Effects of microbial-fertilizers on the morphological parameters and biochemical content of Cow pea Vigna unguiculata (L.) Walp. - A biotechnological approach

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Abstract

The use of chemical fertilizers and agrochemicals has dangerous effects on both environment and human health. Frequently used chemical fertilizers to increase crop yields and reload soil nutrients have been extinguished by the soil for many years resulting in severe soil microbial communities with serious health and environmental risks. They pollute cultivated land and accumulate in the food-webs and infect humanity. One of these strategies is based on the use of microbial fertilizers resulting in the field test to solve problems. For this reason in this field experiment was performed with an objective to assess the effects of different microbial fertilizers on preference parameters of cow pea Vigna unguiculata (L.) Walp. The trial was carried out under the following scheme T₁ – Control, T₂ – Vermicompost (VC), T₃ – Rhizobium, T₄ – AMF, T₅ – Rhizobium + VC, T₆ – VC + AMF, T₇ – Rhizobium + VC + AMF application. There is an end to the use of single and combination of microbial fertilizers gave the energetic expressed as shoot and root length, number of leaves, total leaf area, fresh and dry weight, as well as the chlorophylls, carotinoid, protein aminoacid and carbohydrates of cow pea seedlings. Increasing among the parameters led to a better positive result of the composition of the microbial fertilizers leading to the efficient action of the morphological and all biochemical mechanisms.

Keywords: Rhizobium, Vermicompost, AMF, Growth and biochemical content and Cow pea

1. Introduction

With increase in population, rapid urbanization and industrialization, land area under agricultural production is decreasing day by day. Over the last 150 years, excessive inputs of chemical fertilizers have led to degraded pollution low productivity and serious health and environmental hazards. Organic farming methods such as the use of a microbial inoculation will solve these problems and restore the ecosystems (Mishra, 2014).

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Microbial inoculation or microbial fertilizers can be defined as a natural product of living microorganisms that can be applied to plant roots which are present in the soil in the adjacent area of the root zone (Amutha et al., 2014). Different sources of microbial fertilizers such as biological nitrogen fixers (BNF), plant stimulators, phosphorus solubilizing microorganisms (PSM), plant growth promoting rhizobacteria (PGPR), AMF and others) in microbes restores the soil’s natural nutrient cycle under soil organic matter. Microbial fertilizer contains microorganisms which promote the adequate nutrients for host plants and ensure proper development of physiological growth and regulation. Among the microbial fertilizers showed a significant important in getting yield of high quality and reduce ecological pollution (Shevananda, 2008). And microbial fertilizers improve plant nutrient availability, and stimulated target plant growth when inoculated on the plant surface or on the seeds used in the soil (Muraleedharan et al., 2010; Singh et al., 2014; Song et al., 2015; Dinu et al., 2015; Ghaffari et al., 2018; and Pagnani et al., 2020).

Rhizobium belongs to family Rhizobiaceae, symbiotic in nature, fixes nitrogen 50-100 kg/ha, with legumes only. It is useful for pulse legumes like chickpea, red-gram, pea, lentil, black gram, and oil-seed legumes like soybean and groundnut, and forage legumes like berseem and lucerne. It colonizes the roots of specific legumes to form tumor like growths called root nodules, which act as factories of “ammonia” production. Rhizobium has the ability to fix atmospheric N₂ in symbiotic association with legumes.

AMF form symbiotic relationship with most important plant families. These AMF trafficking of relatively unsustainable nutrients, especially phosphorus, plays an important role in improving soil structure and quality (Soliman et al., 2012). AMF interacts with a wide range of other soil microorganisms in the root rhizosphere, and in total soil. These interactions may be inhibitory or stimulatory some are clearly competitive and others may be mutual. These bio-elictors increase producing primary productions and providing more resources for production of secondary metabolites through increase of available nutrient uptake. AMF it plays a vital role in improving water intake, osmotic adjustment boosting antioxidant activity, functional hormonal relationships, soil fertility and plant nutrition and plant protection against biotic and abiotic stresses (Garcia-Garrido and Ocampo, 2002; Marulanda et al., 2003; Wu et al., 2006; Marulanda et al., 2007; Smith and Read 2008; Ceccarelli et al., 2010; Cekic et al., 2012; Kapoor et al., 2013; Dal Cortivo et al., 2018; and Begum et al., 2019).

Earthworm compost or Vermicompost (VC) contains plant growth hormones, growth regulating substances and humin acids were enhancing plant growth and productivity, when added VC is a stable microorganism that relaxes the soil and improves airflow. The mucus associated with the cast being hydroscopic absorbs water and prevents water logging and improves water-holding capacity. The organic ‘C’ in VC releases the nutrients slowly and gradually into the system and enables the plant to absorb these nutrients (Atiyeh et al., 2002; Sahni et al., 2008; Simsek-Ersahin 2011; Suhag 2016; Kazeminasab et al., 2016; and A. nanthavalli et al., 2019).

Cowpea (Vigna unguiculata (L.) Walp.) belongs to the family Fabaceae is grown in tropical Africa, Asia, is mostly grown as grain, vegetable and forage crop in North and South America. It is preferred because of its wide dietary adaptability and tolerance to many stresses, and is an important food source, and is an important protein source of over 200 million people in sub-Saharan Africa and is among the top 10 fresh vegetables in the People’s Republic of China. There are a number of Mn nutrition factors such as hemagglutinin, tannin, trypsin inhibitors, oxalate, phytate, polyphenols and oligosaccharides (Sreerama, 2012; and Afiuikwa, 2012). Several researchers have undertaken extensive research on how to use microbial fertilizers to see significant improvements in the growth of some agricultural crop plants (Shamseldin et al., 2010; Geetharani and Parthiban 2014; Mary et al., 2015; Pandya et al., 2016; Angadi et al., 2017; and Nazar et al., 2019).

2. Materials and methods

2.1. Seed collection

The seeds of cow pea Vigna unguiculata (L.) Walp. Varity (Paiyur 1) were sourced from locally private agro center, Dharmapuri District, Tamil Nadu, India.
2.2. Seed treatments

The sowing seed get affected with pests and diseases at different stages of its growth by various kinds of microorganisms like bacteria, fungi and others. This following seed purification method can be prevented from seedling stage before cultivation. Collect cow urine in a container for about 15 days. Mix 500 mL of cow urine with 1 L of water and mix well. Add 0.5 kg of seeds to the mixture and stir well. Soak the seeds in the mixture for half an hour and let the chaffy seeds and dust particles float in the solution. Take the soaked seeds out and dry them in the shade. Now the seeds are ready for cultivation. Treatments cover many types of pests and disease and crops that attack root leaves.

2.3. Field preparation

The experimental field was fully plowed, leveled and then divided into test layers according to layout. Pre-irrigation of a sow was provided to ensure effective soil moisture. The field is plotted as $1 \times 1 \text{ m}^2$ in split plot design with three replicates.

2.4. Microbial fertilizers

A variety of microbial fertilizers such as Rhizobium, Azotobacter, AMF and Vermicompost (VC) were utilized for this study from Forest Office (Tree Seedling branch), Dharmapuri District, Tamil Nadu, India.

3. Experimental design and details

3.1. The experimental design and details are as follows:

| Cultivar             | cow pea Vigna unguiculata (L.)Walp. |
|----------------------|-------------------------------------|
| No. of treatments    | 7                                    |
| Sampling days        | 30 DAS (Day After Sowing)            |

3.2. Treatment details

The treatment details are as follows:

- $T_1$ - Control
- $T_2$ - Vermicompost (VC)
- $T_3$ - Rhizobium
- $T_4$ - AMF
- $T_5$ - Rhizobium + VC
- $T_6$ - AMF + VC
- $T_7$ - AMF + Rhizobium + VC

4. Methods of fertilizer in seed inoculation (seed treatments)

4.1. Inoculation of Rhizobium and AMF

Add 150 g jaggery to 1 L water in a container and prepare a jaggery paste by heating it on low flame for 5-10 min. Cool the solution and add the microbial fertilizer into this jaggery paste @100 g of Rhizobium, and AMF to 0.5 kg of seeds. Add the seeds to be treated into this container and mix well so that the microbes are smeared properly over the seeds. These seeds are dried under shade and used for sowing. And the application of 250 g of concentrated VC to soil before sowing directly into soil will provide the plant with the necessary nutrients and also help in the prevention of pests and disease for the seedling stage.

4.2. Irrigation schedule

Pre-sowing irrigation was given to ensure uniform germination. Irrigation was given at two times per day with due care to avoid excess flooding of water. Uniform irrigation was given for three times a week.

4.3. Sampling Collection

Anywhere in the experimental field five plant samples were randomly collected at 30<sup>th</sup> DAS and they were used for observed of morphological parameters like shoot length, root length, root nodules, total leaf area, fresh
weight and dry weight. These plant samples were later used to determine the amount of pigment and biochemical content.

4.4. Weed management
Hand weeding was done two times at 30th DAS after sowing to remove the weeds from the field.

4.5. Germination percentage
The number of sprouted seeds in each treatment was counted up to 7th DAS after each treatment showing the total germination percentage followed by the following formula

$$\text{Germinating Percentage} = \frac{\text{Total Number of Seeds Germinated}}{\text{Total Number of Seeds Sown}} \times 100$$

4.6. Shoot length and root length
Five plants were randomly selected for the field on 30th DAS, to recorded seedling shoot and root length. They were measured by using centimeter scale.

4.7. Fresh weight and dry weight
Five plant samples were randomly selected for 30th DAS in the experimental plot. They were separated into whole seedlings. Their fresh weight was taken by using an electrical single pan balance. The same seedlings were packed in brown pocket cover and they were kept in a hot air oven at 80°C for 24 hr and then their dry weights were also determined.

4.8. The root nodules
With Five plants ingested roots from each plot the roots removed with the help of digging fork were carefully separated by washing the soil gently. The root nodules were calculated.

4.9. Total Leaf Area (Kalra and Dhiman, 1977)
The plant samples were collected at 30th DAS the length and breadth of the leaf samples were measured and recorded. The total leaf area was calculated by using the Kemp’s constant.

$$\text{Total leaf area} = L \times B \times K$$

where, L - length, B - breadth and K - Kemp’s constant (for dicot - 0.66).

4.10. Biochemical content
The photosynthetic pigments like viz., chlorophyll ‘a’ and ‘b’ (Arnon, 1949), and the carotenoid content (Kirk and Allen, 1965). The estimation of protein (Lowry et al., 1951), amino acids (Moore and Stein, 1948), sugars (Nelson, 1944), and carbohydrate content (Dubois et al., 1956) were analyzed and recorded in 30th DAS of experimental plants were presented (Table 2).

5. Results and Discussion

5.1. Germination Percentage
The effects of different type of microbial fertilizers treatment, viz., T₁– Control, T₂– Vermicompost (VC), T₃– Rhizobium, T₄– AMF, T₅– Rhizobium + VC, T₆– VC + AMF, T₇– Rhizobium+VC+AMF on the seed germination (%) and morphological parameters of cow pea were represented in (Table 1). The maximum number of seed germination (100%) and the height value of root length (15.2 ± 0.66 cm/ seedling) and shoot length (31.6 ± 1.680 cm/ seedling), number of nodules (67.5± 3.630), number of leaves (46.4 ± 2.32) total leaf area (18.8 ± 0.94) fresh weight (41.5 ±2.075 g/ seedling) and dry weight (19.7 ±0.985 g/ seedling) was recorded in (T₇), when compared to other six treatments.

The minimum number of seed germination (90.5 %) and lowest value of root length (6.8 ± 0.340 cm/ seedling) and shoot length (16.5 ±0.690 cm/ seedling), number of root nodules (15.3 ±0.765), number of leaves (24.4 ±1.22) total leaf area (8.5 ±0.42) fresh weight (17.9 ±0.895 g/ seedling) and dry weight (9.5 ±0.475 g/ seedling) was recorded in (T₁) treatments.
Table 1: Effects of different microbial fertilizers on growth parameters of cow pea
Vigna unguiculata (L.) Walp

| Treatments | Germination (%) | Root length | Shoot length | Root nodules | Number of leaves | Total leaf area | Fresh weight | Dry weight |
|------------|----------------|-------------|--------------|--------------|-----------------|----------------|--------------|------------|
| T1         | 90.5 ± 4.52    | 6.8 ± 0.340 | 16.5 ± 0.690 | 15.3 ± 0.765 | 24.4 ± 1.22     | 8.5 ± 0.42     | 17.9 ± 0.895 | 9.5 ± 0.475 |
| T2         | 93.0 ± 6.60    | 8.2 ± 0.410 | 17.5 ± 0.875 | 22.8 ± 1.140 | 27.8 ± 1.39     | 12.6 ± 0.59    | 20.5 ± 1.025 | 11.0 ± 0.550 |
| T3         | 95.0 ± 4.75    | 9.0 ± 0.950 | 18.6 ± 0.930 | 40.2 ± 2.010 | 30.8 ± 1.54     | 13.4 ± 0.67    | 22.8 ± 1.140 | 12.9 ± 0.645 |
| T4         | 96.5 ± 4.83    | 10.0 ± 0.500 | 19.0 ± 0.950 | 35.6 ± 1.780 | 35.2 ± 1.76     | 14.1 ± 0.75    | 23.5 ± 1.175 | 15.5 ± 0.775 |
| T5         | 97.5 ± 4.88    | 13.6 ± 0.530 | 22.0 ± 1.30  | 58.6 ± 2.930 | 38.3 ± 1.915    | 15.8 ± 0.79    | 25.2 ± 1.260 | 16.5 ± 0.825 |
| T6         | 98.5 ± 4.93    | 14.8 ± 0.592 | 26.8 ± 1.490 | 39.6 ± 1.980 | 42.4 ± 2.12     | 16.5 ± 0.82    | 38.5 ± 1.925 | 18.6 ± 0.930 |
| T7         | 100 ± 5.00     | 15.2 ± 0.66  | 31.6 ± 1.680 | 67.5 ± 3.630 | 46.4 ± 2.32     | 18.8 ± 0.94    | 41.5 ± 2.075 | 19.7 ± 0.985 |

Note: ± Standard deviation; T1-Control; T2 Vermicompost (VC); T3 Rhizobium; T4 AMF; T5 Rhizobium + VC; T6 VC + AMF; T7 Rhizobium + VC + AMF.

In present study, all the treatments of microbial fertilizers, (T1) were higher germination percentage of cow pea seedlings when compared to (T1) plot. The maximum, seed germination was observed in combined microbial fertilizer (Rhizobium + VC + AMF) treatments. These findings are in agreement with the same results of (Mahakavi et al., 2014 in Arachis hypogaea L.; Sivakumar et al., 2013 in P. haseulos radita L and Vigna sesisis Edhl.; Ghaffari et al., 2018 in Oryza sativa L). Relatively high levels of germination in cow pea seeds are due to AMF, Rhizobium an up take of (Phosphorus and Nitrogen) in the soil, to enhance the metabolic activity in germinating seeds thus, resulted in early and higher seed germination percentage. And another opinion is the maturation of VC to improve the speed of seed germination (Warman and AngLopez, 2010). AMF to colonize the plant roots opening with the first rootlet of the germinating seeds, thereby contributing to plant nutrition and growth (Pagnani et al., 2020; and Dal Cortivo et al., 2020).

That is introducing the microbial fertilizers without adverse effects on the soil condition will increase the soil quality. Raising the respiration rate of the soil indicates that the microbial content of the soil is present in the kinetics and strengthening it. Microbial fertilizers have the potential to contribute to sustainable plant growth, such as root and shoot length of crop plants (Mitra et al., 2019).

In present experiments, among the treatment (T1) was higher shoot and root length of cow pea seedlings when compared to (T1) plot. The role of Rhizobium, VC and AMF is providing necessary nutrients for plant growth. Such microbes are beneficial to plant growth through colonizing plant roots and inducing mechanisms by which plant growth increases, on the basis of present work are in concurrence with the earlier reports of (Mahfouz and Sharaf-Eldin, 2007 in Poenicium vulgare; Abdel-Aziz et al., 2007 on Rosmarinus officinalis; Kumar et al., 2009 on Artemisia pallens; Valadabadi and Farahani, 2011 on Nigella sativa; Al-Fraihat et al., 2011 on Majorana hortensis and Yadegari et al., 2012 on Thymus vulgaris; Herliana et al., 2019 on Glycine max (L) Merril).

The (Table 1) indicates that the inoculation of Rhizobium +VC +AMF results in increase in fresh and dry weights and root nodules of cow pea. The main reason for the increasing shoot and root length, fresh and dry weight and number of root nodules for the application of microbial fertilizers. The microbes are reasonable quantity of N and P uptake by soil, it caused higher biomass production (root, shoot length and fresh weights) which leads to improvement of dry weight of seedlings and also increased the number root nodules (Rashmi et al., 2008; Azzaz et al., 2009; Abdulameer, 2011; Tagore et al., 2013; and A sante et al., 2020). Among the soil microorganisms, Rhizobium form intimate symbiotic relationships with plant root by react chemotactically to flavonoid molecules released as signals by the host. These plant compounds induce the expression of nodulation (nod) genes in rhizobia, which in turn produce lipo-chitooligosaccharide (LCO) signals that trigger mitotic cell division in roots, leading to nodule formation (Matiru and Dakora 2004; Dakora 2003; and Lhuissier et al., 2001).
The field experiment results were found in single and dual inoculation of microbial fertilizers on the biochemical content was presented in (Table 2). In presence study, chlorophyll ‘a’, chlorophyll ‘b’ content of cow pea plants was predictable in field experiments at 30\textsuperscript{th} DAS. The highest chlorophyll content was recorded in \textit{Rhizobium} + VC + AMF plants when compared to control plants. The increasing chlorophyll content was due to the presence of microorganisms present in the soil that colonize in the rhizosphere and stimulate the plant growth and biochemical contents (Varma and Schuepp, 1995; Cabangon et al., 2011; and Yuan et al., 2016). The increase in leaf area can promote photosynthetic rates in AMF treated plants. Higher level of phosphorus (P) in tissues as a result of root colonization by the AMF and \textit{Rhizobium} can be predictable to increase the chlorophyll content, as ‘P’ is one of the important components of increasing chlorophyll content (Adivappar, 2001; Shivaputra et al., 2004; and Arumugam et al., 2010).

| Treatments | 30th DAS of Cow pea (mg/fr.wt.) |
|------------|---------------------------------|
|            | Chl a  | Chl b  | Carotenoid | Total sugars | Amino acid | Proien | Carbohydrate |
| T\textsubscript{1} | 0.72 ± 0.036 | 1.1 ± 0.055 | 0.78 ± 0.038 | 1.41 ± 0.070 | 1.76 ± 0.088 | 2.63 ± 0.131 | 1.41 ± 0.070 |
| T\textsubscript{2} | 1.20 ± 0.060 | 1.98 ± 0.099 | 0.94 ± 0.047 | 1.91 ± 0.095 | 2.45 ± 0.122 | 3.82 ± 0.191 | 2.54 ± 0.127 |
| T\textsubscript{3} | 1.98 ± 0.099 | 2.16 ± 0.108 | 1.48 ± 0.074 | 2.23 ± 0.111 | 3.20 ± 0.160 | 4.64 ± 0.231 | 3.65 ± 0.182 |
| T\textsubscript{4} | 2.16 ± 0.108 | 2.75 ± 0.137 | 1.77 ± 0.089 | 2.86 ± 0.143 | 3.96 ± 0.182 | 5.16 ± 0.258 | 4.45 ± 0.222 |
| T\textsubscript{5} | 2.56 ± 0.128 | 3.57 ± 0.178 | 2.11 ± 0.107 | 3.30 ± 0.165 | 4.41 ± 0.220 | 6.52 ± 0.326 | 5.25 ± 0.262 |
| T\textsubscript{6} | 2.75 ± 0.137 | 4.10 ± 0.020 | 2.32 ± 0.116 | 3.92 ± 0.189 | 5.13 ± 0.256 | 7.10 ± 0.369 | 6.70 ± 0.335 |
| T\textsubscript{7} | 3.00 ± 0.150 | 4.86 ± 0.248 | 2.70 ± 0.138 | 4.61 ± 0.230 | 5.92 ± 0.298 | 8.50 ± 0.420 | 7.22 ± 0.361 |

Note: ± Standard deviation; T\textsubscript{1}-Control; T\textsubscript{2}-Vermicompost (VC); T\textsubscript{3}-Rhizobium; T\textsubscript{4}-AMF; T\textsubscript{5}-Rhizobium + VC; T\textsubscript{6}-VC + AMF; and T\textsubscript{7}-Rhizobium + VC + AMF.

Carotenoid is a scarf pigment in photosynthesis coordination of plants. A maximum value of carotenoid content is registered in (T\textsubscript{7}). The same findings of increase in carotenoid content were reported in Karuppaiah and Kathiravan (2006). A probable mechanism is well known that carotenoids are involved in the protection of the photosynthetic apparatus against photo-inhibiting damage by single oxygen (O\textsubscript{2}). So, carotenoid can directly deactivate (O\textsubscript{2}) and can also slake the excited triple state of chlorophyll (Foyer and Harbinson, 1994; and Singh et al., 2014).

The highest protein content of cow pea plant was recorded in the (T\textsubscript{7}) when compared to control. The same results suggested that the combination of different microbial fertilizers with various crop plants significantly increased soluble protein in spinach \textit{Spinacia oleracea} L. (Song et al., 2015). And also (Hosseinzadeh et al., 2018) statement that increase in protein content, by the soil inoculation of VC in chickpea.

Phosphorus plays an important role in various physiological processes such as root growth and nodulation and nitrogen fixation and specifically for protein synthesis in metabolic activities (Bhardwaj et al., 2014; Kazeminasab et al., 2016; and Omara et al., 2017). The result has been credited to quantitative and qualitative changes in the protein composition of AMF inoculated seedlings (Dumas-Gaudot et al., 2000; Sadhana 2014; and Wang et al., 2018). The significant increasing in protein content (T\textsubscript{7}) respectively when compared to the control is due to the increase the percentage of ‘N’ and ‘P’ in plants (Vazquez et al., 2002). Soil organic acid was found to grow by watering the plant with water soluble ‘P’ the release of organic acids yielded both sequester cations and acidity. The micro environment near the roots in the main means of ‘P’ extraction and Mn, Fe and Zn is transported into by plants and AMF. Increased levels of protein in the inoculated plants may be due to the presence of fungal proteins or post operative stimulation of protein synthesis at the host plants (Krishna and Bagyaraj, 1984; and Cekic et al., 2012). As a result of the nodulation and N\textsubscript{2} fixing, the rape ‘N’ and protein content was increased.
Increase the amino acid and sugar content was observed in cow pea leaves of Rhizobium + VC + AMF inoculated plants. During the catabolic process, proteins are hydrolyzed, producing ammonium and amino acids, mainly in the amide form such as glutamine and asparagine (Ghosh et al., 1995; Hawkins et al., 2000; and Selvaraj and Chellappan, 2006). These microbial fertilizers are a group of independent, self-supporting microbes. They synthesize useful substances from secretions of plant roots, organic matter and harmful gases such as hydrogen sulfide, by using sunlight and the heat of soil as sources of energy (Kim et al., 2004). The useful substances produced by these microorganisms include amino acids, polysaccharides, nucleic acids, bioactive substances, and sugars, all of which promote plant growth and developments (Ranjith et al., 2007; Hosseinazdeh et al., 2018; and Zaferanchi et al., 2019). Increase in protein content may be due to enhanced uptake and translocation of nitrates which provide nitrogen for amino acid synthesis. The results are in close conformity with the findings of (Deshmukh et al., 2005; Sutaria et al., 2010; Jat and Ahlawat, 2010; Dhakal et al., 2016; and Venkataraao et al., 2017). AMF increase the sugar content of the host plant by hydrolysis of starch to sugars and preventing structural changes in soluble protein (Kapoor et al., 2013). AMF significantly increased photosynthetic of host plants and thereby caused an increase in sugar content (Feng et al., 2002).

Carbohydrate and proteins are two main types of biochemical compounds that are important for structural support and physiological functions. In this study we found increases in the carbohydrate content of cow pea determined by their addition to microbial fertilizers (T). The previous studies have shown that bioorganic or microbial fertilizers can improve the maintenance of biochemical content due to high availability of nutrients in the soil (Bhardwaj et al., 2014; and Omara et al., 2017).

6. Conclusion

The conclusion of the experiment, the soil inoculation with specialized microorganisms such Rhizobium, AMF and VC results in better plant growth and biochemical content of cow pea under field condition, therefore, the need to use microbial fertilizers to reduce the use of chemical fertilizers to protect the agricultural community and the environment.

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