Studies on comparative efficacy of botanicals and biochars for management of root and stem rot of cucumber caused by *Fusarium oxysporum* f.sp. *radicis cucumerinum*

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INTRODUCTION

Cucumber (*Cucumis sativus* L.) belongs to family Cucurbitaceae and most important vegetable, which is major source of human edible products and useful fibers. The productivity of the crop is more affected in the polyhouse as well as in field by insects, pest and diseases. Many diseases have been reported on cucumbers from different part of the world, but only few of them cause economic losses. Fusarium wilt and foot rot of cucumber caused by *Fusarium oxysporum* f. sp. *cucumerinum* (Owen) Snyder & Hansen was reported from many parts of the world. Some forma speciales of *F. oxysporum*, cause rotting of roots, lower stems and crowns and rotting of seeds and seedlings (damping-off). 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. Use of natural products like botanical amendments or botanical extracts or biochars for the management of fungal diseases in plants is considered as a substitute method to synthetic fungicides.

Materials and methods

The infected samples were brought from field of RCA Horticulture farm and RCA Polyhouse during *Kharif* 2017 -18 when crop was one month old. Then air dried diseased roots were cut in to 2-3 mm long bits. Bits of infected roots and stems were surface sterilized and placed on Potato Dextrose Agar (PDA) medium and the plates were incubated at 28 ± 2°C in BOD incubator. After five days the white pinkish culture so obtained, was further purified by employing hyphal tip method. The morphological, cultural and formation of macro conidia and micro conidia as chlamydospores were the principle characters to identify the pure cultures.

PATHOGENICITY TEST

The pathogenicity test of *Fusarium oxysporum* f.sp*. radicis cucumerinum* isolate was carried out in cage house in pots using cucumber susceptible variety (cucumber Long desi) by soil inoculation and spore suspension of the fungus having 4.0×10^5 spores/ml was used as inoculum. 10 seeds were sown in a pot containing autoclaved soil. Such three replications were kept in each case with suitable un-inoculated control. The pots were labeled, watered as and when required and left undisturbed in net house for germination and development of the symptoms.

Evaluation of bio-efficacy of botanicals as water and ether extracts against *F. oxysporum* f.sp. *radicis-cucumerinum* by *in vitro* technique.

An experiment was carried out to evaluate eight plant species for their fungicidal activity against root and stem rot pathogen. These are *Ipomea carnea, Calotropis gigantean, Allium cepa, Datura stromonium, Catharanthus roseus, Azadirachta indica, Curcuma longa* and *Piper nigrum*.

$$\textbf{Inhibition percentage} = \frac{C - T}{C} \textbf{x } 100$$

C = Colony diameter in control; T = Colony diameter in treatment

Evaluation of bio-efficacy of different biochars against root and stem rot of cucumber (*F. oxysporum* f.sp. *radicis-cucumerinum*) in pot condition

The effect of biochars on the growth characters like germination percentage, root length and shoot length were evaluated under *In vitro* conditions. Three types of raw materials (Eucalyptus wood, citrus wood, green house waste) were collected and dried under shade for a week. The dried materials were taken to biochar preparation unit. The prepared biochar was collected and filled into pots at four concentrations (1%, 2%, 3% and 4% W/W: Fig. 3). The cucumber susceptible variety (cucumber Long desi) seeds were used for sowing, 8 seeds were planted in each pots and were watered regularly. Three replications of each treatments was maintained and control pots were also employed for comparison.

$$\textbf{Percent plant mortality} = \frac{\textbf{Number of disease plants}}{\textbf{Total number of plants}} \times 100$$

RESULTS

The pathogen was isolated and purified. This pathogen was identified as *Fusarium oxysporum* f.sp. *radicis cucumerinum.* General symptomatology of root and stem rot begins with the tap roots of young plants showing a slightly reddish discoloration. Sudden wilting occurs, especially on lower leaves and in last on the upper leaves.

Pathogenecity test of the culture of *Fusarium oxysporum* f.sp. *radicis cucumerinum* was confirmed on young plants of susceptible variety (Cucumber Long Desi) of cucumber in the pot condition (Fig.1).

Both water and e ther extracts of the botanicals *A. indica* at 30% concentration showed maximum growth inhibition of the mycelial growth of the pathogen against *F. oxysporum* f.sp.*radicis cucumerinum In vitro.* The inhibition per cent as water extract was 77.77% and as ether extract was 82.22% with mycelial growth 20.00% and 16.00%, respectively. The minimum inhibition percentage was noticed with the botanical *C. roseus* in both ether and water extracts (Fig. 2).

Different biochars tested *in vitro* for effects on seed germination, shoot length and root length study at four different concentrations (1, 2, 3 and 4 per cent). Three per cent concentration of the biochar treated seeds exhibited higher germination percentage and the germination was 100 per cent. But at 4% concentration the germination of the seeds decreased and was even less than that of control. The germination of the seeds was hindered at 4% concentration and recorded the lowest germination percentage (33.33%) than control (66.67%).Maximum shoot length 7.73 cm and root length 12.10 cm was observed with 3% Eucalyptus wood (EW) + Citrus wood (CW) + Green house waste (GHW) biochar treated seeds.

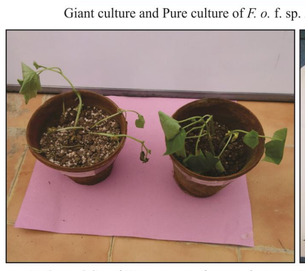
All biochars were effective in reducing the incidence of disease up to 3% concentration with minimum mortality (4.17%) obtained by Eucalyptus wood (EW) + Citrus wood (CW) + Green house waste (GHW), but when the concentration was increased up to 4%, the biochars started to exhibit inhibitory effect on the plant itself and the mortality rate was increased and it was more than the mortality rate that was obtained with 1% concentration of the biochars (Fig. 4 and 5).

CONCLUSION

- Among botanicals, *A. indica* ether extract was highly effective in inhibiting the growth of Pathogen both in water and ether extracts.
- It was found that the germination, shoot and root length was maximum at 3% concentration of Eucalyptus wood (EW) + Citrus wood (CW) + Green house waste (GHW)
- The application of biochar (Eucalyptus wood (EW) + Citrus wood (CW) + Green house waste (GHW)) at 3% concentration was effective in suppressing the disease and exhibited minimum plant mortality.
- The incorporation of the biochars also has the promoting effects on the growth parameters and they also help in build up of the soil microflora by maintaining the soil pH and soil structure.

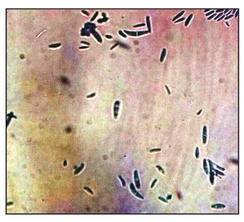
Pathogenicity test

Giant culture and Pure culture of *F. o.* f. sp. *radicis cucumerinum*

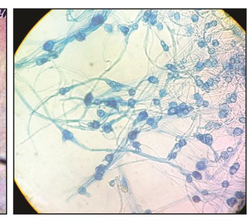


Pathogenicity of *F. oxysporum* f. sp. *radicis cucumerinum* by soil inoculation method

Symptoms developed in seedlings after proving pathogenicity



Conidia of *F. oxysporum* f. sp. *radicis cucumerinum*



Chlamydospore of *F. oxysporum* f. sp. *radicis cucumerinum*

Fig. 1- Pathogenicity test of *Fusarium oxysporum* f.sp. *radicis cucumerinum*

In vitro Evaluation of bio-efficacy of botanicals as water and ether extracts against *F. oxysporum* f.sp. *radicis-cucumerinum*.

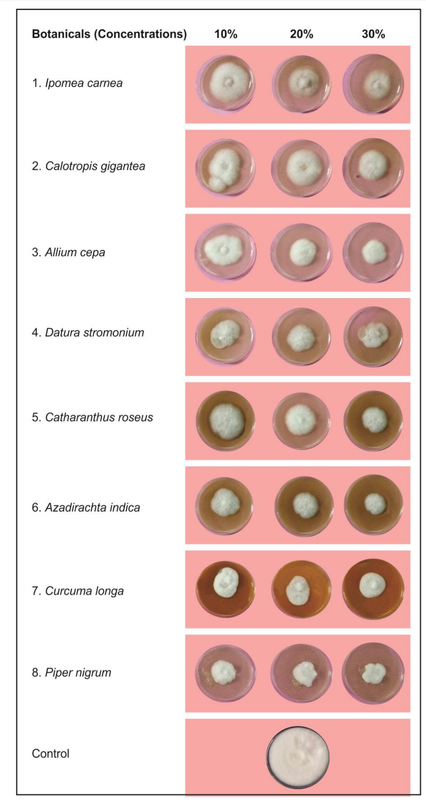


Fig. 2- Bio-efficacy of botanicals as water and ether extracts against *F. oxysporum* f.sp. *radicis-cucumerinum*.

Evaluation of bio-efficacy of different biochars against root and stem rot of cucumber (*F. oxysporum* f.sp. *radicis-cucumerinum*) in pot condition

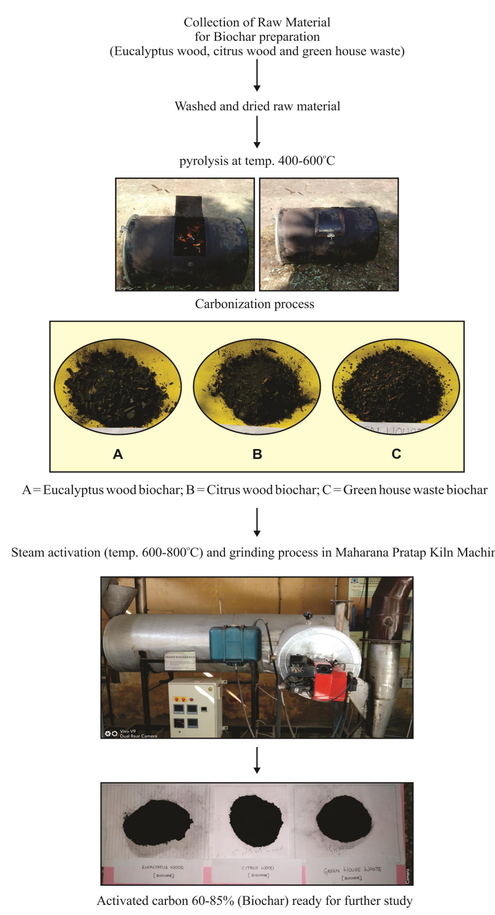


Fig. 3- Flow chart of preparation of Biochar

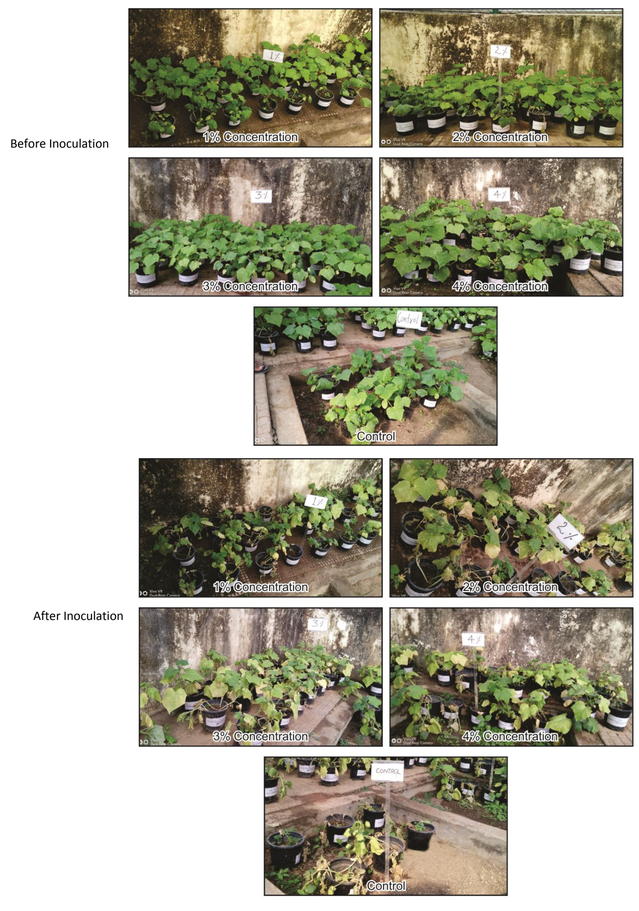


Fig. 4- Efficacy of Biochar on plant mortality

S.No.	Treatments(Bio-chars)	Growth Parameters*							
		1% Con.		2% Con.		3% Con.		4% Con.	
		Shoot Length (cm)*	Root Length (cm)*	Shoot Length (cm)*	Root Length (cm)*	Shoot Length (cm)*	Root Length (cm)*	Shoot Length (cm)*	Root Length (cm)*
1.	Eucalyptus wood	4.97	8.20	5.23	8.47	5.57	9.13	4.23	7.77
2.	Citrus wood	4.63	7.37	4.80	7.67	5.23	8.63	4.06	7.50
3.	Green house waste	5.17	9.00	5.47	9.23	5.83	9.43	4.70	8.23
4.	Eucalyptus wood + Citrus wood	5.57	9.23	5.67	9.50	6.07	9.70	5.10	8.56
5.	Eucalyptus wood + Green house waste	6.47	10.17	6.80	10.63	7.00	11.03	6.10	9.33
6.	Citrus wood + Green house waste	6.03	9.63	6.17	9.70	6.30	10.00	5.60	9.03
7.	Eucalyptus wood + Citrus wood + Green house waste	7.03	11.07	7.27	11.43	7.73	12.10	6.57	10.20
8.	Healthy Control	3.70	5.33	3.60	5.30	3.90	5.83	3.53	5.17
	SEm ±	**0.05**	**0.05**	**0.04**	**0.04**	**0.08**	**0.07**	**0.06**	**0.04**
	CD at 5%	**0.15**	**0.14**	**0.13**	**0.13**	**0.25**	**0.23**	**0.18**	**0.14**

Table 1- Effect of biochar on Growth parameters of cucumber

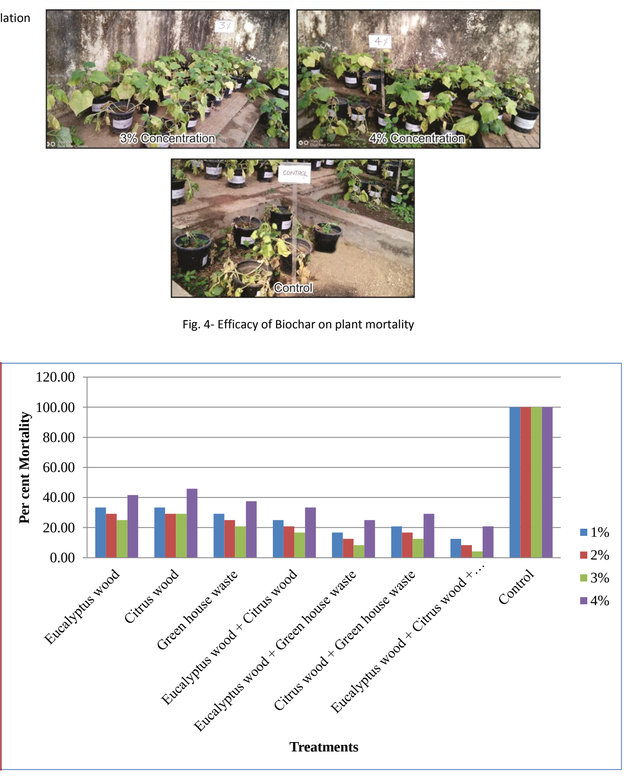


Fig. 5- Efficacy of Biochar on plant mortality at different concentrations