Original Research Article

Bio-efficacy of Biochar's against *Fusarium oxysporum*f.sp. *radicis-cucumerinum*inciting Root and Stem RotDisease of Cucumber in *In vitro* condition

ABSTRACT

To assess *in vitro* evaluation of bio-efficacy different three biochar's *viz.,* Green house waste (GHW), Eucalyptuswood (EW) and Citrus wood (CW) were evaluated at different three concentrations (each @ 1, 2 and 3%) against *F. oxysporum*f.sp*. radicis-cucumerinum* causing root and stem rot of cucumberbyusing poison food technique. Among the three different biochars, Green house waste (GHW) was found most effective by showing minimum mycelial growth of 50.78, 40.10 and 29.88 mm and 43.58, 55.44 and 66.80% per cent growth inhibition at 1, 2 and 3% concentrations, Whereas, Citrus wood (CW) depicted highest mycelial growth 66.26, 55.88, 44.30 mm with 26.38, 37.91 and 50.78% lowest per cent growth inhibition of the pathogen at same above concentrations, respectively.

KEYWORDS: *Cucumber, Biochar, Fusarium oxysporum*f.sp.*radicis-cucumerinum, Root and Stem Rot.*

1. INTRODUCTION

The cucumber (*Cucumis sativus* L.) belongs to family Cucurbitaceae and most important vegetable, which is a major source of human edible products and useful fibershave been domesticated in India and it has been cultivated in Western Asia for 3000 years [1,2]. From India, it spread to Greece and Italia, later into China. It was probably introduced throughout Europe by the Romans and records of cucumber cultivation appear in France in the 9th century, England in the 14th century and North America by the mid 16th century [3].The cucumber is a very popular and widely cultivated vegetable in India. Cucumber popularly known in India as '*khira*' is extensively grown in tropics, subtropics and milder temperate zones of India.Root and stem rot of cucumber is believed to be caused by a new formaespecialis of *F. oxysporum*, presently designated as *F. oxysporum* f. sp. *radicis-*

cucumerinum(FORC) (Vakalounakis, 1996). Root and stem rot is the most destructive disease of glasshouse cucumber crops in Canada in 1994, in France in 1998, in China in 1999 and in Spain in 2000, causing significant losses in the yield [4].When cucumber is infected with the root and stem rot fungus, the primary, secondary and tertiary roots and the basal portion of the stem have brown discolorations. On the stem, this discolouration may extend for 40 to 100 cm above the soil line. *Fusarium* root and stem rot of cucumber has been reported to be favoured at lower soil~~1~~ temperatures (17°C) [5]. Use of resistant varieties is considered as key strategy to control*Fusarium*spp. but their main drawback is instability. Fungicides play a vital role in disease management in various crop ecosystems. Fungicides alsoprevent infection but useof chemical protectants causes heavy burden to environment pollution. Being soil-borne, *Fusarium* spp. is randomly distributed in the soil and is difficult to control by single control strategy. Therefore, it is planned to develop biochars to be more appropriate to manage and suppressthecucumber root and stem rot, soil borne disease.Pyrolyzed biomass waste, commonly called biochar, has attracted interest as a soil amendment. A commercial prototype biochar produced by fast pyrolysis of hardwood dust was examined in soils to determine if it could reduce the damaging effect of allelopathy on arbuscular mycorrhizal (AM) root colonization and on Fusarium crown and root rot of asparagus. In greenhouse studies, biochar added at 1.5 and 3.0% (wt/wt) to asparagus field soil caused proportional increases in root weights and linear reductions in the percentage of root lesions caused by *Fusarium oxysporum*f.sp. *asparagi*and *F. proliferatum* compared with a control. Concomitant with these effects was a 100% increase in root colonization by AM fungi at the 3.0% rate. Addition of aromatic acids (cinnamic, coumaric and ferulic) that are known allelopathic agents affecting asparagus reduced AM colonization but the deleterious effects were not observed following the application of biochar at the higher rate. When dried, ground, asparagus root and crown tissues infested with *Fusarium* spp. were added to soilless potting mix at 0, 1 or 5 g/liter of potting mix and then planted with asparagus, there was a decrease in asparagus root weight and increase in disease at 1 g/liter of potting mix but results were inconsistent at the higher residue rate. However, when biochar was added at 35 g/liter of potting mix (roughly 10% v/v), these adverse effects on root weight and disease were equal to the nontreated controls. A small demonstration was conducted in field microplots. Those plots amended with biochar

[3.5% (wt/wt) soil] produced asparagus plants with more AM colonization in the first year of growth but in the subsequent year, biochar treated plants were reduced in size, possibly due to greater than average precipitation and the ability of biochar to retain moisture that, in turn, may have created conditions conducive to root rot. These studies provide evidence that biochar may be useful in overcoming the deleterious effects of allelopathic residues in replant soils on asparagus [6]. Biochar is the product of organic material, like wood, that is burned in a low oxygen environment. This results in a charcoal that acts as a carbon sink for high concentrations of environmental carbon dioxide. It is thought to serve a myriad of functions that aid in plant growth and development such as housing microorganisms and fostering their growth and assisting in water and nutrient absorption and retention. The effect of three concentrations of biochar (1.5, 3 and 6% by weight) on the root growth of corn and soybean plants in Minnesota soil and sand was investigated. The dry root biomass of the corn and soybean plants was measured at 27 days of growth. Soil pH and electrical conductivity was measured in the soil and sand samples. Of the four trials conducted for each percentage of biochar, the corn in sand at 3% biochar resulted in the greatest dry root biomass weight. The trend for corn was an increase in root biomass with an increase in biochar up to 3.0% followed by a decrease in root biomass with a further increase in biochar to 6.0%. The 1.5% biochar trials in sand had the greatest dry root biomass in soybean. The trend for soybean was similar to corn in MN soil, but differed in sand with the peak root biomass occurring at 1.5% biochar, followed by a decline with 3.0 and 6.0% biochar to below control root biomass. Soil and sand pH levels increased with biochar due to its basic nature. Root biomass for both soybean and corn increased in accordance with pH up to a level of 3.0% biochar then decreased with an increase in biochar and pH levels. As the electrical conductivity in the soil and sand increased with an increase in biochar, root biomass increased up to a biochar level of 3.0% and then decreased as the biochar level increased to 6.0% and electrical conductivity increased as well. The exception was corn in MN soil where the electrical conductivity decreased in tandem with the decrease in root biomass peaking at 3.0% biochar [7].

2. MATERIAL AND METHODS

In vitro evaluation of bio-efficacy three different biochar's were evaluated at three different concentrations (each @ C_1-1%, C_2-2% and C_3-3%) against *F. oxysporum*f.sp*. radicis-cucumerinum*byusing poison food technique [8].

Treatments details:

T_1 - Green house waste (GHW)

T_2 - Eucalyptuswood (EW)

T_3 - Citrus wood (CW)

T_4 - Control

Pay attention to text alignment on the page(same setting at the page paragraph)! The technique includes cultivation of test organism on a medium that contains the test biochar. PDA was employed as the basal medium in all the studies. The required quantity of each biochar at different three concentrations was incorporated aseptically in 100 ml PDA in 250 ml flasks at the time of pouring the media in petri plates. The medium was vigorously shaken to ensure that the biochar was distributed evenly. After that 20 ml of medium was poured in each petri plate aseptically and allowed to solidify. Five mm diameter mycelial discs were cut from periphery of 10 days old fungus cultures and inoculated into petri plate. The mycelial disc was inverted in the center of the plates to establish direct contact with biochar medium and incubated for 7-8 days at 28±1^{0}C. In Factorial Complete Randomized Design (FCRD) five replications of each treatment were kept. At the same time a control was also maintained by growing on biochar free PDA. Observations on linear growth were taken, when the fungus reached maximum development in control petri plate.

The per cent inhibition of the mycelial growth of the fungus in each treatment was calculated by using formula [9].

$$I = \frac{C - T}{C} \times 100$$

Where, I = Per cent inhibition

C = Area of test fungus in control (mm)

T = Area of test fungus in respective treatment (mm)

3. EXPERIMENTAL RESULTS

Pay attention to text alignment on the page(same setting at the page paragraph)! *In vitro* evaluation of bio-efficacy different three biochar's *viz.,* Green house waste (GHW), Eucalyptuswood (EW) and Citrus wood (CW) were evaluated at different three concentrations (each @ 1, 2 and 3%) against *F. oxysporum*f.sp*. radicis-cucumerinum*byusing poison food technique. Among the three different biochars, Green house waste (GHW) was found most effective by showing minimum mycelial growth of 50.78, 40.10 and 29.88 mm and 43.58, 55.44 and 66.80% growth inhibition at 1, 2 and 3% concentrations, followed by Eucalyptus wood (EW) with 59.68, 50.18, 38.66 mm mycelial growth and 33.69, 44.24 and 44.99% growth inhibition 1, 2 and 3% concentrations. Whereas, Citrus wood (CW) depicted highest mycelial growth 66.26, 55.88, 44.30 mm with 26.38, 37.91 and 50.78% lowest per cent growth inhibition of the pathogen at same above concentrations, respectively.Dataarerepresented in Table1 & Fig. 1 and Plate 1.

Table 1: Evaluation of bio-efficacy of different biochar's against *Fusarium oxysporum*f.sp. *radicis-cucumerinum*in *In vitro* conditionTry to frame the columns of the table as well as possible!

Sr. No.	Treatments	Mycelial growth/Colony diameter at different concentrations (mm*)				Per cent growth inhibition at different concentrations			
		C_1 (1%)	C_2 (2%)	C_3 (3%)	Mean	C_1 (1%)	C_2 (2%)	C_3 (3%)	Mean
1.	T_1 - Green house waste (GHW)	50.78	40.10	29.88	40.25	43.58 (41.31)	55.44 (48.13)	66.80 (54.82)	55.27 (48.09)
2.	T_2 - Eucalyptuswood (EW)	59.68	50.18	38.66	49.51	33.69 (35.48)	44.24 (41.69)	57.04 (49.05)	44.99 (42.07)

3.	**T_3 - Citrus wood (CW)**	66.26	55.88	44.30	55.48	26.38 (30.90)	37.91 (38.00)	50.78 (45.45)	38.36 (38.12)
4.	**T_4 - Control**	90.00	90.00	90.00	90.00	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Concentration Mean		**66.68**	**59.04**	**50.71**	**58.81**	**25.91 (26.92)**	**34.40 (3196)**	**43.66 (37.33)**	**34.66 (32.07)**
		S.Em ±	**C.D. at 5%**	**C.V. (%)**		**S.Em±**	**C.D. at 5%**	**C.V. (%)**	
Treatments		**0.43**	**1.22**			**0.43 (0.25)**	**1.22 (0.71)**		
Concentrations		**0.37**	**1.06**			**0.37 (0.22)**	**1.06 (0.61)**		
T × C		**0.74**	**2.11**	**2.82**		**0.83 (0.48)**	**2.35 (1.36)**	**5.33 (3.33)**	

*Meanoffivereplications

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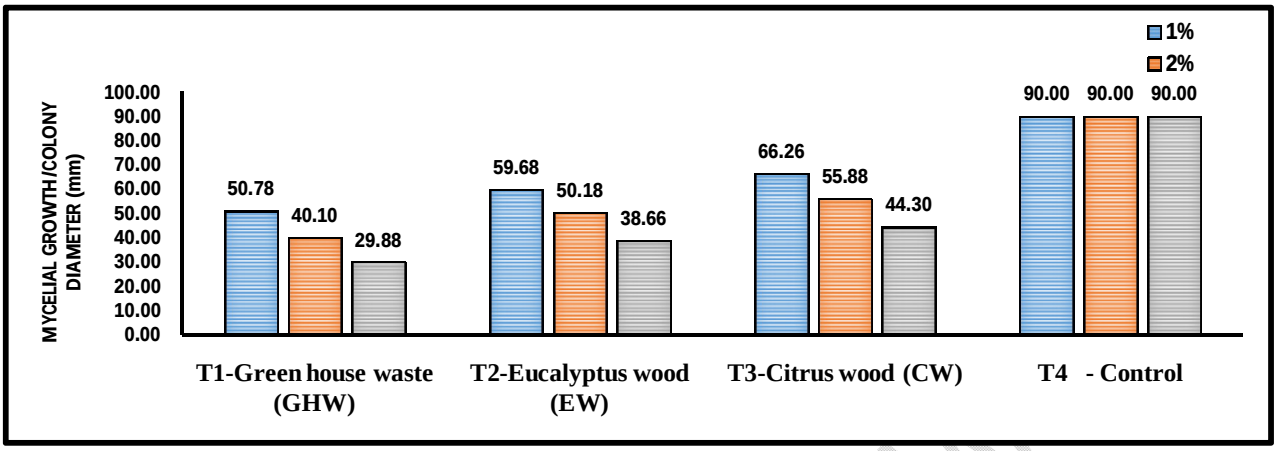

Figure 1: Evaluation of bio-efficacy of different biochar's against *Fusarium oxysporum*f.sp. *radicis-cucumerinum*in *In vitro* condition

4. DISCUSSION

Among the three different biochars, Green house waste (GHW) was found most effective by showing minimum mycelial growth of 50.78, 40.10 and 29.88 mm and 43.58, 55.44 and 66.80% per cent growth inhibition at 1, 2 and 3% concentrations. Whereas, Citrus wood (CW) depicted highest mycelial growth 66.26, 55.88, 44.30 mm with 26.38, 37.91 and 50.78% lowest per cent growth inhibition of the pathogen at same above concentrations. A commercial prototype biochar produced by fast pyrolysis of hardwood dust in soils to determine if it could reduce the damaging effect of allelopathy on *arbuscular mycorrhizal* (AM) root colonization and on *Fusarium* crown and root rot of asparagus. In greenhouse studies, biochar added at 1.5 and 3.0% (wt/wt) to asparagus field soil caused proportional increases in root weights and linear reductions in the percentage of root lesions caused by *Fusarium oxysporum*f.sp*. asparagi* and *F*. *proliferatum*compared with a control. Concomitant with these effects was a 100% increase in root colonization by AM fungi at the 3.0% rate [6].The effectiveness of biochar application to promote plant growth and suppress plant diseases is usually dependent on the application dose of the biochar. Here, we evaluated the effects of biochar supplied at 0, 1, 2 and 3% (w/w) on tomato growth, Fusarium wilt disease severity and rhizosphere microbial community diversity and found that biochar applied at 1 and 2% promoted tomato growth and decreased the severity of Fusarium wilt disease. High-through put amplicon sequencing indicated that 1% biochar decreased the alpha diversity and altered the composition of the bacterial and fungal community in the tomato rhizosphere, increasing the abundance of potential plant-beneficial microorganisms. Quantitative PCR confirmed that all doses of biochar increased the abundance of rhizosphere bacteria; biochar applied at 1 and 2% decreased the abundance of rhizosphere fungi and *Fusarium oxysporum*f.sp. *lycopersici* (FOL), while biochar applied at 3% increased abundance of FOL. Our results indicated that biochar applied at 1 and 2% suppressed tomato Fusarium wilt disease, which might be linked to the change of the rhizosphere microbial community structure and increased the abundance of potential plant-beneficial microorganisms such as *Pseudomonas* sp. within the microbiome [10]. The effects of biochar on leaf and soil borne diseases of plants can be seen in addition to its ability to sequester carbon, improve soil quality, and enhance plant performance. However, the mechanisms by which soil borne pathogens are suppressed and plant performance is enhanced are not well

understood. The present work aimed to comprehensively establish the links between biochar induced changes in the richness of the rhizosphere microbial population, in association with the reduction of soil borne Fusarium wilt disease (*Fusarium oxysporum*f.sp. *vasinfectum*), in cotton (*Gossypiumhirsutum*), with improved plant performance. Biochar made from organic waste significantly decreased the colonization and survival of Fusarium in soil, raised the cultureable counts of numerous microbes with biocontrol potential (microorganisms that boost plant growth and development) and inhibited Fusarium wilt of cotton. The biochar amendment significantly enhanced the cotton plant development and physiological parameters such as chlorophyll content etc. Overall, 9% organic waste biochar had shown a significant impact on cotton growth as compared to other treatments with or without biochar. Compared to the soil only control, the disease index was considerably reduced in all biochar-amended treatments. In terms of the plant's resistance to Fusarium wilt, biochar-induced increases in the level of overall chlorophyll content and biochemicals such as phenolics, flavonoids, etc. Additionally, cotton plants grown with a 9% biochar composition had considerably greater NPK levels than other treatments with or without biochar. The biochar addition resulted in increased counts of *Pseudomonas* spp., *Actinomycetes* spp. and *Trichoderma* spp., while *Acidobacteriales*, *Rhodospirillales* and *Frankiales*were less when compared with an un-amended (without biochar) soil control [11]. Thus, the composition of rhizosphere bacteria in the treatments with and without modified biochar was found to differ significantly. The findings were in line and similar to the current studies.

5. CONCLUSION

Among the three different biochars, Green house waste (GHW) was found most effective by showing minimum mycelial growth of 50.78, 40.10 and 29.88 mm and 43.58, 55.44 and 66.80% per cent growth inhibition at 1, 2 and 3% concentrations, Whereas, Citrus wood (CW) depicted highest mycelial growth 66.26, 55.88, 44.30 mm with 26.38, 37.91 and 50.78% lowest per cent growth inhibition of the pathogen at same above concentrations, respectively.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interest exist.

Statistical Analysis:

The data from various experiments were subjected to analysis for coefficient of deviation. For laboratory, completely randomized design was followed. Means of the experiments were used to compare for efficacy of treatments.

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