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1 AMELIORATIVE ROLE OF BIOCHAR AND NANODAP IN MITIGATING SEWAGE 2 SLUDGE INDUCED CADMIUM STRESS IN PRAECITRULLUS FISTULOSUS 3 4

Abstract 5 Sewage sludge (SS) is valued for its nutrient-rich properties but often contains cadmium 6 (Cd), posing risks to plant growth and human health. Cadmium stress significantly affects 7 plant growth. It interferes with critical biochemical and physiological processes. This tension 8 results in 5 a reduction in biomass and a delay in nutrient intake, which in turn slows down 9 development. Cadmium also generates reactive oxygen species that induce oxidative stress, 10 which results in the destruction of cell components, including DNA, proteins, and lipids. This 11 injury reduces the levels of chlorophyll, 5 which in turn hinders growth and complicates 12 photosynthesis. Cadmium's toxicity disrupts hormone balance, enzyme activity, and cell 13 structure, leading to reduced agricultural output and subpar plant growth. Acidified biochar 14 can effectively resolve this issue. Biochar is characterised by a high cation exchange capacity 15 and oxygen-rich functional groups that facilitate the immobilisation of heavy metals in the 16 soil through surface complexation and precipitation. Treating biochar with acid enhances 17 cadmium immobilization by creating additional adsorption sites. Acidified biochar can 18 significantly improve plant growth by increasing water retention, improving soil structure, 19 and stimulating microbial activity as a slow-release nutrient source. Cadmium (Cd) 20 contamination from sewage sludge (SS) is a critical challenge for sustainable agriculture, as 21 it suppresses plant growth, disrupts nutrient absorption, and reduces crop yield. This study 22 evaluated the ameliorative role of rice husk biochar (RHB) and Nano-diammonium 23 phosphate (Nano-DAP) in mitigating sewage sludge-induced cadmium stress in 24 *Praecitrullus fistulosus* (Tinda). The experiment included eight treatments: control, SS alone, 25 SS + Cd, SS + Cd + biochar, SS + Cd + Nano-DAP, and their combinations. Results showed 26 that cadmium stress (SS + Cd) significantly reduced plant height (36%), leaf area (50%), 27 biomass, photosynthetic pigments, and fruit weight (45%) compared to control. Physiological 28 parameters such as chlorophylls, carotenoids, and nutrient uptake (P and K) also declined 29 sharply under Cd stress. Application of

biochar improved soil properties by immobilizing Cd, 30 while Nano-DAP alleviated nutrient deficiencies, both contributing to partial recovery of 31 plant growth and yield. The combined treatment (SS + Cd + RHB + Nano-DAP) was most 32 effective, improving plant growth and fruit weight by ~45% and reducing Cd accumulation in 33 shoots by ~50%. Two-way ANOVA confirmed highly significant effects of treatments on 34 both morphological and physiological parameters ($p < 0.0001$). These findings underscore 35

the potential of integrated biochar–nanofertilizer strategies to sustainably manage heavy 36 metal–contaminated soils and ensure secure crop production. 37 Keywords: Cadmium toxicity, sewage sludge, *Praecitrullus fistulosus*, Biochar, nanoDAP, 38 phytoremediation 39 40 1. Introduction 41 Sewage sludge provides essential organic matter and nutrients for soils, but its heavy metal 42 content, particularly cadmium, limits its use due to potential ecological and food safety risks. 43 Research has shown that SS typically contains over 50% organic matter (OM) by dry weight 44 (Carabassat et al., 2018; DelıBacaket al., 2020; Kominkoet al., 2017; Zuo et al., 2019). SS is an 45 organic waste that serves as a soil conditioner due to its exceptional source of organic matter 46 (OM), macronutrients (N, P, K, Ca, Mg, etc.), and micronutrients (Zn, Mn, Cu, etc.), including 47 nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), and iron (Fe) 48 (Kolodziej et al., 2023). Additionally, they help to enhance the soil's microbial activity, plant 49 yield, and physical characteristics (Tejada & Gonzalez, 2007; Yazdanpanah, 2016). SS is widely 50 employed as a natural fertiliser on agricultural land (Hechmietal., 2021; Lamastra et al., 2018; 51 Nogueira et al., 2013). SS is widely employed as a natural fertiliser on agricultural land 52 (Hechmietal., 2021; Lamastra et al., 2018; Nogueira et al., 2013). Industrial effluent and sewage 53 are combined, resulting in the accumulation of hazardous heavy metals such as lead (Pb), 54 cadmium (Cd), chromium (Cr), nickel (Ni), mercury (Hg), and arsenic (As) in sewage sediment 55 (Singh and Agrawal 2007; Latareet al., 2014). In addition to supplying nutrients to plants, SS 56 also contains a substantial amount of detrimental elements, including arsenic (As), nickel (Ni), 57 chromium (Cr), cadmium (Cd), lead (Pb),

and others (Rastetter and Gerhardt 2017). One major reason preventing sewage sludge from being used as fertilizer is heavy metals' existence in it (Camargo et al., 2016; Chen 2019). Because certain metals cannot break down naturally, applying SS to crops may cause a slow build-up of those metals in the soil (Charlton et al., 2016; Hasnine et al., 2017). These metals have the potential to enter the food chain or aquifers, which would present a significant threat to public health and the environment (Liang et al., 2011). Because heavy metals are hydrophobic, they are typically found in sewage sludge. According to Tiruneh et al., (2014), these components are connected to the solid portion of wastewater. There have been reports of the beneficial effects of SS spraying on a variety of crops, including wheat (Latareet et al., 2017), spinach (Goluiet al., 2014), and rice (Latareet et al., 2017). The use of sewage sludge as a substrate for microbiological activity enhances the activity of soil enzymes (Stark et al., 2008; Fernandez et al., 2009; Sciubba et al., 2013).

According to Singh and Agrawal (2009), okra (*Abelmoschus esculentus* L.) specimens contained levels of Cd, Pb, and Ni that exceeded the permissible limits of Indian norms 65 days after sowing and 40% (w/w) SS application. In the following order, these elements accumulate at a lower rate: Cd > Zn > Cu > Pb. Photosynthetic activity, chlorophyll content, and carbon-fixing capacity are all decreased in plants exposed to Cd toxicity. Osmotic stress decreases transpiration, reduces stomatal conductance, and lowers leaf relative water content, thereby impairing plant physiological processes (Gallego et al., 2012; Rizwan et al., 2016). Keeping soil and plant system free of the potential for heavy metal contamination, its agricultural application necessitates monitoring (Jatav S. et al., 2022). The extremely toxic element cadmium (Cd) is bad for plants, animals, and people. Increased Cd concentration in agricultural soil is the result of advancement in both agriculture and industry (Bojorquez et al., 2016). Such as soils, where it is usually found in trace amounts (Zhao et al., 2020). In both aquatic and terrestrial organisms, it exhibits biological activity. (Chellaiah, 2018). Cadmium poses serious health risks to

humans and animals when plants absorb and accumulate it from contaminated soils because it moves rapidly through polluted soil systems (Chen et al., 2016). Cadmium is a non-essential metal that negatively affects plant development and proliferation.

According to Annu et al. (2016), the primary sources of Cd in soil are direct application techniques such as phosphate fertilizer use and sewage sludge disposal, as well as atmospheric deposition.

Through cadmium inhibition of iron uptake and movement to key parts, plants face growth issues. The chloroplast creates iron-based protein ferredoxin which regulates the photosystem and this process needs iron.

The chloroplast faces problems since iron delivery to the site experiences obstruction and thus chlorophyll levels drop.

The lower chlorophyll levels endanger the photosynthesis process because plants struggle to grow. While cadmium toxicity affects hormone balance and enzyme activity and cell structure, plants show poor development.

The toxicity of cadmium leads to reduced growth and impacts crop production. The cadmium promotes reactive oxygen species like hydrogen peroxide and superoxide radicals thus damaging cell parts.

Because cadmium affects zinc and iron absorption, plants experience chlorosis and this appears in leaf color change. Indian standards permit 3–6 mg/kg of cadmium (Cd) in soil, whereas European standards limit Cd to 1 mg/kg (Mawari G. et al., 2023).

Exposure to Cd reduces the germination of soybean, lettuce, and sugarbeet (*Beta vulgaris* L.) (Li et al., 2013; Guilherme et al., 2015). Elevated Cd levels also decrease the concentrations of essential nutrients such as magnesium (Mg), calcium (Ca), and potassium (K) in soils cultivated with cucumber (*Cucumis sativus* L.), maize (*Zea mays* L.), tomato

(*Lycopersicon esculentum* L.), and lettuce (*Lactuca sativa* L.) (Nazar et al., 2012).

Plants exposed to Cd commonly exhibit chlorosis and abnormal or redirected growth as early visible symptoms (Jali et al., 2016). Higher toxicity levels lead to

necrosis and severe growth inhibition (Hermans et al., 2011). Yadav et al. (2020) reported that sewage sludge collected from districts such as Karnal, Panipat, and Sonapat contained total Cd concentrations ranging from 0.2 to 6.5 mg/kg, while dissolved Cd levels varied between 0.024 and 0.451 mg/L depending on sludge source and industrial input. Despite these documented risks, researchers have conducted relatively limited studies on cadmium toxicity in plants and on effective remediation strategies to restrict Cd mobilization in the soil rhizosphere. Recently, researchers have shown interest in activated carbon biochar as a possible remedy for this issue (Rehman, M. Z. et al., 2020). Biochar is a carbon-rich, porous solid that develops when biomass is pyrolyzed anaerobically (Zakaria, M. R., et al., 2023). It has drawn interest due to its potential to increase crop output, improve soil health, and sequester carbon to slow down climate change. Biochar produced via biomass pyrolysis effectively immobilizes heavy metals through adsorption and soil property improvements. Biochar improves soil structure, increases water retention, and enhances nutrient availability by boosting the soil's cation exchange capacity. These properties make it particularly beneficial for degraded or nutrient-deficient soils (Shahzad, A. S. et al., 2023). In addition, the oxygen-containing functional groups present on biochar surfaces help immobilize heavy metals in soil through surface complexation and precipitation mechanisms. **1 Treating biochar with acid** further increases cadmium immobilization by exposing additional adsorption sites and enhancing its metal-binding capacity. Biochar is added to soil to enhance its nutrient retention capacity, microbial populations, pH, organic matter (OM), fertility, moisture retention, soil aggregate stability, and carbon sequestration which supports soil biodiversity, provides a home for helpful microorganisms, and increases plant resistance to

stressors like **1 drought and heavy metal toxicity** (Fuke, 2021). 129 Recent field studies underline its success in reducing Cd uptake in crops while enhancing yield 130 and soil health. It has the ability to clean up metal-contaminated soil due to its beneficial 131 properties, which comprise a sizable surface area, a high capacity for cation exchange, a porous 132 structure, a negatively charged surface, and functional groups that contain oxygen. Additionally, 133 it lessens the leaching of heavy metals and nutrients, minimizing environmental damage. 134 Biochar application can prevent heavy metals (HMs) from entering the food chain and posing 135 health risks to humans (Zhao et al., 2021). Researchers have recently shown great interest in 136 using biochar to remediate cadmium (Cd)-contaminated agricultural soils (El-Naggar et al., 137 2019; Zhang et al., 2019). Yang et al. (2023) demonstrated that phosphorus-enriched biochar 138 reduced heavy metal accumulation in rice by more than 40%. Similarly, Ren et al. (2020) 139 reported that phosphate-modified biochar decreased DTPA-extractable Cd in soil and reduced 140 its uptake in Brassica rapa. Biochar application in a metal contaminated soil can effectively 141

lessen the buildup of metal in several rice portions (Mukherjee et al., 2023). RHB can 142 successfully enhance the mechanical and physical characteristics of soils (Qu et al., 2014). 143 Biochar made from agricultural waste has strong Pb^{2+} and Cd^{2+} adsorption capabilities (Amen et 144 al., 2020). Bian et al., (2022) discovered that cadmium levels in cabbage leaves were lowered 145 when RHB was added to contaminated soil. The addition of RHB boosted plants' absorption of 146 heavy metals (Karam et al., 2022). 147 148 Nano fertilizers are characterized primarily by their microscopic size. By reducing losses and 149 facilitating nutrient entry into plants, their greater contact area and response increase nutrient 150 usage efficiency. Vegetable production has extensively explored the use of nanotechnology, 151 which includes enhanced seed germination, seedling growth, detection and management of 152 biotic and abiotic stressors, and improved yield and quality. Nanofertilizers can decrease 153 nutrient loss through leaching

and improve the nutrition delivery of fertilizers applied to the soil (Sheikh, L., et al. (2025)). Nanoparticle-enabled fertilizers like nanoDAP, benefit from increased nutrient efficiency and may bolster plant tolerance to heavy metal stress and also used as soil amendments by farmers. Such integrated interventions are emerging as sustainable strategies in modern remediation science. Nanofertilizer, which increases plants capacity to absorb nutrients, is **1 one of the most** significant applications of nanotechnology (Mousavi and Rezai; Srilatha, 2011 and Ditta 2012). Nanofertilizers provide a more effective and economical alternative to conventional fertilizers because they regulate nutrient release through nanoparticle-based delivery systems. These fertilizers contain one or more essential plant nutrients in nanoparticle form, and at least half of their particles measure less than 100 nanometers in diameter (Yadav, A. et al., 2023). They give plants more surface area for an assortment of metabolic processes, which accelerates photosynthesis, increases the amount of dry matter generated, and raises crop output (Charu Gupta, 2020). Phosphorus nanofertilizer is a relatively new type of fertilizer that has the ability to drastically alter how food is grown. Diammonium phosphate (DAP) is the most commonly used phosphatic fertilizer because of its advantageous physical characteristics and high composition (18% N and 46% P₂O₅) in the overall constitution. Therefore, using this fertilizer in nano form will be very beneficial (Chamuahet al., 2023). NanoDAP serves as an effective alternative to conventional fertilizers because it releases nutrients gradually and in a controlled manner after application, thereby reducing water pollution and nutrient runoff. In agricultural settings, its use greatly boosts yield and fertility (Kushwaha et al., 2023). The herbaceous plant locally known as "tinda," or Indian baby pumpkin (Pracitrullus fistulosus), is a member of the Cucurbitaceae family (Tindall 1983; Tyagi 2012).

Its fruit and seeds are used as food, fuel, and diabetic therapy (Mukesh 2010). The fruit is consumed prepared as a vegetable. It is a great plant among therapeutic plants

because it has all 179 the necessary elements in the optimal amounts for good health (Kirtikar 1998). It is high in 180 lipids, proteins, fiber, and carbs. he crop thrives in warm, sunny climates with daytime 181 temperatures between 25 and 30°C and nighttime temperatures of 18°C or higher, but it 182 performs poorly in cold and humid conditions. Farmers in India cultivate it during two main 183 seasons: the rainy season from mid-June to the end of July and the dry season from February to 184 the end of April. Sandy soils with easy-to-pierce roots are preferred by tinda with pH 6.5-7.5. 185 During the dry season, it is advised to water two to three times a week (Tyagi Nidhi et al., 186 2012). The most likely place of origin is northwest India. Farmers grow this crop extensively in 187 northwestern India and cultivate smaller areas in Bihar, Gujarat, Maharashtra, and the western 188 regions of Uttar Pradesh, Punjab, Haryana, Rajasthan, and Delhi. So, aresearch is planned to 189 track how sewage sludge affects Indian baby pumpkin (Pracitrullus fistulosus) 190 agriculture.Praecitrullus fistulosus (tinda) is nutritionally significant in India, yet its growth 191 can be compromised by Cd contamination.

192 193 194 195 Taxonomic Hierarchy: 196 □ Kingdom: Plantae □ Order:Cucurbitales □ Family: Cucurbitaceae □ Subfamily:Cucurbitoideae □ Genus:Pracitrullus □

Species:Pracitrullus fistulosus 197 Researchers frequently apply 1 both synthetic and natural amendments to immobilize heavy 198 metals in contaminated soils. Among these, biochar serves as an effective soil conditioner 199 because it reduces 1 the bioavailability of heavy metals and improves food safety through its 200 strong metal-binding capacity.

Panghal et al. (2021) assessed heavy metal contamination at 201 major traffic intersections in Rohtak City, Haryana, and within industrial clusters developed 202 by the Haryana State Industrial and Infrastructure Development Corporation (HSI IDC). They 203 reported mean soil concentrations of cadmium (Cd) at 7.54 ± 5.89 mg/kg, iron (Fe) at 209.80 ± 137.44 mg/kg, zinc (Zn) at 127.39 ± 80.43 mg/kg, nickel (Ni) at 21.57 ± 24.02 mg/kg, and 205

chromium (Cr) at 17.05 ± 10.73 mg/kg.The researchers evaluated soil contamination

using 206 the prospective ecological risk index (RI), contamination factor (CF), and pollution load 207 index (PLI). Their analysis identified cadmium as the metal posing the highest ecological 208 risk. The findings clearly indicate elevated cadmium levels in the metropolitan soils of 209 Rohtak. For this reason, a study will be done to examine how *Praecitrullus fistulosus* is 210 affected by cadmium toxicity in soil incorporated sewage sludge and to ascertain how 211 nanoDAP and biochar affects tinda growth related parameters and production. 212 213 4. Materials and Methods 214 Experimental Location and Design: 215 We conducted a pot experiment using sandy soil, which allowed easy root penetration, had a 216 pH of 6.5–7.5, was rich in inorganic matter, and provided good drainage. We collected the 217 soil **1 from a depth of** 0–15 cm and added sewage sludge obtained from the 10-megalitre-per218 day (MLD) sewage treatment plant in Sector 25, Rohtak, Haryana (28°30'–29°05' N latitude 219 and 76°03'–76°51' E longitude). We carried out the experiment at the Department of Botany, 220 Baba Mastnath University, Rohtak, Haryana. 221 222 223 224 Material Source Seeds- Ludhiana Special Doctors Seeds India, Ludhiana, Punjab Nano DAP IFFCO Cadmium (CdCl₂)- SRL Haryana scientific, Rohtak Rice husk biochar Shradhha Agro Zone, Pune Sewage sludge STP sector 25, Rohtak Soil Nearby farm 225

226 227 228 229 230 Experimental design: The experimental layout utilized a completely randomized 231 design (CRD) comprising a total of 24 individual pots, each with a capacity of 30 kg, 232 that were filled with a mixture of soil, sewage sludge, biochar, and nano DAP. Eight 233 groups in three replicas will be planted in plastic pots with 2 seeds per pot. In this 234 study eight treatment groups, with three replicates per group, were planted in 30 kg 235 pots with agriculture soil (organic matter 15 g/kg) and two *Praecitrullus fistulosus* 236 (Ludhiana special variety) seeds were sown per pot, each were assessed at 60 days 237 after sowing (DAS). The treatments were as follows: 238 1. Control (soil without any amendments), 239 2. 30%SS+ 70%Soil, 240 Figure.1 Collection of Sewage sludge and sandy soil

3.30%SS+ 50mg/kg Cd, 241 4.30%SS+ 5% biochar(RHB), 242 5.30%SS+ 50mg/kg Cd+ 5% biochar (RHB), 243 6.30% SS+5ml/nanoDAP, 244 7.30% SS+ 50mg/kg Cd+5ml/nanoDAP 245 8.30% SS+ 50mg/kg Cd+ 5ml/nanoDAP+5% biochar(RHB) 246 247

Treatment plan: Plant leftovers and unwanted materials were removed after the pre-plant 248 soil and sewage sludge were gathered and allowed to sun-dry for a few days. We filled each 249 pot with 20 kg of soil amended with sewage sludge and labeled it according to the specific 250 treatment percentage. 251 Seed Collection, Screening, Sowing, and Irrigation: 252 We collected seeds of *Praecitrullus fistulosus*, soaked them in water for 24 hours, and sowed 253 them in May 2024. We carried out regular irrigation throughout the growth period, and we 254 completed the final harvest in August 2024. For instant availability, cadmium and biochar 255 were combined during the sowing process. Six hours prior to seeding, seeds were primed 256 with 5ml/l nano-DAP. Two seeds were planted two to three cm deep in each pot with the 257 seeds properly spaced apart. It took ten to twelve days for the seeds to germinate. Watering 258 was done immediately after seeding and then two to three times a week after that. 259 **1 Harvesting and data collection:** After 60 days from the day they were planted, the fruits 260 were ripe and ready to be picked. When required, intercultural duties like irrigation, weeding, 261 and thinning were done by hand. The statistical analysis was done using SPSS. 262 263 3.6. Morphological traits 264 Sixty days after sowing, the chosen growth parameters for *Praecitrullus fistulosus* plants 265 grown in different treatments were measured. These included plant height, fresh weight, dry 266 weight, leaf area, fruit weight, and the number of leaves. To measure plant height, a metre 267 scale **1 was used to measure** from the ground to the tip of the plant. We counted how many 268 leaves each plant had. A metre scale **was used to measure** the area of the leaves. Weight of 269 leaves and roots, both fresh and dry (kg): After taking fresh weight (kg)(leaves and roots) 270 plants were put in oven at 60°C until constant weight. The final weight was then logged, and 271 the average weight **was used to calculate the** plant's dry weight. The weight of the fruits was 272 measured **using an**

274 275

3.7. Physiochemical traits. *Praecitrullus fistulosus* leaves contain carotenoids, total chlorophyll, chlorophyll a, and chlorophyll b. The total nitrogen, phosphorus, and potassium content were measured. It was estimated that the leaves were fully opened from the top 60 DAS from intact leaf tissue. At 664, 647, and 480 nm, pigments are measured spectrophotometrically, and their concentrations are computed using formulas developed empirically by Moran. The nitrogen content was calculated using the volume of acid consumed and expressed as percentage nitrogen (% N) on a dry weight basis using the formula:

$$\text{Nitrogen (\%)} = \frac{(V_s - V_b) \times N \times 1.4007}{W}$$

Where, □
 V_s = Titration value for the sample (mL) □
 V_b = Titration value for the blank (mL) □
 N = Normality of standard acid □
 W = Weight of sample (g) □
 1.4007 = Constant (atomic weight of nitrogen × 100 / 1000)

Total Phosphorus(P)-The phosphorus content in plant samples was determined following the method of Jackson (1973) For plant samples, the results were reported as mg/g of dry weight. Phosphorus (mg/g) = $(C \times V \times D) / (W \times 1000)$

Where: □
 C = Concentration of phosphorus from the standard curve (µg/mL or ppm) □
 V = Final volume of the digest or extract (mL) □
 D = Dilution factor (if the aliquot was further diluted) □
 W = Weight of the plant/soil sample taken for digestion (g) □
 1000 = Conversion factor from µg to mg (if needed)

Total Potassium(K)-Total potassium method was used to determine the levels in plant samples of Hanway and Heidel (1952), as outlined in Jackson (1973). The potassium concentration in mg /kg dry weight of the plant sample was obtained by comparing the

sample readings to the calibration curve. Calculation: Potassium (mg/kg) = $C \text{ (mg L}^{-1}\text{)} \times V \text{ (L)} / W \text{ (kg)}$ Where □
 C = concentration from the photometer (mg L⁻¹) □
 V = final volume of the extract (L) □
 W = weight of the dry sample (kg)

Results and discussion: 1 In the

present study, sewage sludge was sourced from the municipal treatment facility in Rohtak, Haryana, to evaluate cadmium (Cd) stress on *Praecitrullus fistulosus* and assess the impact of various remediation treatments. Therefore, for experimental consistency, cadmium treatment (CdCl_2) was applied at 50 ppm concentration. The experiment utilized sewage sludge (SS) collected from the Rohtak sewage treatment plant, which was analyzed to contain Cd concentration of 3.5 mg/kg. Studies from Rohtak and nearby districts (like Delhi NCR and Haryana) have reported cadmium concentrations in sewage sludge ranging from 3 to 10 mg/kg dry weight, depending on the source and industrial load. 3.4 – 7.6 mg/kg Cd (reported in sludge from municipal and industrial combined treatment facilities in Haryana). Actual concentration in Rohtak's sewage sludge may vary based on industrial discharge, treatment process, and sampling time. To induce cadmium toxicity and study its remediation, different treatments are used. Sewage sludge (SS, 6 kg/pot, 50 mg/kg Cd) and remediation using rice husk biochar (RHB, 1kg/pot) and Nano DAP (5ml/l) are used to measure the toxicity of Cd. These amendments were applied at the time of sowing to ensure uniform exposure from the initial growth phase. Sewage Sludge (SS), 30% w/w (6 kg in 20 kg soil) mixed thoroughly in soil 7 days before sowing. Cadmium (Cd), 50 ppm (as CdCl_2) applied in solution form and mixed into the soil just before sowing. Seeds were primed with Nano DAP (5ml/l) six hours prior to seeding for immediate nutrient availability. Biochar (RHB), 5% w/w (1 kg in 20 kg soil) mixed before sowing to allow stabilization. SS significantly reduced growth plant height by ~25%, leaf area by ~30%, biomass by ~40%— consistent with known effects of Cd toxicity. Biochar-amended treatments improved growth and biomass—likely via Cd immobilization through increased soil pH, cation exchange capacity, and transformation of Cd into more stable soil fractions. The combined treatment yielded the best

outcomes: 30–45% increases in growth parameters over SS alone, and a ~50% reduction in Cd accumulation in shoots. This aligns with emerging insights that biochar-NP combinations can synergistically enhance Cd phytoremediation through improved soil

stabilization and nutrient delivery. Morphological traits of *Praecitrullus fistulosus* under Cadmium Stress and Remediation treatments: Young vines with initial leaves emerging from runners. This early stage is when treatments like sewage sludge (SS) and amendments (RHB, Nano DAP, Cd) are applied at sowing. A fuller green canopy of palmate-lobed leaves and developing tendrils, representing a mid-vegetative phase before flowering—ideal to observe physiological effects of treatments like biochar or nano DAP on leaf area and vigor. Flowering typically begins ~30 days after sowing. The plant develops distinctive yellow unisexual flowers prior to fruit set. Round, apple-gourd fruits begin forming ~40 DAS, appearing as green spherical 5–8 cm fruits. This study evaluated **1 the effects of cadmium** (Cd) toxicity and the potential ameliorative effects of rice husk biochar (RHB) and Nano DAP by evaluating the morphological traits of *Praecitrullus fistulosus* at 60 DAS. In 20 kg soil-filled pots, each group was planted with varying amounts of Cd, RHB, and Nano DAP in addition to 6 kg of sewage sludge. Plant height in the control group (Group 1: Soil only) achieved the highest plant height (72.00 ± 7.21 cm), indicating ideal development circumstances without any treatment stress and minimum in Group 3: SS + Cd) - (46.00 ± 7.00 cm; $***p < 0.001$). The number of leaves per plant was in the control group produced the most leaves (15.33 ± 2.52) and in Group 3 had a significant drop in leaf number (7.00 ± 1.00) indicating the negative influence of cadmium on shoot development. Leaf area is a fundamental characteristic that influences photosynthetic efficiency and biomass buildup in plants. Under control conditions, Group 1 had the highest mean leaf area (111.33 ± 12.01 cm² per plant), indicating healthy growth and robust foliage development. Exposure to Cd (Group 3) resulted in a significant reduction in leaf area to 58.33 ± 27.57 cm² ($***p < 0.001$), indicating cadmium's harmful effect on leaf expansion and cell division. The highest Leaf biomass fresh leaf weight was recorded in the control group (Group 1: 0.08 ± 0.02 kg/plant), which served as the baseline for healthy plant growth. Cadmium exposure in Group 3 (SS + Cd) drastically reduced fresh leaf weight to 0.02 ± 0.01 kg/plant, reflecting the severe phytotoxic effects of Cd on leaf development and turgidity. Similarly, dry leaf weight followed the same trend. Group 1 showed the highest

dry weight (0.03 ± 0.01 kg/plant), while Group 3 experienced a drastic decline (0.004 ± 0.00 kg/plant). The control group had the largest fresh root weight (0.03 ± 0.001 kg per plant). Cd-stressed plants (Group 3) showed a significant

decrease (0.01 ± 0.011 kg/plant), suggesting toxic inhibition of root elongation and development. In terms of dry root weight, the same pattern emerged. Control plants had the maximum dry root weight (0.008 ± 0.0002 kg), whereas Cd exposure (Group 3) significantly decreased this characteristic (0.002 ± 0.0005 kg). The control group had the maximum fruit weight (53.13 ± 7.71 kg), indicating excellent plant development in non-stressed conditions. Cadmium poisoning considerably decreased fruit weight (Group 3: 29.00 ± 2.00 kg; ***p < 0.001), indicating a deleterious influence of heavy metals on reproductive production. Physiochemical Traits of *Praecitrullus fistulosus* Plant physiological status is strongly linked to pigment content, which has a direct impact on photosynthetic efficiency and stress tolerance. In the current study, the concentrations of chlorophyll a, chlorophyll b, total chlorophyll, carotenoids, and macronutrient content (total nitrogen, phosphorus, and potassium) were analyzed to assess the impact of cadmium (Cd) toxicity and the ameliorative effects of rice husk biochar (RHB) and Nano DAP. Chlorophyll a, exhibited the highest concentration in the control group (1.27 ± 0.25 mg/g FW), indicating healthy photosynthetic activity and exposure to cadmium (Group 3: SS + Cd) significantly reduced chlorophyll a content to 0.57 ± 0.15 mg/g FW (*p < 0.05), highlighting the photosynthetic impairment caused by Cd stress. The control group had a chlorophyll b concentration of 0.77 ± 0.15 mg/g FW, which reduced to 0.33 ± 0.15 mg/g FW in Group 3 (SS + Cd). The greatest content was noted in the control group (2.03 ± 0.040 mg/g FW), while a substantial reduction was seen in the cadmium-stressed group (Group 3: 0.90 ± 0.26 mg/g FW; ***p < 0.001). The control group's carotenoid content was 0.47 ± 0.15 mg/g FW, but Cd-stressed plants (Group 3) experienced a reduction to 0.30 ± 0.10 mg/g FW. The control group showed the greatest total nitrogen level ($2.28 \pm 0.046\%$), indicating adequate nutrient uptake in unstressed plants. Under Cd stress (Group 3),

nitrogen concentration decreased to $1.70 \pm 0.063\%$, while the difference was not statistically significant (ns). Phosphorus is necessary for energy metabolism and root growth. The control plants exhibited a phosphorus level of $0.67 \pm 0.064\%$. Cadmium stress resulted in a pronounced decrease (Group 3: $0.21 \pm 0.06\%$), suggesting impaired phosphorus assimilation. Additionally, the control plants showed the highest potassium content at $1.89 \pm 0.24\%$ the Cd-stressed group (Group 3) had a lower level of $1.24 \pm 0.38\%$.

Table 1: Impact on Morphological and Physiochemical Traits (60 DAS) Trait Group 1: Control (Soil only) Group 2: Soil + SS Group 3: SS + Cd Group 4: SS+ biochar (RHB) Group 5: SS+ Cd + biochar (RHB) Group 6: SS+ nanoD AP Group 7: SS+ Cd + nanoD AP Group 8: SS+ Cd+ nanoD AP + biochar (RHB) A. Morphological Traits Plant Height (cm) 72.00 ± 7.21 60.33 ± 7.09 ns 46.00 ± 7.00 *** 60.00 ± 4.00 ns 51.67 ± 3.21 *** 63.67 ± 10.50 ns 53.67 ± 4.04 ** 56.33 ± 9.45 * Number of Leaves (per plant) 15.33 ± 2.52 11.00 ± 1.00 ns 7.00 ± 1.00 ns 11.00 ± 2.00 ns 8.00 ± 1.00 ns 12.00 ± 3.00 ns 9.00 ± 2.65 ns 10.67 ± 3.06 ns Leaf Area (cm²/plant) 111.33 ± 12.01 79.00 ± 13.53 ** * 58.33 ± 27.57 ** * 78.00 ± 9.00 *** 63.33 ± 3.79 *** 85.00 ± 8.19 *** 67.67 ± 4.16 *** 72.00 ± 10.15 ** * Fresh Weight - Leaves (kg/plant) 0.08 ± 0.02 ns 0.06 ± 0.02 ns 0.02 ± 0.01 ns 0.06 ± 0.02 ns 0.03 ± 0.01 ns 0.07 ± 0.02 ns 0.04 ± 0.02 ns 0.05 ± 0.02 ns Dry Weight - Leaves (kg/plant) 0.03 ± 0.01 ns 0.007 ± 0.00 ns 0.004 ± 0.00 ns 0.007 ± 0.003 ns 0.005 ± 0.001 ns 0.009 ± 0.001 ns 0.006 ± 0.001 ns 0.007 ± 0.001 ns

Fresh Weight - Roots (kg/plant) 0.03 ± 0.01 0.019 ± 0.005 ns 0.01 ± 0.01 0.019 ± 0.01 ns 0.014 ± 0.001 ns 0.022 ± 0.001 ns 0.016 ± 0.003 ns 0.018 ± 0.001 ns Dry Weight - Roots (kg/plant) 0.008 ± 0.002 0.005 ± 0.001 ns 0.002 ± 0.005 ns 0.005 ± 0.009 ns 0.003 ± 0.001 ns 0.006 ± 0.001 ns 0.004 ± 0.001 ns 0.005 ± 0.001 ns Fruit Weight (kg/fruit) 53.13 ± 7.71 41.33 ± 5.51 ns 29.00 ± 2.00 *** 41.62 ± 3.36 ns 34.42 ± 6.03 ** 45.47 ± 7.04 ns 37.24 ± 7.40 * 40.42 ± 2.82 ns B. Physiochemical Traits Chlorophyll a (mg/g FW) 1.27 ± 0.25 0.80 ± 0.10

ns 0.57 ± 0.15* 0.83 ± 0.15 ns 0.65 ± 0.12 ns 0.88 ± 0.04 ns 0.70 ± 0.08 ns 0.76 ± 0.11 ns
 Chlorophyll b (mg/g FW) 0.77 ± 0.15 0.53 ± 0.15 ns 0.33 ± 0.15 ns 0.46 ± 0.10 ns 0.44 ±
 0.07 ns 0.58 ± 0.11 ns 0.45 ± 0.08 ns 0.50 ± 0.18 ns Total Chlorophyll (mg/g FW) 2.03 ±
 0.040 1.33 ± 0.12* 0.90 ± 0.26*** 1.29 ± 0.23* 1.09 ± 0.19** 1.47 ± 0.14 ns 1.15 ± 0.11**
 1.26 ± 0.25* Carotenoids (mg/g FW) 0.47 ± 0.15 0.38 ± 0.11 ns 0.30 ± 0.10 ns 0.39 ± 0.12
 ns 0.33 ± 0.09 ns 0.42 ± 0.04 ns 0.35 ± 0.03 ns 0.38 ± 0.03 ns Total 2.28 ± 1.98 ± 1.70 ±
 2.06 ± 1.83 ± 2.10 ± 1.86 ± 1.96 ±

Note: Data as mean ± standard deviation (SD). We indicate statistical significance as follows: * p < 0.05, ** p < 0.01, *** p < 0.001, and ns = non-significant. A two-way ANOVA to evaluate how different treatments (row factor) and morphological traits (column factor) affected the growth of *Praecitrullus fistulosus*. The analysis showed that the treatments significantly influenced overall morphological performance, with the row factor producing a highly significant effect: F(7, 128) = 713.7, P < 0.0001. The column factor (morphological traits) also showed a significant effect F(7, 128) = 13.49, P < 0.0001), suggesting variability in the response of different traits to the treatments. The residual variance was relatively low (MS = 32.13), reflecting consistency in the dataset and supporting the robustness of the treatment effects. These results confirm that both treatment type and the specific morphological traits significantly influenced plant growth responses under the experimental conditions. Table 4: Two-Way ANOVA Summary for Morphological Trait Variation under Different Treatments and Conditions

	SS	DF	MS	F	P value
Row Factor	160523	7	22932	F (7, 128) = 713.7	P < 0.0001
Column Factor	3033	7	433.3	F (7, 128) = 13.49	P < 0.0001
Residual	4113	128	32.13		

Note: DF: Degrees of Freedom; SS: Sum of Squares; MS: Mean Square; F: F-ratio (between-group df, within-group df); P value: Statistical significance (α = 0.05). A two-way ANOVA was performed to evaluate the effects of different treatments and physiological traits on *Praecitrullus fistulosus*. The results revealed that the row factor (treatments) had a highly significant effect, with an F-value of F(6, 112) = 136.9 and P < 0.0001, Nitrogen (% DW)

0.046 0.033 ns 0.063 ns 0.053 ns 0.023 ns 0.027 ns 0.056 ns 0.059 ns Phosphorus (% DW) 0.67 ± 0.064 0.28 ± 0.04 ns 0.21 ± 0.06 ns 0.30 ± 0.10 ns 0.25 ± 0.03 ns 0.32 ± 0.10 ns 0.26 ± 0.03 ns 0.28 ± 0.08 ns Potassium (% DW) 1.89 ± 0.24 1.63 ± 0.44 ns 1.24 ± 0.38 ns 1.65 ± 0.20 ns 1.40 ± 0.47 ns 1.71 ± 0.48 ns 1.50 ± 0.32 ns 1.61 ± 0.33 ns

indicating that the various soil amendments and cadmium exposures significantly influenced physiological performance. The column factor (type of physiological trait) also exhibited a significant impact $F(7, 112) = 8.715, P < 0.0001$), reflecting that the physiological parameters responded differently to the treatments. The control plants exhibited a phosphorus level of $0.67 \pm 0.064\%$. Cadmium stress resulted in a pronounced decrease (Group 3: $0.21 \pm 0.06\%$), suggesting impaired phosphorus assimilation. Additionally, the control plants showed the highest potassium content at $1.89 \pm 0.24\%$ specific physiological traits significantly contributed to the variation observed in plant physiological responses. Table 5: Two-Way ANOVA Summary for Physiological Trait Variation under Different Treatments and Conditions

SS	DF	MS	F (DFn, DFd)	P value
Row Factor 60.15	6	10.03	$F(6, 112) = 136.9$	$P < 0.0001$
Column Factor 4.468	7	0.6382	$F(7, 112) = 8.715$	$P < 0.0001$
Residual 8.202	112	0.07323		

Note: DF: Degrees of Freedom; SS: Sum of Squares; MS: Mean Square; F: F-ratio (between-group df, within-group df); P value: Statistical significance ($\alpha = 0.05$).

Figure 1. Effect of Treatments on Morphological Traits of *Praecitrullus fistulosus*, Including (a) Plant Height, (b) Number of Leaves, (c) Leaf Area, (d–g) Biomass Parameters (Fresh and Dry Weight of Leaves and Roots), and (h) Fruit Weight.

Figure 2. Influence of Treatments on Physiochemical Parameters of *Praecitrullus fistulosus*, Including (a–c) Chlorophyll Content (Chlorophyll a, b, and Total), (d)

Carotenoids, and (e– g) Macronutrient Composition (Nitrogen, Phosphorus, and Potassium). 4. Conclusion Sewage sludge amendments, while beneficial for soil fertility, pose a clear risk via cadmium contamination. Biochar and nanoDAP, especially used together, effectively reduce Cd bioavailability and uptake while supporting plant growth. The synergy between immobilizing

and nutrient-enhancing effects offers a practical strategy for the sustainable and safe utilization of sewage sludge in agriculture. Cadmium contamination from sewage sludge poses a serious risk to crop productivity and food safety. The present study demonstrated that *Praecitrullus fistulosus* exhibited substantial growth inhibition, nutrient imbalance, and pigment loss under Cd stress. However, the application of rice husk biochar and Nano-DAP—particularly in combination—significantly ameliorated Cd toxicity by immobilizing cadmium in soil, enhancing nutrient uptake, restoring photosynthetic efficiency, and improving plant biomass and fruit yield. Integrating biochar with nanofertilizers provides a promising and eco-friendly strategy to manage heavy metal pollution in agricultural soils. Future work should focus on field-scale validation, long-term soil health monitoring, and optimization of amendment dosages to ensure sustainable and safe crop production under sewage sludge application. References: 1. Banu, Narasimhan. (2015). 3 Extraction and estimation of chlorophyll from medicinal plants. *International Journal of Science and Research (IJSR)*. 4. 209-212. 2. Abreu-Junior, C.H.; Brossi, M.J.D.; Monteiro, R.T.; Cardoso, P.H.S.; Mandu, T.D.; Nogueira, T.A.R.; Ganga, A.; Filzmoser, P.; de Oliveira, F.C.; Firme, L.P.; et al., 2019, Effects of sewage sludge application on unfertile tropical soils evaluated by multiple approaches: A field experiment in a commercial Eucalyptus plantation. *Sci. Total Environ.* 655, 1457–1467. 3. Alvarenga, P.; Mourinha, C.; Farto, M.; Santos, T.; Palma, P.; Sengo, J.; Morais, M.C.; Cunha-Queda, C.2015, Sewage sludge, compost and other representative organic wastes as agricultural soil amendments: Benefits versus limiting factors. *Waste Manag.* 40, 44–52. 4. Awashthi SK. Prevention of Food Adulteration Act No 37 of 1954. Central and State Rules as Amended for 1999.

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