BREWED TEA WASTE PROMOTE GROWTH OF *VIGNA UNGUICULATA* L. SEEDLINGS: SCOPE OF TEA WASTE USAGE IN GERMINATION BED INDUSTRY

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Abstract

The conversion of tea wastes in to a commercial asset ensures sustainable agriculture. In the present study, the growth of *Vigna unguiculata* L. seedlings were monitored on germination media contains black tea and milk tea wastes respectively. The addition of tea waste helped to increase soil pH whereas there was no change in conductivity of soil solution. The presence of tea waste resulted in an increase in size of vegetative parts, lateral roots and biomass respectively. Plant growth promoting effects of tea waste at molecular level was reflected as an increase of chlorophyll a content. However, there was no change in accumulation of chlorophyll b and carotenoids. Similar results noticed after treatment with vermicompost too. It predicted that more plant available nitrogen account for above changes. Thus, we concluded that tea waste can be an alternative for vermicompost to prepare germination beds.

Keywords: Tea waste, Germination bed, Seedlings vigor, Lateral roots, Photosynthetic pigments

Introduction

Healthy seedlings are prerequisite for crop productivity. Therefore, the seedlings are very often raised in germination bed. Germinating bed preparation is a bio-safe process in which soil is mixed with vermin compost, cow dung etc. Addition of organic matter in germination bed not only improves physicochemical properties of the soil but also attract plant growth promoting microbes (Sebastian and Prasad, 2013). Secondly, the usage of organic matter helps to improve nutrient status of the soil as well as humic substances in the soil. So raising plantlets in germinating beds offers a controlled rhizosphere environment that ensures development of healthy seedlings.

Germination cannot occur unless and until the seed is provided with an external supply of water. Water is absorbed by a dry seed through the micropyle and the seed coat. Water performs a number of functions during the germination of seeds (Zhang et al. 2015). It softens seed coat and makes it permeable for better gaseous exchange. Aeration of both the soil and seed is absolutely necessary for the germination because oxygen is necessary for the aerobic respiration by which the seeds get the requisite energy for the growth of the embryo (Li et al. 2019). Water also activates the protoplasm of the seed cell via converting insoluble food materials such as starch to soluble sugar. It is noteworthy that several enzymes which are essential for germination become active only in the presence of water. Seeds germinate under a wide range of temperature (Motsa et al. 2015; Zhang et al. 2015). But freshly harvested seeds of several plants germinate only within a narrow temperature range which widens only when after ripening has taken place. Similarly, plants differ as to the effect of light on their germination (Motsa et al. 2015). Seeds of many plants are non photoplastic because germination of these plants are not depend on light. Generally, photo assimilates stored in the plants support growth of tissues such as root, leaves, flower initials etc. with a negative carbon balance (Lemoine et al. 2013). The ability of a seed to germinate when provided with optimum condition is described as viability of the seeds. It depends upon type of stored food, hardness of seed coat etc. Most of the crop plants lose their viability after longer periods. However, seed dormancy is due to the internal factors such as accumulation of ABA in the seed (Ryu et al. 2014).

India occupies second position in terms of consumption, export, and production of tea (Lutgendorf, 2011). It estimated that more than 1.2 million tons of tea is produced annually in India. However, most of the industries dispose their tea wastes in open dumps or land area which results in pollution of land and water with phytochemicals (Kumar et al. 2017). Proper disposal of tea waste from industries and kitchens is necessary for maintaining healthy environment. Preparing germinating beds from the tea waste is one of the cheapest ways for the proper disposal of this waste without doing any harm to the environment. The usage of tea waste for germinating bed is a cheap and eco-friendly way to provide nutrients that boost plant growth. Tea waste also attracts beneficial creatures to seed beds. Earthworm are attracted by tea waste, and it serve many purposes in the planting such as turning organic matter in to plant food and increasing the amount of oxygen and water in the soil (Kaur et al. 2015). A growing media made up of tea waste can provide adequate anchors or support to the seedlings. It also would be a reservoir for nutrients and water. Addition of tea waste may facilitate oxygen to the roots, and

gas exchange between roots and the rhizospere. Therefore, in the present study, the utilization of tea waste for the preparation of germination bed is tested.

Materials and methods

Plant material

Vigna unguiculata L. is a herbaceous plant belong to genus Vigna. This plant is an important crop in the semiarid regions across Asia and Africa. The plant can either be short and bushy (20.0cm) or acts like a climber growing to a height of 7.0 m. The plant had taproot which can penetrate to a depth of 2.4m.The crop is mainly grow for its seeds which are high in protein (23.0 g per100.0 g). The mature plants thrive in poor dry conditions and it grows well in soils having 85.0 % sand. However, germination and the growth of seedlings of cow pea plants dependant on the availability of water and nutrient status of the soil. Vigna seeds used for the experiment in the present study were purchased from a local market at Erattupetta.

Germination media

Growing medium have following major functions such as supply roots with nutrients, air and water, allow plants for maximum root growth and physically support the plant respectively. The health of seedling is greatly affected by the growth media. In the present study germination bed is prepared by adding tea waste generated after the brewing of tea. Both black tea and milk tea waste were used for the present study. The soil for the culture was air-dried and sieved (3.0mm) for the removal of root debris and stones. The germination bed for the seedling culture is prepared by mixing 5.0 g of dry tea waste in 25.0 g of soil. A group of seedlings maintained in soil (30.0 g) and soil (25.0 g) plus vermicompost (5.0 g) as negative and positive controls respectively.

Growth conditions

The seeds were sown on the surface of the germinating media. Each type of growth media inoculated with 6 seeds to monitor the germination process. The plant culture performed at light intensity of 50.0 ± 5 μmol photons $m^{-2}s^{-1}$, photoperiod of 18 h light/6 h dark, temperature of 32 ± 2 °C, and water supply 5.0 mL/day respectively during the growth period of 21.0 days.

Measurement of soil pH

Soil pH is one of the important factors which determine the plant available nutrients present in soil. The soil pH decreases when there is an increase in the

amount of hydrogen ions in the soil. A drop in pH increases acidity and causes dissolution of cations in soil solution. In the present study, soil pH measured from soil solution using a standard calibrated pH meter where soil to water ratio was 1:2 (Sebastian and Prasad, 2014).The soil solution obtained after stirring of soil-water mixture for about 30 minutes.

Analysis of soil conductivity

Soil conductivity is a measure of the amount of salts in soil. It indicates soil health as well as nutrient availability of plants. In the present study, soil electrical conductivity measured from soil solution using a potassium chloride standard calibrated digital conductivity meter. The ratio of soil to water was 1:2 and conductivity measured after 30 minutes of stirring of soil and water mixture (Cambardella et al. 1994). The conductivity expressed in units of µ siemens.

Quantification of biomass

Analysis of biomass will show efficacy of various treatments performed in the study that will improve crop productivity. To quantify biomass, plants cleaned with water, and the fresh weight of the individual plants measured with the help of a weight balance. The biomass expressed as mg fresh weight.

Morphological analysis

Morphological characters such as length of plant parts and number of lateral roots were analyzed by direct observation of specimen at the time of harvest. Length or width of leaves, stem and roots measured using a cm scale. The lateral root number was calculated by counting number of lateral roots arise from the tap root.

Estimation of photosynthetic pigments

Plant pigments measured from acetone – DMSO (1:1) extract of leaf. Leaf discs placed in test tubes containing extraction solvent, and kept in dark for 24.0 hrs. The absorbance (A) of extract recorded and the content of pigments (µg/ml) determined using Arnon's equations for photosynthetic pigments (Lichtenthaler and Wellburn, 1983). The amount of photosynthetic pigments expressed in mg per gram fresh weight of leaves.

Results

Soil properties

Soil is a complex mixture of minerals, organic matter, water, air and microbes which degrade remains of living things. The physicochemical properties of soil will change in the course of addition of soil amendment. The soil pH and conductivity

of soil solution are the key soil properties which changes up on addition of soil amendments. In the present study, mixing of tea waste to the soil caused an increase of soil pH. It noticed that soil pH increase from 6.8 to 7.2 (0.4) units after the addition of both black and milk tea wastes. However, the increase in soil pH after the addition of vermicompost was about 0.1 units only. Soil conductivity is closely related to presence of mineral salts in the soil. But there was no change in conductivity (0.066 Siemens/m) of soil solution after the addition of both vermicompost and tea waste.

Morphological changes

Mophological analyses help to identify the threshold of plant growth responses. The changes in vegetative structures are observed for the comparison of effectiveness of various treatments on plant growth (Figure.1, Table.1). Treatment with milk tea was effective to increase length of root, stem and leaves. Secondly, black tea waste amendment found to be superior over other additives to increase length of the stem. However, the vermicompost amendment which is used as a positive control was more effective to increase the number of lateral roots.

Biomass

Biomass production depends on photosynthetic activities of leaves. In the present study, it noticed that addition of organic amendments such as vermicompost and tea waste increase biomass of Vigna seedlings up to 13.0-21.0 % (Table.2). The highest increase of biomass noticed among of plants reared in germination bed with milk tea waste. The milk tea waste treatment increased stems and leaf biomass up to 33.0 % and 18.0 % respectively. However, there was no effect on root biomass after treatment with milk tea waste. It is noteworthy that seedlings reared with black tea waste amendment had 33.0 % more root biomass compared to those grow with milk tea waste amendment.

Photosynthetic pigments

Photosynthetic pigments are involved in light harvesting process during photosynthesis. Secondly, content of pigments such chlorophyll considered as an indicator of nitrogen status of the plant. It found that total chlorophyll content increased up to 9.0 % after the growth of seedlings in germination bed contain milk tea waste (Figure.2). The increase in chlorophyll was more pronounced with regard to accumulation of chlorophyll a where there was an increase of 20.0 % during application of milk tea waste (Figure. 3). However, black tea waste amendment had no effect on the accumulation of chlorophyll in seedlings. Milk tea waste was more effective than vermicopost to increase accumulation of chlorophyll in

seedlings (Figure. 3). Also, addition of vermicompost decreased chlorophyll b content in seedlings unlike tea waste where there was an increase of chlorophyll b content up to 4.0 % (Figure. 4). There was also a decrease in carotenoids content among seedlings grow in germination bed contain tea waste and vermicompost amendments (Figure. 5).

Discussion

A germination bed is a local soil environment to raise seedlings from seeds. Seeds are embryonic phase of a plant. Germination occurs as soon as moist warm conditions available. The seed coat becomes permeable to O_2 and water during the course of imbibitions of water (Debeaujon et al. 2000). After imbibitions, the inner content of the seed coat leads the rupture of the seed coat, and it is followed by emergence of plumule and radicle (Northam and Callihan, 1994). Seeds confront changes in colour and water content during germination. Germination ends when the embryo has developed into a seedling which is able to perform photosynthesis. In the present study, addition of organic matter content in the form of vermicompost and brewed tea waste to the germination bed found to enhance vigor of seedlings. These seedlings had more biomass as well as photosynthetic efficiency in terms of pigment composition.

Organic matter in the soil helps to decrease soil compactness which in turn enhances aeration (Shah et al. 2017). Also, organic matter reported to increase plant available nutrients in the soil (Ding et al. 2020). So it assumed that addition of tea waste enhanced aeration and nutrient availability for seedlings growth. But there was no change in conductivity of soil solution after the treatment of vermicompost and brewed tea waste. The inherent factors affecting soil conductivity are soil minerals, nutrients, and soil texture (Ding et al. 2020). Above results points that increase in amount of cations such as K^+, Mg^{2+}, Ca^{2+} etc. in the soil was not significant after the addition of organic amendments chosen for the study. However, the addition of vermicompost and brewed tea waste made soil pH slightly alkaline which is ideal for uptake of chemical species of nitrogen (Bruce et al. 2013). It is also reported that organic matter enhances cation exchange capacity of the soil (Shah et al. 2017). So the addition of organic amendments increased plant available nutrients in the soil which in turn promoted seedlings growth in the present study.

Morphological analysis pointed that vegetative parts attain more size and biomass after the addition of vermicompost and brewed tea waste. It also noticed that seedlings forms more lateral roots during growth in germination bed contain organic amendments. The number of lateral roots in *Arabidopsis thaliana* increased

during organic matter treatment as a response to accumulation of nitrogen (Walch-Liu et al. 2006). It is also reported that photosynthesis efficacy as well as more nitrogen uptake result an increase in size and biomass of plants grow in soil contain organic matter (Guo et al. 2019). The results of present study corroborate with above reports, and the analysis of photosynthetic pigments support the finding that increase in plant available nutrients, especially nitrogen account for higher biomass and more lateral roots production during growth of Vigna seedlings in both vermicompost and tea waste amended soil.

Chlorophyll is an indicator of nitrogen status of the plant (Prost and Jeuffroy, 2007). The increase of chlorophyll a content during treatment with vermicompost and milk tea waste indicates that addition of these amendments in soil results more nitrogen for plant uptake. It is noteworthy that both these amendments are rich in protein as well as amino acids which are a source for nitrogen. However, black tea waste had relatively low protienaceous compounds compared to milk tea waste which account for no change in accumulation of chlorophyll content after treatment with black tea waste. Chlorophyll b and carotenoids are accessory pigments which play important role in photoprotection when the light reaction of photosynthesis is under stress (Kume et al. 2017). A decreasing trend in accumulation of these pigments found in the experimental plants with respect to control plants points that addition of organic amendments in the germination bed enhance photosynthetic efficiency of Vigna seedlings. In conclusion, the results of the present study point that addition of tea waste could be an alternative for vermicompost used in germination bed. Secondly, milk tea waste found to be superior to black tea waste for the stimulation of growth of Vigna seedlings.

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Table. 1 Morphological changes in seedlings

Treatments	Leaf length	Leaf width	Root length	Lateral roots	Stem length
S	3.0	2.6	3.0	4.0	10.0
S + C	3.7	3.0	5.0	8.0	10.4
S + BT	3.8	2.5	4.8	6.0	13.2
S + MT	3.8	2.7	5.1	6.0	10.6

*Abbreviations : S –Soil, S+V- Soil with vermicompost, S + BT- Soil with black tea waste, S + MT - Soil with milk tea waste

Table. 2 Biomass of plant parts after treatments

Treatments	Root weight	Leaf weight	Stem weight
S	0.06	0.18	0.22
S + C	0.08	0.22	0.24
S + BT	0.08	0.18	0.26
S + MT	0.06	0.24	0.26

*Abbreviations :S –Soil, S+V- Soil with vermicompost, S + BT- Soil with black tea waste, S + MT - Soil with milk tea waste

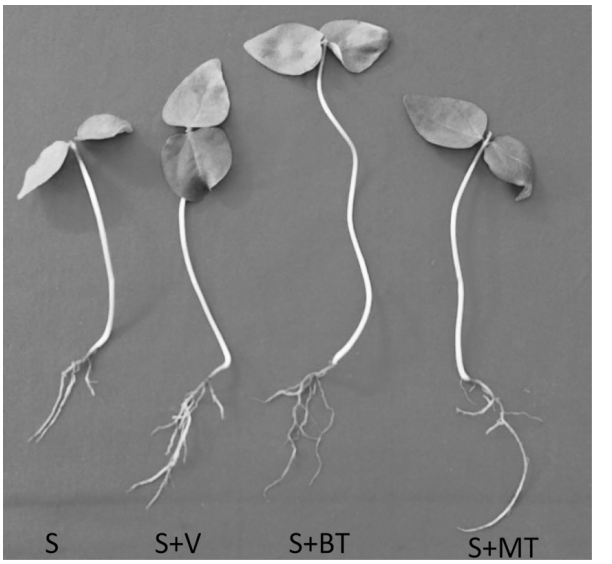

Figure. 1

Morphological appearance of plants at the time of harvest. Abbreviations: S - Soil, S+V - Soil with vermicompost, S + BT - Soil with black tea waste, S + MT - Soil with milk tea waste

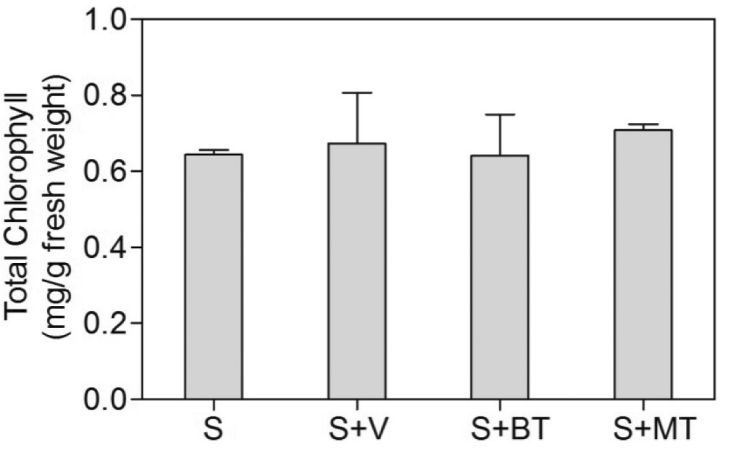

Figure. 2

Total chlorophyll contents in leaves. Abbreviations: S - Soil, S+V - Soil with vermicompost, S + BT - Soil with black tea waste, S + MT - Soil with milk tea waste.

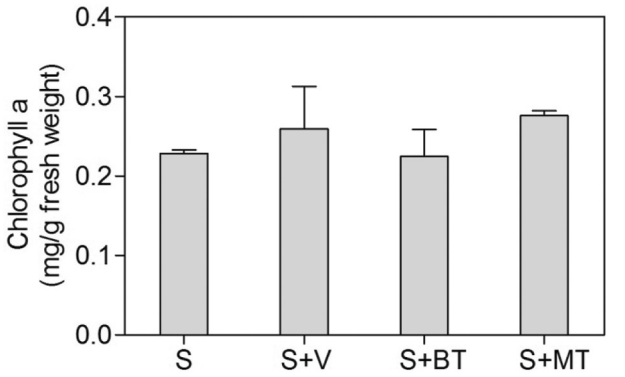

Figure. 3

Chlorophyll a content in plants. Abbreviations: S - Soil, S+V - Soil with vermicompost, S + BT- Soil with black tea waste, S + MT - Soil with milk tea waste.

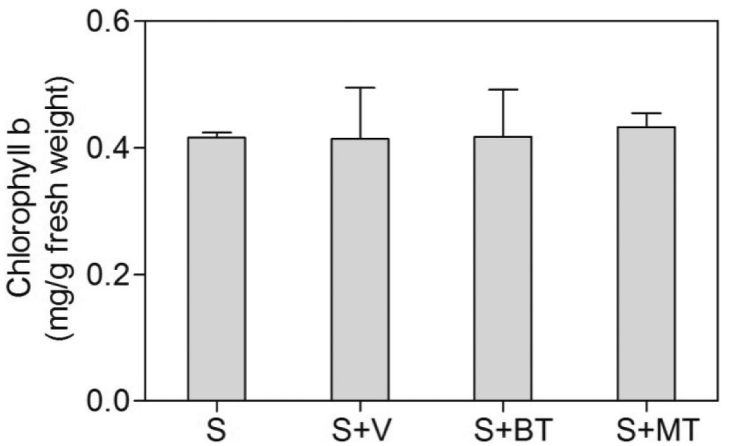

Figure. 4

Chlorophyll b content in leaves. Abbreviations: S - Soil, S+V - Soil with vermicompost, S + BT - Soil with black tea waste, S + MT - Soil with milk tea waste.

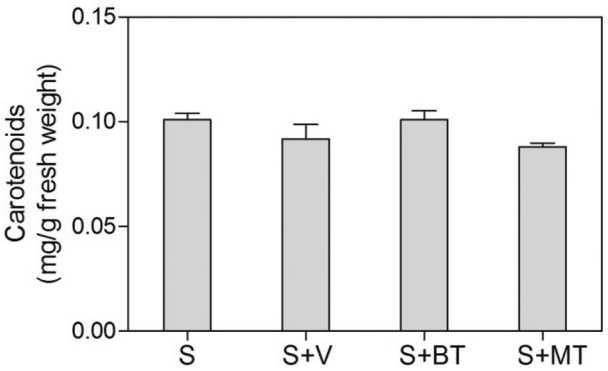

Figure. 5

Carotenoid content in plants. Abbreviations: S - Soil, S+V - Soil with vermicompost, S + BT - Soil with black tea waste, S + MT - Soil with milk tea waste.