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Original Research Article

Comparative Study of Toxicity Attenuation of Phorate & Chlorpyriphos on *Eisenia fetida* by Rice Straw Biochar

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ABSTRACT:
Biochar is an external organic input added in soil as ameliorative agent against soil pollution. In this study, the impact of biochar on Eisenia fetida's mortality (LC50) of two organophosphates (Phorate and Chlorpyriphos) in the artificial OECD soil was assessed. Acute test was applied to calculate the LC50 of Phorate and chlorpyrifos on the earthworm Eisenia fetida by using artificial soil test. Biochar was derived from rice straw, a major agricultural crop residue at two different pyrolysis temperature 300°C and 500°C with the heating rate of 10°C/min for 3 hours in an inert gas environment with limited supply of oxygen in a tubular muffle furnace. Rice straw biochar was then analyzed physiochemically and morphologically. Results demonstrated that the biochar produced is consistent with available literature. Experiment was set up as per OECD guidelines. Two different biochar RSB300 and RSB500 treatments were applied from 1.5%, 3%, 5%, 6.5%, 8% and 10% application rate for both organophosphates separately to nullify the earthworm's mortality at LC50. The mortality changes were noted after 14 days exposure of treatments at median lethal concentration of pesticides.LC50 of Phorate and CPF were calculated by probit analysis (p value <.005) and found to be 27.436mg/kg and 99.806 mg/Kg respectively. The outcome of correlation analysis between rate of application of biochar vs mortality (LC50) in Eisenia fetida for Phorate and Chlorpyrifos reported that RSB500 Phorate and RSB500 CPF showed statistically significant difference with p values close to 0 and R square values than RSB300 Phorate and RSB300 CPF.

Keywords: *Application rate of biochar, Eisenia fetida, Organophosphates, Earthworm's uptake, Remediation of soil*

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INTRODUCTION

Biochar is the porous carbonaceous product obtained thermochemically from biomass in deficiency of oxygen. Recently, biochar has gained significant attention for its multipurpose applications (Singh *et al.,* 2015). Biochar possesses specific chemical and physical properties which makes it carbon negative and ecofriendly in nature. It can sequester carbon (climate change mitigation) (Lehmann *et al*. 2006), adsorb contaminants, immobilizes heavy metals (Ahmad *et al.,* 2014), surpasses greenhouse gases, applied as soil amendment to enhance soil fertility by incorporating nutrients, increases water holding capacity (soil and water remediation), biofuel production and waste management (Stubble burning like problems) Lehmann, 2007, Lehmann and Joseph 2009, Simmons *et al*., 2021). The success of biochar largely depends on the various physicochemical properties it possesses. These properties directly depend on the feedstock and heating conditions -residence time, heating rate and pyrolysis temperature (Chandra, & Bhattacharya, 2019; Itoh *et al.,* 2020). Figure 1 summarizes the structural and functional integrity of Biochar as a multidimensional application for improving ecosystem balance.

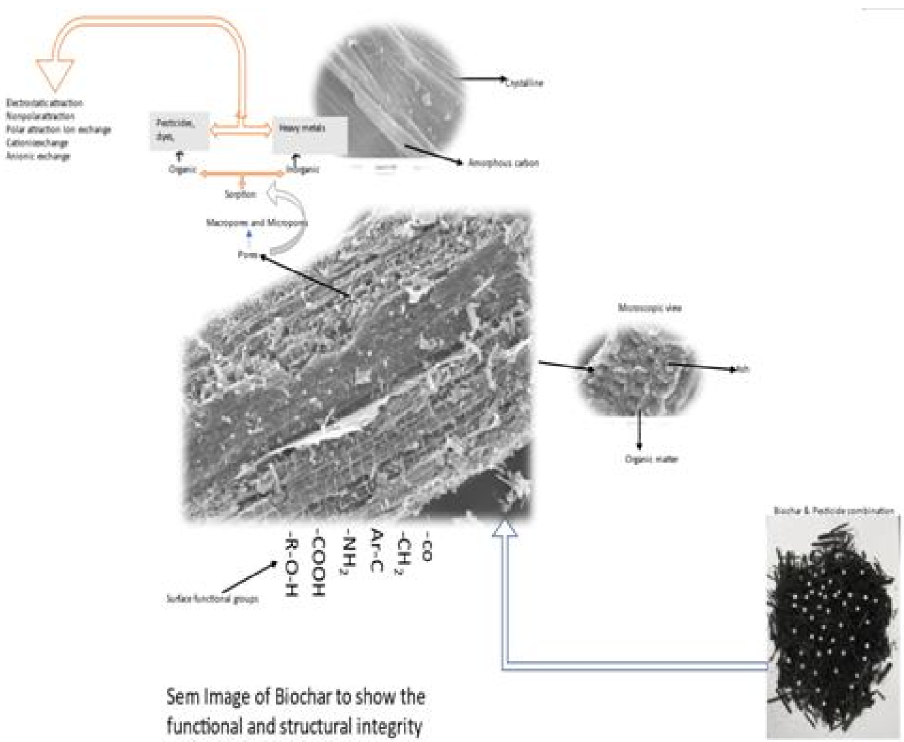


Figure 1: Summarizes the Structural and Functional Integrity of Biochar as a Multifaceted Strategy

Soil pollution by various anthropogenic and natural activities is making the environment hostile for its inhabitant's especially excessive use of pesticides in the fields. Pesticide contamination not only reaches to our plate but also destroying the non-target organism like earthworms (Bakırcı *et al.,* 2014; Soydan *et al.,* 2021). Studies have been reported on sorption and desorption of pesticides by using biochar in soil, in plants and in nontarget organisms like earthworms (Yang *et al*., 2010; Kookana *et al.,* 2009;Mohan *et al*., 2014; Wang *et al*. 2014; Khorram *et al.,* 2016; Zhang *et al.,* 2019; Chen *et al*., 2020). Fomesafen dissipated in biochar amended soil as compared to control. As studied by Khorram *et al*., 2016.Fomesafen concentration was reduced drastically in soil, in plants as well as earthworms after biochar amendment. Half-life was significantly increased from 34 days to 160 days in 2% biochar amendment in soil. Bioavailability of fomesafen (herbicide) in earthworms were declined in soil pore water and further in plants and earthworms in 2% biochar-amended soil to 0.38–45 and 0.47–0.50% compared to the level of the control, respectively. Concluding that biochar amendment can be a suitable option for immobilizing the chemical from soil and safeguarding the non-target organisms. Concentrations of PCB was studied in *Eisenia fetida* tissue and it was reduced by 52% and 88% at 2.8% and 11.1% biochar, respectively. The above-ground biomass and worm survival rates were also increased in biochar amended industrial PCB soil. Hence, concluding that biochar amendment serves the purpose of decreasing the bioavailability of contaminants like PCB in soil and thereby reducing the threat of excessive use of chemicals to humans, environment and non-target organisms (Denyes *et al*., 2012). CPF and carbofuran bioavailability to spring onions (*Alium cepa*) was reduced to 10 % and 25% as compared to control by 1 % biochar produced at 850°Celsius respectively (Kookana *et al*., 2009). Wheat straw biochar sorped HCB 42 times higher than that by soil and reduced uptake of HCB by earthworm (Song *et al*., 2012).

But direct application of biochar in soil to study earthworm's health and on pesticide sorption is scanty. There is a need to study the various parameters that are being studied for the sublethal doses toxicity on earthworms after adding Biochar as soil amendment. The most important is to decide the dose of biochar that needs to apply to maximize the advantages of biochar as amendment. In this study two very toxic organophosphates –Phorate and CPF are taken. *Eisenia fetida* the most recommended species for ecotoxicology by OECD for studying ecotoxicology of soil.

Bioavailability and sorption have enough literature available to prove the success of Biochar to dissipate the contaminants organic or inorganic in soil, plants and earthworm. Comparative study of damage caused by pesticides at sub-lethal doses and pesticide mixed with fixed dose of biochar at the same sub-lethal dose to earthworm is the need of hour. Many pesticides are banned because of their extremely toxic nature but other pesticides with the same chemical formulation are available in the market. Sublethal doses are also causing damage to earthworms as reported in literature (Pelosi *et al*., 2013; Singh *et al*., 2015; Qui *et al*., 2018; Pereira *et al*., 2019). This study recommends the need to investigate rate of application of biochar in soil as amendment to nullify the effect of sublethal doses of pesticides in soil, plants and earthworm. A study was conducted to evaluate the concentration of RSB to nullify mortality at LC50 for Phorate and CPF for the species *Eisenia fetida.* Biochar derived from rice straw at two different pyrolysis temperature RSB300 and RSB500 were added in separate experimental set up in doses 1.5%, 3%, 5%, 6.5%, 8% and 10%. No such studies have been done previously but addition of biochar at different doses to check survival, avoidance, reproduction, other biomarkers and to increase enzymatic activities of earthworms have been done. We hypothesized that there is significant difference between mortality at LC50 of pesticide and at different doses of RSB300and RSB500 for earthworms below 10%. Also, the comparative analysis for the two different RSB will be done and effectiveness will be assessed. Our hypothesis is supported the positive results shown for survival and sorption of contaminants by adding biochar as amendment (Zhang *et al*., 2010; Denyes *et al*., 2012; Mohan *et al*., 2014; Kookana *et al*., 2009. Below 10% biochar rate of application was taken because high dose of biochar leads to the genotoxicity and even death of earthworms (Zhang *et al*., 2019; Khorram *et al*., 2016). Han *et al.,* 2021 studied the direct action of RSB on earthworms in a 14-day incubation experiment in field soil. It was reported that 0.3% to 5% RSB levels in soil were not avoided by earthworms but high doses 7.5% & 15% resulted in avoidance. Below 5% no impact on survival, weight and DNA damage was found. Low doses 0.5 and 1% biochar as soil amendment recommended safe for soil macrofauna.

MATERIALS AND METHOD

The experiment was conducted using commercial formulations of organophosphates and following OECD guidelines for the non-target organism earthworms. The LC50 (median lethal concentration) of both the pesticides were calculated using Probit method by using artificial soil method. The different doses of RSB produced at different pyrolysis temperatures i.e. 300 and 500 were applied in different doses to reduce the mortality (LC50) to below 50% for both the pesticides.

Chemicals and test animals

Commercial formulation of Phorate 10% CG and CPF 20% EC were purchased from local market with permission, of brands Insecticide Indian Ltd. and Krish Agro Industries respectively. Other chemicals were of reagent grade and were purchased from Sigma

Aldrich.

Earthworms are the most commonly used model organisms for ecotoxicological studies. As per OECD guidelines, 1984 and ISO, 1993 *Eisenia fetida* is the recommended species for studying the toxicological aspect of soil. Earthworms were procured from Bhoojevan Organic Firm, Najafgarh (New Delhi) and then were cultured in laboratory conditions at 20°C, 80% humidity with a 12 h dark and 12 h light cycle. Artificial soil was prepared as per OECD guidelines.

Experimental Design

Determination of LC50 median lethal concentration of Phorate and CP

The experiment was conducted as per OECD no. 207 (1984) guidelines for testing of chemicals. The one of the sensitive species *Eisenia fetida* was taken for the experiment.

As per OECD guidelines, artificial soil consisted of 70% industrial quartz, 20% kaolinite clay, 10% organic matter (coconut peat) and calcium carbonate were added to maintain pH in optimum range.

All the components in the mixture are mixed and after that water is added. Moisture content of about 35% dry weight is maintained. Phorate was added in soil on dry weight basis. The doses added 5, 10, 20, 40, 80 mg/kg. The 4 replicates were prepared for each concentration. Adult and well clitellate earthworms weighing 350 -400 mg (ISO,1993) were separated from the culture beds, washed with deionized water and rested in a dish on moist filter paper for 3 hours to get their gut clean. All worms separated were acclimatized in the artificial soil under test conditions for 24 hours. After acclimatization and gut cleaning of earthworms, 10 adults well clitellate worms were released in each pot kept under laboratory conditions for 14 days. Mortality percentage was checked 14th day of the study.

Regularly food was given and moisture was maintained in the pots. Mortality was checked and dead worms were removed on urgent basis. The LC50 of Phorate on *Eisenia fetida* was calculated using log dose/probit regression line method as per Kumar and Singh (2016) with slight modifications. A preliminary range-finding test was done for both pesticide and then concentration at which 100% mortality was observed was evaluated. It was based on treatments in the range 0.01, 0.1, 1.0, 10, 100, 1000 mg/kg (dry weight of artificial soil).

For estimating the LC50, five concentrations in a geometric series are used. The concentration selected were at the range of 0 to 100 mg/kg (dry weight)-5mg, 10mg, 20mg, 40 mg, 80mg/kg soil. Each concentration was prepared in 4 replicates and simultaneously 4 controls were prepared. The mortality was noted at each concentration on the 14th-day interval. Similarly, for CPF the 100% mortality concentration was evaluated on the 14th day. For final estimation of LC50, five different geometric sequences from zero mg/kg to 200 mg/kg were set up in 4 replicates.

CPF contaminated soils were prepared by adding an acetonitrile-CPF solution to artificial soil. Different experimental concentrations of Phorate and CPF were used in acute toxicity tests. Proper mixing was done to ensure the even distribution of insecticide in the soil. Control set up was prepared by adding soil with the solvent only. Set up was left undisturbed for acetonitrile vaporization. Moisture was adjusted 35% and 10 well clitellate mature earthworms were added in each replicate. With the help of plastic wraps beakers were covered to reduce moisture loss and gravimetrically monitored over the experiment duration.

Estimating the concentration of biochar to reduce the mortality at LC50

Once LC50 was evaluated artificial soil was prepared and rice straw derive biochar was added at different doses in the LC50 of both the pesticides. The 14 days artificial soil test was conducted to evaluate the effect of different doses of biochar on the LC50 of the pesticides. Mortality was noted on the 14th day and dose with maximum reduction in mortality was noted. A control was set up only with LC50treatment in the two independent experiments with 3 replicate one for Phorate and other for CPF. Different concentrations of RSB300 and RSB500 were combined with LC50contaminated artificial OECD soil to check the effect on the mortality at LC50from 50% to below.

Biochar Production

Rice straw was collected in harvesting season of rice from the district Rohtak, Haryana (28.8955°N, 76.6066°E). The collected straw was washed with tap water to remove any adherent dust and dirt particles. After cleaning with tap water, the rice straw was oven-dried for 12 h at 80°C. Rice straw cut into pieces and filled in a metal container to be placed in tubular electric muffle furnace to follow the process of slow pyrolysis. As stated by Tripathi *et al*. 2016, the method of slow pyrolysis gives the maximum yield of biochar. The dried rice straw was subjected to pyrolysis at 300°C and500°C for 3 h in a metal container and placed under N_2 atmosphere in a muffle furnace. The heating rate and residence time for the entire study were maintained at 10°C/min. After it gets cooled to room temperature, the biochar were collected and homogenized to pass through a 0.15 mm sieve (Yang *et al* ., 2010) The product of pyrolysis was characterized to confirm its structural integrity and physical properties.

Biochar characterization

The characterization of RSB300 and RSB500 were done as per standard protocol. The physical and chemical characterization as stated by Nartey and Zhao, 2014 were followed. The chemical characteristics analyzed were pH, CEC, Zeta potential at Sophisticated Analytical Instrumentation facility at All India Institute of Medical Science in New Delhi (SAIF, AIIMS New Delhi), FTIR (Alpha, Burker Optics, Ettinngen, Germany) using a working wave number range 400-4000 cm^{-1} analyzed by Origin pro 2018. The physical characteristics were scanning electron microscopy, transmission electron microscopy at SAIF, AIIMS New Delhi and X-ray diffraction (Table Top Miniflex-II, Rigaku, Japan). The pH (ESICO Model 1010 Microprocessor based pH meter) and CEC were measures by soaking the biochar and deionized water in 1: 10 (w/v) for 60 min in a rotatory shaker at 40 rpm. The suspension was then left undisturbed for an hour. After that pH and CEC were measured (Salam *et al.,* 2020). The moisture and ash content were estimated by heating the biochar at 105°C for 24 hours and at 700°C for 1 hour respectively (Aller et al., 2017) in tubular electric muffle furnace.

Statistical Analysis

The LC50 of both the pesticides were obtained by plotting the mortality percentage vs concentration of both the pesticides using Probit analysis. Two-way ANOVA was conducted to compare the main effects of treatment of biochar and mortality (LC50) of pesticides as well as their interaction effects on the treatment of different rates of application of biochar and concentration of pesticide on the mortality. 7x2x2 factorial scheme with three replications, being 7 doses of biochar (0; 1.5; 3; 5; 6.5; 8 and 10%), two pyrolysis temperature (300ºC and 500ºC) and two pesticides (Pest 1-Phorate and Pest 2- CPF), totaling 28 treatments, individual effect of each factor, as well as their interactions on the mortality of organisms (LC50) through the correlationand the mean comparison test (Tukey's test)

RESULTS AND DISCUSSION

LC50 (median lethal concentration) of Phorate and CPF.

Dose and duration relationship has been confirmed during the exposure period. Mortality has been increasing with the increasing concentration of both organophosphates. No mortality was observed in control for any of the experimental setups. The mortality for Phorate was found to be 20% at 10mg/kg and 100% for 100mg/kg. Similarly, 100% mortality was reported at 1000 mg/kg for CPF. Then for CPF second range determining test was done 0, 100, 200, 300, 400, 500, 600, 700, 800, 900 and 1000mg/kg. Hence, the probable range for LC50 is 10mg/kg to 100mg/kg for Phorate and 0mg/kg to 200mg/kg.

For analyzing the LC50of both the pesticides Probit analysis was done. Results were recorded after 14 days of exposure and estimates are cited in Table 1 for Phorate and CPF respectively. At 10mg/kg Phorate mortality is 27.5%. The lowest dose causing 100% mortality is 100mg/kg for Phorate and 200mg/kg for CPF. The LC50 of Phorate and CPF were found to be 27.436mg/kg and 99.806 mg/Kg respectively. Related trends were reported by Kumar & Singh, 2016 for Phorate where LC50 was 22.5mg/kg concluding that Phorate is an “extremely toxic” to non-target organisms in soil. The LC50 of CPF was found to be in accordance with the previous studies in the literature Striping Zhou *et al.,* 2011 mentioned the LC50 to be 116mg/kg and Zhou *et al.,* 2007 reported 91.78 mg/kg for 14 days acute test.

Table 1: RSB physicochemical properties and Literature Reference-Values expressed as mean ±S.D. (N=3). (Wu *et al.,* 2012; Salam *et al.,* 2020)

	RSB300	RSB500
pH	7.43 ± .655	10.45 ± .481
CEC	95.67 ± 2.08	56± 2.65
EC	2.557 ± .295	3.593 ± .342
Ash content	23.933 ± 1.626	32.453 ± 1.105
Biochar yield	33.923 ± .557	29.457 ± .631

Acute toxicity is one out of the many tools to assess the toxic level of any pesticides in ecological niches as it gives results very quick (Alves *et al.,* 2013). Mortality being a close indicator of toxicity along with biomass and reproduction. Some parts of the earthworms were damaged, ravaged, melted, and broken for Phorate. Wang *et al.,* 2012; Gupta & Chakravorty, 2010; Jovana *et al.,* 2014 reported that organophosphates are very detrimental to earthworms at sublethal doses and below RAD (recommended agricultural doses). The results concluded that Phorate is more toxic than CPF to *Eisenia fetida.*

Production of Biochar from Rice straw (Agricultural Residue)

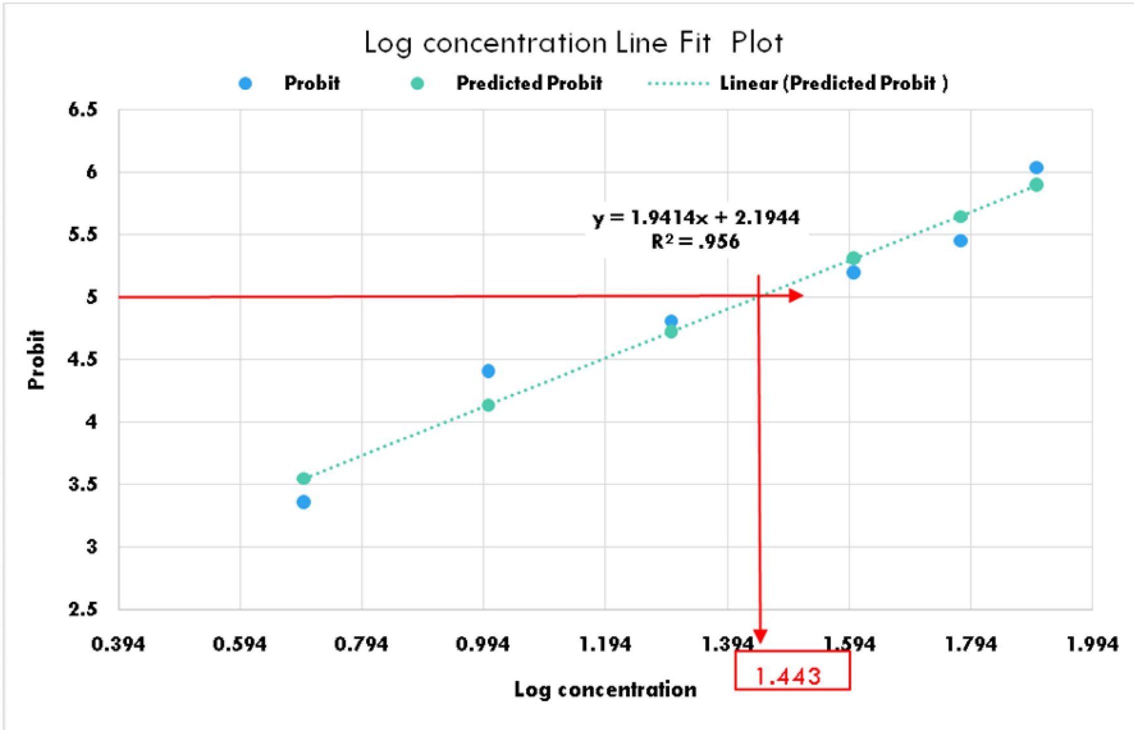
Various methods can be used to produce biochar but slow pyrolysis method is the best suiting method for lignin and hemicellulose-rich feedstock like rice straw (Park *et al.,* 2014). The heating technology is electricity as it was produced in tubular electric muffle furnace with the peak temperature for slow pyrolysis are 300°C and 500°C, heating rate 10°C/min, residence time 3hr to reach the peak temperature and inert gas nitrogen gas at the rate of 1ml/min. The peak temperature was maintained for 3 hours for complete pyrolysis. As reported by Wu *et al.,* 2012 300-700°Cat heating rate of 10°C /min is a typical range to carry out slow pyrolysis. Also, slow pyrolysis produces the maximum yield. The main aim of the production was to study biochar properties and estimate the yield for its successful implementation as an ameliorator. The RSB yield reduces with the gradual decrease in the pyrolysis temperature (Wu *et al.,* 2012). Previous studies have proved that biochar produced at higher temperature is more beneficial in carbon sequestration as a soil amendment.

Characterization of Biochar

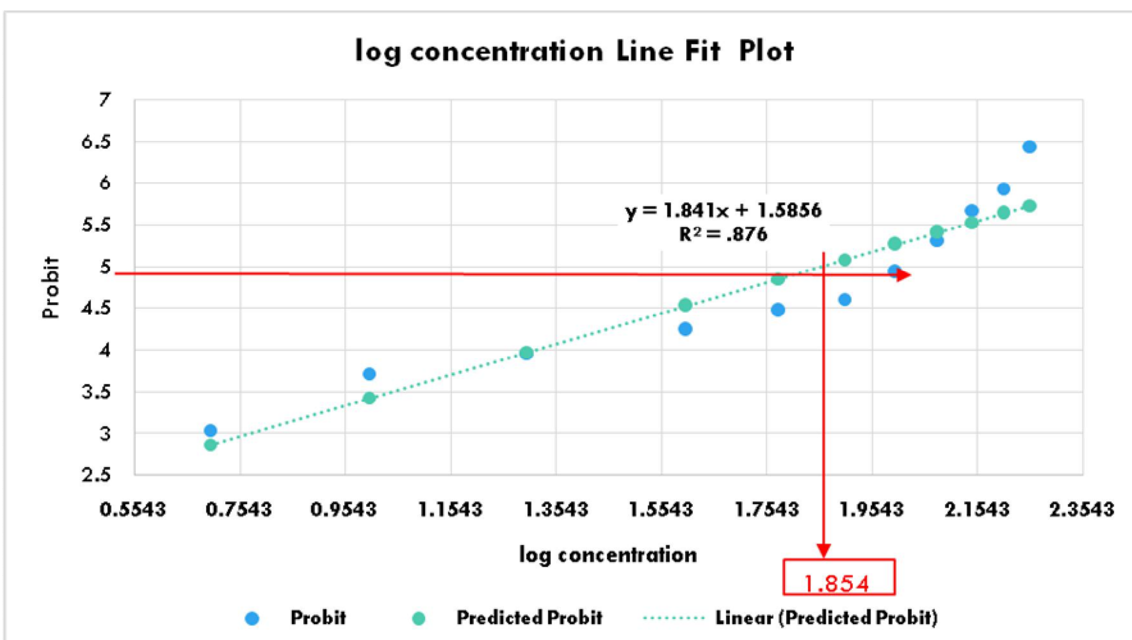
Biochar have been produced by a variety of feedstock of agricultural residues like from rice waste (Hossain *et al* ., 2020) wheat straw (Song *et al.,* 2012) corn straw (Intani*et al.,* 2018), corn stalk (Zheng *et al.,* 2010) peanut shell (Wang*et al.,* 2019) bamboo shoot (Ye *et al.,* 2015) rice straw (Wu *et al*., 2012) Agricultural residue production in the world is 500 -550 million tons annually in India (Devi *et al.,* 2017) from different crops including 122 million tons of rice. Hence, rice straw was selected as feedstock for producing biochar. All the physicochemical properties of RSB300 and RSB500 are cited in Table 2 and Graph 1 & 2. All details are in accordance with the work done by Salam *et al*., 2020; Park *et al*., 2014 and Wu *et al*., 2012. RSB produced at 300°C and 500°C showed physical, chemical and morphological properties largely as per literature.

Table 2: Statistical analysis of Mortality (LC_{50}) of *Eisenia fetida* at Phorate and CPF vs Rate of application of RSB300 and RSB500

Correlation	Correlation coefficient	95% Confidence interval	R square value	P value	Statistically significant	F value
Mortality (LC_{50}) Phorate vs RSB300	0.357	(-.541, 0.875)	12.8%	0.431	NO	0.73
Mortality (LC_{50}) Phorate vs RSB500	-.779	(-.966, -.064)	60.7%	0.039	YES	7.73
Mortality (LC_{50}) CPF vs RSB300	.080	(-.716,.786)	.6%	.865	NO	0.03
Mortality (LC_{50}) CPF vs RSB500	.357	(-0.541, .875)	59.7%	0.042	YES	7.41



Graph 1: LC_{50} probit Phorate



Graph 2: LC_{50} probit CPF

The yield of RSB were consistent with that reported by Wu *et al*., 2012, Park *et al*., 2014; Salam *et al*., 2020 and Yakout, 2017. The pH recorded was high due to heating at a higher temperature. The loss of acidic groups and increase in aromaticity was supported by earlier studies (Wu *et al.,* 2012). As reported by Chandra and Bhattacharya, 2019 up to 500°C the available nutrients achieve maximum gain but after 500°C the reduction starts. Hence, 500°C was considered to be more advantageous as pyrolysis peak temperature and also suitable as soil amendment. Also, Erdem (2021) supported the results for different pyrolysis temperature as obtained in the present studies.

Zeta potential

With the increasing pyrolysis temperature, the zeta potential decreases (Jaing *et al.,* 2015). The value of zeta potential for RSB300 and RSB500 were found to be -32.6 mV and -36.6 mV respectively (Figure 4)

FTIR analysis

The biochemical composition and functional group on the surface of the biochar can be revealed by this tool FTIR. Figure 2 shows the FTIR spectra of RSB300 and RSB500. The aromaticity and biochemical constituents with functional groups are confirmed by FTIR analysis for RSB300 and RSB500 (Fig. 2). There is decrease in the peaks as temperature increase from 300 to 500 similar to Salam *et al.,* 2020. The peaks at 3377.17 cm-1 confirm the presence of the -OH group due to the hemicellulose, cellulose and lignin content of biochar. This peak is not noticed in RSB500. The peaks observed for RSB300 are as follows: Vibration peak at 1439 cm-1 and 780.74 cm-1 corresponds to aliphatic -CH3 bend and aromatic CH bend. Peak at 1613.87 supports the aromatic ring-like structure. Peak 1103.75, 780.74 and 462.48 corresponds to -C-O, P-O, Si-O-Si and C-O stretch (Wu *et al.,* 2012). 1440 cm-1 indicated the functional group C=C in the biochar at 300C (Salam *et al.,* 2020).For RSB500 peaks were observed at 1397cm-1 due to stretching of the C-O bonds. The peak at 803.09 and 875 corresponds to aromatic C-Hbonds. Peaks at 1107 and 459.86 correspond to Si-O-Si, P-O bond of phosphate, C-O bond of carbonates. Peak at 1597 revealed the C=C bonds present on the RSB 500. No peak was observed at 3200 cm-1 or above. Different groups were detected in both the biochars produced from rice straw. Si-O-Si being the key component of rice straw

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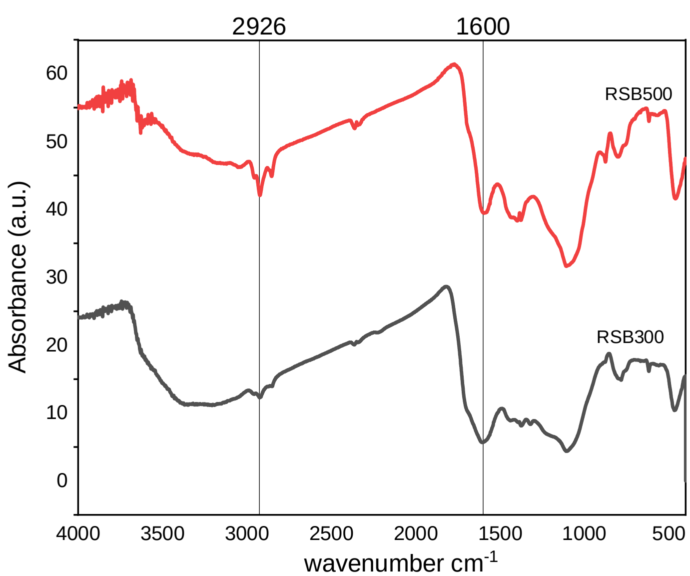


Figure 2: **FTIR spectra for rice straw derived biochar at 300° and 500°**

XRD analysis

XRD confirms the amorphous nature of biochar due to the loss of crystallinity at high temperature and shown the presence of minerals like silica, quartz, sylvite and calcite for RSB500 and RSB300 (Figure 3). Sharp peaks were observed 3.1493 and 2.2269 corresponding to Sylvite, 3.3453_A and 2.4579_A reflecting Quartz and small but sharp peaks at 2.1884, 2.3830 and 3.9816_A corresponding to Silica dioxide for RSB300. The peaks were in accordance with the results reported by Wu et al., 2012. In RSB500 peaks for calcite was intensified at 3.037 A which was absent in RSB300. Peaks at 3.372 and 4.27_A correspond to SiO_2, 3.1815 and 2.249_A revealed Sylvite (KCl) and 3.3434_A for Quartz. The results showed similar trends with the work done by Wu *et al.,* 2012, Salam *et al.,* 2020.With increasing pyrolysis temperature, the peaks get intensified and decomposition of cellulose takes place.

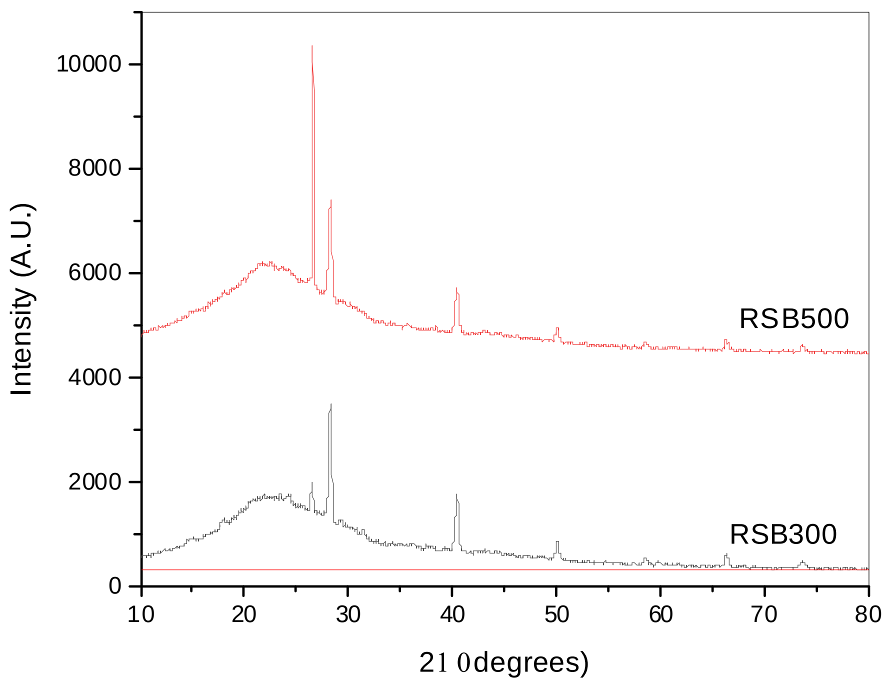


Figure 3: XRD spectra for RSB300 and RSB500

The ash content as estimated by ASTM was found to be 23.933% for RSB300 and 32.453 % for RSB500 and proving that with rise in temperature, volatilization of constituents the ash content increases (Wu *et al.,* 2012; Salam *et al.,* 2020). This high ash content increase the pH, and CEC due to soaring temperature and more readily soluble compounds supported by Zhao *et al*., 2013. The sorption tendency of biochar is expressed by the electrophoretic mobility the zeta potential of RSB300 and 500 (Figure 4.1 & 4.2) depicts the electrophoretic mobility of the biochar for sorption of the contaminants and hold nutrients. As pH increase so increases the negative surface charge which is depicting by the recorded more negative zeta potential of RSB supporting the role of soil ameliorator and decontaminator. Similar trends were shown by Jiang *et al.,* 2012.

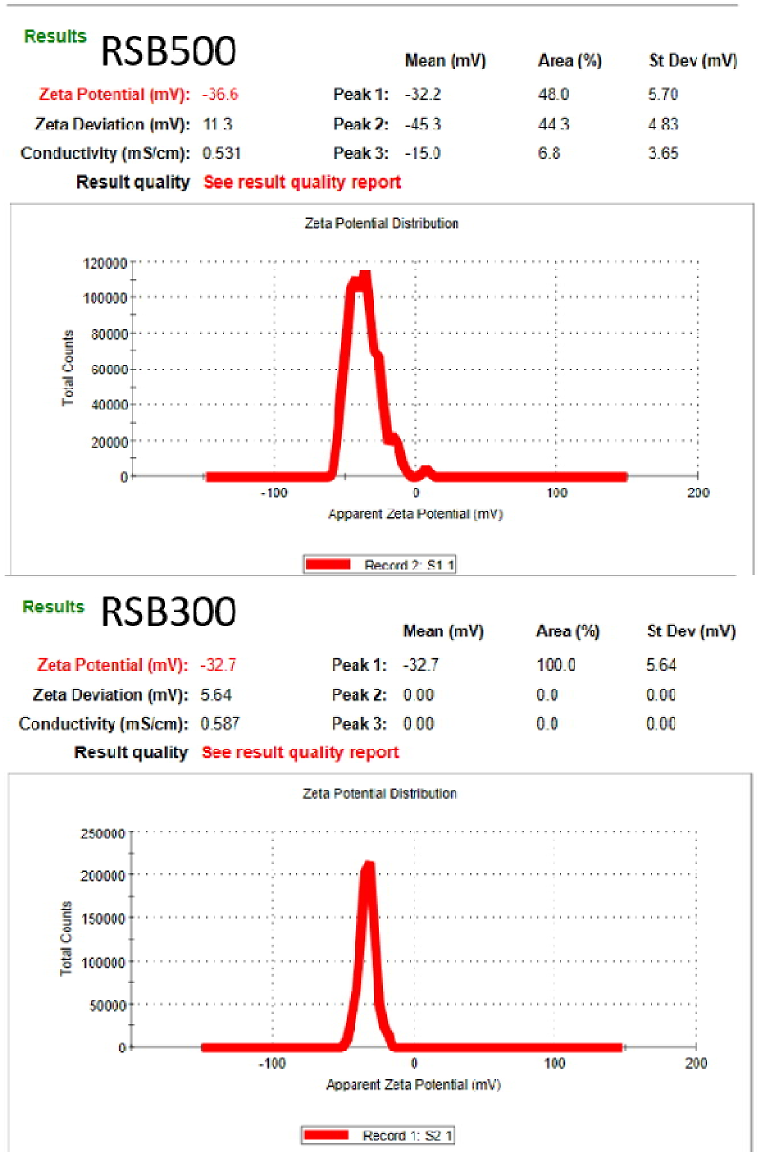
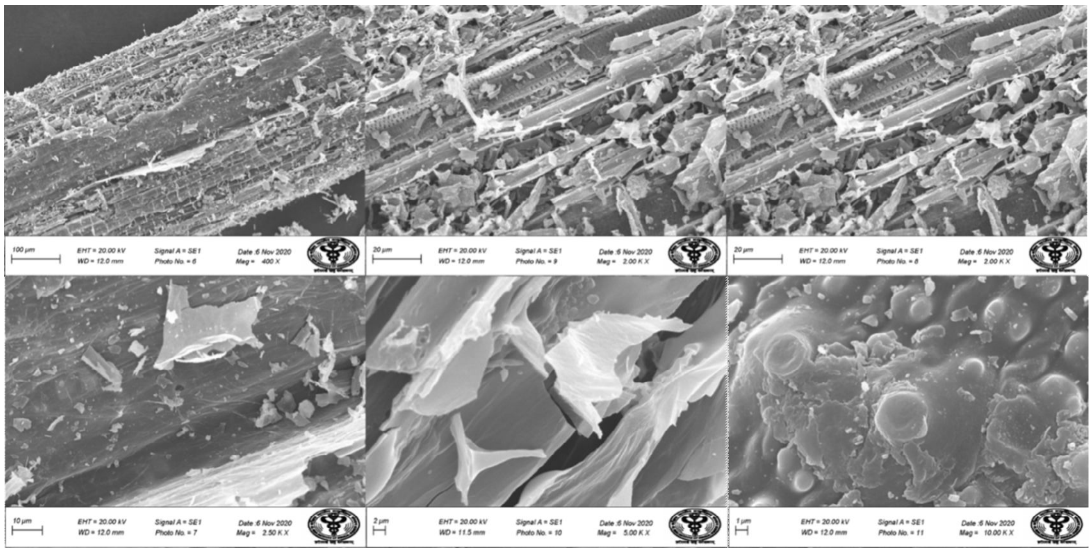


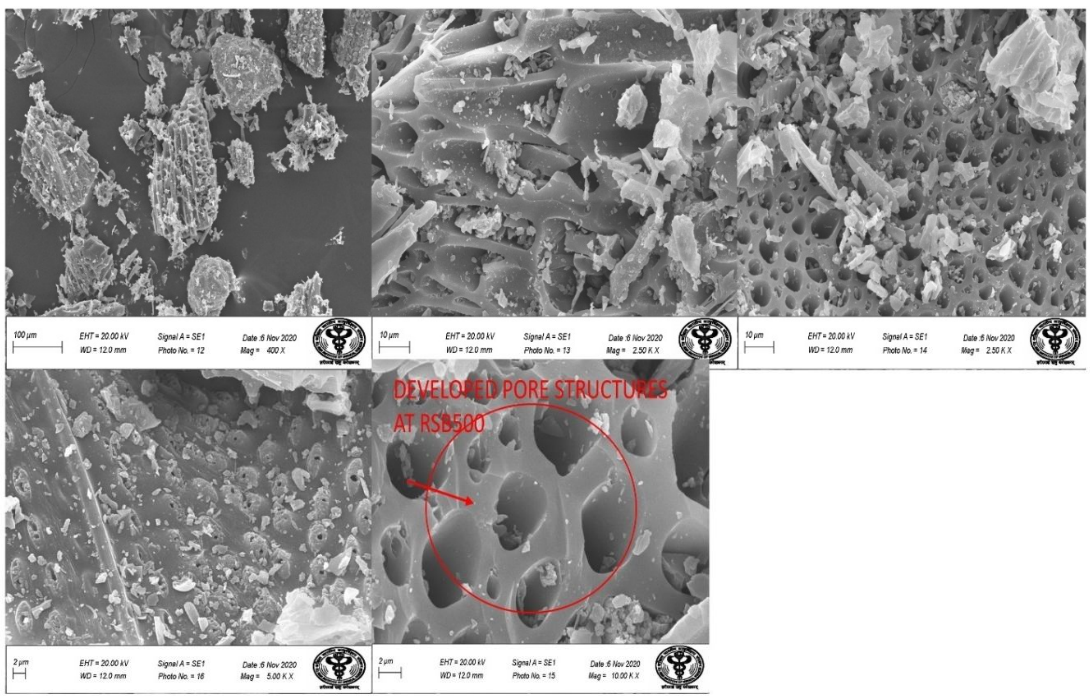
Figure 4: Zeta potential of RSB300 and RSB500

SEM analysis

The morphological analysis was done with the images of SEM at different magnifications at 400X, 2XX and 2.5X and TEM (Figure 5.1 & 5.2). The conclusion drawn from SEM / TEM were confirmed the amorphous, porous structure of RSB with disintegrated, irregular plates and more active sites for adsorbing similar to the results reported by Cox *et al.*,1999; Jiang *et al.,* 2012.



5.1 RSB300



5.2 RSB500

Figure 5: SEM photographs of rice straw biochar at different magnifications: 5.1 RSB300 and 5.2. RSB500

Estimation of the rate of application of biochar for maximum reduction of mortality at LC50 of both the pesticides

As stated by Erdem, 2021 the application rate is the deciding factor for the action of biochar. It's the dose and exposure that determines the toxic or beneficial impact of biochar amendment in soil.

Different concentration of biochar was applied starting from 0%, 1.5%, 3% ,5% 6.5%, 8% to10 % in artificial soil with lethal median concentration of pesticides. The mortality (LC50) of earthworms exposed to different doses of biochar is shown in Figure. Finding cited that RSB500 added at 3% and 5% shown reduction in mortality from 50% below. It was reduced from 50% to 32.5%and 40%, for 3% and 5% for Phorate RSB500 and 37.5 % and

30% at 3% and 5% for CPF RSB500. The higher rates of application at 6.5% to 10% shown a rise in mortality in *Eisenia fetida* signifying the conclusion drawn by Zhang at al., 2019 that the high doses of biochar impact negatively. At high concertation rise in pH may have resulted in a rise in mortality as reported by Malev *et al*., 2015. Phorate being more toxic reported less reduction in mortalities as compared to CPF. The rate of application of biochar is recommended at low doses in soil for better survival of the non-target organisms and benefitting soil quality as reported by Li *et al.,* 2011; Hale *et al.,* 2013; Zhang *et al.,*2019.

Biochar application: At the 0.05 level, the data was significantly drawn from a normally distributed population. Statistical data of mortality at LC50 of Phorate and CPF vs RSB300 and RSB500 biochar different level of application in table 2. Data reflects that the mortality reduction is not significant in RSB300 for Phorate as well as CPF with *p values greater than 0.05* for the given 95% confidence interval (C.I.). Whereas RSB500 for Phorate and CPF, results are statistically significant for the given 95% C.I. of mean with p values closer to zero and less than 0.05. Correlation values for RSB500 Phoratemortality (LC50) vs level of biochar application lie in the range of (-0.966, -0.064) and for RSB500CPF is (-0.541, 0.875). RSB500 is proved to be more beneficial due to high pyrolysis temperature and also low dose of biochar application successfully nullifying the mortality below 50%as compared to RSB300. The results show that there is a treatment and concentration of pesticide exhibited statistically significant $P < 0.05$. A factorial ANOVA two-way was conducted to compare the main effects of treatment of biochar dose on the mortality (LC50) as well as their interaction effects on the treatment of biochar dose and concentration of pesticide on the mortality. The results show that there is a treatment and concentration of pesticide exhibited statistically significant $P < 0.05$. The main effect of treatment yielded an effect size of 0.642, indicating that 64.4% of the variance in the mortality was explained by treatment ($F=50.148$, $P < 0.05$). The main effect of concentration of pesticide yielded an effect size of 0.678, indicating that 67.8% of the variance in the mortality was explained by pesticide ($F=29.541$, $P<0.05$). The results also show that there is an interaction effect of biochar dose on mortality (LC50) of pesticide 1 and pesticide 2 ($F=5.126$, $P < 0.05$).

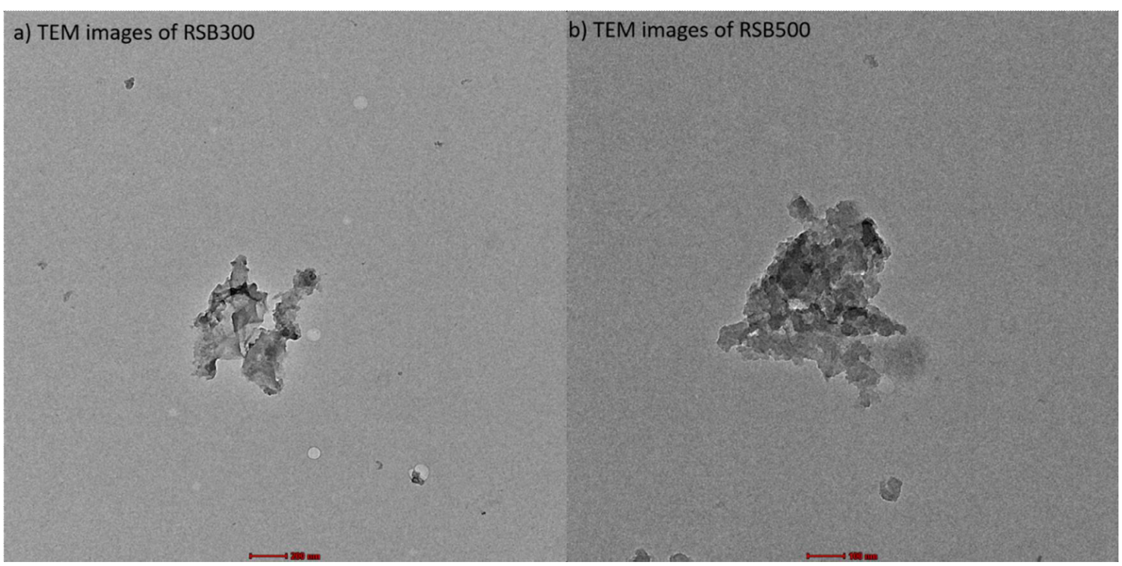
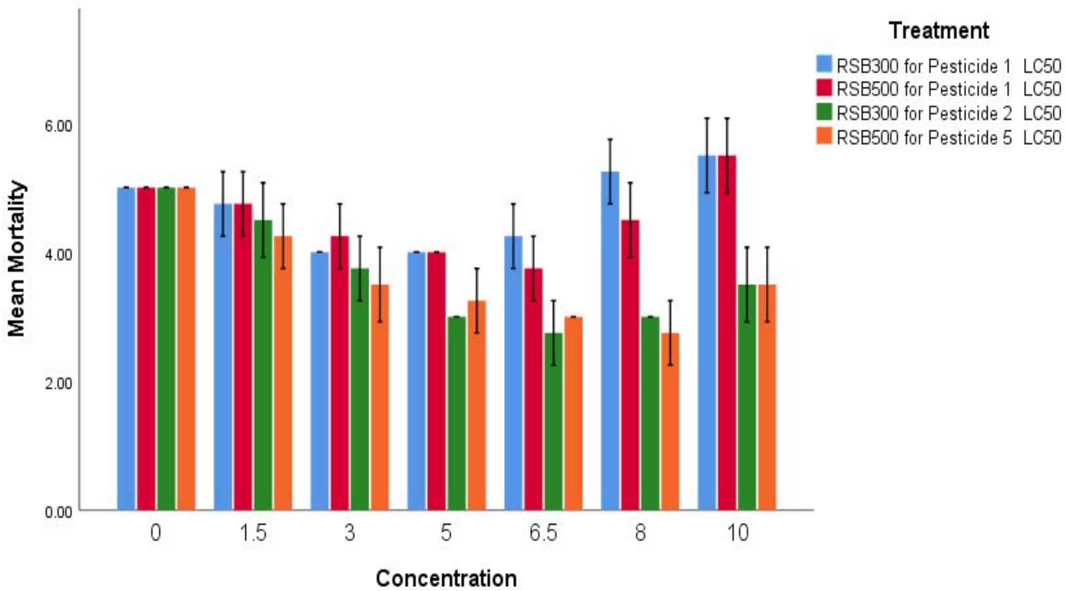


Figure 6: TEM images of RSB300 and RSB500.

The main effect of treatment yielded an effect size of 0.642, indicating that 64.4 % of the variance in the mortality was explained by treatment ($F=50.148$, $P < 0.05$). The main effect of concentration of pesticide yielded an effect size of 0.678, indicating that 67.8% of the variance in the mortality was explained by pesticide ($F=29.541$, $P < 0.05$). The results also show that there is an interaction effect of treatment of different levels of biochar application and mortality (LC50) (F=5.126, $P < 0.05$).Graph 3 Graphical representation of means mortality vs concentration of biochar application for (RSB300 and RSB500) for Pesticide 1-Phorate and Pesticide 2 - CPF. Error bars indicates class intervals. The results were in accordance with the studies reported by Chandra and Bhattacharya, 2019 up to 500°C the available nutrients achieve maximum gain but after 500°C the reduction starts. Hence, 500°C was considered to be more advantageous as pyrolysis peak temperature and also suitable as soil amendment.



Graph 3: Graphical representation of mean mortality vs concentration of biochar application for (RSB300 and RSB500) for Pesticide 1-Phorate and Pesticide 2 - CPF. Error bars indicates class intervals.

CONCLUSION

Stubble burning and pesticide pollution are two major threats with a common contemporary sustainable and eco-friendly solution i.e. Biochar production and using it as ameliorative agent in degrading the bioavailability of organophosphates i.e. Phorate and CPF to earthworms. Biochar produced from rice straw helps to manage organic waste and also reduces the mortality caused by the pesticide's contamination in soil. The RSB produced at high pyrolysis temperature and applied in low doses results in increased mortality reduction amendment atLC50 of pesticides in artificial OECD soil. It is advisable to estimate the level of application of biochar in order to minimize the toxic impact of high doses of biochar. At low doses of biochar application in combination with earthworms work mutually beneficial for soil.

Future studies can be conducted to understand the mechanism for level of damage caused at doses above 5%, understanding the mechanism of fraction of toxic contaminants presenting biochar which may be responsible for increasing mortality at a high rate of application 6.5%, 8% and 10%.

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Declaration of competing interest

The authors declared that they have no conflicts of interest to this work.

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Abbreviations

°C- Degree Celsius
ACR- Agricultural crop residue
ASTM- American Society for Testing and Materials
CEC- Cation exchange capacity
CG- Concentration granules
cm^{-1}- centimeter inverse
CPF- Chlorpyrifos
EC- Electrical Conductivity
FTIR- Fourier-transform infrared spectroscopy
HCB- Hexachlorobenzene
ISO- International Organization for Standardization
LC50 -Median Lethal Concentration
mg- milligram
mg/Kg- milligram per kilogram
mV- millivolt
OECD - Organization for Economic Co-operation and Development
PCB- Polychlorinated biphenyls
pH- potential of hydrogen
RSB- rice straw derived biochar
SEM- Scanning electron microscopy
TEM- Transmission electron microscopy
XRD- X-ray powder diffraction

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