INNSPUB

Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print) 2222-3045 (Online)
Vol. 10, No. 5, p. 148-154, 2017
http://www.innspub.net

RESEARCH PAPER OPEN ACCESS

Assessment of selected culture media for competent growth characteristics of *Ceratocystis manginecans* (Cause of Mango Sudden Death)

Kokab Jabeen*, Shahzad Asad

Department of Plant and Environmental Protection, PARC Institute of Advanced Studies in Agriculture, NARC, Islamabad, Pakistan

Article published on May 29, 2017

Key words: *Ceratoc ystismanginecans,* Mango sudden death (MSD), Culture Media

Abstract

Comprehensive and critical facts of aspects which influence patterns of growth and nutrition of the fungi is precondition for research related to the relationship of host and pathogen. A number of media compositions likewise influence the colony morphology of fungus. Most conjoint and distressing disease in mango trees is Mango sudden death (MSD) caused by *Ceratocystis manginecans*. Present study is aimed to evaluate the influence of selected six different culture media (agar and broth media) on sporulation; colony characteristics and growth of the *C. manginecans* isolated from MSD infected mango trees. Statistical analysis of the data gathered revealed that all the treatments were significantly different from each other. Among solid media, Malt Extract Agar and Carrot Juice Agar supported best growth of *C. manginecans*89.53mm and 85.31mm respectively. Among broth media Carrot Juice supported better growth (503.33mg dry weight) than other media. The Characteristics of growth like color of substrate, colony color and margins, mycelia topography as well as sporulation of the *C. manginecans* were studied. Best sporulation of *C. manginecans* was shown in Malt Extract Agar media. Selection of most effective culture media for efficient growth characteristics of *C. manginecans* is necessary for its physiological and taxonomical study as well as for suitable disease management strategies.

***Corresponding Author:** Kokab Jabeen ✉ kkj_satti@yahoo.com

Introduction

Mango sudden death (MSD) is menace to cost-effective farming of mango. MSD cause drastic reduction in the production quantity and quality of mango. Early visible disease symptoms include bark splitting; gummosis from bark; vascular discoloration; and streaking underneath gummosis. Diseased mango tree leaves wither out, but stay attached with the tree. When the severely infected trunk is scrapped, rotted cankers are produced or exudation of foul smelled liquid occurs in some cases (Masood *et al.*, 2010).

In Pakistan (2007) a wilt epidemic of *Magnifier indica* (mango trees) was stated. According to Wyk *et al.* (2007) and Asma *et al.* (2014), the confirmed pathogen of mango sudden death in Pakistan was *Ceratocystis manginecans*. The name *Ceratocystis manginecans* was derived from a Latin word neck means "to kill, slay/put to death". This refers to the fact that *C. manginecans*is responsible for a serious disease of the mango trees. Colony colure of *C. manginecans*is grayish olive. It has banana odor (Wyk *et al.*, 2007).

For*an in vitro* study, fungus is isolated as pure culture in a particular media for the studies relating physiology, nutrition, growth as well as management of that particular fungus. A wide range of media can help in the isolation, the diametric growth, dry weight growth and sporulation of the fungus. Several media compositions can also influence the diverse colony morphology of fungus. A classical approach to distinguish any fungal species is morphological characterization, which is one of the foremost requisite of the fungal taxonomy (Diba *et al.*, 2007).

Carbohydrates exist in plants in both forms (simple and complex).Before utilization, fungi convert carbohydrates of complex forms into simpler one (water soluble and low molecular weight sugars). Different fungal species retort differently to a specific compound. Fungi have noticeable variation in consumption of varied carbohydrate sources (Zain *et al.*, 2009).

Comprehensive and critical information of aspects which influence patterns of growth and nutrition of the fungi is precondition for research related to the relationship of host and pathogen. A number of media compositions likewise influence the colony morphology of fungus. For growth parameters of a pathogen, much attention is not given to culture media (Mishra and Mishra, 2012).

Present study is aimed to evaluate the influence of selected 06 different culture media on sporulation; colony characteristics and growth of the *C. manginecans* isolated from MSD infected mango trees. Selection of most effective culture media for efficient growth characteristics of *C. manginecans* is necessary to be developed for suitable disease management strategies. It may aid to the physiological and taxonomical traits of *C. manginecans*.

Materials and methods

Present study was conducted following methodology described by Zain *et al.* (2009) and Koley *et al.* (2015) with slight modification.

Culture Media preparation

06 different solid/agar and liquid/broth media of Potato Dextrose, Malt Extract, Czapek's solution, Sabouraud's, Mango Stem Decoction Glucose and carrot Juice were used to culture the *C. manginecans*. These selected media comprise of a number of elements (in 500ml distilled H_2O), e.g., PDA (potato starch10 g, dextrose 10g, agar 20g), MEA (malt extract 12.5g, agar 10 g), Czapek's solution Agar ($NaNO_3$ 1.5g, K_2HPO_4 0.5g, $MgSO_4$. $7H_2O$0. 25g,KCl 0.25g, $FeSO_4$. $7H_2O$ 0.005g, Sucrose 15g, Agar 10g), SA (Maltose 20g, peptone 5g, agar 10g), Mango Stem Decoction Glucose Agar (Boiled Mango wood log chips 100g, Glucose10g, agar 10g), and Carrot Juice Agar (Fresh Carrot juice 250ml, agar 10g). (Zain *et al.*, 2009 and Koley *et al.*, 2015).

In all cases, the general preparation of culture medium was similar. For preparation of solid/agar media, initially agar was added into 500ml of distilled H_2O.

Then other required ingredients were also added in it. Finally it was autoclaved. Antibiotic ampicillin (25mg/500ml) was added. For preparation of all above mentioned broth/liquid media, similar method was trailed using same constituents but without addition of agar. Media pH was set at 7.0.

Incorporation of the C. manginecans Inoculum

The pure culture of the *C. manginecans* was obtained from Mango Research Laboratory, National Agricultural Research Centre, Islamabad, Pakistan. Laminar flow chamber was initially sterilized by spraying 4% formaldehyde solution and then with the ultra violet radiation. For both solid and liquid media, mycelial discs were taken from 7 days old culture of *C. manginecans* using a sterilized cork borer. 25ml of each of the agriculture media was dispensed into each 90mm sterilized Petri plate. 25ml of each of the autoclaved broth media was poured into 100ml flask separately. All the experiment was conducted in aseptic conditions. 5mm disc of solidified culture media was taken out from center of the plate using sterilized inoculating needle. Same sized disc of fresh culture of *C. manginecans* was transferred in each plate except control plate. 5mm disc was added in each flask except control flask. Control of each media was kept without adding fungal plug. Ten replicates of each treatment were used. All the plates and flasks were incubated at 25±2°C. (Zain *et al.*, 2009 and Koley *et al.*, 2015).

Fungal growth measurement technique

For all Agar media treatments, linear growth of the fungus was determined from back of Petri plates. Direct measurement of the colonies was done in the same axis on daily basis after first two days of inoculation. Fine transparent plastic scale in millimeter was used to measure linear growth of colony. For broth media incubation period was 7 days. After one week of incubation, the mycelia mat was harvested and filtered using what man's filter paper #1. Initial weight of each filter paper was measured before use. Mycelia mat on filter paper was dried at 60°C for 2 days in a hot air oven. After 2 days, this was taken out and was placed inside a desiccators containing $CaCl_2$ to avoid moisture absorption. Until a constant weight was attained, the heating and weighing was sustained. (Munde *et al.*, 2013 and Koley *et al.*, 2015).

Observation on growth characteristics

Color of substrate and colony, colony margin, mycelium topography of *C. manginecans* were witnessed with naked eye. For measurement of the sporulation on as elected media, 5mm disc was cut out from the *C. manginecans* colony from periphery with sterilized cork borer. This disc was moved to test tube containing 5ml autoclaved distilled water. It was thoroughly mixed to obtain an even suspension of spores. Single drop of suspension of spores was poured on slide. At three fields of light microscopic, using 10X objective, an average of spore count was recorded. (Saha *et al.*, 2008 and Koley *et al.*, 2015).

Statistical Analysis

Completely Randomized Design (CRD) of experiment was used. The data was analyzed using Statistics 8.1 software.

Results and discussion

The objectives of the study were influence and comparison among 06 different culture media in solid and liquid form on the growth of *C. manginecans*. These media are synthetic, semi-synthetic and natural. The data of the diametric growth of *C. manginecans* on selected media revealed that Malt Extract agar supported significantly highest diametric growth (89.53mm), followed by carrot juice agar (85.31mm). The host stem extract (Mango stem decoction glucose agar) supported least growth (55.40mm) of the *C. manginecans*. (See Fig. 1). Statistical analysis of the data shows that all the selected culture media treatments were significantly different from each other, (See Table 1-2).

Table 1. The spore suspension concentration assessment using following representations.

Spores count for each microscopic field	Description
0	(nil) -
1-15	(poor) +
16-30	(moderate) ++
31-45	(good) +++
46-60	(excellent) ++++

Table 2. Completely Randomized AOV for the Diametric Growth Measurement by Treatment.

Source	DF	SS	MS	F	P
Treatment	5	10942.2	2188.43	1815	0.0000
Error	54	65.1	1.21		
Total	59	11007.3			

Grand Mean 72.260 CV 1.52.

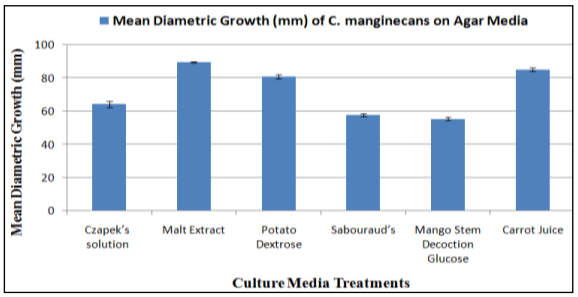

Fig. 1. Growth rate of *Ceratocystis manginecans* on selected agar media.

The growth of dry weight of *C. manginecans* was also calculated on the same 06 broth/liquid culture media. Carrot juice broth supported significantly highest growth with 503.33mg dry weight of *C. manginecans*, followed by 498.55mg of malt extract broth and 361.93mg potato dextrose broth. Host broth medium (mango stem decoction glucose) supported the least dry weight growth (80.960mg) of *C. manginecans*. (See Fig. 2). All treatments (growth media or substrate) showed significant difference of growth from each other, (See Table 3). While studying the growth of the *C. manginecans* in the selected broth media, it was revealed that MEB was observed to be producing less dry weight growth of the *C. manginecans* than Carrot juice, being the liquid medium of 2nd choice. It also specifies apparently that the growth quality in the broth culture media at all times does not draw a parallel to growth quality of *C. manginecans* in agar media.

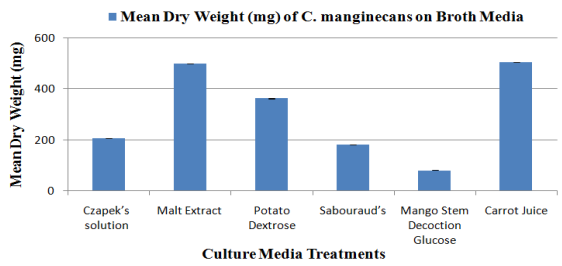

Fig. 2. Growth rate of *Ceratocystis manginecans* on selected broth media.

The characteristics of growth i.e. color of substrate and colony, colony margins, mycelial topography and the sporulation strength of *C. manginecans* were also considered on selected agar media, (See Table 4). Substrate colors of different media with growth of *C. manginecans* were dark brown (in case of MEA), light brown (in case of PDA, Czapek's solution Agar), Reddish brown (in case of Carrot Juice Agar) and yellowish white (in case of Mango Stem Decoction Glucose). The colony color of *C. manginecans* was a yellowish white tinge in Czapek's solution Agar.

Light grey colony color appeared on Mango Stem Decoction Glucose and SA medium. Whereas the mouse grey colony color appeared in case of MEA. Grayish brown colony appeared in Carrot Juice agar medium. (See Fig. 3). The colony margins were wide-ranging from regular and smooth to irregular and wavy in different compositions of media. Mycelia topography of *C. manginecans* was sub-merged, merged and aerial in different media. (See Fig. 4).

Excellent sporulation (above 46 spores per microscopic field) of *C. manginecans* was detected on MEA medium. Good sporulation (per microscopic field 31-40 spores) was observed on Carrot Juice agar, and PDA. Whereas moderate sporulation with per microscopic field 16-30 spores was seen on Czapek's solution Agar.

A poor sporulation with per microscopic field 1-15 spores was witnessed on SA and Host media. Deviation in colony and substrate color, colony margins and mycelia topography on selected agar media adds substantial information that can help in the taxonomic identification of *C. manginecans*. Major morphological characters of *C. manginecans* studied include: Hyphae-smooth and segmented; ASCII-evanescent; Ascosporic-hyaline and hat-shaped; Anamorphic- Thielaviopsis; Conidiophores- of two morphological forms; Primary conidiophores- philander, lagenifor and hyaline; Secondary conidiophores- tube-like, flaring at mouths, short and hyaline; Chlamydospores- brown, thick-walled, and globes to sub-globes. Wyk *et al.* (2007) described same morphological characters of *C. manginecans* as well.

Table 3. Completely Randomized AOV for Dry Weight Measurement by Treatment.

Source	DF	SS	MS	F	P
Treatment	5	1554835	310967	177778	0.0000
Error	54	94	2		
Total	59	1554930			

Grand Mean 305.25 CV 0.43.

Table 4. Growth characteristics of *Ceratocystis manginecans* on selected media.

Sr. #.	Culture media	Colony characters				Sporulation
		Substrate color	Colony color	Mycelia features	Colony margin	
1	Czapek's solution Agar	Light brown	Light grey	Merged	Slightly thick flat, Irregular	++ (moderate)
2	Malt Extract Agar	Dark brown	Mouse grey	Aerial	Smooth flat regular	++++ (excellent)
3	Potato Dextrose Agar	Light brown	Dark grey	Submerged	Thin flat, regular	+++ (good)
4	Sabouraud's Agar	Yellow	Light grey	Merged	Irregular, wavy	+ (poor)
5	Mango Stem Decoction Glucose Agar	Yellowish white	Yellowish brown	Merged	Strongly thin flat, Irregular	+ (poor)
6	Carrot Juice Agar	Light reddish brown	Grayish brown	Arial	Slightly thick flat, regular	+++ (good)

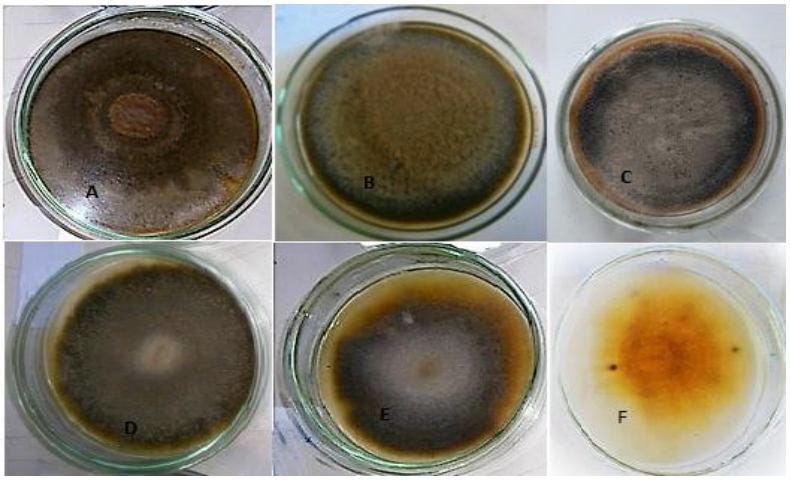

Fig. 3. Growth of *C. manginecans* on the selected culture media of: a) Malt Extract Agar; b) Carrot Juice Agar; c) Potato Dextrose Agar; d) Czapek's solution Agar; e) Sabouraud's Agar; f) Mango Stem Decoction Glucose Agar.

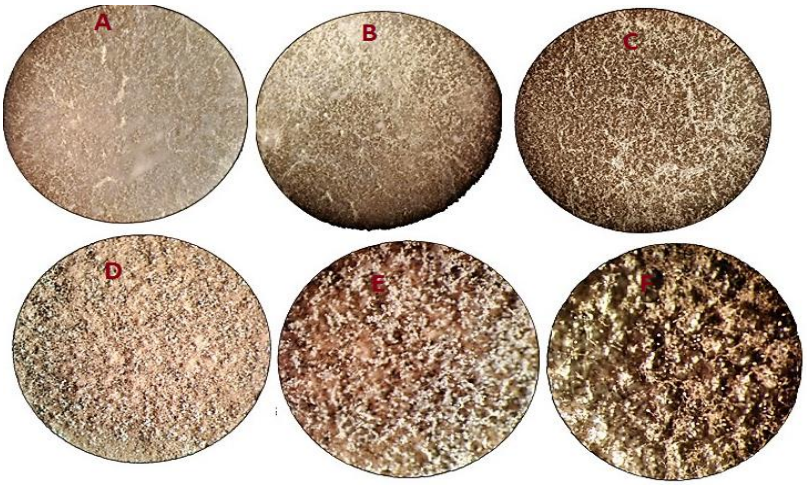

Fig. 4. Under Stereomicroscope observation of growth characteristics of *C. manginecans* on selected culture media of: a) Mango Stem Decoction Glucose; b) Sabouraud's; c) Czapek's solution; d) Potato Dextrose; e) Carrot Juice; f) Malt Extract.

The first step in any pathological research is to get culture of pathogen in most appropriate media (Koley *et al*., 2015). Saha *et al*. (2008) studied the influence of culture media and environmental factors on mycelia growth and speculation of *Lasiodiplodia theobromae*. Koley *et al*. (2015) evaluated12 different culture media for growth characteristics of *Alter aria salami* (causing the early blight in tomato).

They found PDA as best culture media for *A. salami*. For the growth study of *C. manginecans*, researchers used different culture media. Asma *et al*. (2014) did experiments on *C. manginecans* using MEA as culture media, whereas (Khuhro *et al*., 2005; and Masood *et al*., 2010) used PDA culture media for *Ceratocystis* sp. during their research work. Present study revealed that MEA media support best mycelia growth and best sporulation of *C. manginecans*, followed by Carrot Juice agar, and PDA.

Conclusion

Present study reveals that Malt Extract Agar culture medium provides excellent sporulation and best diametric growth, Whereas Carrot juice broth medium shows maximum dry weight growth of *C. manginecans*. It is concluded that simple formulation of MEA and more nutrient contents which support the best mycelia growth as well as excellent sporulation of the *C. manginecans*. This study will be helpful to understand the growth parameters and reproduction of the *C. manginecans* and its management to combat the mango sudden death. Likewise, this study can supplement the knowledge related to the taxonomic behavior of *C. manginecans*.

Acknowledgements

First of all praises and thanks to Allah Almighty for His showers of blessings throughout this research work. This research was supported by Higher Education Commission, Pakistan. We thank our Faculty members and colleagues of PARC Institute of Advanced Studies in Agriculture, NARC, Islamabad. They provided insight and expertise that greatly assisted this research.

References

Diba K, Kordbacheh P, Mirhendi SH, Rezaie S, Mahmoudi M. 2007. Identification of *Aspergillus* species using morphological characteristics. Pakistan Journal of Medical Sciences **23,** 867-872.

Khuhro RD, Nizamani SM, Abbasi QD, Solangi GS, Jiskani MM. 2005. Mango Tree Mortality due to Asian ambrosia beetle, *Xylosandrus crassiusculus* Mot. (Coleoptera: Scolytidae) Pak. J. Agri. Agril. Engg., Vet. Sc **21(1),** 39-42.

Koley S, Mahapatra SS. 2015. Evaluation of culture media for growth characteristics of *Alternaria solani*, causing early blight of tomato. Journal of Plant Pathology and Microbiology **S1,** 2-5.
DOI: 10. 4172/2157-7471. 1000S1-005.

Masood A, Saeed S, Iqbal N, Malik MT, Kazmi MR. 2010. Methodology for the evaluation of symptoms severity of mango sudden death syndrome in Pakistan. Pakistan J. Bot. **42(2),** 1289-1299.

Mishra PT, Mishra V. 2012. Effect of media, temperature and pH on growth of *Alternaria alternata* causing leaf spot of cotton. Annals of Plant Protection Sciences **20,** 246-247.

Munde VG, Diwakar MP, Thombre BB, Dey U. 2013. Cultural and morphological characters of *Alternaria solani* on different media. Bioinfolet. **10,** 984-986.

Rashid. A,Iram S, Ahmad I. 2014. Molecular characterization of *Ceratocystis Manginecans* sp. from Mango in Pakistan. Pakistan Journal of Agricultural Sciences **51(3),** 1-5.

Saha A, Mandal P, Dasgupta S, Saha D. 2008. Influence of culture media and environmental factors on mycelial growth and sporulation of *Lasiodiplodia theobromae* (Pat.) Griffon and Maubl. Journal of Environmental Biology **29,** 407-410.

Wyk VM, Al Adawi AO, Khan IA, Deadman ML, Al Jahwari AA, Ploetz R, Wingfield BD, Al Subhi AM, Wingfield MJ. 2007. *Ceratocystis manginecans* sp. Nov., causal agent of a destructive mango wilts disease in Oman and Pakistan. Fungal Diversity **27,** 213-230.

Zain ME, Razak AA, El-Sheikh HH, Soliman HG, Khalil AM. 2009. Influence of growth medium on diagnostic characters of *Aspergillus's* and *Penicillium* species. African Journal of Microbiology Research **3(5),** 280-286.