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SHORT COMMUNICATION

EFFECT OF SOIL APPLIED ORGANIC AMENDMENTS AND MICROBIAL INOCULANT ON THE IMPROVEMENT OF GROWTH AND YIELD OF SOYBEAN (*Glycine max* L.)

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ABSTRACT

The usage of organic amendments and microbial inoculant together can be a reliable solution to avoid soil heavy metal pollution and to increase crop production in sustainable farming. This study was done to determine the effectiveness of organic amendments and Bradyrhizobium sp. in improving soybean (Glycine max L.) growth and yield. Control (T_1) and the other seven treatments were comprised as, field soil + compost (15% by weight) (T_2), field soil + wood biochar (15% by weight) (T_3), field soil + synthetic chemical fertilizer [0.01 kg m^{-2} urea, 0.01 kg m^{-2} P_2O_5 (triple super phosphate) and 0.008 kg m^{-2} K_2O (muriate of potash)] (T_4), field soil + Bradyrhizobium inoculum. (10^8 CFU ml^{-1}) (T_5), field soil + compost (15% by weight) + Bradyrhizobium inoculum (10^8 CFU ml^{-1}) (T_6), field soil + wood biochar (15% by weight) + Bradyrhizobium inoculum. (10^8 CFU ml^{-1}) (T_7), field soil + synthetic chemical fertilizer (111.5 kg N ha^{-1}, 111.5 kg P ha^{-1}, and 82.9 kg K ha^{-1}) + Bradyrhizobium inoculum. (10^8 CFU ml^{-1}) (T_8), The experiment was arranged in a completely randomized block design with ten replicates. In wood biochar amendment together with Bradyrhizobium sp. inoculation significantly ($p< 0.05$) enhanced plant height and the soybean yield compared to all the other treatments. All the organic amendments added treatments were positively influenced by the inoculation of Bradyrhizobium sp. The present study concludes that soybean yield can be significantly enhanced by the application of Bradyrhizobium sp. and wood biochar amendment.

Keywords: Biochar, *Bradyrhizobium* sp., Compost, *Glycine max*

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1.0 INTRODUCTION

The agriculture sector is the foundation in Sri Lanka's economy with more than 70% of the population living in rural areas depending on farming for their livelihoods. The extensive use of chemical fertilizers has led lowering the crop nutrient composition, soil fertility degradation due to surface runoff of excess nutrients and hence, reduces the agricultural sustainability [1]. Further, contamination of underground and surface water due to leaching out of nitrates and phosphates causes eutrophication of water bodies [2]. and consequently, shows harmful effects on human health [2] [3]. Sustainable agriculture is a production system that sustains the health of soil, ecosystems and people [1]. It relies on ecological processes, biodiversity and nutrient cycles adapted to local conditions rather than the use of inputs with diverse effects [4]. Ecological agricultural practices are very important in increasing and maintaining soil fertility. The supply of nutrients all macro and micro elements is more balanced in organic fertilizer, which helps to keep plants healthy [5]. Furthermore, organic natural amendments enhance soil biological activity, which enhances nutrient mobilization from organic and chemical sources and decomposition of toxic substances [6].

Introducing plant growth promoting bacteria (PGPR) and fungi (PGPF) into soil has been practiced in agriculture for decades for many functions such as supplying nutrients to crops, stimulating plant growth, inhibiting the activity of plant pathogens and improving soil structure etc. [7]. *Rhizobium* spp. has been successfully used worldwide to permit an effective establishment of the nitrogen fixing symbiosis with leguminous crop plants [8]. Biochar is a type of soil amendment and it is a kind of charcoal made from the pyrolysis of a range of biomass feedstocks, including crop, wood and yard wastes and manures [9]. This carbon rich product is being used to improve soil conditions and to sequester soil carbon [10] [11]. It is rich in a stable form of carbon which is not oxidised by soil microorganisms [12]. The predominant aromatic structure of biochar is resistant to biological and chemical decomposition. Unlike chemical fertilizers biochar has an extremely long life in soils and remain in soil from hundreds to thousand years [13]. For successful soil management, maintaining a suitable level of soil organic matter and biological cycling of nutrients is critical in the humid tropics [13].

Compost application can improve soil quality and productivity of crop production by replenishing soil organic matter and supplying nutrients [14]. Organic matter is a vital component of a healthy soil as it plays an important role in soil physical, chemical and biological fertility [13] [14]. Even soils under intensive agricultural management need an external supply of organic matter, as compost, in order to counteract progressive soil organic matter decline [15]. Mature compost has C/N ratio of less than 25, acidic or slightly acidic pH, and discrete concentrations of soluble P or K [15]. Similar to most organic amendments, the use of compost often leads to an increase in microbial population, diversity and microbial activity [16]. Therefore, the objective of the study was to determine the effectiveness of biochar, compost and *Bradyrhizobium* inoculant in the growth and yield of soybean.

2.0 MATERIALS AND METHODS

2.1 Study site

The present study was carried out in the greenhouse at the Rajarata University of Sri Lanka in Anuradhapura district, dry zone of Sri Lanka (8° 21' 0" North, 80° 30' 0" East). The average temperature inside the greenhouse during this period was 32.0 °C. Annual precipitation was between 1000-1500 mm and received monsoon rains mostly. The soils were red yellow podzolic soils [17].

2.2 Biochar production

Dried wood chips of *Cinnamomum zeylanicum*, *Mangifera indica*, *Michelia champaca*, *Hevea brasiliensis* and *Anacardium occidentale* were used for the production of wood biochar. Biochar was produced at a 450 -550 °C pyrolysis temperature using the two-barrel nested design [18].

2.3 Inoculum preparation and application

Bradyrhizobium japonicum, host specific for soybean was isolated from soybean root nodules which were collected from the field by following standard procedures. For the preparation of inoculum soybean nodules were surface sterilized by washing a series of disinfectants such as 95% ethanol and 3% (v/v) sodium hypochlorite (NaOCl) and sterile distilled water. After surface sterilization, nodules were crushed on a sterile Petri plate with 1 ml of sterile distilled water. Streak plates were prepared on congo red yeast mannitol agar (CRYMA) medium, which enabled to separate *Rhizobium* and *Bradyrhizobium* from other microbes within 3 – 7 days of incubation at 28 ºC. *Bradyrhizobium* sp. was re-isolated and maintained on a medium of yeast mannitol agar (YMA) and subsequently transferred to a density of 10^8- 10^9 CFU ml^{-1} in yeast mannitol broth to be used as *Bradyrhizobium* inoculum for soybean seed coating before sowing respective to the treatment [19].

A pot experiment was designed as Randomized Complete Block Design (RCBD) with ten replicates. Control; field soil only (T_1) and the other seven treatments were comprised of field soil + compost [15% by weight] (T_2), field soil + wood biochar [15% by weight] (T_3), field soil + synthetic chemical fertilizer [111.5 kg N ha^{-1}, 111.5 kg P ha^{-1}, and 82.9 kg K ha^{-1}] (T_4), field soil + *Bradyrhizobium* inoculum [10^8 CFU ml^{-1}] (T_5), field soil + compost (15% by weight) + *Bradyrhizobium* inoculum [10^8 CFU ml^{-1}] (T_6), field soil + wood biochar (15% by weight) + *Bradyrhizobium* inoculum [10^8 CFU ml^{-1}] (T_7), field soil + synthetic chemical fertilizer [111.5 kg N ha^{-1}, 111.5 kg P ha^{-1}, and 82.9 kg K ha^{-1} (urea, triple super phosphate and muriate of potash were used as N, P and K respectively)] + *Bradyrhizobium* inoculum [10^8 CFU ml^{-1}] (T_8). Soils were sampled from the field in Anuradhapura, Sri Lanka, at depth 0- 15 cm, air dried and then ground to pass a 2 mm sieve and prepared for a total weight of 3 kg pot^{-1} and mixed with various amendments accordingly. Air dried 100 g of ground *Gliricidia sepium* leaves and cow dung were added to each pot as organic supplementing mixture. One seed per pot was sown and irrigated with 100 ml of water daily.

Plant growth parameters, number of nodules and yield components were measured accordingly. Data were subjected to analysis of variance (ANOVA) and mean separation using the Tukey's Studentized Range Test (SAS version 6.12) at a significance level of $p<0.05$ [20].

3.0 RESULTS AND DISCUSSION

Table 1: Plant growth parameters and yield components of biochar, compost, biofertilizer and synthetic fertilizer amended treatments

Treatment	Plant height (cm)	Number of nodules	Root dry weight (g)	Shoot dry weight (g)	Number of pods	Dry weight of 100 seeds (g)
T_1	$56.6^{d} \pm 2.62$	$54.5^{d} \pm 4.11$	$4.27^{d} \pm 1.28$	$8.80^{c} \pm 1.28$	$28^{e} \pm 1.64$	$10.94^{b} \pm 0.76$
T_2	$69^{bc} \pm 4.34$	$73^{c} \pm 8.73$	$5.85^{c} \pm 0.56$	$10.58^{b} \pm 0.6$	$51.2^{cd} \pm 2.24$	$12.99^{ab} \pm 0.22$
T_3	$79.8^{b} \pm 0.19$	$98.5^{b} \pm 11.07$	$6.81^{a} \pm 0.51$	$11.25^{ab} \pm 0.27$	$61^{b} \pm 1.73$	$12.55^{ab} \pm 0.92$
T_4	$70.2^{bc} \pm .0.59$	$44.5^{e} \pm 8.53$	$5.37^{c} \pm 0.39$	$10.86^{b} \pm 0.25$	$59.2^{c} \pm 2.75$	$11.31^{b} \pm 0.52$
T_5	$64.6^{c} \pm 3.85$	$90^{b} \pm 9.24$	$5.33^{c} \pm 0.81$	$10.78^{b} \pm 0.78$	$56.4^{c} \pm 2.29$	$13.39^{a} \pm 1.43$
T_6	$71^{bc} \pm 3.39$	$90^{b} \pm 8.63$	$6.47^{ab} \pm 0.27$	$12.06^{a} \pm 1.14$	$57.4^{c} \pm 2.06$	$12.88^{ab} \pm 0.20$
T_7	$87.2^{a} \pm 0.08$	$132.5^{a} \pm 17.39$	$6.84^{a} \pm 0.28$	$13.75^{a} \pm 0.82$	$71^{a} \pm 1.76$	$13.87^{a} \pm 1.49$
T_8	$70.6^{bc} \pm 4.39$	$46^{e} \pm 7.59$	$5.45^{c} \pm 1.25$	$10.26^{b} \pm 0.25$	$60.4^{b} \pm 3.36$	$13.93^{a} \pm 1.81$

Field soil only (T_1), field soil + compost [15% by weight] (T_2), field soil + wood biochar [15% by weight] (T_3), field soil + synthetic chemical fertilizer [111.5 kg N ha^{-1}, 111.5 kg P ha^{-1}, and 82.9 kg K ha^{-1}] (T_4), field soil + *Bradyrhizobium* inoculum [10^8 CFU ml^{-1}] (T_5), field soil + compost (15% by weight) + *Bradyrhizobium* inoculum [10^8 CFU ml^{-1}] (T_6), field soil + wood biochar (15% by weight) + *Bradyrhizobium* inoculum [10^8 CFU ml^{-1}] (T_7), field soil + synthetic chemical fertilizer [111.5 kg N ha^{-1}, 111.5 kg P ha^{-1}, and 82.9 kg K ha^{-1}] + *Bradyrhizobium* inoculum [10^8 CFU ml^{-1}] (T_8).

Considering the soybean plant height, a significant difference ($p< 0.05$) was observed in different treatments and the highest was recorded in T_7 treatment which consisted of field soil, wood biochar and *Bradyrhizobium* spp., followed by biochar only treatment (T_3) (Table 1). This growth improvement could be explained by the higher nutrient availability in biochar treated soil, especially N and K [18]. Several studies also showed that biochar enhances nitrogen retention in compost and reducing emissions of ammonia as much as 65% [18]. The addition of biochar to soil could provide a potential sink for carbon and increase soil nutrient availability due to high water retention and nutrient sorption ability [21]. Further, the efficiency of biochar as an amendment for crop production depend on the soil type [21], wood species [22], other soil inputs [21] [22], or combination of these factors. Studies were also shown that the characteristics of biochar most important to plant growth can improve over time after its incorporation into soil [23], suggested that biochar may not be provided a significant source of plant nutrients, but they can improve the nutrient assimilation capability of crops where they are applied, by positively influencing the soil environment. *Bradyrhizobium* spp. inoculants also could increase biological nitrogen fixation inside soybean nodules and hence plant growth can be improved [24].

Nodule number also significantly different ($p< 0.05$) among the treatments (Table 1). The highest nodule number was observed in T_7 followed by T_3 and T_5. Interestingly, only biochar amended treatment (T_3) has shown increased nodule number same as *Bradyrhizobium* spp. added treatment (T_5). All biochar amended treatments showed a higher number of nodules than the chemical fertilizers added treatments. The low nitrogen content of most biochar and the exchange of NH_4^+ between the biochar surface and soil solution are likely to modify nitrogen availability to plant roots and hence stimulate nodulation and nitrogen fixation in legumes [25]. The number of nodules were higher in *Bradyrhizobium* inoculums added treatments than its respective un- inoculated treatments, except the treatment consisted of chemical fertilizers. This is because high N fertilization could inhibit symbiotic N fixation by *Bradyrhizobium* spp. Significantly increased ($p< 0.05$) root dry weight of soybean was observed in biochar amended treatments [T_3 and T_7] (Table 1). Porous nature of biochar facilitates profound root growth could be the reason for higher root dry weight [21].

Number of pods per plant also significantly different ($p< 0.05$) among the treatments of the present study. The number of pods per plant is economically important because it directly affects the profit of soybean farmers. Significantly higher pod number was observed in T_7 treatment of the present study, while the lowest was observed in T_1 which was not consisted of any amendment. Biochar and its highly porous structure can provide a suitable habitat for *Bradyrhizobium* spp. by providing carbon, energy and mineral nutrient needs [24] [25]. It was observed that although, *Bradyrhizobium* spp. inoculants are enhanced the number of pods in the plant, such inoculants are not much responsive with synthetic fertilizer [26]. Dry weights of 100 seeds (g) of the differently amended treatments revealed that the treatments of T_5, T_7 and T_8 showed the highest 100 seeds dry weights, while T_1 showed the significantly different ($p< 0.05$) the lowest dry weight, compared to the other treatments (Table 1). This also revealed that wood biochar and *Bradyrhizobium* spp. have potential to increase seed dry weight and even synthetic fertilizer amendment alone (T_4) it could not be higher.

4.0 CONCLUSION

It can be concluded that the native *Bradyrhizobium* spp. could be the most suitable inoculant for soybean cultivation practices in dry zone, Sri Lanka. Thus, wood biochar (15% by weight) with the native *Bradyrhizobium* spp. inoculant (10^8 CFU ml^{-1}) could be successfully used as an alternative to synthetic chemical fertilizer in soybean farming in dry zone, Sri Lanka.

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