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RESEARCH ARTICLE

EFFECT OF BIOFERTILIZER, VERMICOMPOST AND CHEMICAL FERTILIZER ON DIFFERENT BIOCHEMICAL PARAMETERS OF *ARACHIS HYPOGAEA* L.

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ABSTRACT

The objective of any agricultural research program is to increase the level of crop productivity. The strategy to boost the level of cropproductivity would be through the adoption of package of practices comprising use of seeds of high yielding varieties, adequate doses of manures and fertilizers and plant protection chemicals. Seed germination is one of the important factors for progressive farming. In the present investigation, the effect of plant fertilizers on growth and productivity of *Arachis hypogaea* were studied at the various concentrations of fertilizers. We used Biofertilizer (B), Vermicompost (V), Chemical fertilizer (C) and combination of these three. It was found that, the seed germination percentage was high in Vermicompost treated soil in *Arachis hypogaea*. Groundnut protein content was found high in B+C treated soil, while carbohydrate and phenol content increased in B+V treatedsoil. High seed germination was observed in vermicompost treated soil.

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INTRODUCTION

India has made spectacular break through in production and consumption of fertilizers during the last four decades. But consumption of renewable form of energy (chemical fertilizers) will be quite a limiting factor for increasing agriculture production in future. Because of escalating energy cost, chemical fertilizers are not available at affordable prices to the farmers. Moreover, the unbalanced and continuous use of chemical fertilizers is leading to a reduction in the crop yields and results in imbalance of nutrients in the soil which has adverse effects on soil health. Good quality farm yard manure (FYM) is more valuable organic manure. The long term manurial studies conducted at many places have revealed the superiority of integrated nutrient supply system in sustaining crop productivity in comparison to chemical fertilizer alone (Gaur, 1991). The beneficial effect of vermicompost was first highlighted by Darwin (1881). Vermciompost contains micro site rich in available carbon and nitrogen (Sudhakar *et al.*, 2002). Worm cast incorporated soils are also rich in water soluble P (Gratt, 1970) and contained two to three times more available nutrients than surrounding soils (Sudhakar *et al.*, 2002) which encourages better plant growth. Bano and Kale (1987) reported that application of vermicompost along with chemical fertilizers recorded higher yield of brinjal.

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Savalagi and Savalagi (1991) found increased germination percentage, shoot length and dry matter of hybrid sorghum (CSH-5) upon seed treatment with vermicompost. Jasvir Singh *et al.* (1997) registered higher fruit yield per plant in chilli with the application of vermicompost. Sharma and Mahendra (1963) stated that application of 27 kg nitrogen and 27 kg phosphorus in addition to a basal dose of FYM @ 10 car loads per acre, recorded significantly highest fruit yield of tomato. Natarajan (1990) noticed higher plant height and number of branches per plant in chilli when FYM was applied @ 25 t per ha as a basal dose along with 75:33:35 kg NPK per ha. Surlekov and Rankov (1989) reported greater plant height, number of branches and number of leaves per plant in chilli with the application of farmyard manure @ 20 t per ha along with 100:80:100 kg N, P2O5 and K2O per ha. Okon (1985) reported that it is possible to increase plant height, leaf size and early flowering by use of *Azospirillum*. Shashidhara (2000) noticed that *Azospirillum* + phosphobacteria recorded higher 1000-seed weight (5.93 g) which was significantly superior over 50 per cent RDF (5.40 g) in chilli. Chandrashekar (2003) observed that the plant growth parameters viz., shoot and rootlength and number of leaves per plant in green gram plants at 45 days were significantly increased due to inoculation of P-solubilizing fungal strains along with rock phosphate application as compared to rock phosphate alone (control).
Mahendran and Kumar (1998) studied effect of biofertilziers on quality parameters of potato and observed that application of two equal split doses of 100 per cent recommended dose of

NPK with *Azospirillum* and phosphobacterium increased the ascorbic acid content significantly over control. Seetha (1999) obtained an early flowering in gerbera plants (100.60days) when inoculated with *Azospirillum* and VAM in addition to 50 per cent nitrogen and phosphorus dose. Vermicompost suppress parasitic attacks dramatically and also have shown to increase germination rates, growth etc in wide ranges of crops (Arancon *et al.*,2004). Similar results were also reported by application of Vermicompost on seed germination in mung bean by Nagavallemma *et al.* (2004). El-Saht (1995) observed a greater increase in reducing sugars associated with progressively greater decreases in the content of sucrose, polysaccharides and total saccharides in groundnut plants with the increase in concentration of urea fertilizer Sugiyama *et al.* (1984) stated that the soluble proteins are increased with better N supply and favorable growth condition. Greef (1994) reported that high values of the reduced N fraction (protein fraction) were found in photo synthetic active leaf tissue. Sasikumar and Sivakumar (2017) reported that enhances the growth and yield of *A. hypogaea*.

MATERIALS AND METHODS

Plant material - Two plants *A. hypogaea* were used for sowing and field experiment was conducted at Chellankuppam Village, Cuddalore District of Tamil Nadu. Treatment of soil – The soil contained in five plant pots treated with different type fertilizer along with the control one.

$T_{1:}$	Biofertilizer + Vermicompost (B+V) - 1gm of biofertilizer and 1gm of vermicompost used for 4gm of soil
$T_{2:}$	Biofertilizer +Chemilical fertilizer (B+C) – 4gm of soil contain 1gm of biofertilizer and 0.5 gm urea spread over the soil.
$T_{3:}$	Chemical fertilizer + Vermicompost – 4gm of soil contain 1gm of vermicompost and urea.
$T_{4:}$	Biofertilizer + Vermicompost +Chemical fertilizer – 4gm soil contain 1gm of vermicompost and biofertilizer +0.5gm of chemical fertilizer spread over the soil.
Control	Soil contain no fertilizer.

Sowing of seeds – 50seeds of each *Arachis hypogaea* were sown in the pots: This contains different types of fertilizer and combination of fertilizers. The seeds were sown in the pots filled with the normal fertile soil as well as the soil treated with different fertilizer like chemical fertilizer, Biofertilizer, Vermicompost and combination of different Fertilizer. We used eight pots for each of the plant.

Percentage of seed germination: Germination test was conducted in three replications of 100 seeds each by adopting between paper towel methods as described by ISTA rules . The temperature of 28 ± 1°C and RH of 95 per cent was maintained during the germination test. The first and final germination counts were recorded on fifth and eighth days of germination test respectively for normal seedlings and germination was expressed in percentage.

$$\text{Germination (\%)} = \frac{\text{Number of seeds germinated}}{\text{Number of seeds put for germination}} \times 100$$

Isolation of carbohydrate from leaves of plants grown on treated and controlled soil: One gm of finely powdered oven dried sample was taken in 4 ml of distilled water, heated in boiling water bath for 30 min. and centrifuged at 3000 rpm. for 20 min. Supernatant was collected & transferred in to test tubes. Volume to 25 ml. was made with distilled water.

Estimation of carbohydrates of sample by phenol - sulphuric acid assay: Several test tubes containing 10 ml of neutral hexose such as D-glucose in 10 ml of distilled water in order of 10 µl, 20 µl….170 µl were set. 190 µl, 180µl…30 µl. of phenol in each test tube to make final vol. 200µl was added. Six test tubes of sample containing 50 µl carbohydrates were also set and then added 150 µl of phenol to make final vol. 200 µl.

Blank test tube (containing no carbohydrate) of 200 µl phenol were also set and added 1 ml of conc. Sulphuric acid rapidly in all test tubes wait for 10 min. shaked vigorously 30 min. and absorbance at 490 nm was recorded. Then concentration of carbohydrate of unknown samples from a standard curve plot was determined.

Isolation of protein from plant: Took 500 mg of leaf from both (treated & controlled) plants separately and washed well to remove unwanted impurities. Then the leaf sample were grinded with distilled water using pestle and mortar separately. Grained mixture of leaf was centrifuged at 1000rpm for 10 minutes, supernatant was taken out and used as leaf extract.

Total protein estimation from leaves of plant grown on treated and controlled soils: Suitable aliquot (1ml) of extracted protein solution was taken and added 5ml of freshly prepared alkaline copper sulphate reagent. Mixed properly and after 10 min added 0.5ml of Folin's reagent. Mixed the contents instantaneously. Allowed the colour to develop for 30 min. Recorded the absorbance at 660 nm after setting the instrument with reagent blank which contains 1ml of phosphate buffer instead of sample aliquot. In another set of tubes took suitable aliquot of BSA solution (in a range of 20-200µg) , made the total volume to 1ml with phosphate buffer and allowed to develop the colour as described in steps 1-3. A standard curve of absorbance at 660nm verses µg of BSA was drawn. From this standard curve the amount of protein in the sample tube was determined.

Isolation of total phenol content of leaf: Weighed 0.5g of leaf samples of different genotypes and grinded with a pestle and mortar in 10-time volume of 80% ethanol. Centrifuged the homogenate at 10,000 rmp for 20min.and saved the supernatant. Re-extracted the residue with five times the volumes of 80% ethanol, centrifuge and pool the supernatants evaporated the supernatant to dryness.

Estimation of total phenol content of leaf: Pipetted out the aliquots (0.2ml to 1.0ml) into test tubes. Made the volumes in each tube to 3ml with double distilled water. Added 0.5ml of folic -ciocalteau reagent. After 3min, added 2ml of 20% Na_2CO_3, solution to each tube. Mixed thoroughly the tubes in boiling water bath for 1min, cooled and measured the absorbance at 650nm against a reagent blank. Prepared a standard curve using different concentration of phenols in the test samples were estimated. Total phenol content was expressed in term of gallic acid (mg/gm of dry mass) which is used as reference.

RESULTS

Percentage of seed germination after 30 days: After 30 days in groundnut (*Arachis hypogaea*) maximum seed germination percentage observed by vermicompost (88%) and minimum by chemical fertilizer (64%).

Table 1. Percentage of seed germination in groundnut plants

S.No.	Fertilizer	Ground nut
1.	Biofertilizer	74%
2.	Vermicompost	88%
3.	Chemical fertilizer	64%
4.	B+V	72%
5.	V+C	68%
6.	C+B	68%
7.	C+V+B	74%
8.	Control	76%

Biofertilizer treated *A.hypogaea* (40th day): In the field studies, plants treated with combination of biofertilizer vermicompost and chemical fertilizer T_1, T_2, T_3, T_4 and control of biofertilizer respectively showed that T_1 and T_2 treated *Arachis hypogaea* showed enhanced growth compared to control. Better result was observed in the plants treated with T_2 SLF (Fig 1).

Carbohydrate content in the leaves of plants: After 30 days in groundnut plant maximum carbohydrate concentration was obtained by control (0.900mg/g) and minimum in V+B treated soil 90.049mg/g).

Table 2. Total carbohydrate content of plants after 30 for groundnut

S.No.	Fertilizers	Carbohydrates content after 30 days(mg/g)
1.	Biofertilizer	0.280 ± 0.5
2.	Vermicompost	0.360 ± 0.3
3.	Chemical fertilizer	-
4.	B+V	0.090 ± 0.4
5.	V+C	0.310 ± 0.5
6.	C+B	0.010 ± 0.3
7.	C+V+B	0.530 ± 0.2
8.	Control	0.470 ± 0.3

Total protein content in the leaves of plants: After 30 days in groundnut plant maximum protein concentration was observed by C+B treated soil (6.10µg/ml) and minimum by biofertiizer treated soil (3.80µg/ml).

Table 3. Total protein content in the leaves of groundnut plants

S.No.	Fertilizers	Protein content after 30 days (µg/ml)
1.	Biofertilizer	3.80 ± 0.4
2.	Vermicompost	4.90 ± 0.5
3.	Chemical fertilizer	5.10 ± 0.3
4.	B+V	5.70 ± 0.4
5.	V+C	4.70 ± 0.5
6.	C+B	6.10 ± 0.4
7.	C+V+B	4.80 ± 0.3
8.	Control	5.90 ± 0.3

Total phenol content of plants: The value of phenol content after 30 days was observed in groundnut plant maximum phenol content observed in biofertilizer treated soil (0.930mg/g)and minimum observed by B+V treated soil (0.230mg/g).

Table 4. Total phenol content in the leaves of groundnut plants

S.No.	Fertilizers	Phenol content after 30 days (µg/ml)
1.	Biofertilizer	0.930 ± 0.3
2.	Vermicompost	0.720 ± 0.2
3.	Chemical fertilizer	0.370 ± 0.1
4.	B+V	0.230 ± 0.2
5.	V+C	0.250 ± 0.3
6.	C+B	0.350 ± 0.1
7.	C+V+B	0.370 ± 0.1
8.	Control	0.420 ± 0.2

DISCUSSION

Laboratory experiments were carried out with the objective of studying the effect of Biofertilizer, Vermicompost and Chemical fertilizer on seed germination and Biochemical aspect of *Arachis hypogeae*. In case of groundnut high seed germination percentage (88%) was observed in Vermicompost treated soil. In groundnut plant maximum carbohydrate concentration was obtained by control (0.900mg/g) and minimum in V+B treated soil 90.049mg/g). in groundnut plant maximum protein concentration was observed by B+C treated soil (6.10µg/ml) and minimum by biofertiizer treated soil (3.80µg/ml). The value of phenol content after 30 days was observed maximum in soil in groundnut plant maximum phenol content observed in biofertilizer treated soil (0.930mg/g)and minimum observed by B+V treated soil (0.230mg/g). Agriculture plays a pivotal role in the growth and survival of nations; therefore, maintaining its quantity and quality is essential for feeding the population and economic exports. Over the years, agriculture has undergone various scientific innovations in order to make it more efficient (Ajmal, 2018). Modern agriculture involves usage of pesticides and chemical fertilizers with an essence of increasing the world's food production, as these serve as a fast food for plants causing them to grow more rapidly and efficiently. Continuous application of chemical fertilization leads to the decay of soil quality and fertility and might lead to the collection of heavy metals in plant tissues, affecting the fruit nutritional value and edibility (Farnia and Hasanpoor, 2015).

Hence, in the recent years, many organic fertilizers have been introduced that act as natural stimulators for plant growth. A particular group of organic fertilizers includes outcomes based on plant growth-promoting microorganisms identified as 'Biofertilizers'. These biofertilizers comprised efficient strains of nitrogen fixing or phosphate solubilizing microorganism. Organic farming has appeared as a prime concern area globally in aspect of the growing demand for safe and healthy food, durable sustainability and issue on environmental pollution associated with random use of agrochemicals (Ghany *et al.*, 2013). Biological fertilization is based on the supply of organic inputs including fertilizers, organic wastes, domestic sewage, animal manure, and microorganisms, such as fungi and bacteria. They are used to enhance fixation of nutrients in the rhizosphere, produce plants of growth stimulants, effective in soil stability, offer biological control, biodegrade substances, recycle nutrients, support mycorrhiza symbiosis, and evolve bioremediation processes in soils contaminated with toxic, xenobiotic and recalcitrant substances.

The bio-fertilizers supply also enhance the productivity per area in a comparatively short time, consume smaller amounts of energy, reduce contamination of soil and water, increase soil fertility, and encourage antagonism and biological control of phytopathogenic organisms. We concluded that-in groundnut plant protein content increased through B+C treated soil, while carbohydrate and phenol content increased through B+V treated soil and high seed germination was observed in vermicompost treated soil.

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