



RESEARCH ARTICLE

Effect of moss extract and organic manures on the development of groundnut *Arachis hypogaea* L.

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Abstract

Soil amendment of organic manure promotes nutrient cycling and enriches soil that eventually leads to improved crop health, nutritional quality and productivity. Peat moss serves as an ideal growth medium for crops and therefore moss extract can be used for soil amendment. The present study evaluates the effect of soil amendment using moss extract (ME) and its combination with farm yard manure (FYM), vermicompost (VMCP), and cow urine (CU) on groundnut *Arachis hypogaea*. The experiment was consisted of eight treatments (control, ME, FYM, VMCP, CU, FYM+ME, VMCP+ME, and CU+ME) and carried out in the earthen pots filled with farm soil. Growth parameters such as root length shoot length, number of leaves, leaf surface area, root nodules, and pod numbers were recorded after 30, 60, and 90 days of intervals. Chlorophyll, carbohydrate, and protein content of leaves and seed oil content were analysed after 90 days of sowing. ME had positive influence on the growth of plants after 30 and 60 days of treatment. Combined treatments of different manures with moss resulted in better growth and productivity. Protein and carbohydrate content was significantly increased in VMCP+ME and FYM+ME treatments. Oil content in the seed was not affected by ME alone or its combination with other manures. These results suggest that ME can be used as nutrient source for organic soil amendment.

Keywords: Moss Extract; Groundnut; Plant Growth; Chlorophyll Content; Seed Oil Content

1. Introduction

Soil amendment using various organic fertilizers is important for improving crop health, nutritional value, and productivity (Indoria et al., 2018). Currently, farm yard manure, vermicompost, and poultry manure are some of the common manures employed in Indian agriculture systems (Rashmi et al., 2023). The farm yard manure used for soil amendment is mainly collected from the livestock including milking cows, bulls, goats, etc. These farm animals are mostly being fed with a few types of food crops (Mottet et al., 2017; Van Zanten et al., 2019). Soil amendment using only animal manures may not be sufficient to fulfil the natural fertility of the soil and also the nutrient requirement of crops (Bayu et al., 2005; Jensen, 2013; Rayne and Aula, 2020). Additional organic nutrient supplementations containing natural metabolites are necessary to improve soil fertility (Indoria et al., 2018).

In addition to the soil amendment using farm yard manures, over-cropping is also a major cause for soil fertility degradation (Cassman, 1999; Lal, 1998). Since India is a traditionally agricultural country, the quality of the soil in several regions is degrading due to continuous cropping over the time (Bhattacharyya et al., 2015; Dhanda et al., 2022; Sarkar et al., 2011). The combined impact of over-cropping and soil amendment using regular farmyard manures can have negative impact on nutritional quality of the crop and yield. As chemical fertilizers have several disadvantages and are not sufficient to promote microorganism growth and soil nutrient cycling (Kolay, 2007; Paharvi et al., 2021). Therefore, the usability of alternate natural nutrient sources needs to be investigated in order to improve crop nutrition and production (Indoria et al., 2018).

Peat moss seems to be an excellent addition to organic soil amendments (McKeon-Bennett and Hodkinson, 2021). Peat moss has long been used in agriculture and horticulture as a growth medium owing to their high-water holding capacity, slow degradation rate, and nutrient recruitment capacity (Pacé et al., 2018; Wang et al., 2022). The moss and moss extracts have been

reported for their role as a plant growth stimulant (Taskila et al., 2016). In the present study, we tested the effect of *Hypnum* moss extract on *Arachis hypogaea*. The experiment was designed in order to study the effect of moss extract alone and the combination of moss extract and FYM, vermicompost, and cow urine. The effects of soil amendment using moss extract were evaluated using growth, biochemical and productivity parameters.

2. Material and method

2.1. Moss collection and extract preparation

Hypnum moss was collected from the Lavasa city situated in the northern Western Ghats and brought to the laboratory. The moss was kept under shade for drying. After fifteen days of shed drying, the moss was crushed into powder using grinder. The powder was sieved through strainer with mesh size 0.5 mm. 100 gm powder was mixed with 1L distilled water and kept in shaker overnight at 25° and 60 rpm for 24 h. Then the solution was filtered through Whatman filter paper no. 1. The filtrate was termed as moss extract and directly used for the experiment.

2.2. Experimental set up

To study the effect of moss extract (ME) on *A. hypogaea*, we mixed 100 ml moss extract in 1 kg farm soil. Dry soil was collected from the farm. Soil chemical composition was analysed (Table 1). The experiment was conducted in 24 cm diameter pots (height 17 cm). The pots were filled with soil pre-mixed with moss extract. In addition, the combined effect of moss extract with farm yard manure (FYM), vermicompost (VMCP), and cow urine (CU) was studied on *A. hypogaea*. For individual FYM and VMCP treatments, 200 g of manure (either FYM or VMCP) was mixed in 1 kg of soil. For the combined treatment of moss extract with FYM and VMCP, 200 g of manure (either FYM or VMCP) mixed in the soil (pre-mixed with moss extract 100ml/L). For CU treatment, 10 L of cow urine was collected from Killar cow (and stored at least for 20 days in shade) was used. The cow urine was mixed in the soil at 100 ml/kg proportion. For the combined treatment of ME and CU,

cow urine (100 ml) was mixed in 1 kg soil premixed with moss extract (100 ml/kg). The experiment was consisted of eight groups (control, ME, FYM, VMCP, CU, FYM+ME, VMCP+ME, and CU+ME). Twelve pots were assigned for each treatment and two seeds were sown in each pot.

We used the seeds of Phule Chaitaya variety (KDG 160) for the experiment. The variety is developed for the production in summer season and in dry region by Mahatma Phule Agriculture University, Rahuri. Before sowing, the seeds were treated with 0.02% HgCl₂ for 5 min (Pawar et al., 2020). The plants were irrigated regularly.

Table 1. List of chemicals present in the soil and their concentrations.

Chemical constituents in soil	
pH	7.56
Organic carbon	0.68%
Nitrogen (Kg/ha)	185
Phosphorus (Kg/ha)	10.1
Potassium (Kg/ha)	410
Sodium (mg/L)	260
Free lime (%)	16.2
Ferrous (ppm)	1.11
Manganese (ppm)	0.31
Zink (ppm)	0.07
Copper (ppm)	0.49
Calcium (ppm)	33.1
Magnesium (ppm)	41.56
Boron (ppm)	0.11
Sulfur (ppm)	10.35

2.3. Plant growth assessment

Plant growth assessment was done after 30, 60, and 90 days of sowing. At each time-point, three random pots were selected for the growth analysis. Root length and shoot length of the plants were measured using scale at each sampling point. Leaf numbers, root nodules, and pod numbers were recorded for each plant. Leaf surface area of three largest leaves of each plant was measured manually using graph paper. For each group, a total of six plants from three pots (randomly selected) were considered for growth assessment.

2.4. Biochemical assessment

At each sampling point (30, 60, and 90 days), total chlorophyll content in the leaves was estimated following the method described by (Arnon, 1949). One gram of finely cut fresh leaves was used for Chlorophyll extraction. Chlorophyll was extracted using 80% acetone (Merck) and centrifuged at 5000 rpm for 10 min. supernatant was transferred to another centrifuge tube and the procedure was repeated till the residue becomes colourless. Chlorophyll A and Chlorophyll B were estimated from the supernatant at 663 and 645 nm using UV spectrophotometer (UV-1800 Shimadzu, Japan) and expressed in mg/g.

After 90 days of sowing, total proteins in leaves were estimated following Lowry et al. (1951) method. The proteins were extracted in pH 7.2 phosphate buffer (25 mM Potassium phosphate, 2 mM MgCl₂, 2 mM EDTA, 15% (v/v) glycerol, and 0.2% (v/v) 2-mercaptoethanol. Optical density of protein samples was taken at 660 nm using UV spectrophotometer. Bovine serum albumin (BSA) was used as a standard for the determination of protein concentrations.

Total carbohydrates were estimated following colorimetric Anthrone method (Sadashivam and Manickam, 1996). Carbohydrates were digested using diluted Hydrochloric acid (HCl) into simple sugars (monosaccharaides) and dehydrated into

furfural. Freshly prepared Anthrone reagent was added to the digested sugars and digested sugars concentration was determined at 630 nm (UV spectrophotometer) using glucose as a standard.

Oil content in the seeds from different groups was estimated after 90 days of sowing following the previously described method (Harwood, 1984; Sadashivam and Manickam, 1996). The oil in groundnut seeds was extracted in petroleum ether using Soxhlet apparatus and oil percent was calculated.

2.5. Statistical analysis

The data obtained was arranged and visualised in tabular and graphical format using Microsoft excel. Graphs were prepared using GraphPad Prism (version 6). Differences in morphological and biochemical parameters among different treatment groups were analysed by ANOVA and individual group comparisons with the control were made using Duncan's post-hoc test. Statistical analyses were carried out in SPSS version 17. Significance level for each test was set at 0.05.

3. Result

Root length and shoot length of *A. hypogaea* in all treatment groups including control increased gradually after 30, 60, and 90 days of sowing (Table 2). After 30 days of sowing root and shoot lengths in ME treatment groups were increased significantly as compared to the control. In the combined groups of ME and FYM, VMCP, and CU had significant increase in root and shoot length when compared with their respective individual treatments. After 60 days of sowing, root and shot length were increased significantly as compared to the control (Table 2). After 90 day of treatment, there was no significant difference in root length between control and individual treatments of organic manures. Also, there was no significant difference in root length between individual manure treatments (FYM, VMCP, and CU) and their combined treatments with ME (Table 2). After 60 days of sowing root length in the individual treatment groups (except CU) was significantly increased as compared to the control. Combined treatment of FYM, VMCP and CU with ME resulted in significant increase in shoot length as compared to their respective individual treatments. However, after 90 days of sowing shoot length in ME and VMCP groups was increased significantly as compared to the control. There was no significant difference in the shoot length of combined treatments with ME and their respective individual treatments (Table 2).

Leaf numbers and their surface area increased over the time (Table 3). After 30 days of sowing leaf number in FYM individual group was significantly more as compared to the control (Table 3). In the remaining individual manure treatments leaf number was comparable with the control. However, leaf numbers in all combined treatment of moss with different manures were significantly larger as compared to their respective individual treatments. After 60 days of sowing there was no significant difference in the leaf numbers between the groups receiving individual treatments of different manures and control. Notably, leaf number in the group receiving combined treatment of moss along with VMCP was increased as compared to individual VMCP treatment. After 90 days of sowing leaf numbers in FYM and VMCP groups were significantly increased as compared to the control. Interestingly, among combined treatments, leaf number in the group treated with CU and ME was significantly increased as compared to CU treatment (Table 3). After 30 days of treatment, leaf area in all individual manure treatment groups increased significantly when compared with the control (Table 3). Notably, leaf area in CU+ME group was increased as compared to individual CU treatment. After 60 days of sowing only ME treatment stimulated the increase in leaf area as compared to the control. However, there was no significant difference in the leaf area of different combined treatments and their respective individual treatments. After 90 days of sowing leaf area among various treatments did not differ (Table 3).

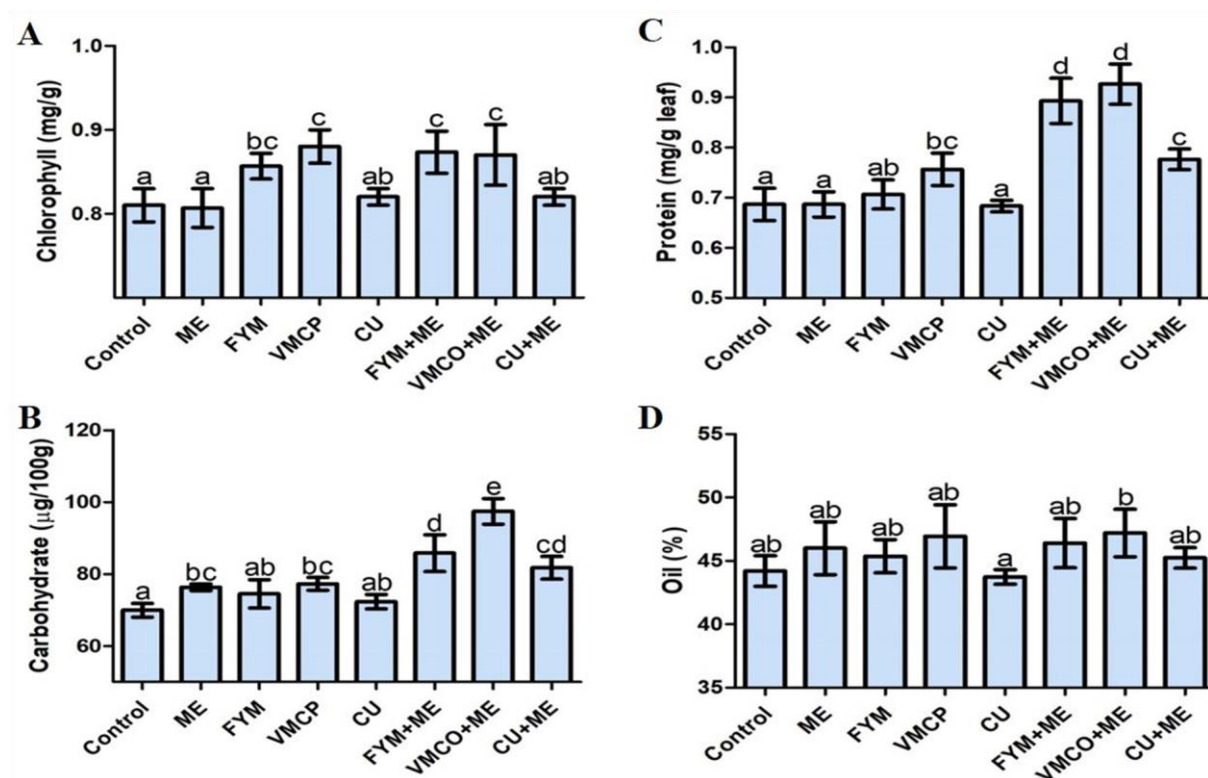


Figure 1. Chlorophyll (A) and carbohydrate (B) content of the leaves of *Arachis hypogaea* after 90 days of sowing and protein content of the leaves (C) and oil content in seeds (D) (summer) after 90 days of sowing. Column and error bars represent Mean±SD. ME: Moss extract, FYM: Farmyard manure, VMCP: Vermicompost, CU: Cow urine.

Table 2. Root length and shoot length (cm) of *Arachis hypogaea* after 30, 60, and 90 days of sowing. Values in the table indicate Mean±SD. ME: Moss extract, FYM: Farmyard manure, VMCP: Vermicompost, CU: Cow urine.

	Root length			Shoot length		
	30 Days	60 Days	90 Days	30 Days	60 Days	90 Days
Control	21.2±2.1a	29.4±3.2a	36.16±1.95a	21.56±4.54a	31.36±3.28a	42.2±2.25a
ME	28.6±3.19b	33.33±3.72ab	37.2±2.42a	28.63±1.59b	41.76±1.12d	49.33±2.82bc
FYM	22.4±2.06a	36.7±3.14bc	38.8±0.87ab	24.83±1.23ab	37.13±2.22bc	47.83±2.93abc
VMCP	24.76±1.04ab	36.9±3.24bc	39.06±1.51ab	26.63±2.01ab	36.76±1.73bc	49.56±4.7bc
CU	23±0.79a	30.6±2.4ab	37.3±1.01a	23.53±2.18ab	34.16±2.61ab	44.53±0.9ab
FYM+ME	37.06±3.91c	40.23±5.92c	38.83±2.21ab	36.8±3.45c	43.4±2.38d	53.3±2.94c
VMCP+ME	39.66±4.25c	42.8±2.47bc	42.23±3.1b	43.26±2.71d	45.43±2.77d	51.86±4.87c
CU+ME	36.96±1.4c	37.03±1.64c	37.1±1.75a	35.96±2.76c	41.13±3.08cd	47.6±0.86abc

Root nodules and pods appeared after 30 days of sowing and their numbers increased as treatments progressed (Table 4). After 30 days of sowing MO, FYM, and VMCP treatments stimulated the development of multiple root nodules as compared to the control. Interestingly, in all combined treatments of different manures and moss significantly more root nodules were developed. After 60 days of sowing VMCP and ME treatments significantly increased root nodules as compared to the control. All combined treatments stimulated the development of multiple root nodules when compared with their respective individual treatments. After 90 days of sowing, VMCP treatment stimulated the development of more of root nodules. However, only FYM+ME treatment

stimulated the development of more root nodules as compared to the control (Table 4). After 30 days of sowing there was no significant difference in the numbers of pods between individual manure treatments and control (Table 4). However, there was no significant difference in the pod numbers between combined treatments of manures and their respective individual treatments (Table 4). After 60 days of sowing pod numbers were increased in VMCP group. After 90 days of sowing only ME induced significantly a greater number of pods as compared to the control. However, there was no significant difference in the number of pods between combined treatments and their respective individual treatments (Table 4).

Table 3. Leaf number and leaf area (cm²) in *Arachis hypogaea* after 30, 60, and 90 days after sowing. Values in the table indicate Mean±SD. ME: Moss extract, FYM: Farmyard manure, VMCP: Vermicompost, CU: Cow urine.

	Number of leaves			Leaf area		
	30 Days	60 Days	90 Days	30 Days	60 Days	90 Days
Control	22.4±2.46a	36.73±1.75ab	54.8±8.51a	8.73±0.32a	12±0.45a	13.06±0.28
ME	26.2±2.45ab	36.86±2.54ab	65.33±6.3ab	10.63±0.25d	12.63±0.25bc	13.43±0.3
FYM	30.5±0.88bc	37.66±2.65ab	73.76±3.98bc	9.93±0.45c	12.4±0.26abc	13.23±0.25
VMCP	27.2±2.59ab	39.5±2.36ab	70.03±1.27bc	10.4±0.26cd	12.46±0.15abc	13.26±0.32
CU	24±2.19a	36.16±2.66a	57.6±1.86a	9.36±0.2b	12.26±0.11ab	13.2±0.1
FYM+ME	37.7±3.27de	42.3±3.08bc	76.93±4.46bc	10.43±0.5cd	12.8±0.3c	13.5±0.26
VMCP+ME	38.6±2.85e	46.13±5.0c	81.26±4.30c	10.8±0.1d	12.86±0.2c	13.43±0.3
CU+ME	33.23±3.48cd	41.6±3.68abc	72±2.19bc	10.6±0.2d	12.7±0.17bc	13.3±0.17

Table 4. Number of root nodules and number of pods in *Arachis hypogaea* after 30, 60, and 90 days after sowing. Values in the table indicate Mean±SD. ME: Moss extract, FYM: Farmyard manure, VMCP: Vermicompost, CU: Cow urine.

	Number of nodules			Number of pods		
	30 Days	60 Days	90 Days	30 Days	60 Days	90 Days
Control	12.36±1.1a	19.7±0.45a	33.5±2.26a	1.66±0.47a	4.66±0.47a	8±0.81a
ME	22.9±2.98c	26.36±1.75c	35.76±2.86ab	2.33±0.47ab	6.33±0.47b	10.66±1.24bc
FYM	18±2.55b	21.86±2.35ab	37.16±3.1ab	2.33±0.47ab	5.33±0.47ab	10.33±1.24abc
VMCP	22.36±1.91c	23.8±2.77bc	40.1±3.5bc	2.66±0.47ab	6±0.81ab	10±1.41abc
CU	15.26±1.04ab	22.36±2.25ab	35.66±0.73ab	2±0.81ab	5.66±0.94ab	8.33±0.47ab
FYM+ME	29.2±1.15d	33.33±2.25d	42.46±3.75c	3.33±0.47b	6.66±0.47b	11.66±1.24c
VMCP+ME	31±2.88d	36.96±1.91e	40.13±2.66bc	3.33±0.47b	6.66±0.47b	12.33±1.24c
CU+ME	27.73±1.64d	31.2±2.08d	36.6±1.32ab	3±0.81ab	6.33±0.47b	12.33±0.94c

Chlorophyll content in the leaves of in FYM and VMCP treatment increased significantly as compared to the control (Figure 1A). However, individual treatment of ME or its combination with other manures did not influence chlorophyll content (Figure 1B). Carbohydrate content in ME and VMCP groups was increased when compared with the control (Figure 1B). Interestingly, all combined treatments with ME stimulated increase in carbohydrate content in the leaves when compared with their respective controls (Figure 1B).

Protein content of the leaves was increased only in individual VMCP treatment as compared to the control (Figure 1C). Interestingly, protein content of the leaves in all combined treatments with moss was significantly increased when compared with their respective controls (Figure 1C). Although there was increase in the seed oil content in the individual manure groups (except CU) and the combined groups with ME, the difference was not significant (Figure 1D).

4. Discussion

Peat moss is a widely used plant growth medium in agriculture and horticulture (Kitir et al., 2018). Therefore, moss has been proposed to be an excellent source of nutrients necessary for soil health (Mahrup et al., 2019) and improves microbial diversity (Jiang et al., 2024). In the present study, we demonstrated the positive influence of ME on *A. hypogaea* growth and biochemical parameters. For the present experiment, we used Phule chaitanya variety (*A. hypogaea*) especially developed for arid region. Agriculture in the arid zones of India is facing several issues related to soil health and water management (Rao, 2002). Moreover, groundnut contributes a significant portion of oil seed production in India. Groundnut oil and several other products are integral part of the food in Indian population (Bansal et al., 2017; Reddy and

Immanuelraj, 2017). Since, soil amendment using moss extract along with commonly used organic manures have positive effect on the growth of *A. hypogaea*, it can be developed further for on field application. Further, study of the biochemical or nutrient composition of the moss extract can help to understand the plant growth promoting potential of moss extract (Pipes and Yavitt, 2022). In the present study, we used *Hypnum* moss collected from the Western Ghats for extract preparation in the present study. We tested growth promoting potential of *Hypnum* moss extract for the first time for any oilseed crop.

In the present study, we observed positive influence of moss extract on the growth of *A. hypogaea* mainly after 30 and 60 days of sowing. This suggests that moss extract is beneficial for *A. hypogaea* during the early stages of development. After 90 days of sowing, there was no significant effect of either moss extract or its combination with other manures on the root length, shoot length, number of leaves. The possible reason for the low effect of moss extract after 90 days could be limited space for the plant growth in pots. Previous studies have demonstrated stunted growth of plants in limited space (Fikre and Boto, 2024; Poorter et al., 2012). We also observed no difference in the leaf surface area among different treatment groups. Possibly, the plants in all groups might have achieved maximum growth of leaf surface.

Chlorophyll content of the leaves in different treatment groups was not affected by moss extract treatment. However, carbohydrate and protein contents of the leaves in moss extract treated group and the groups receiving combined treatments of moss with FYM and VMCP were significantly increased. These observations imply that moss extract stimulates the protein and carbohydrate metabolism in *A. hypogaea*. Carbohydrate and protein metabolism in plants can be associated with vegetative growth and stress response (Pelleschi et al., 2006; Steward et al., 1956). However, stimulated

vegetative growth in *A. hypogaea* could not result in the increased seed oil content as there was no significant difference in the oil content of the seeds from different treatment groups.

Currently, several organic plant growths promoting agents (including seaweed extract, mycorrhizal fungi, etc.) are being used in agriculture (Backer et al., 2018; Khan et al., 2009). In the present study, we used *Hypnum* moss extract as a growth promoting agent which can have further beneficial implications. The present study demonstrated that the moss extract can help to enhance the growth in *A. hypogaea*. Moss extract treatment stimulates carbohydrate and protein metabolism. Further on field studies need to be carried out to test the growth promoting potential of *Hypnum* moss extract. Additionally, chemical constituents in the moss extract also need to be investigated for bioprospection purpose.

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Author's contributions

Both authors were involved in study design, analysis of the results and writing of the manuscript. SK performed the experiment and field data.

Conflict of Interest

Authors have no conflict of interest to declare.

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