

**“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, green house waste). The prepared biochar were collected and filled into pots at four concentrations (1%, 2%, 3% and 4% W/W). All biochars were effective in reducing the incidence of 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 Mortality.*

**1. INTRODUCTION**

Cucumber (*Cucumis sativus* L.) popularly known in India as 'khira' and gherkins are extensively grown in tropics, subtropics and milder temperate zones of India and it belongs to family *Cucurbitaceae* and most important vegetable, which is 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 total production 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 field by insects, pest and diseases. Among them, diseases are one of the major constraints affecting quality and quantity of the crop. Many diseases have been reported on cucumbers from different part of the world, but only 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 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 (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 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 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 environmental friendly and human friendly nature. Howard *et al.* (2011) studied that 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. Biochar can be effective against both airborne (e.g. *Botrytis cinerea*, different species of powdery mildew) and soil borne pathogens (e.g. *Fusarium* spp., *Phytophthora* spp., *R. solani*) (Bonanomial *et al.*, 2015). Amit *et al.* (2014) reported reduction in soil borne pathogens *Rhizoctonia solani*, in cucumber after treating with biochars.. Drew *et al.* (2012) found that biochar amendment induces resistance to canker causing *Phytophthora* pathogens. Biochars may affect 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 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 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; Koltone *et al.*, 2011). Pan *et al.* (2017) used combination of biochar and wood vinegar as a new type of fertilizer to replace the traditional fertilizer.. With respect to 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
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Figure 1. Infected root and stem rot caused by *F. oxysporum* f. sp. *radicis-cucumerinum*



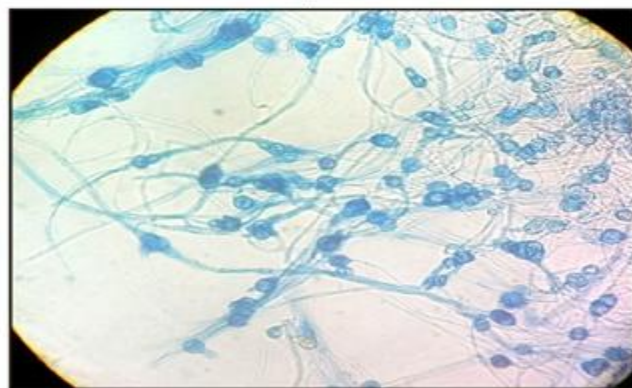
Giant culture and Pure culture of *F. o. f. sp. radicis cucumerinum*



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 Pure culture, pathogenicity, symptoms and chlamydospore.

### Preparation of biochar

Three types of raw materials (Eucalyptus wood, citrus wood, green house waste) were collected and dried under shade for a week. Biochar was prepared by following 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 pots and were watered regularly. Three replications of each

treatment was maintained and control pots were also employed for comparison (Fig. 3).

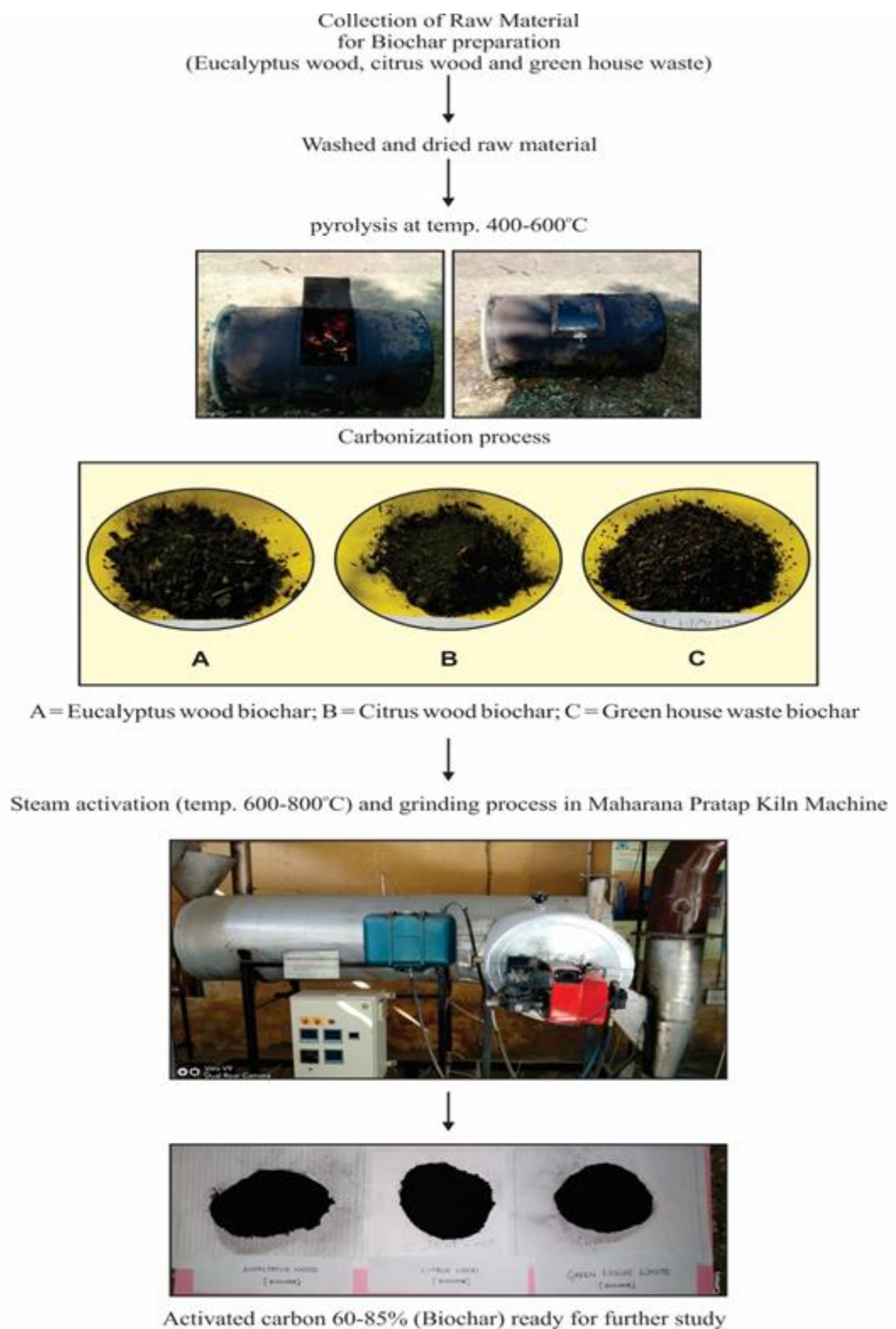


Figure 3. Flow chart of biochar preparation at CTAE, MPUAT, Udaipur

### **Inoculation of inoculum and observations:**

For the expression of disease, sorghum grain inoculum was put to the soil for pathogen multiplication and dry conditions were maintained for disease expression. The dose of inoculum was used as spore suspension  $5 \times 10^5$  spores/ml. After 7 days of the inoculation the observations of per cent Plant mortality were recorded. Counted number of wilted plant and calculated using the formula,

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

### **Evaluation of impact of biochar on growth parameters of cucumber:**

The effect of biochars on the growth characters 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 room temperature for germination. After 5 days of experiment, observations were taken for number of seeds germinated and after 10 days, shoot length and root length were recorded in centimetres. (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 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 significantly 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 significantly 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 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
	<b>SEm ±</b>	<b>0.05</b>	<b>0.05</b>	<b>0.04</b>	<b>0.04</b>	<b>0.08</b>	<b>0.07</b>	<b>0.06</b>	<b>0.04</b>
	<b>CD at 5%</b>	<b>0.15</b>	<b>0.14</b>	<b>0.13</b>	<b>0.13</b>	<b>0.25</b>	<b>0.23</b>	<b>0.18</b>	<b>0.14</b>

\*Mean of three replications.

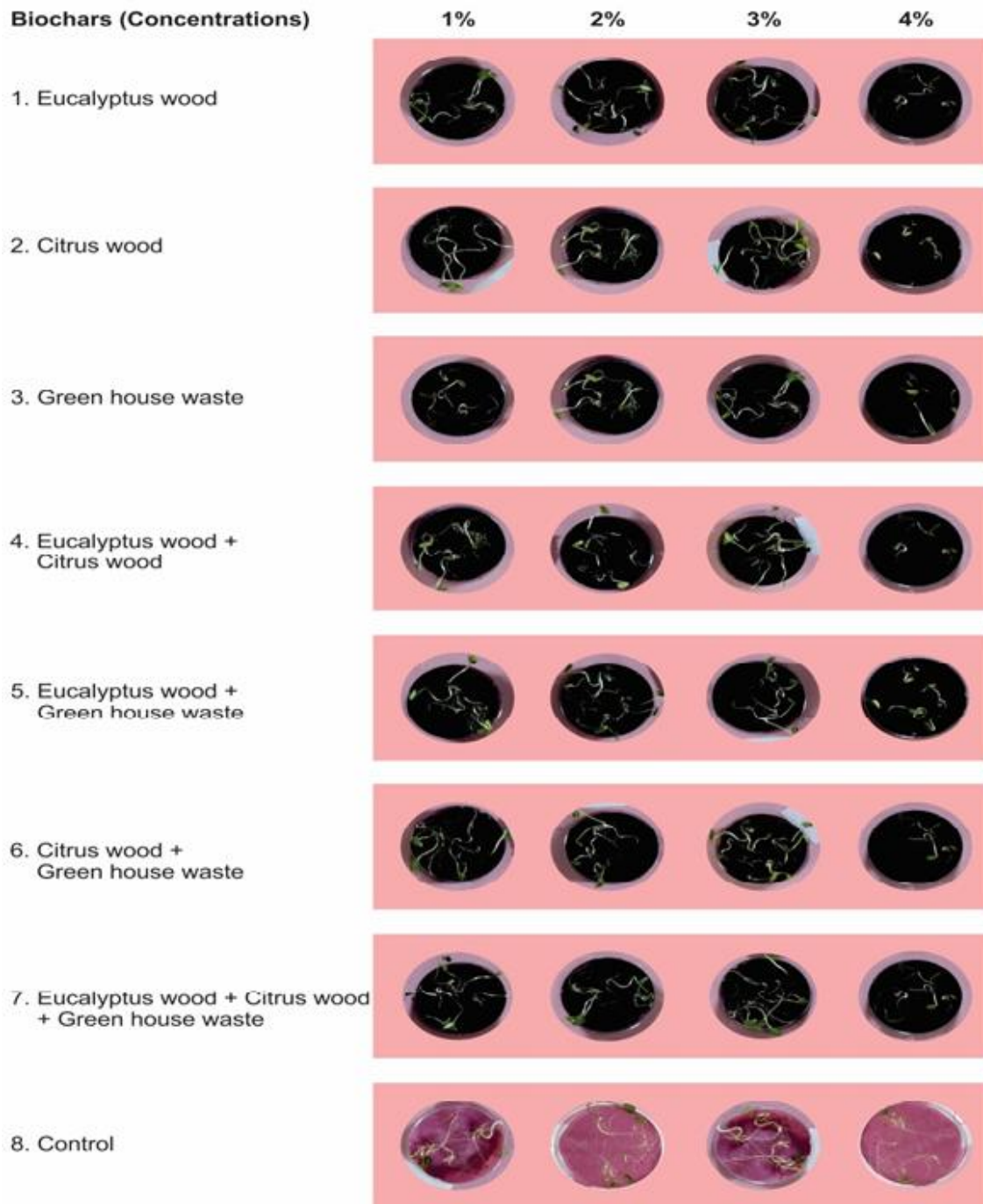


Figure 4. Effect of different biochars on germination of cucumber seeds

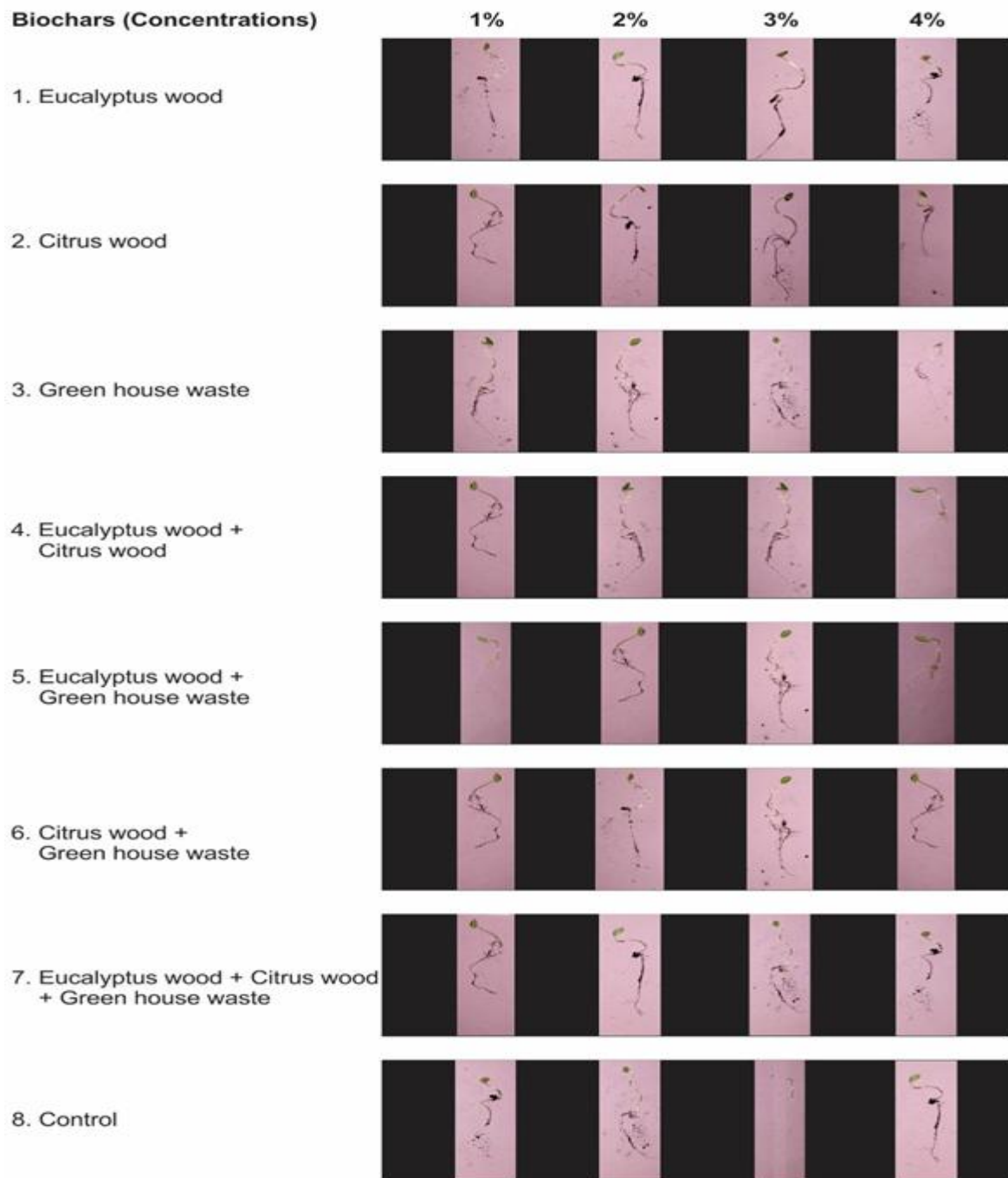


Figure 5. Effect of different biochars on shoot and root length of cucumber seedlings

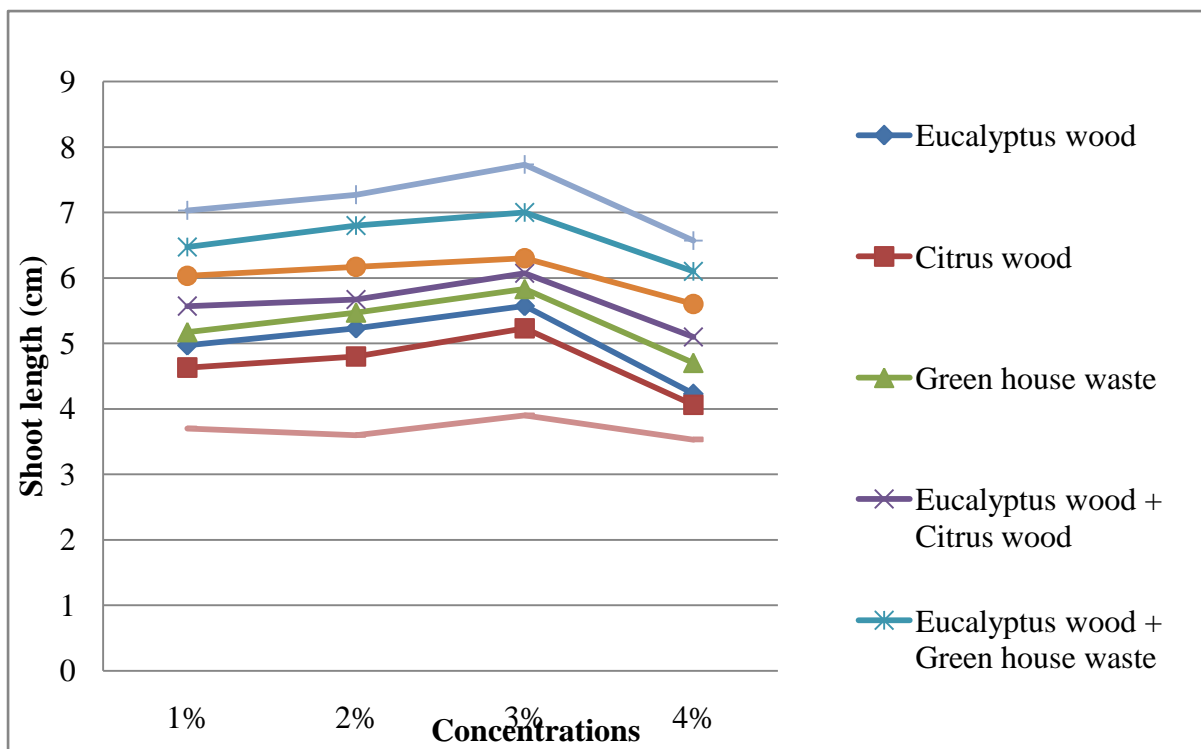


Figure 6: Effect of different Biochars on growth parameter (Shoot length) at different concentrations

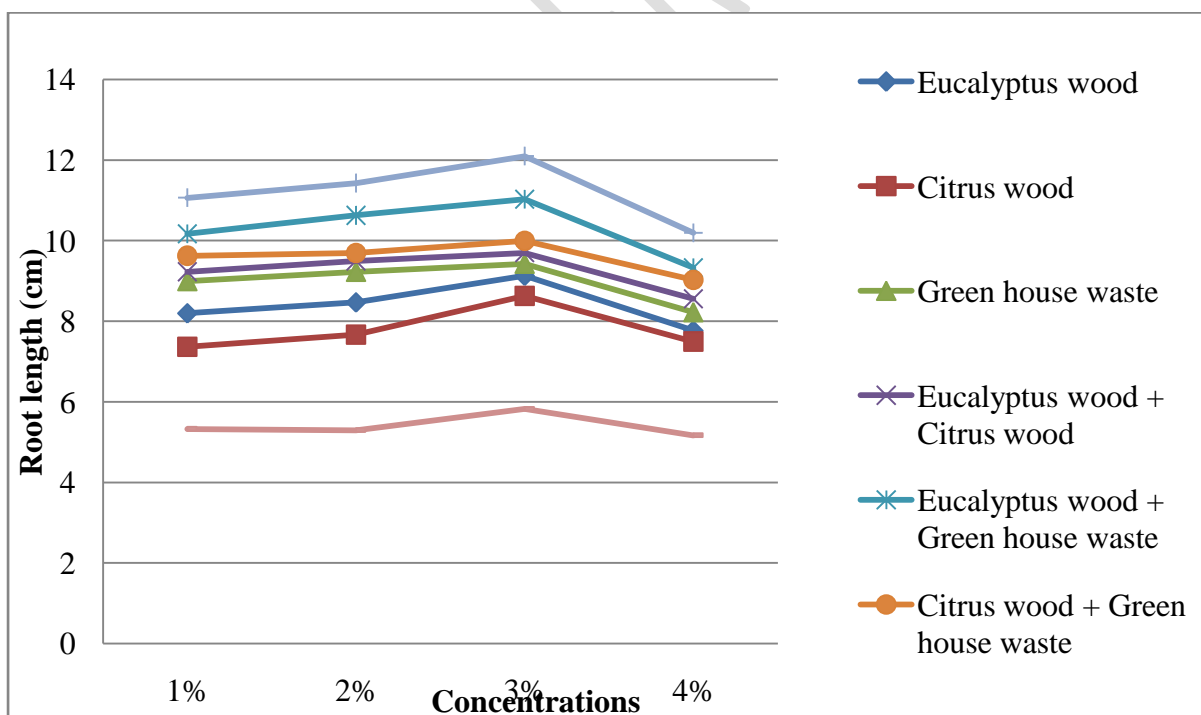


Figure 7: Effect of different Biochars on growth parameter (Root length) at different concentrations

## **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. oxysporum* f. sp. *radicis-cucumerinum* at four concentrations viz., 1, 2, 3 and 4 per cent by *wt/wt*. Data 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 maximum per cent plant mortality as (33%) compared to 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 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. Eucalyptuswood (EW) and Citrus wood (CW) both of them recorded maximum per cent mortality as (29.17%) compared to 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 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 maximum per cent mortality as (29.17%) compared to 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 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 satisfied 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 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 maximum per cent mortality as (45.83%) compared to 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 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)
	<b>SEm ±</b>	<b>2.21</b>	<b>3.16</b>	<b>3.89</b>	<b>2.08</b>
	<b>CD at 5%</b>	<b>6.69</b>	<b>9.57</b>	<b>11.76</b>	<b>6.29</b>

\*Mean of three replications; Figures in parentheses are arcsine  $\sqrt{\text{per cent}}$  angular transformed values

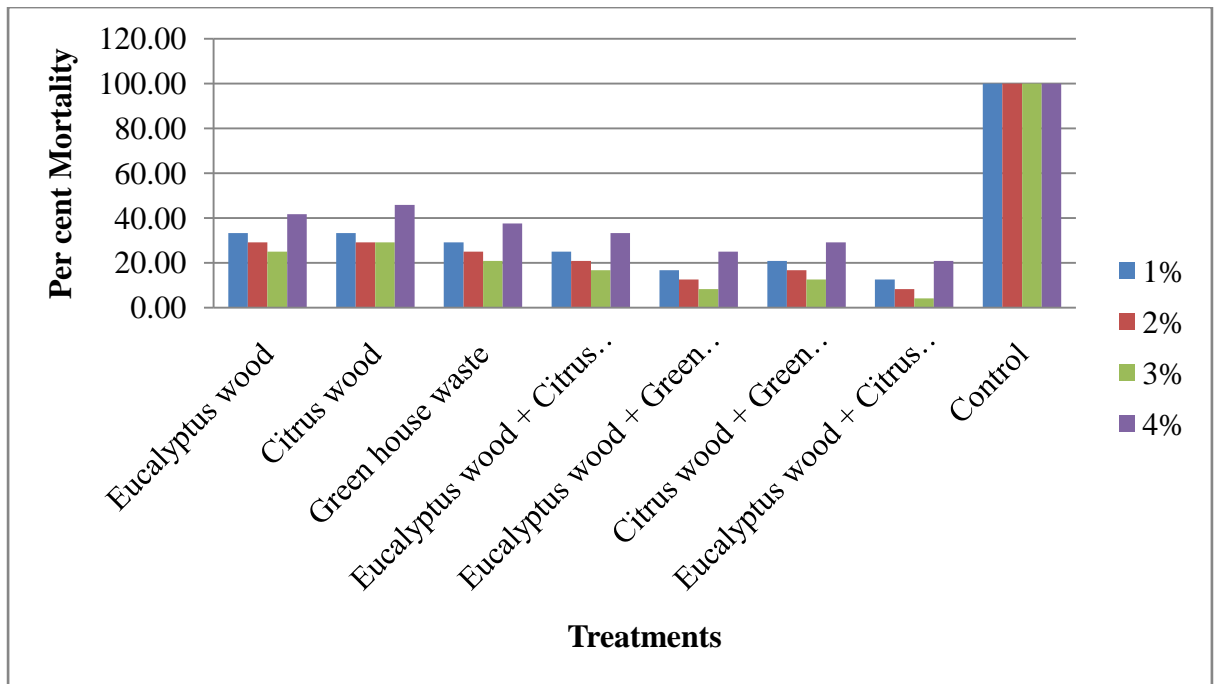
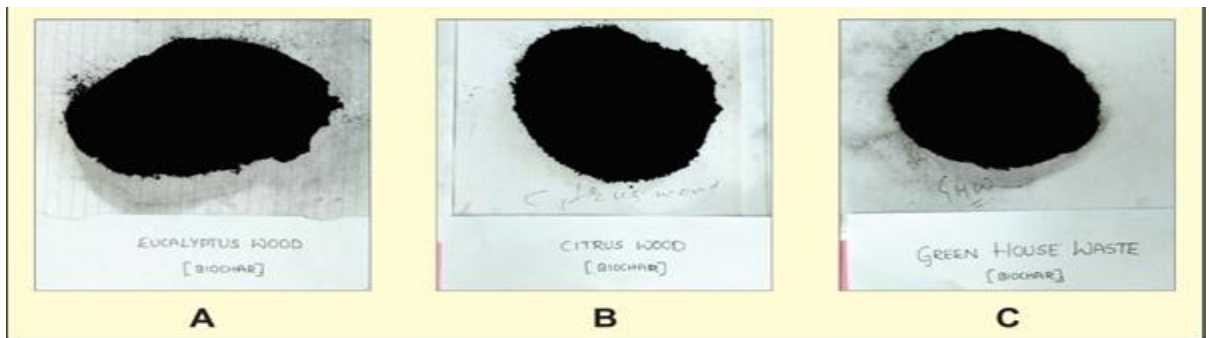


Figure 8: Effect of different Biochars on per cent plant mortality in the management of *F. oxysporum f. sp. radialis-cucumerinum*

UNDER PEER REVIEW



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 treated seeds exhibited 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 Shoot growth parameters increased significantly after low and medium dose of biochar application in infertile soil in 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 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 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 found to have maximum mortality rate 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. Frenkelet *al.* (2017) reported that >3% concentration of the biochars were 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 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 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 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 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 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 coefficient of deviation. For laboratory and pot trials, completely randomized design was followed. Means of the experiments were used to compare for efficacy of treatments.

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