ASSESSMENT OF BIOCHAR AND COMPOST ANTIFUNGAL POTENTIAL AGAINST *BOTRYODIPLODIA THEOBROMAE* PAT.

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ABSTRACT

Biochar and compost were used to appraise their antifungal activity against *Botryodiplodia theobromae* Pat. causing die back disease in several cost-effective vital crops. Various concentration of aqueous and methanolic extract (1-3%) of biochar and compost were applied against the test fungus. Highest antifungal activity was shown by biochar extracts in contrast to compost extracts. Different organic fractions viz. *n*-hexane, chloroform, ethyl-acetate and *n*-butanol from biochar extracts were separated. Minimum Inhibitory Concentration (MIC) assay was also conducted with these isolated fractions by using serial dilution method. The MIC (10 - 0.019 mg mL^{-1}) for each fraction was noted at 24, 48 and 72 hours intervals. *n*-butanol and Puslan (synthetic fungicide) were effectively inhibited the spore germination of *B. theobromae* with MIC of 0.019 mg mL^{-1}. This study concluded that biochar and compost possess antifungal potential against *B. theobromae*. So, they may be exploited for the isolation of nature friendly antifungal compounds.

Keywords: Antifungal activity, Dieback, Organic fractions, MIC, Methanolic extracts.

INTRODUCTION

Botryodiplodia theobromae Pat., belongs to Ascomycetes family Coelomycetes, is a common phytopathogenic fungus in the tropics and subtropics of world (Farr and Rossman, 2012). *B. theobromae* caused gummosis, soft rot, crown rot and die back on a large number of host plants (Khanzada *et al.,* 2004a; Muniz *et al.,* 2011).

Chemical application is a highly effective way to manage plant diseases. Fungicides have become the most important means of controlling fungal pathogens (Khanzada *et al.,* 2004b; Bester *et al.,* 2007). However fungicides impose negative effect on environment. The use of natural plant products is an ecologically safe way to manage fungal plant pathogens. Several plant species contain antifungal substances and have been used to treat fungal diseases.

Organic amendments like use of biochar and compost are an alternative way towards safer environment and production of disease free plants. Biochar is a carbon-rich product of pyrolysis or thermal decomposition of organic material under controlled conditions (da Silva *et al.,* 2012). Biochar application improves the soil remediation process and used in a number of agricultural practices (Lehmann, 2007).

Compost is a dense mature organic product that obtained by precise natural break down of biological matter via microorganisms like bacteria and fungi. The organic matter decomposed by such microorganisms changed into a stable product comprises processed organic material and nutrients which easily taken by plants. Soil-borne infectious diseases in crop plants can be inhibited by amending soil with compost (Noble and Coventry, 2005; Danon *et al.,* 2007; Rebollido *et al.,* 2008).

Thus, this study aimed to evaluate the antifungal properties of compost and biochar material against the destructive fungus *B. theobromae.*

MATERIALS AND METHODS

The current study was conducted in Department of Botany, Lahore College for Women University, Lahore, Pakistan in Plant Physiology & Fungal Biotechnology Lab. The compost material was collected from Lahore Compost Company Private, Limited, while biochar was procured from the Agroclimatology Lab, Department of Agronomy, University of Agriculture, Faisalabad, Pakistan. Test fungal specie culture was obtained from Department of Agriculture, Bahauddin Zakriya University, Multan, Pakistan. Sub-culturing of this culture was accomplished on PDA (Potato Dextrose Agar) medium and preserved in fridge (4^0C) for future use.

Antifungal bioassay

One hundred milliliter methanol and distilled water were used to soak twenty grams each of both compost and biochar separately for 1 week at room temperature. Each immersed extracts were filtered with an autoclaved muslin cloth after seven days. Evaporation of the filtrates was done at room temperature to obtain their gummy masses.

In vitro antifungal bioassay was performed with methanolic and aqueous extract of biochar and compost. PDA 2% solution was made by adding 1.2 g of PDA into 60 mL of distilled water in a 250 mL conical flask. The solution was autoclaved for 30 minutes at 121^0C and 15 lb $inch^{-2}$ pressure. Different concentrations viz. 1%, 2% and 3% of biochar and compost aqueous and methanolic extract were prepared for experimental treatment. To make v/v concentration 0.1%, 0.2% & 0.3%, 59.1, 59.4 and 59.7 mL of each stock solution was added respectively to 250 mL conical flask having 2% PDA medium. Control treatment contained no biochar and compost extracts. Chloromycetin capsule was added in every concentration to prevent bacterial contamination. Each treatment was replicated three times.

Mycelial discs (5 mm diam.) were made from the pure culture of *B. theobromae* by using a cork borer. After solidification of PDA medium mycelial discs were put in the center of every petri dish.
The test fungus growth was calculated by applying the following formula (Karim *et al.,* 2015).

$$\text{Growth inhibition (\%)} = \frac{\text{(Growth in control - Growth in treatment)}}{\text{Growth in control}} \times 100$$

Partitioning of biochar material

Methanol (100 mL) was added in twenty gram biochar at room temperature and left for three days. After filtration the obtained extract was kept to evaporate at room temperature which gave 0.3 grams gummy mass. This gummy mass was then further partitioned with *n*-hexane, chloroform followed by ethyl acetate and *n*-butanol. This partitioning gave a gummy mass of *n*-hexane 0.01 g, chloroform 0.011 g, ethyl acetate 0.08 g and 0.015 g of *n*-butanol fraction, respectively (Sherazi *et al.,* 2016).

Minimum Inhibitory Concentration (MIC) assay

Serial dilution micro assay was used for the investigation of minimum inhibitory concentration of the various separated fractions and Puslan (72 WP) a synthetic fungicide (Jabeen *et al.,* 2014). Isolated fractions were solubilized in Dimethyl sulfoxide (DMSO) and serially diluted (10 mg mL^{-1} – 0.19 mg mL^{-1}) with distilled water in test tubes of 15 cm long having 1.6 cm diameter. Seven days old fungal culture of *B. theobromae* was suspended with fresh malt extract medium to get a final conidial concentration $1x10^5$ mL^{-1}, then 100 µL of this suspension were poured in test tubes. Control treatments contain only DMSO and distilled water. This experiment was kept at room temperature 25-30 $^{\circ}$C and after 24, 48 and 72 h the MIC values were noted. The mycelial growth and spore germination of *B. theobromae* was checked with the help of an inverted light microscope.

Statistical analysis

All the data were statistically analyzed using ANOVA followed by Duncan's Multiple Range Test (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

In the present investigation aqueous and methanolic extracts of biochar and compost were investigated against the target fungus *B. theobromae*. The test fungus growth was greatly inhibited by each applied concentration of aqueous extract of biochar. However, 3% concentration was highly effective as it caused 96% reduction in biomass of *B. theobromae*. Other concentrations viz. 2% and 1% also retarded the radial growth of *B. theobromae* upto 77%-79% as compared to control (Fig. 1).The methanolic extracts of Biochar also significantly retarded the growth of *B. theobromae* at all the applied concentrations. However 3% concentration reduced diameter of test fungus 80% while 2% and 1% also inhibited the growth of *B. theobromae* upto 56%-59%, respectively. Biochar showed antifungal potential due to its some important properties such as it improves the uptake of nutrient and minerals which enhanced plant growth and provides protection against soil-borne pathogens. Biochar associated organic compounds have ability to suppress soil microorganisms that resulted in multiplication of resistant microbial colonies and induce systemic plant defense mechanism (Silber *et al.,* 2010). Tiilikkala *et al.* (2010) also reviewed the potential of biochar in agricultural use particularly in pesticide applications as an alternative in lieu of sustainable use of pesticides and plant protection strategies.

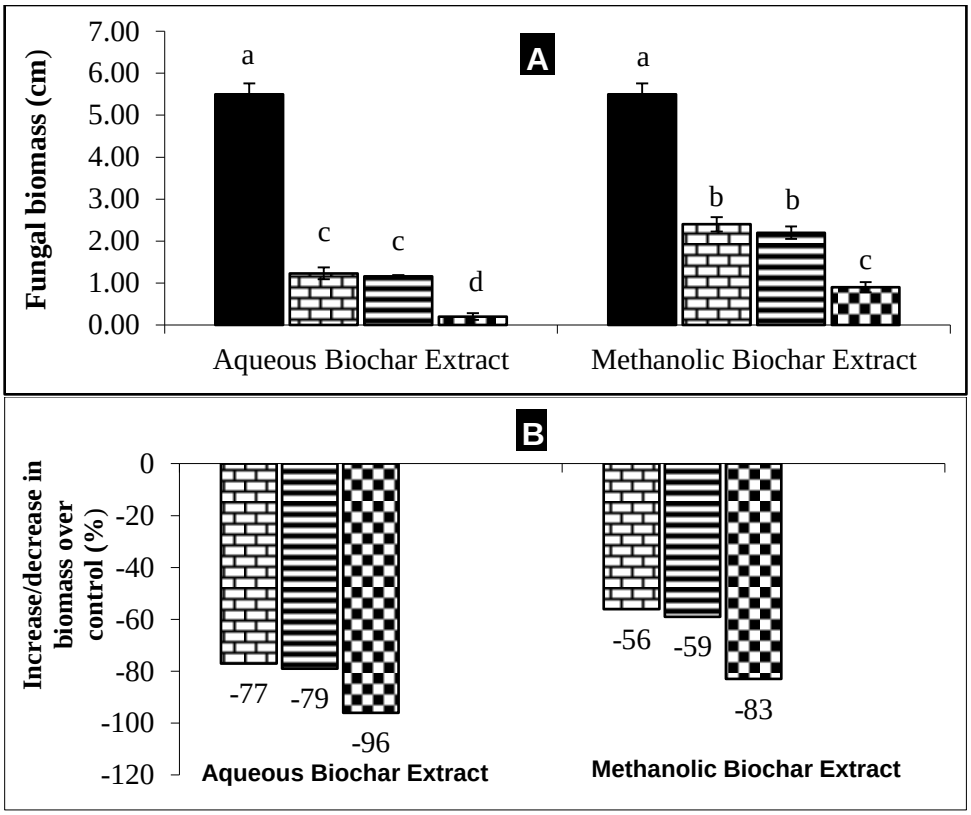

Fig. 1 A & B. *In vitro* antifungal effect of biochar aqueous and methanolic extracts on *Botryodiplodia theobromae*. Vertical bars show standard error and alphabetical letters show significant difference.

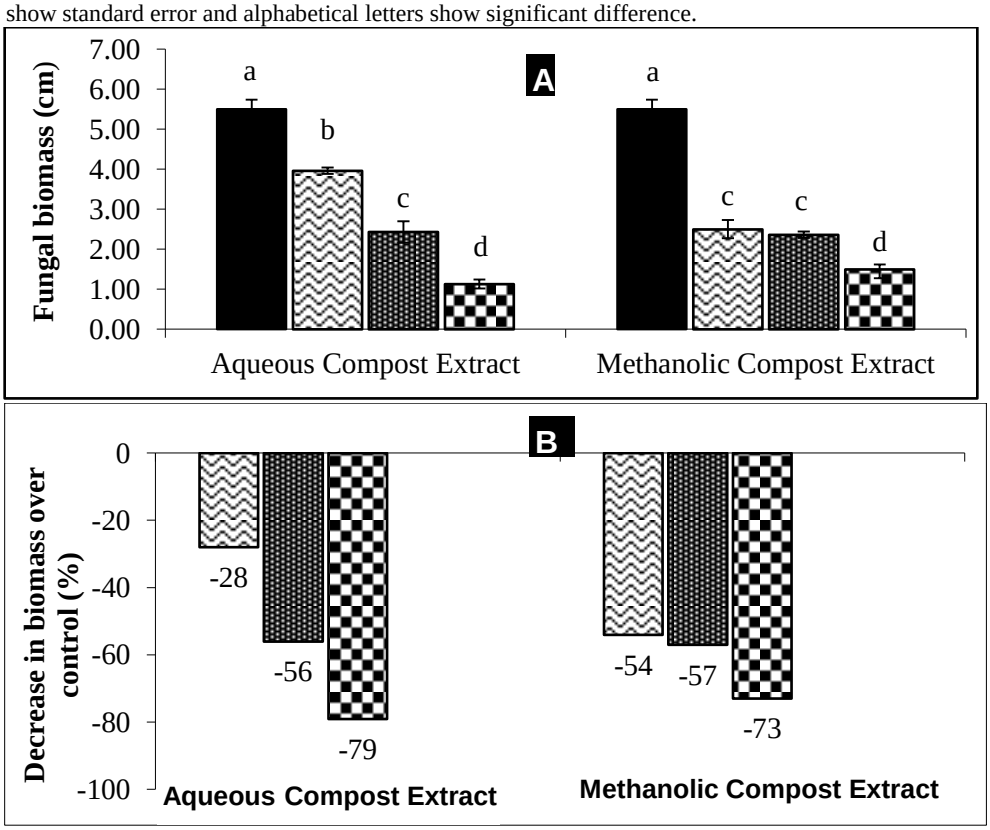

Fig. 2 A & B. *In vitro* antifungal effect of compost aqueous and methanolic extracts on *Botryodiplodia theobromae*. Vertical bars show standard error and different alphabetical letters show significant differences.

Table 1. MIC values of various fractions isolated from methanoilc Biochar extract and Puslan against *B. theobromae* after 24, 48 & 72 h incubation period.

Con. mg mL^{-1}	Fractions																				
	24 hours after incubation							48 hours after incubation							72 hours after incubation						
	(H_2O)	(DMSO)	*n*-hexane	Chloroform	Ethyl acetate	*n*-butanol	Puslan	(H_2O)	(DMSO)	*n*-hexane	Chloroform	Ethyl acetate	*n*-butanol	Puslan	(H_2O)	(DMSO)	*n*-hexane	Chloroform	Ethyl acetate	*n*-butanol	Puslan
10	+	-	-	-	-	-	-	+	+	-	-	-	-	-	+	+	-	-	-	-	-
5	+	-	-	-	-	-	-	+	+	-	-	-	-	-	+	+	-	-	-	-	-
2.5	+	-	-	-	-	-	-	+	+	-	-	-	-	-	+	+	+	+	+	-	-
1.25	+	-	-	-	-	-	-	+	+	-	-	+	-	-	+	+	+	+	+	-	-
0.625	+	-	-	-	-	-	-	+	+	-	-	+	-	-	+	+	+	+	+	-	-
0.3125	+	-	-	-	-	-	-	+	+	-	+	+	-	-	+	+	+	+	+	-	-
0.156	+	-	-	-	-	-	-	+	+	-	+	+	-	-	+	+	+	+	+	-	-
0.078	+	-	-	-	-	-	-	+	+	+	+	+	-	-	+	+	+	+	+	-	-
0.039	+	-	-	-	-	-	-	+	+	+	+	+	-	-	+	+	+	+	+	-	-
0.019	+	-	-	-	-	-	-	+	+	+	+	+	-	-	+	+	+	+	+	-	-

Mycelium present :(+); Mycelium absent :(-)

The aqueous extract of compost was found highly effective in retarding fungal growth i.e., 79%, followed by 2% concentration with a reduction upto 56%. While 1% concentration was least effective and suppressed the test fungal radial diameter upto 28% (Fig. 2). Each applied concentration of methanolic extract of compost also significantly retarded *B. theobromae* growth but as compared to biochar it showed less antifungal activity. In methanolic compost treatment, 3% concentration showed maximum reduction in radial colony growth of fungus upto 73%, while 1% and 2% concentrations also retarded the diameter of fungal colony upto 54- 57% respectively. Previously available data suggested that compost can be used as an alternative to fungicide and showed great antifungal activity especially against soil-borne plant pathogenic fungi (De Ceuster and Hoitink, 1999; Suárez-Estrella *et al.,* 2007). Hoitink and Boehm (1999) also reported that compost application can control infectious plant diseases by activation of disease resistance gene. Use of compost has great advantage over fungicides as it is cheap, safe to apply and biodegradable.

Minimum inhibitory concentration values were estimated of the four isolated fractions viz. *n*-hexane, chloroform, ethyl acetate and *n*-butanol and a reference fungicide (Puslan) against the test fungus. The test tubes were incubated for 24, 48 and 72 h time period (Table 1). Of all the applied fractions, Puslan and *n*-butanol were found most effective as their highest and lowest (10 mg-0.019 mg mL^{-1}) concentrations entirely inhibited the test fungus mycelium formation even after 72 hours of incubation. Control treatments (DMSO and Water) promoted the mycelial growth and mycelium became visible in these treatments after 24 hours incubation period. Earlier literature reports (Elad *et al.,* 2010; Chen *et al.,* 2016) suggested that biochar applications and induced system resistance is plants against soil borne fungal pathogens.

So, the present study concluded that biochar and compost have significant antifungal potential against *Botryodiplodia theobromae.*

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