Original Research Article

"Evaluation of Biochars against *Fusarium Oxysporum* f. sp. *radicis-cucumerinum* and their Impact on Growth Parameters of Cucumber in Pot Condition"

ABSTRACT

The present study was conducted under greenhouse conditions to evaluate the efficacy of biochars prepared from different plant parts against the pathogen *Fusarium oxysporum* f. sp. *radicis-cucumerinum* causing root and stem rot of cucumber. Three types of raw materials were used to prepare the biochars (Eucalyptus wood, citrus wood, and greenhouse waste). The prepared biochar was collected and filled into pots at four concentrations (1%, 2%, 3%, and 4% W/W). All biochars were effective in reducing the incidence of the disease up to 3% concentration with minimum mortality (4.17%) obtained with Eucalyptus wood (EW) + Citrus wood (CW) + Greenhouse waste (GHW). The biochars also positively influenced the plant growth parameters like seed germination, root length, and shoot length. Maximum shoot length 7.73 cm and root length 12.10 cm was observed with 3% Eucalyptus wood (EW) + Citrus wood (CW) + Greenhouse waste (GHW) biochar treated seeds.

KEYWORDS: *Cucumber, Biochar, Fusarium oxysporum f. sp. radicis-cucumerinum, Growth parameters ~~Mortility~~Mortality.*

1. INTRODUCTION

Cucumber (*Cucumis sativus* L.) popularly known in India as '*khira*' and gherkins ~~are~~ is extensively grown in the tropics, subtropics, and milder temperate zones of India and it belongs to the family *Cucurbitaceae* and most important vegetable, which is a major source of human edible products and useful fibers. In India, major cucumber growing states are Karnataka, Andhra Pradesh, Assam, Bihar, Jammu Kashmir, Telangana, Madhya Pradesh, Orissa, Kerala, Jharkhand and almost all states with a total production of 1.14 million tons in 78 thousand hectare area (Anon., 2017). The productivity of the crop is more affected in the polyhouse as well as in the field by insects, pests, and diseases. Among them, diseases are one of the major constraints affecting the quality and quantity of the crop. Many diseases have been reported on cucumbers from different parts of the world, but only a few of them cause economic losses. Although an accurate estimate is difficult to obtain, the annual crop loss is probably between 20 and 30% (Anon. 2017). Root and stem rot of cucumber is believed to be caused by a new formae specialis 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 (Punja & Parker, 2000). 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 discolo~~u~~ration 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 soi1 temperatures (17°C) (Vakalounakis, 1996).

Generally, chemical fungicides are used to control Fusarium wilt of cucumber. Unfortunately, these chemical fungicides are not readily biodegradable; tend to persist for years in the environment and few fungi have developed resistance to them. Now a day natural products or extracts are gaining much importance in controlling the fungal diseases of plants because of their environmentally friendly and human human-friendly nature. Howard *et al.* (2011) studied that biochar is the product of organic material, like wood, that is burned in a low low-oxygen environment. This results in a charcoal that acts as a carbon sink for high concentrations of environmental carbon dioxide. Biochar can be effective against both airborne (e.g. *Botrytis cinerea*, different species of powdery mildew) and soil soil-borne pathogens (e.g. *Fusarium* spp., *Phytophthora* spp., *R. solani)* (Bonanomi*et al*., 2015). Amit *et al*. (2014) reported a reduction in soil soil-borne pathogens *Rhizoctonia solani*, in cucumber after treating treatment with biochars.. Drew *et al.* (2012) found that biochar amendment induces resistance to canker canker-causing *Phytophthora* pathogens. Biochars may affect the growth of roots or *mycorrhizae* in the soil and thereby the extent to which the plant takes up water or nutrients from the soil surrounding the biochar particles that is are not directly influenced by the added biochar. In addition, a growing number of studies point to positive influences of biochar addition to soil, such as improvement in the pH of acidic soils (Yamato *et al.,* 2006; Chan *et al.,* 2007), increased cation exchange capacity in organic matter-poor soils (Silber *et al.,* 2010), increased nutrient retention in light soils (Glaser *et al.,* 2002) and enrichment in beneficial soil microorganisms (Kolb *et al.,* 2009; Kolton*et al.,* 2011). Pan *et al.* (2017) used a combination of biochar and wood vinegar as a new type of fertilizer to replace the traditional fertilizer.. With respect toFor the above context some of the plant parts were used to prepare biochars and these were evaluated against the pathogen causing root and stem rot of cucumber under greenhouse conditions in pots.

2. MATERIAL AND METHODS

Isolation and Pathogenicity test:

The infected samples were collected from Horticulture Farm and Polyhouse, Rajasthan College of Agriculture, Udaipur during *Kharif* season-2019 after 30 days of sowing Standard protocol was followed for isolation of test pathogen The pathogenicity test was conducted on the susceptible variety (Cucumber Long Desi) and pathogenicity was confirmed and the inoculum was prepared using sorghum grains for mass production which was used for further studies (Fig. 1 & Fig. 2).

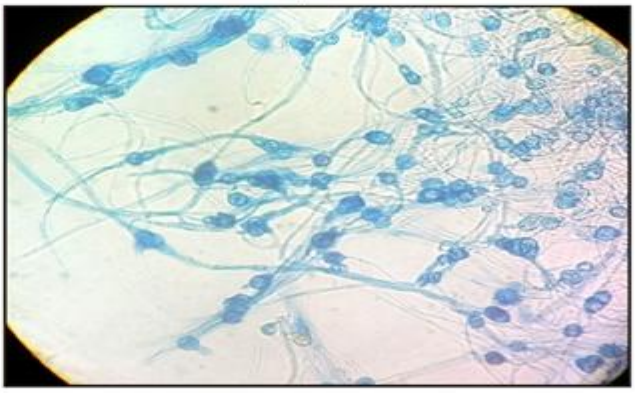
Infected field view | Typical symptoms on stem | Infected root

Figure 1. Infected root and stem rot caused by *F. oxysporum* f. sp. *radicis-cucumerinum*

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

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

Symptoms developed in seedlings after proving pathogenicity



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

Figure 2. Giant culture and ~~P~~pure culture, pathogenicity, symptoms and chlamydospore.

Preparation of biochar

Three types of raw materials (Eucalyptus wood, citrus wood, and green~~-~~house waste) were collected and dried under shade for a week. Biochar was prepared by following the standard protocol (mention reference). The prepared biochar was collected and filled into pots at four concentrations (1%, 2%, 3% and 4% W/W). The cucumber susceptible variety (cucumber Long desi) seeds were used for sowing, 8 seeds were planted in each pot~~s~~ and were watered regularly. Three replications of each treatment ~~was~~ were maintained and control pots were also employed for comparison (Fig. 3).

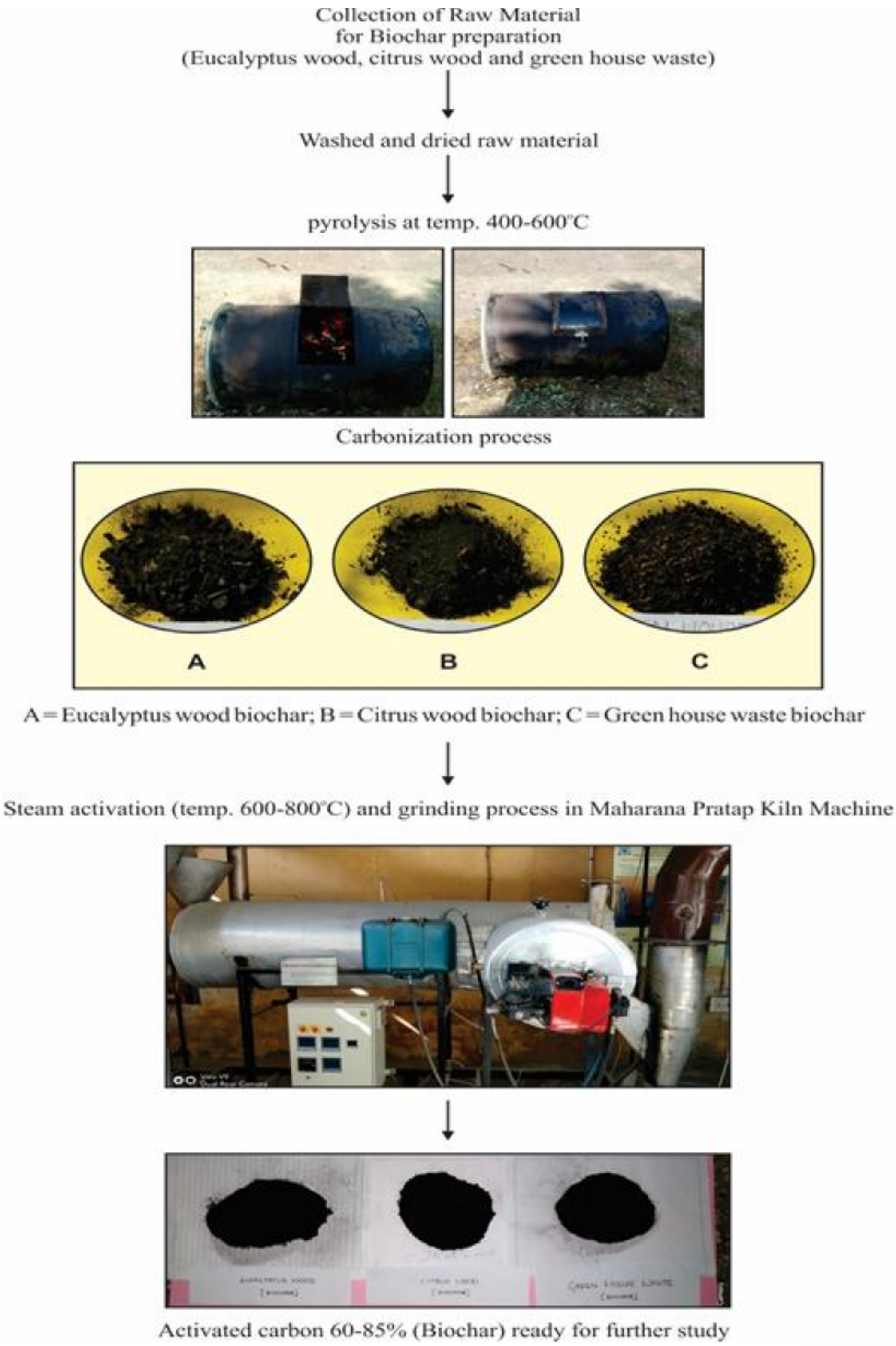


Figure 3. Flow chart of biochar preparation at CTAE, MPUAT, Udaipur

Inoculation of inoculum and observations:

For the expression of disease, sorghum grain inoculum was put into the soil for pathogen multiplication and dry conditions were maintained for disease expression. The dose of inoculum was used as spore suspension $5x10^5$spores/ml. After 7 days of the inoculation, the observations of per-cent Plant mortality were recorded. Counted the number of wilted plants and calculated using the formula,

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

Evaluation of the impact of biochar on the growth parameters of cucumber:

The effect of biochars on the growth characteristics like germination percentage, root length and shoot length were evaluated under *in-vitro* conditions. Four seeds were placed in the Petri plate held with two layered sterilized blotter papers and moistened regularly and kept ~~for~~ at room temperature for germination. After 5 days of the experiment, observations were taken for the number of seeds germinated and after 10 days, shoot length and root length were recorded in ~~centimetres~~centimeters. (Figure-4 & Figure-5).

3. RESULTS

Effect of biochar on seed germination:

The germination of the seeds was stable and found to be increasing up to 3% concentration of the biochars and was in the range 66.67-91.67% at 1% concentration and 75.00-100% at 2 and 3% concentration. The germination of the seeds was hindered at 4% concentration and recorded the lowest germination percentage (33.33%) than the control (66.67%) exhibited in Fig.4.

Effect of biochars on shoot length:

The significantly maximum shoot length of the seedlings (7.73cm) was observed when they were treated with 3% Eucalyptus wood (EW) + Citrus wood (CW) + Greenhouse waste (GHW), followed by the treatments Eucalyptus wood (EW) + Greenhouse waste (GHW), Citrus wood (CW) + Greenhouse waste (GHW), Eucalyptus wood (EW) + Citrus wood (CW), Green-house waste (GHW), Eucalyptus wood (EW) and Citrus wood (CW) which recorded shoot length 7.00, 6.30, 6.07, 5.83, 5.57 and 5.23 cm, respectively presented in Table 1, Fig. 5& Fig. 6.

The significant~~ly~~ maximum shoot length of the seedlings (7.27cm) was also observed with 2% Eucalyptus wood + Citrus wood + Greenhouse waste, followed by the treatments Eucalyptus wood + Greenhouse waste-, Citrus wood + Greenhouse waste, Eucalyptus wood + Citrus wood, Green-house waste, Eucalyptus wood and Citrus wood by exhibited shoot length 6.80, 6.17, 5.67, 5.47, 5.23 and 4.80 cm, respectively.

At lower 1% concentration significant~~ly~~ maximum shoot length of Eucalyptus wood + Citrus wood + Greenhouse waste seedling(7.03cm), followed by the treatments Eucalyptus wood + Greenhouse waste, Citrus wood + Greenhouse waste, Eucalyptus wood + Citrus wood, Green-house waste, Eucalyptus wood and Citrus wood by exhibited shoot length of 6.47, 6.03, 5.57, 5.17, 4.97 and 4.63 cm, respectively.

The same treatments exhibited the inhibitory effect on the growth parameters, when the concentration was increased to 4%. The maximum shoot length (6.57cm) was recorded with Eucalyptus wood + Citrus wood + Greenhouse waste, followed by the treatments Eucalyptus wood + Greenhouse waste, Citrus wood + Greenhouse waste, Eucalyptus wood + Citrus wood, Green house waste, Eucalyptus wood and Citrus wood which recorded shoot length 6.10, 5.60, 5.10, 4.70, 4.23 and 4.06 cm, respectively.

Effect of biochars on root length:

The results are presented in Table 1, Fig.5 & Fig.7 that significantly maximum root length of the seedlings (12.10cm) was recorded when they were treated with 3% Eucalyptus wood (EW) + Citrus wood (CW) + Greenhouse waste (GHW), followed by the treatments Eucalyptus wood (EW) + Greenhouse waste (GHW), Citrus wood (CW) + Greenhouse waste (GHW), Eucalyptus wood (EW) + Citrus wood (CW), Green-house waste (GHW), Eucalyptus wood (EW) and Citrus wood (CW) which recorded root length 11.03, 10.00, 9.70, 9.43, 9.13 and 8.63 cm, respectively.

Significantly maximum root length of the seedlings (11.43cm) was also observed when they were treated with 2% Eucalyptus wood (EW) + Citrus wood (CW) + Greenhouse waste (GHW), followed by the treatments Eucalyptus wood (EW) + Greenhouse waste (GHW), Citrus wood (CW) + Greenhouse waste (GHW), Eucalyptus wood (EW) + Citrus wood (CW), Green-house waste (GHW), Eucalyptus wood (EW) and Citrus wood (CW) by exhibited root length 10.63, 9.70, 9.50, 9.23, 8.47 and 7.67 cm, respectively.

At lower 1% concentration significantly maximum root length of Eucalyptus wood (EW) + Citrus wood (CW) + Greenhouse waste (GHW) seedlings (11.07cm), followed by the treatments Eucalyptus wood (EW) + Greenhouse waste (GHW), Citrus wood (CW) + Greenhouse waste (GHW), Eucalyptus wood (EW) + Citrus wood (CW), Green-house waste (GHW), Eucalyptus wood (EW) and Citrus wood (CW) by exhibited root length 10.17, 9.63, 9.23, 9.00, 8.20 and 7.37 cm, respectively.

The same treatments exhibited the inhibitory effect on the growth parameters, when the concentration was increased to 4%. The maximum root length (10.20cm) was also recorded with Eucalyptus wood (EW) + Citrus wood (CW) + Greenhouse waste (GHW), followed by the treatments Eucalyptus wood (EW) + Greenhouse waste (GHW), Citrus wood (CW) + Greenhouse waste (GHW), Eucalyptus wood (EW) + Citrus wood (CW), Green-house waste (GHW), Eucalyptus wood (EW) and Citrus wood (CW) which recorded root length 9.33, 9.03, 8.56, 8.23, 7.77 and 7.50 cm, respectively. Data is presented in Table 1, Fig.6 Fig.7 & Fig. 5.

Table 1. Effect of different Biochars on growth parameters at different concentrations

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 + Greenhouse waste	6.47	10.17	6.80	10.63	7.00	11.03	6.10	9.33
6.	Citrus wood + Greenhouse waste	6.03	9.63	6.17	9.70	6.30	10.00	5.60	9.03
7.	Eucalyptus wood + Citrus wood + Greenhouse 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**

*Mean of three replications.

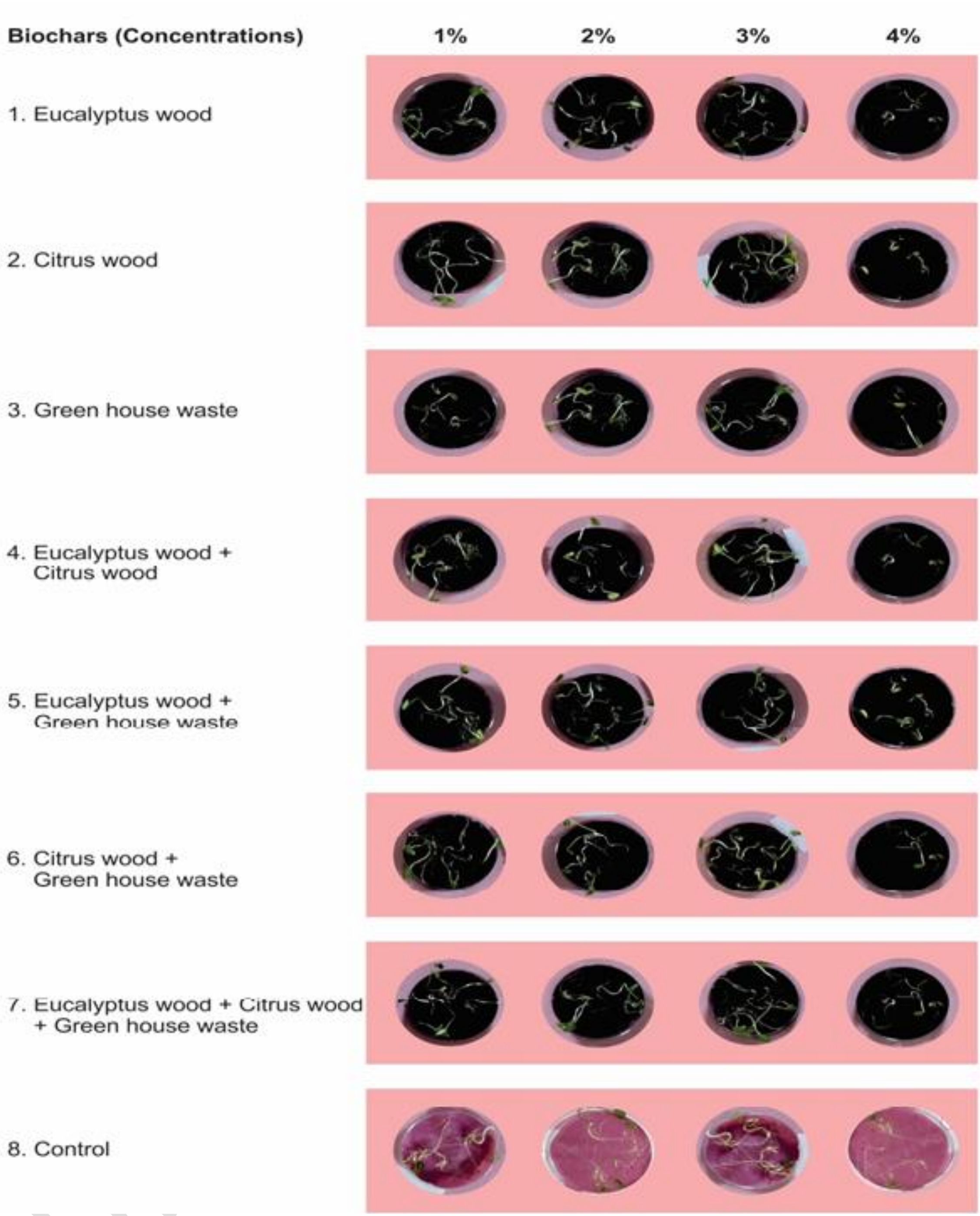


Figure 4. Effect of different biochars on germination of cucumber seeds

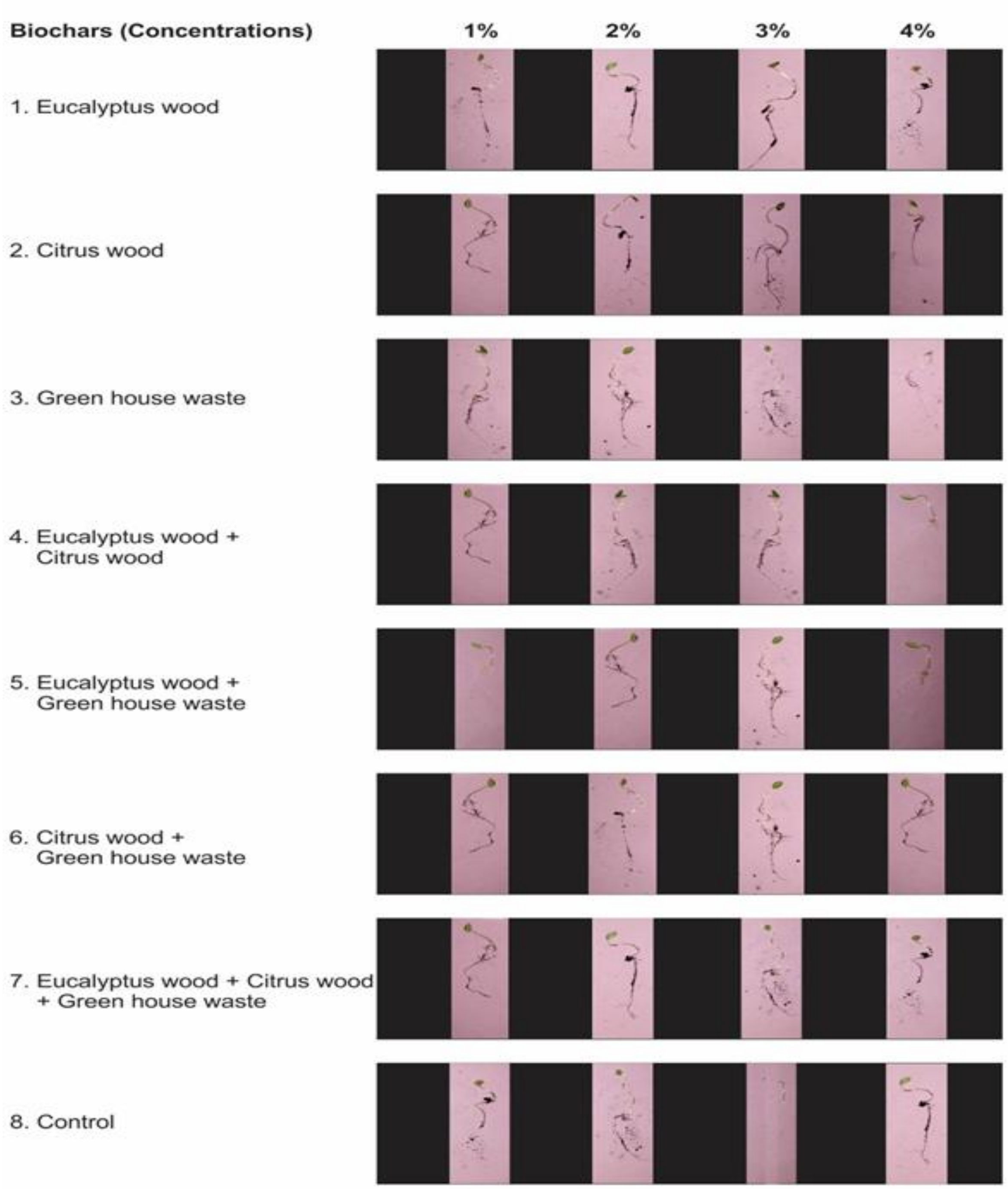


Figure 5. Effect of different biochars on shoot and root length of cucumber seedlings

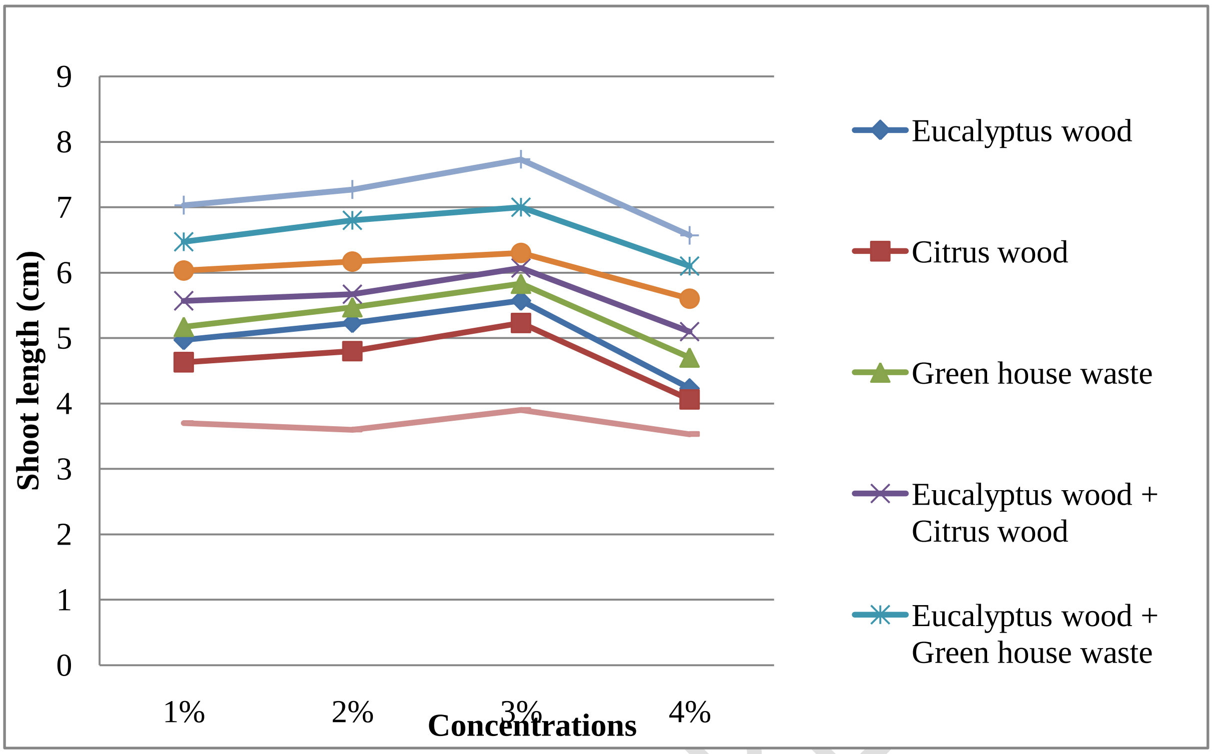


Figure 6: Effect of different Biochars on growth parameter (Shoot length) at different concentrations

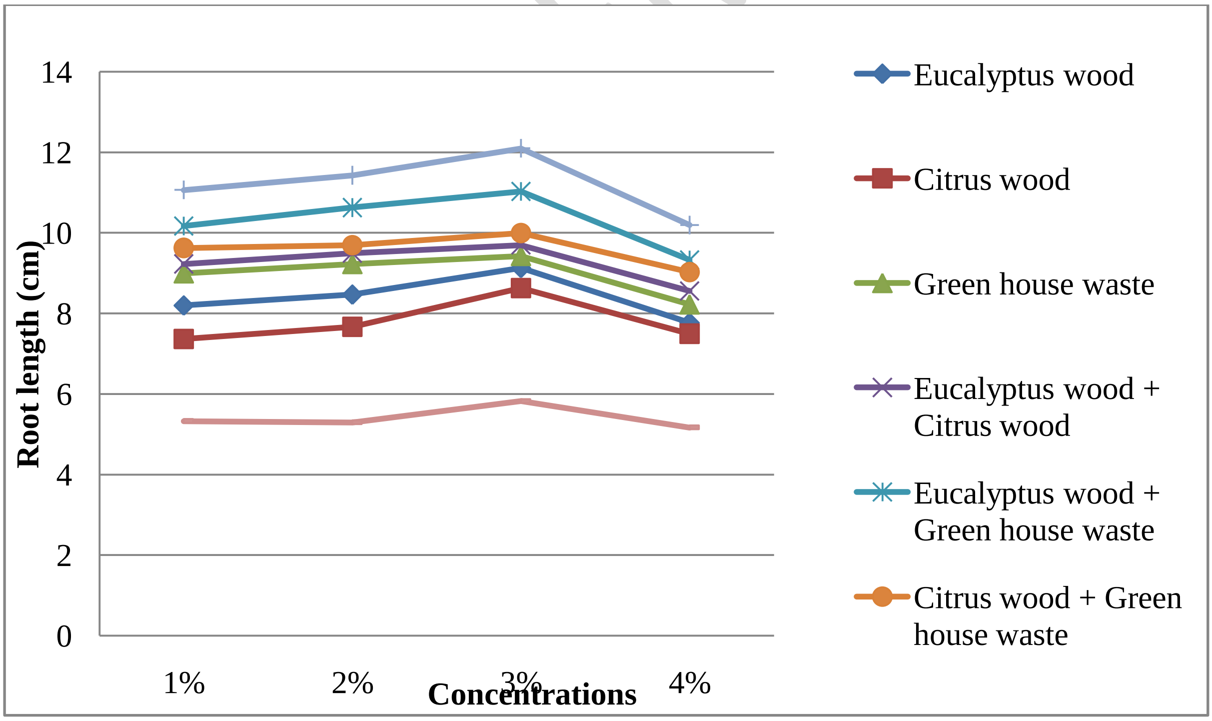


Figure 7: Effect of different Biochars on growth parameter (Root length) at different concentrations

Evaluation of bio-efficacy of different biochars against *F. oxysporum* f. sp. *radicis-cucumerinum* in pot condition

Seven treatments of biochar were evaluated in pot condition against *F. oxy*sporum f. sp. *radicis-cucumerinum* at four concentrations *viz*., 1, 2, 3 and 4 per cent by *wt/wt*. Data are presented in Table 2, Fig.8& Fig. 9,10 and 11.

At 1 per-cent

The treatment, Eucalyptus wood (EW) + Citrus wood (CW) + Greenhouse waste (GHW) was found very effective in inhibiting the disease root and stem rot of cucumber with minimum mortality of 12.5%, followed by Eucalyptus wood (EW) + Greenhouse waste (GHW), Citrus wood (CW) + Greenhouse waste (GHW), Eucalyptus wood (EW) + Citrus wood (CW) and Green-house waste (GHW) with mortality rate 16.7, 20.8, 25.0 and 29.2 percent, respectively. Eucalyptus wood (EW) and Citrus wood (CW) both of them recorded a maximum per-cent plant mortality as of (33%) compared to the rest of the treatments.

All the treatments were significantly effective in controlling the disease over control, but treatment with Eucalyptus wood (EW) + Citrus wood (CW) + Greenhouse waste (GHW) was significantly superior over the rest of the treatments and recorded minimum per-cent mortality (12.5%) among all the treatments.

At 2 per-cent

The treatment, Eucalyptus wood (EW) + Citrus wood (CW) + Greenhouse waste (GHW) was also found very effective in inhibiting the disease root and stem rot of cucumber with minimum mortality of 8.33%, followed by Eucalyptus wood (EW) + Greenhouse waste (GHW), Citrus wood (CW) + Greenhouse waste (GHW), Eucalyptus wood (EW) + Citrus wood (CW) and Green-house waste (GHW) with mortality rate 12.5, 16.67, 20.83 and 25.00 percent, respectively. Eucalyptus wood (EW) and Citrus wood (CW) both of them recorded a maximum per-cent mortality as of (29.17%) compared to the rest of the treatments. All the treatments were significantly effective in controlling the disease over control, but treatment with Eucalyptus wood (EW) + Citrus wood (CW) + Greenhouse waste (GHW) was significantly superior over the rest of the treatments and recorded minimum per-cent mortality (8.33%) among all the treatments.

At 3 per-cent

The treatment, Eucalyptus wood (EW) + Citrus wood (CW) + Greenhouse waste (GHW) was also found very effective in inhibiting the disease root and stem rot of cucumber with minimum mortality of 4.17%, followed by Eucalyptus wood (EW) + Greenhouse waste (GHW), Citrus wood (CW) + Greenhouse waste (GHW), Eucalyptus wood (EW) + Citrus wood (CW), Green-house waste (GHW) and Eucalyptuswood (EW) with mortality rate 8.33, 12.50, 16.67, 20.83 and 25.00 percent respectively. Citrus wood (CW) recorded a maximum per-cent mortality as of (29.17%) compared to the rest of the treatments. All the treatments were significantly effective in controlling the disease over control, but treatment with Eucalyptus wood (EW) + Citrus wood (CW) + Greenhouse waste (GHW) was significantly superior over the rest of the treatments and recorded minimum per cent mortality (4.17%) among all the treatments.

At 4 per-cent

The increase in the concentration of the treatments didn't give satisified results instead the mortality rate increased and was more than that at 1%. The treatment, Eucalyptus wood (EW) + Citrus wood (CW) + Greenhouse waste

(GHW) recorded a mortality of 20.83%, followed by Eucalyptus wood (EW) + Greenhouse waste (GHW), Citrus wood (CW) + Greenhouse waste (GHW), Eucalyptus wood (EW) + Citrus wood (CW), Green-house waste (GHW) and Eucalyptuswood (EW) with mortality rate 25.00, 29.17, 33.33, 37.50 and 41.67 percent, respectively. Citrus wood (CW) recorded a maximum per-cent mortality ~~as~~ of (45.83%) compared to the rest of the treatments. All the treatments were significantly effective in controlling the disease over control, but treatment with Eucalyptus wood (EW) + Citrus wood (CW) + Greenhouse waste (GHW) was significantly superior over the rest of the treatments and recorded minimum per cent plant mortality (20.83%) among all the treatments.

Table 2. Effect of different biochar on per-cent plant mortality in the management of root and stem rot of cucumber in pot condition

S.No.	Treatments(Bio-chars)	Plant Mortality per-cent (%)*			
		1%	**2%**	**3%**	**4%**
1.	Eucalyptus wood	33.3 (35.16)	29.17 (32.57)	25.00 (29.99)	41.67 (40.16)
2.	Citrus wood	33.3 (35.16)	29.17 (32.57)	29.17 (32.57)	45.83 (42.57)
3.	Green-house waste	29.2 (32.57)	25.00 (29.99)	20.83 (26.89)	37.50 (37.75)
4.	Eucalyptus wood + Citrus wood	25.0 (29.99)	20.83 (26.89)	16.67 (23.79)	33.33 (35.16)
5.	Eucalyptus wood + Greenhouse waste	16.7 (23.79)	12.50 (20.70)	8.33 (13.80)	25.00 (29.99)
6.	Citrus wood + Greenhouse waste	20.8 (26.89)	16.67 (23.79)	12.50 (20.70)	29.17 (32.57)
7.	Eucalyptus wood + Citrus wood + Greenhouse waste	12.5 (20.67)	8.33 (13.80)	4.17 (6.90)	20.83 (26.89)
8.	Healthy Control	100.0 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
	SEm ±	**2.21**	**3.16**	**3.89**	**2.08**
	CD at 5%	**6.69**	**9.57**	**11.76**	**6.29**

*Mean of three replications; Figures in parentheses are arcsine √ per-cent angular transformed values

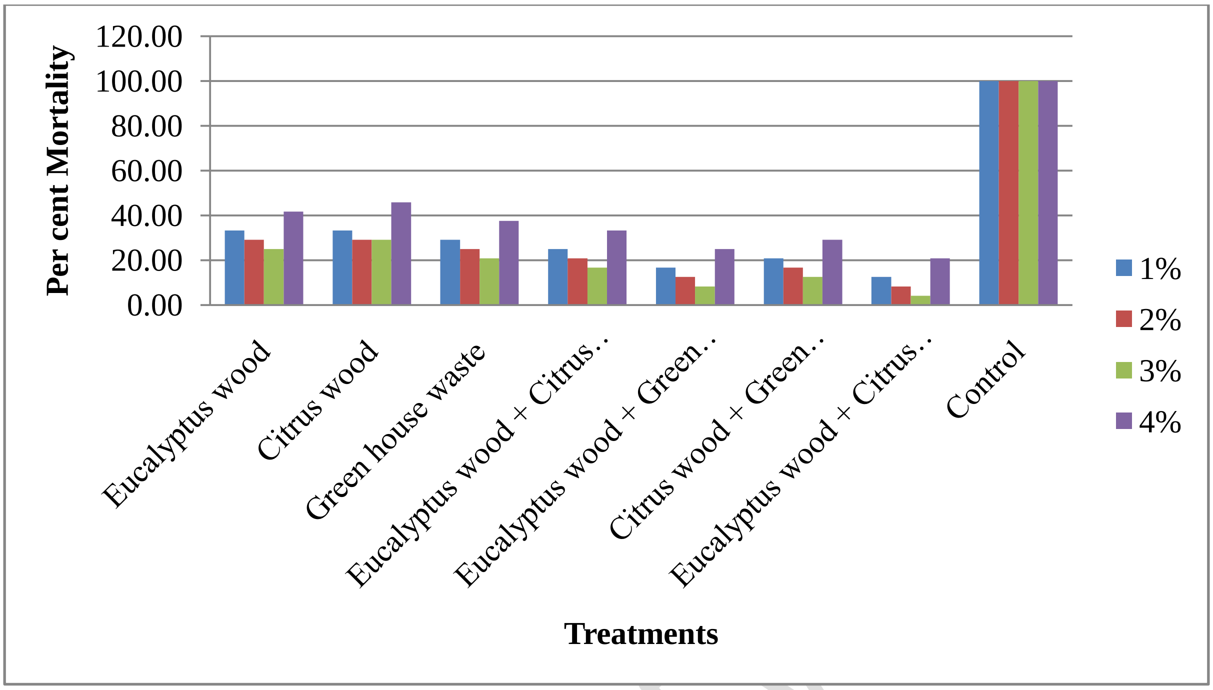
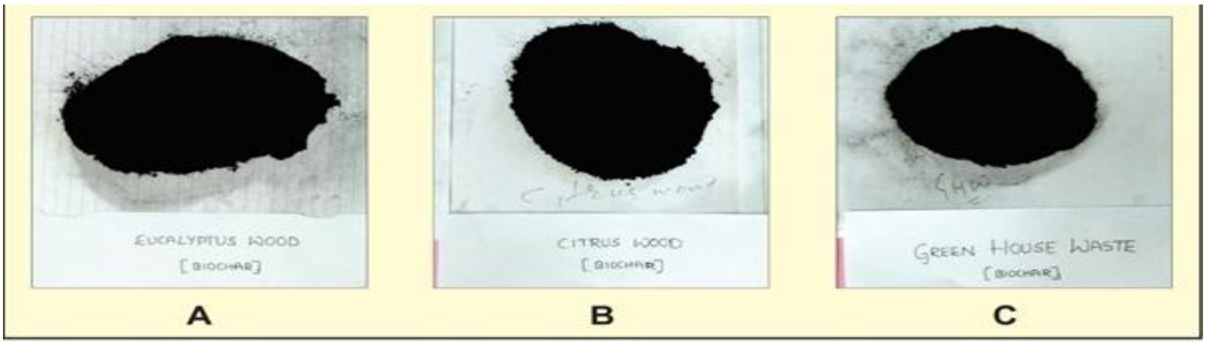


Figure 8: Effect of different Biochars on per-cent plant mortality in the management of *F. oxysporum* f. sp. *radicis-cucumerinum*



A = Eucalyptus wood biochar; B = Citrus wood biochar; C = Green house waste biochar

Figure 9. Preparation of Pot mixture into different concentrations and sowing

Figure 10. Plant stand before inoculation of the pathogen *F. oxysporum* f. sp. *radicis-cucumerinum*

Figure 11. Effect of biochars at different concentrations on plant mortality in the management of root and stem rot of cucumber

4. DISCUSSION

The biochars exhibited their effect on the growth parameters of cucumber. Three per-cent concentration of the ~~biochar~~ biochar-treated seeds exhibited a higher germination percentage and the germination was 100 per-cent. The germination of the seeds was hindered at 4% concentration and recorded the lowest germination percentage (33.33%) than control (66.67%). Kucukakyuz *et al*. (2016) found that ~~S~~shoot growth parameters increased significantly after low and medium doses of biochar application in infertile soil in the case of wheat. The shoot and root length of the seedlings of cucumber were also influenced by the biochar concentrations. Maximum shoot length 7.73 cm and root length 12.10 cm was observed with 3% Eucalyptus wood (EW) + Citrus wood (CW) + Greenhouse waste (GHW) biochar treated seeds. Pan *et al.* (2017) showed that the combined addition of biochar with wood vinegar had the greatest promotion effect on the plant growth of cucumber. Compared to the control treatment, the biochar addition with wood vinegar significantly increased the plant height, root length, root volume and root tips by 29.7, 117, 121 and 76.1%, respectively.

All biochars were effective in reducing the incidence of the disease up to 3% concentration with minimum mortality (4.17%) obtained by Eucalyptus wood (EW) + Citrus wood (CW) + Greenhouse waste (GHW), but when the concentration was increased up to 4%, the biochars started to exhibit an 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. The Citrus wood biochar was found to have a maximum mortality rate of 45.83% at 4% concentration. Amit *et al.* (2014) observed suppression of damping off disease by treatment with 1% Eucalyptus biochar and 0.5% greenhouse waste biochar. Frenkel *et al.* (2017) reported that >3% concentration of the biochars were was ineffective in controlling the disease.

5. CONCLUSION

The germination, shoot and root length was maximum at 3% concentration of Eucalyptus wood (EW) + Citrus wood (CW) + Greenhouse waste (GHW). Similarly, a 4% concentration of the biochars was toxic and inhibited the growth of seedlings with minimum germination, shoot and root length. The application of biochar (Eucalyptus wood (EW) + Citrus wood (CW) + Greenhouse waste (GHW)) at 3% concentration was effective in suppressing the disease and exhibited minimum plant mortality of 4.17% but at 4% concentration the biochars exhibited toxic effects and the mortality rate was increased. The possible reason for the increase in mortality on an increase in the concentration of the biochar may be the toxic effect of the chemicals in the biochar or the alteration of the soil characters like p^H, EC, etc. which may have affected the survivability of the beneficial microorganisms present in the soil. The biochars Biochars can also be included in the management of the disease up to the concentration of 3% as they are effective in reducing the mortality of plants. The incorporation of the biochars also has the promoting effects on the growth parameters and they also help in the build-up of the soil microflora by maintaining the soil pH and soil structure.

Statistical Analysis:

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

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