences*. 2017 Oct 20; 18(10):2086.

Cite this Article: Garuma NR (2019). Isolation and Characterization of Mango Shoot Dieback Pathogen. Greener Journal of Agricultural Sciences 9(1): 86-89, http://doi.org/10.15580/GJAS.2019.1.012119024.