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# Evaluation of antifungal potential of Wood Biochar against *Fusarium oxysporum* Schlecht

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**ABSTRACT**

Antifungal potential of biochar produced from wood was evaluated against *Fusarium oxysporum* Schlecht. Methanol was used to prepare biochar extract of which various concentrations were experienced *in-vitro* against tested fungal strain. All the applied concentrations of methanol extract i.e. 1%, 2%, 3%, 4%, 5%, 6%, 7% and 8% effectively inhibited *F. oxysporum* growth. However, 7% and 8% concentrations were proved to be most efficient in retarding the test fungus growth upto 15%. Partitioning of biochar methanolic extract was done using some organic solvents such as *n*-hexane, chloroform, ethyl acetate and *n*-butanol in increasing the polarity order. These secluded fractions were further tested to evaluate their *in vitro* antifungal activity along with a reference fungicide (Mancozeb). Data obtained after 7 days of incubation depicts that maximum antifungal activity was observed in fungicide with 89% inhibition in the radial diameter of the test fungus. *n*-hexane and chloroform also effectively retarded the growth of *F. oxysporum,* whereas *n*-butanol and ethyl acetate fractions gave minimum activity . So it can be concluded that wood biochar has the potential to control *F. oxysporum*.

**Keywords:** Bioassay, *in vitro*, Organic fractions, Methanolic extract, Phytochemical analysis.

## INTRODUCTION

*Fusarium oxysporum* Schlecht belong to Ascomycetes, caused wilt disease in large number of economically important crop plants. It mostly penetrates in the infected crop residues, soil and seeds through its chlamydospores and mycelium. It also resides even in absence of host on stem and root tissue which are buried for more than 6 years (Singh et al., 2008). The appearance of banana shaped conidia is the main distinguishing character by which it can be recognized (Summerell et al., 2010). *F. oxysporum* colonize in the xylem tissue of its host which results in blockage and breakdown of tissue, followed by yellowing and wilting of leaves and ultimately leads to tissue death (Bennett et al., 2008)

Several methods like cultural, chemical, biological and use of resistance varieties are documented in the literature to control this disease (Mcgovern, 2015). Application of thick layer of mulch on the soil surface is helpful in reducing the growth of soil borne fungal pathogens. The use of opposed microorganisms is one of the most beneficial alternative measure in order to establish an ecofriendly atmosphere devoid of disease causing fungal strains (Lugtenberg & Kamilova, 2009). *F. oxysporum* can greatly be reduce by using synthetic fungicides which include fuberidazole, thiabendazole, carbendazim, prochloraz, benzimidazoles, propiconazole, benomyl and thiophante preferably (Nel et al., 2007).

Soil improvement using compost and biochar of plants have vast potential to limit the growth of disease causing pathogenic fungal specimens like *Fusarium* species. Because compost is a natural product of organic residues known to be derived by decomposition of aerobic microorganisms (Bonanomi et al., 2007). On the other hand, along with the enhancement of soil environment by increasing carbon levels, biochar is recommended as a low-cost preference (Yao et al., 2011; Elaigwu et al., 2014). Biochar is rich in carbon produced during a process named as “pyrolysis” in the oxygen deficient environment (Sohi et al., 2010; Elad et al., 2011). During this process, carbonization level of feedstock increased

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followed by high temperature, which indicated by reduced hydrogen and oxygen amount, whereas increased carbon content yield more biochar (Harvey et al., 2011; Uchimiya et al., 2011). A mixture of biochar and compost resulted in attributing more positive effects on plant growth (Schulz & Glaser, 2012) due to availability of more beneficial microorganisms in the rhizosphere followed by enhanced reproduction rate (Graber et al., 2010). Different biochar products have different physical and chemical properties depending on the type of feedstock available, for example, herbaceous or grass species has lesser amount of biochar as compared to woody biomass as it has higher concentration of lignin, cellulose and hemicelluloses (Lupoi & Smith, 2012). The application of biochar plays an important role in retarding disease rigorousness of foliar pathogens by activating plant defense mechanism (Elad et al., 2010). Biochar is well known to boost cation exchange, bulk density, water holding capacity, pH and nutrient retention in soil (Atkinson et al., 2010). Due to these beneficial characteristics of biochar, the present study is designed to investigate antifungal activity of wood biochar against *Fusarium oxysporum*

## MATERIALS AND METHODS

### Collection of test material

Biochar produced from wood was selected to evaluate the antifungal potential against *F. oxysporum.* Biochar of wood was collected from Agro climatology lab, University of Agriculture, Faisalabad, Pakistan. Biochar was ground thoroughly to fine powder through electric blender.

### Procurement of test fungus species

*F.oxysporum* was procured from Fungal Culture Bank, Institute of Agricultural Sciences, University of the Punjab, Lahore, Pakistan. The culture was maintained on 2% MEA (Malt Extract Agar) medium and stored in refrigerator at 4°C for future use.

### Antifungal bioassay

Medium comprising Malt Extract Agar (MEA) was primed using 250 mL of conical flask by the addition of 2 g of both agar as well as malt extract in 100 mL distilled water. For the extraction of biochar, 100 mL of methanol was taken in which 20g of grinded biochar was soaked at room temperature for one week and after that filtration was carried out by means of muslin cloth which has been autoclaved for sterilization purposes. In order to minimize the volume upto 2g the filtrate was subjected for evaporation at room temperature. Gummy extract was then diluted for the purpose of making 20% stock solution by the addition of sterilized distilled water. Various concentrations viz.1%, 2%, 3%, 4%, 5%, 6%, 7%, 8% from biochar methanolic extract were prepared. Control treatment was without any extract. In order to avoid contamination from bacterial growth chloromycetin capsule was added.

Five millimeter discs were cut with sterilized cork borer from the margins of seven days old culture of test fungus and mycelial discs were then transferred to each flask. Three replicates were made for each treatment. After inoculation the flasks were allowed to incubate for 7 days at room temperature for fungus to grow (Hanif et al., 2017). Percentage growth inhibition was calculated by using the given below formula for fungal colonies:

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

### Phytochemical analysis

Phytochemical analysis of biochar methanolic extract was carried out by using the protocol of (Parekh & Chanda, 2007) for identification of various secondary metabolites.

### Partitioning of test material

Twenty grams of biochar was extracted with 100mL methanol at room temperature. This extraction yielded the gummy methanolic mass of 0.624g. The gummy mass was partitioned with *n*-butanol, ethyl acetate, chloroform and *n*-hexane in increasing polarity order (Wheed et al., 2016).

### *In vitro* antifungal activity of organic fractions

The isolated fractions *n*-hexane, chloroform, ethyl acetate and *n*-butnaol along with a reference fungicide were tested against *F. oxysporum.* From each organic fraction and fungicide three concentrations viz. 1%, 2% and 3% were made to evaluate their antifungal potential (Sherazi et al., 2016).

### Statistical Analysis

Analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) was used to analyze data statistically using the protocol of Steel & Torrie, 1980.

## RESULTS AND DISCUSSION

In the present study the probable antifungal activity of wood biochar methanolic extract was studied against soil borne wilt causing detrimental plant pathogen *F. oxysporum.* According to the gained results all the applied concentrations of methanolic extract of biochar retarded the test fungal growth. However, 7% and 8% were found potentially good as compared to control against *F. oxysporum* with 15% inhibition. While other tested fractions were found less affective as compared to control treatment (Figure 1). As far as biochar antifungal potential is concerned little work from the literature is available. Although the present findings were in agreement with the results of Harel et al. (2012) that the use of biochar activates the plant defense mechanism which enables them to face challenges resulting from infections caused by harmful fungal specimens like *Colletotrichum acutatum, B. cinerea* and *Podosphaera aphanis.*

**Table I:** Phytochemical analysis of Biochar

| Sr. No. | Phytochemicals constituents | Observations |
|---|---|---|
| 1. | Tannins | - |
| 2. | Phlobatannins | - |
| 3. | Saponins | - |
| 4. | Alkaloids | - |
| 5. | Glycosides | - |
| 6. | Terpenoids | - |
| 7. | Flavonoids | - |

+ Presence
- Absence

The phytochemical tests of this methanolic extract of wood biochar were performed (Table 1). The results indicated that all the phytochemicals including glycosides, alkaloids, tannins, saponins, terpenoids, phlobatannins and flavonoids were found absent in test material. Biochar is produced as a result of high heat pyrolysis, which can be a reason of absence of phytochemical. This might be the first report on phytochemical analysis of biochar as there was no previous literature available.

Partitioning of biochar methanolic extract resulted in the gummy masses of *n*-hexane (1.6 g), chloroform (1.1 g), ethyl acetate (0.4 g), *n*-butanol (1.2 g). *In vitro* bioassay with each organic fraction and fungicide was performed. Results showed that fungicides fraction was found most effective against *F. oxysporum* in comparison to other fractions (Figure 2). Highest growth reduction was given by fungicides and its all applied concentrations i.e., 1%, 2% and 3% reduced test fungal colony growth upto 86% and 80%, respectively. Other fractions also effectively inhibited the test fungal growth; among them 1%, 2% and 3%, concentration of chloroform showed 28%, 34% and 26% inhibition respectively. Tested concentrations of *n*-hexane fraction also showed 22-34% inhibition while *n*-butanol fraction concentrations was found least effective.

Elad *et al*. (2010) described that soil amended with biochar activated plant defense mechanism against soil microbes. This amendment effectively suppressed the infections caused by foliar pathogens, *Botrytis cinerea* and *Leveillulat aurica* in tomato and pepper, respectively. Elmer & Pignatello (2011) reported that biochar also proved beneficial in minimizing root infections caused by *F. proliferatum* and *F. oxysporum*. On the basis of above findings the present study, can be concluded that wood biochar contain antifungal properties against *F. oxysporum.*

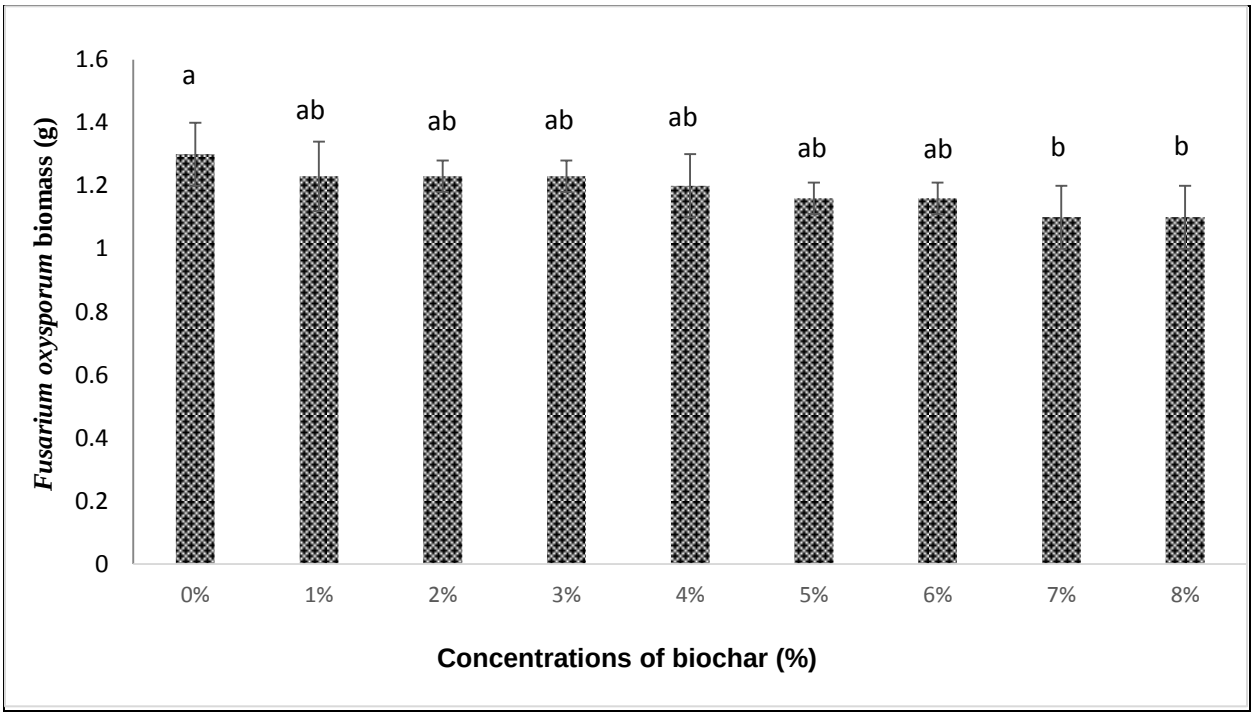


**Fig. 1:** Effects of methanolic extract of Biochar on *in vitro* growth of *F. oxysporum* Schelecht. Vertical bars show standard errors different letters show non-significant difference

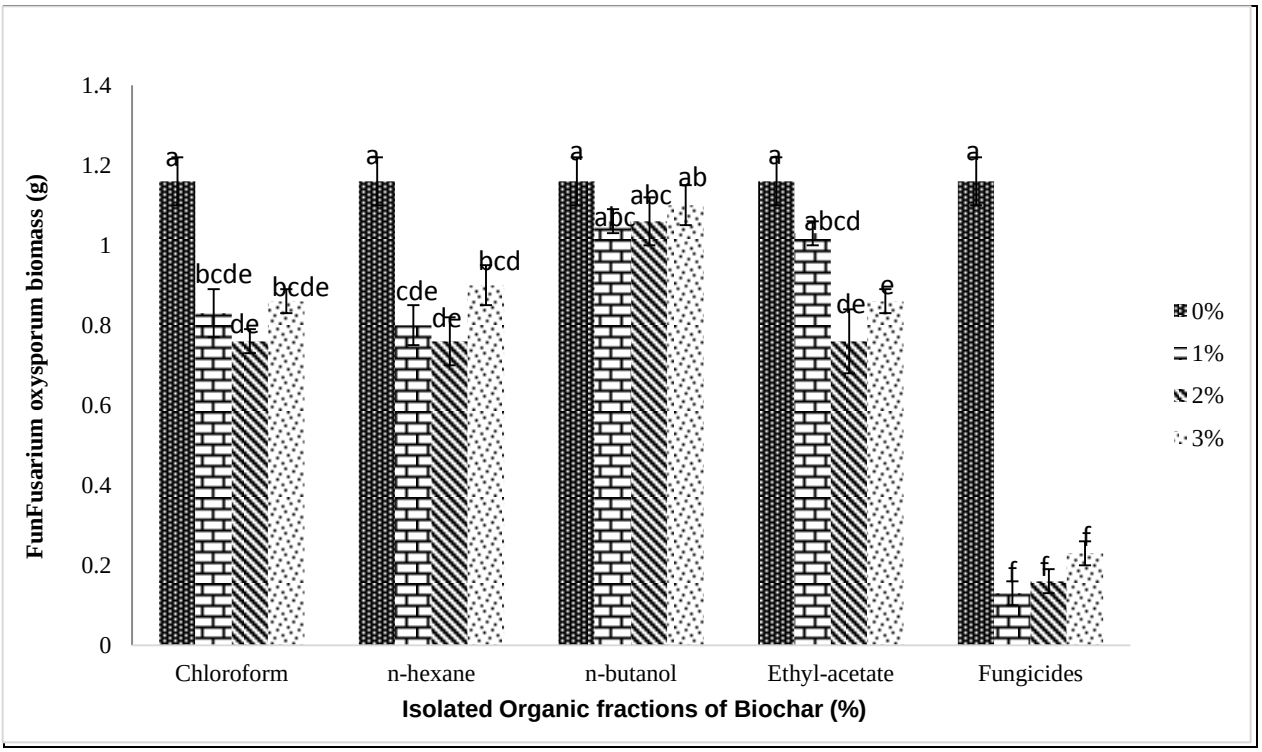


**Fig. 2:** Effect of different concentrations of Biochar extract on *in-vitro* growth of *F. oxysporum* Schelecht

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