`Original Research Article

Evaluation of Synergistic Effects of Vermicompost and Beneficial Microbes on Pea

ABSTRACT

This study was conducted during *Rabi* 2017-18 under net house condition, Department of Plant Pathology, IAS, BHU, Varanasi to evaluate the synergistic effects of vermicompost and beneficial microbes on pea plants. The experiment was laid out in completely randomized design with three replication and fifteen treatments. The result indicated that among all treatment, a treatment V4 was found to be best in terms of all growth parameters and plant defense-related compounds. The treatment combination of M1V3 has given best results followed by treatment M2V2. The results from this experiment demonstrated the strong synergistic effects between vermicompost and two microbes, as they improved the soil nutrients status by supporting growth and development of plants by producing growth hormones and also protected the plants from diseases by enhancing the production of defense - related compounds.

Key words: *Pea, growth parameters, vermicompost, Trichoderma harzianum, Pseudomonas fluorescens, defense-related compounds.*

1. INTRODUCTION

Pea (*Pisum sativum*) is among the four important cultivated leguminous crops next to soya bean, groundnut and beans [1]. The importance of this crop is many folds as it is used as pulse, vegetable, fodder and industrial crop. Pea is the rich source of protein, carbohydrate, vitamins and fibers. Besides these, it is an excellent source of calcium, phosphorus, iron, sodium and potassium [2]. In India, pulse crops are cultivated extensively because it requires very low farm inputs and are very much adaptable to extreme stressed environmental conditions. India is the largest producer (25% of global production), consumer (27% of world consumption) and importer (14%) of pulse in the world [3]. Recently the growing population and climate change have emerged as a great challenge for the farmers and researchers. For higher production, farmers rely mainly on the excessive use of chemical fertilizers and chemical pesticides that leads to environment degradation, human health problems and deterioration of crop quality. So, there is a growing demand for using ecologically sound and sustainable agricultural practices by utilizing available renewable resources.

Vermicomposting is an efficient technology that can reduce the ecological risk of toxic compounds and pathogens by converting large amount of solid organic waste into biodegradable

and stabilized by-products with the combined action of earthworm and other microorganisms. Earthworms act as natural bioreactor as it stimulates the rate of decomposition of organic waste materials by increasing both surface area and aeration of the substrate [4]. Vermicompost, is used as a potential source of organic inputs for sustainable agriculture, contains beneficial microorganisms, both the major (NPK) and the micro nutrients, enzymes and hormones [5]. Vermicompost is an excellent plant growth promoter as it provides all nutrients that are essential for better plant growth and establishment like nitrates, phosphates, exchangeable calcium and soluble potassium in easily absorbable form for plants. Vermicompost improves soil aeration, water holding capacity, enriches soil with microorganisms, and enhances germination, plant growth and crop yield by improving root growth.

Well studied beneficial microbes such as *Trichoderma harzianum* & *Pseudomonas fluorescens* offer an environment friendly alternative to the use of synthetic chemical pesticides for controlling various plant diseases. So, the addition or enrichment of vermicompost with microbial inoculants enhanced the efficiency of vermicompost on plant growth as well as defense related compounds in pea plants was the intension of the current work was to study. By judicious use of organic manures and biofertilizers, there is not only sustained crop productivity and better soil health but it also helps in supplementing chemical fertilizers for crop.

2. MATERIALS AND METHOD

The present investigation was conducted to access the synergistic effects of vermicompost and beneficial microbes (*T. harzianum* & *P. fluorescens*) on pea plants (Azad P-3 variety) during *Rabi* 2017-18 under net house condition, Department of Plant Pathology, IAS, BHU, Varanasi (UP), India. The experiment was conducted in plastic pot of 500 g capacity.

2.1. Evaluation of effects of vermicompost and beneficial microbes on plant growth response

2.1.1. Maintenance of microbes

Two microbes selected for this study was obtained from the Department of Plant Pathology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi. *Trichoderma harzianum* was sub cultured on PDA media and *Pseudomonas fluorescens* on nutrient agar media in Petri plates and also in slants for further use.

2.1.2. Preparation of soil and Vermicompost mixtures

Cattle dung added with earthworms (*Eisenia foetida*) was used to prepare vermicompost. Vermicompost was obtained from vermicompost unit of the IAS, BHU. Soil collected from BHU farm and gravels, grasses and other stubbles or plant-debris was removed. Then soil was mixed with different concentrations (1%, 2%, 3% and 4%) of vermicompost. Soil without vermicompost was served as control (V0). Soil was wetted by spraying water at two hours interval to maintain sufficient moisture. Then soil and vermicompost was mixed thoroughly.

About 500 gram of soil was filled in each plastic pot. These pots were then arranged treatment wise in net house.

2.1.3. Treatment details

The experiment consists of fifteen treatments *viz*., T_1 - Control (V0), T_2 - Vermicompost 1.0% (V1), T_3 - Vermicompost 2.0% (V2), T_4 - Vermicompost 3.0% (V3), T_5 - Vermicompost 4.0% (V4), T_6 - Seed treatment with *T. harzianum* (M1), T_7 - Seed treatment with *T. harzianum* + 1.0% Vermicompost **(**M1V1), T_8 - Seed treatment with *T. harzianum* + 2.0% Vermicompost **(**M1V2), T_9 - Seed treatment with *T. Harzianum* + 3.0% Vermicompost **(**M1V3), T_{10} - Seed treatment with *T. harzianum* + 4.0% Vermicompost **(**M1V4), T_{11} - Seed treatment with *P. fluorescens* **(**M2), T_{12} - Seed treatment with *P. fluorescens* + 1.0% Vermicompost **(**M2V1)**,** T_{13} - Seed treatment with *P. fluorescens* + 2.0% Vermicompost **(**M2V2), T_{14} - Seed treatment with *P. fluorescens* + 3.0% Vermicompost **(**M2V3), T_{15} - Seed treatment with *P. fluorescens* + 4.0% Vermicompost (M2V4)**.** These treatments were replicated thrice in completely randomized design.

2.1.4 Seed treatment with beneficial microbes, seed sowing and caring of plants

Seeds of uniform shape, size and colour were selected for the experiment. The seeds were surface sterilized with 1% sodium hypochlorite for 30 seconds, and then washed 3-4 times with distilled water and air dried. Seeds were then treated separately with suspension of two selected microbes (*T. harzianum* and *P. fluorescens*) prepared in 1% CMC (Carboxymethyl cellulose - used as adhesive). Seeds were then allowed to dry overnight. Four seeds per pot were sown. Plastic pots were kept in net house and soil moisture was maintained by watering at regular interval. All other cultural practices, including watering and weeding were carried out uniformly.

2.1.5. Observation of Plant Growth Parameters

Observation of various plant growth parameter *viz*. Shoot length (cm), root length (cm), fresh shoot and root weight (g), dry root and shoot weight (g) were recorded at 30 days after sowing. Two pots were taken from each treatment. Soil was first removed from root surface, to protect roots from breaking. Plants were rinsed with running tap water , and root length and shoot length was measured with the help of meter scale and their average was taken for statistical analysis. After measuring shoot length and root length, roots and shoot were then separated for measuring fresh shoot and root weight by an analytical balance. Dry weight of shoots and roots was calculated by drying them separately in a tray dryer at 60^{o}C till constant weight was observed.

2.2. Estimation of effects of vermicompost and beneficial microbes on defense-related compounds

2.2.1. Collection of samples for biochemical analysis

Plants from each treatment were collected at 30 DAS. Collected samples were then stored in a deep freezer (- 80^0C) until it is used for biochemical analysis.

2.2.2. Total phenol content (TPC)

The total phenol contents (TPC) was determined by following the method of Zheng and Shetty. [6], by using Folin-Ciocalteau reagent and Gallic acid (GA) as standard. Fresh leaf tissues (0.5 g) were taken in a mortar pestle and grinded in 10 ml of 50% methanol, incubated for 1 hour, extract was obtained. To 1ml of enzyme extract, 1 ml of 95% methanol, 5ml of sterile distilled water and 0.5ml of 50% Folin-Ciocalteau reagent were added and the content of each test tube was vertexed thoroughly. After 5 minutes, 1 ml of 5% sodium carbonate was added, and again the reaction mixture was vertexed and allowed to stand for 1 hour and the optical density (OD) was taken at 725 nm. Absorbance values were converted to GA equivalents (GAE) per g fresh weight (FW).

2.2.3. Phenylalanine ammonia lyase (PAL) assay

PAL is an important plant enzyme that eliminates ammonia from phenylalanine to form *trans*-cinnamic acid, a precursor of lignins, flavanoids, and coumarins. Leaf sample (0.1g) was taken and crushed in 2 ml of 0.1Molar sodium borate buffer (pH-7) containing 1.4 mM β-mercaptoethanol (4 µL) and then centrifuged at 16000 g for 15 minutes at 4^oC. Supernatant obtained was used as the enzyme source. To the reaction mixture 0.2 ml of supernatant, 0.5 ml of 0.2 M borate buffer (pH - 8.7) and 1.3 ml distilled water were added. The reaction was started by adding 1ml of 0.1 M L - phenylalanine (pH - 8.7) and incubated for 30 minutes at 32^0C. The reaction was terminated by adding 0.5 ml of 1M trichloroacetic acid (TCA). Reaction mixture without enzyme extract served as blank. The absorbance was taken at 290 nm. PAL was measured by formation of *Trans* - cinnamic acid at 290 nm as described by Brueske [7] and was expressed in terms of µ mol TCA per gram of fresh weight (FW).

2.2.4. Peroxidase (PO) assay

The slightly modified method of Hammerschmidt et al. [8] was adapted to measure the peroxidase activity by 0.1 g of pea leaf crushed in 2.0 ml of chilled 0.1 M phosphate buffer (pH -7.0), centrifuged at 16000 g for 15 minutes at 4^oC and the supernatant obtained was used as enzyme source. For the assay mixture pipette out 0.05 ml enzyme extract, 1.5 ml of 0.05 M pyrogallol and 0.5 ml of hydrogen peroxide (1% v/v). The changes in absorbance were recorded at 420 nm after 30 seconds interval for 3 minutes. The peroxidase activity was expressed as U/g/min fresh weight.

2.3. Statistical analysis

All statistical analyzes were carried out using computer software Statistical Package for the Social Sciences (SPSS) version 16.0. Data were subjected to one-way ANOVA (analysis of

variance) followed by Duncan's multiple range test to differentiate the significant difference between different treatments at the probability level of $P \leq 0.05$. Results are expressed as means of three replicates, and vertical bars indicate standard deviations of the means.

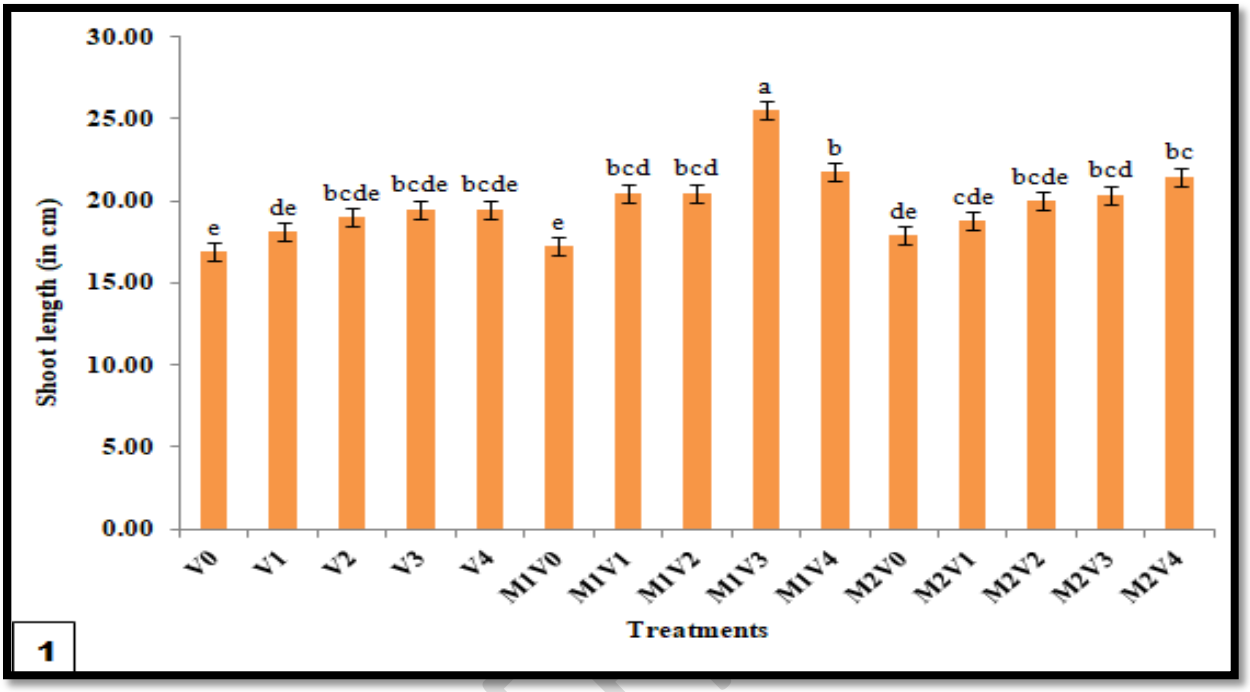
3. RESULTS AND DISCUSSION

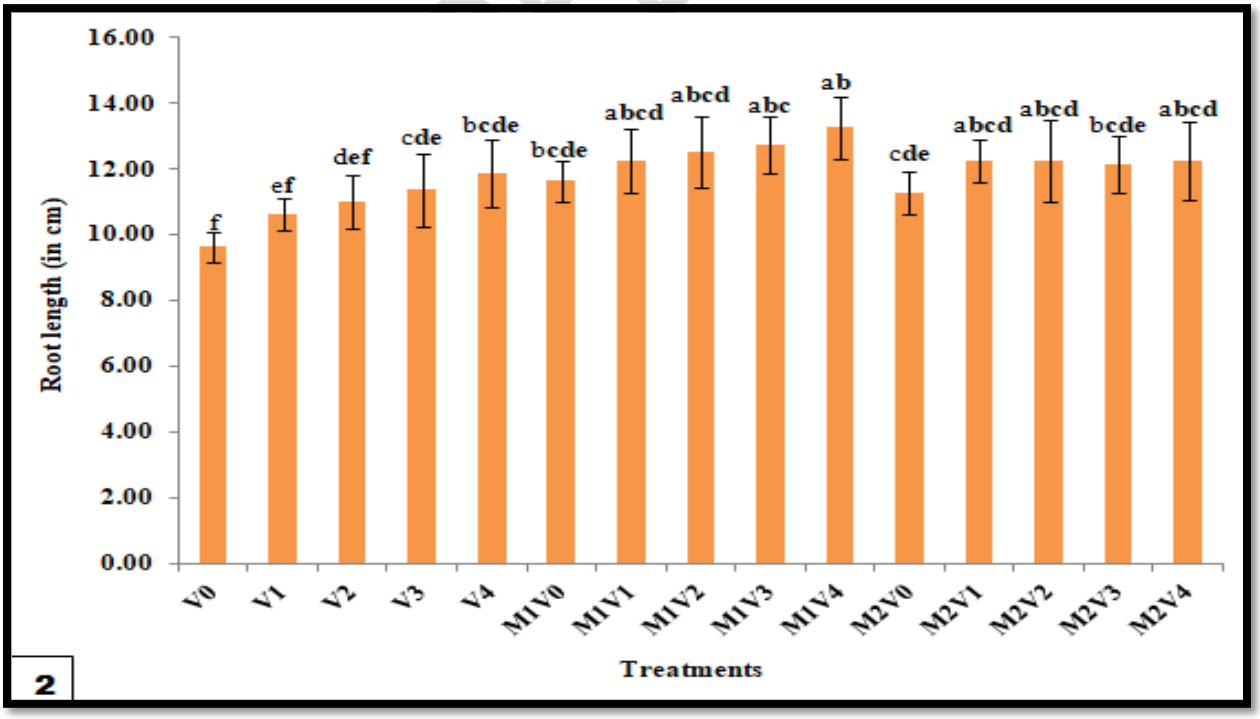
3.1. Growth parameters

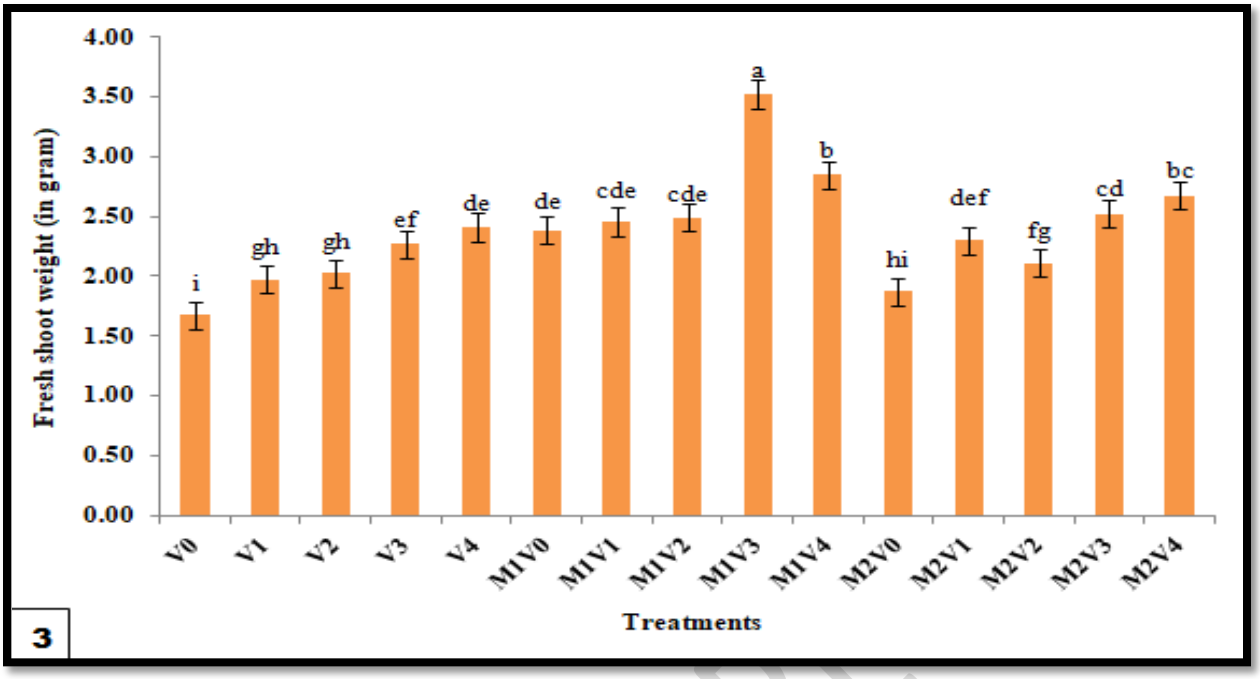
In this study, the results showed a significant effect of increased shoot and root lengths, and total fresh and dry weights of shoot and roots. Among all treatments highest shoot length was observed in M1V3 (25.50 cm) and M1V4 (21.75 cm), while the lowest in control (16.88). Shoot length increased from V0 to V4 with the percentage of vermicompost in the soil [Figure A. (1)]. Maximum root length was observed in M1V4 (13.25 cm) followed by M1V3 (12.75 cm) and M1V2 (12.50 cm). Overall maximum root length was recorded in plants grown from seeds treated with *T. harzianum* [Figure A. (2)]. While studying the effects of *Trichoderma* on host plants Kleifeld and Chet [9] obtained similar growth promotion results and reported that some *Trichoderma* spp. can also penetrate and live endophytically with plant roots similar to mycorrhizal fungi, and root length and total root area may be enhanced by IAA production by these fungi [10]. The highest value of fresh shoot weight (3.54 g) was noticed in M1V3 followed by M1V4 (2.85g) and control (1.68 g). Almost similar result was obtained in M1 (2.38g) and V4 (2.41 g) [Figure A. (3)]. Dry shoot weight was recorded maximum in M1V3 (0.38 g) followed by M1V4 (0.33 g) and minimum in V1 (0.20 gram). Almost similar result was observed in M1 (0.27 g) and V4 (0.28 g) [Figure A. (4)]. Data shown in Figure A. (5) indicate the maximum fresh root weight in M1V2 (1.969 g) followed M1V1 (1.92 g) and control (0.74 g). Overall maximum fresh root weight was recorded in plants obtained from seed treatment with *T. harzianum*, followed by plants from seed treatment with *P. fluorescens* and minimum in control. So, we can say that *T. harzianum* promotes plant growth and enhances its biomass production. From the Figure A. (6), the maximum dry root weight is obtained in M1V2 (0.16 g) followed by M1V1 (0.15 g). From M1V0 to M1V2 dry root weight was increased but after that it starts decline from M1V3 (0.12 g) to M1V4 (0.10 g). Similar effects were observed in treatment with different doses of vermicompost (V0 to V4) and plants obtained from seed treated with *P. fluorescens* (M2V0 to M2V4).

The improvement in soil environmental condition resulted in higher proliferation of plant roots, which helped plants to draw water and nutrients from larger area and deeper layers and thus owing to higher nutrients availability, production of more photosynthates and their translocation to different plant parts resulted in increased vegetative growth. These findings are in accordance with the results of [11-15]. Growth promotion activities might be due to the synthesis of phytohormones, N_2 – fixation, synthesis of some enzymes such as 1- aminocyclopropane -1-carboxylate (ACC) deaminase that regulate the levels of plant hormones (lowers the plant

ethylene levels under certain stress conditions and helps in the efficacious functioning of the PGPR) , as well as the solubilisation of inorganic phosphates and mineralisation of organic phosphates, which makes phosphorus available to the plants as the finding confirmed by Rodriguez and Fraga [16].



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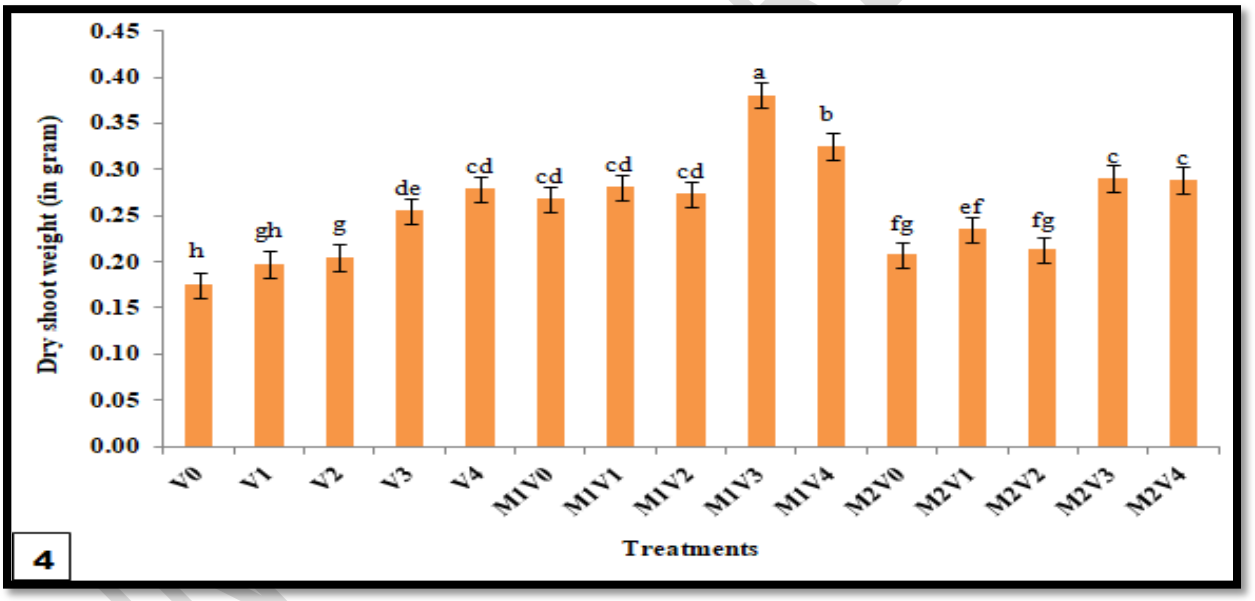
Treatments

3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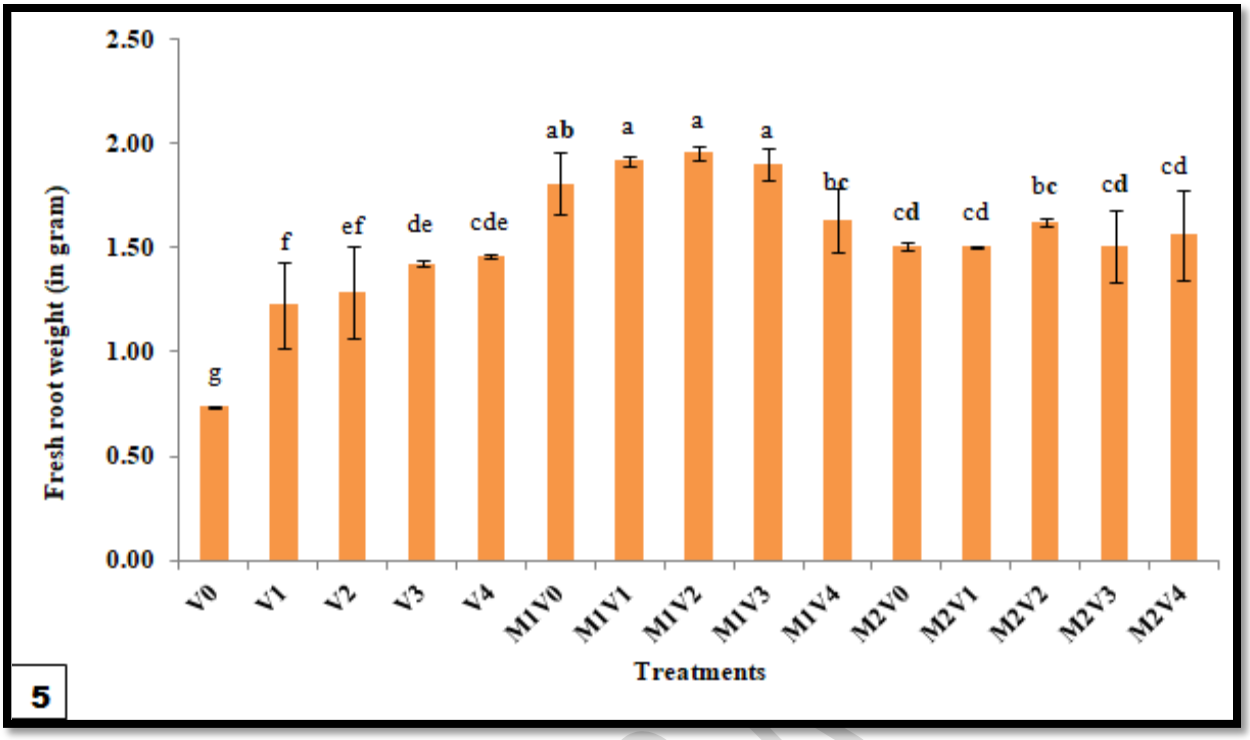
Treatments

4



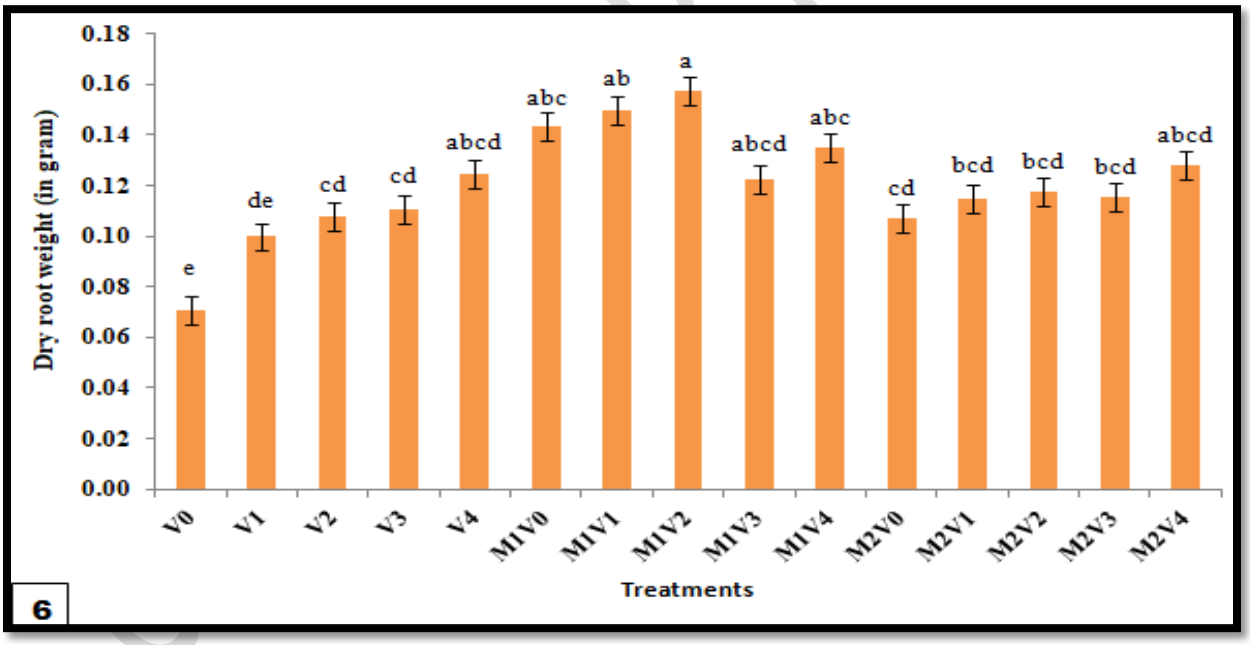
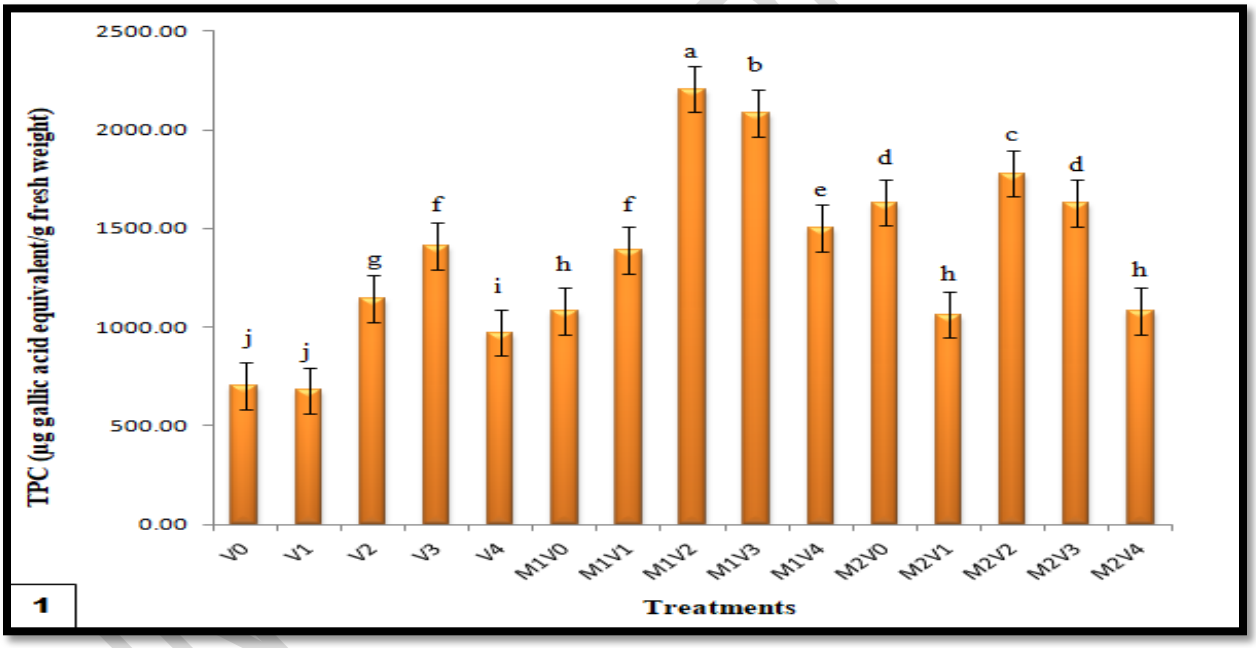


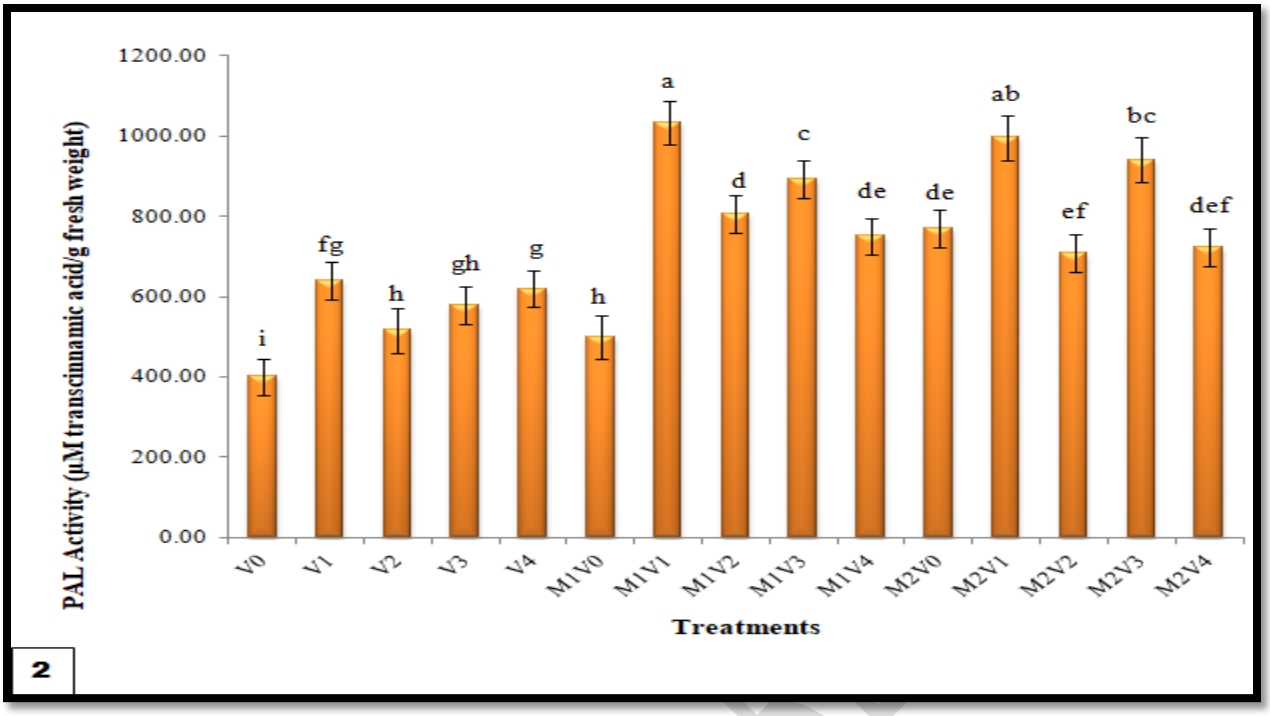
Figure. A. Effects on plant growth parameters, raised in soil substituted with different doses of vermicompost singly and in combination with seed treatment with *Trichoderma harzianum* and *Pseudomonas fluorescence* in different treatments at 30 days after sowing (DAS): **1)**. Shoot length; **2)**. Root length; **3)**. Fresh shoot weight; **4)**. Dry shoot weight; **5)**. Fresh root weight; **6)**. Dry root weight. Results are expressed as means of three replicates, and vertical bars indicate standard deviations of the means. Different letters indicate significant differences among treatment within the results at the same time interval according to Duncan's multiple range test at $P \leq 0.05$.

3.2. Synthesis of defense-related compounds

Plants contain different ranges or arrays of the cellular mechanisms that help them in defending themselves from various invading pathogens. Applications of different kinds of beneficial microbes reprogramme the plant metabolism by altering their cellular mechanisms, in response to the specific stimuli, which would be visible when host plants are challenged with a pathogen [17]. Inducing the plants own defense mechanism by prior application of a biological inducer is thought to be a novel plant protection strategy. Plants treated with biocontrol agents responds quickly and show a strong activation of the cellular defense responses after challenged with pathogen as compared to untreated plants [18]. Such cellular responses also include an earlier oxidative burst and stronger upregulation defense genes [19].

Results from this study shown that the pea plants treated with microbial agents exhibited higher activities of defense-related enzymes and accumulation of phenols in leaves. The data presented in Figure. B. (1-3). In this study the increased accumulation of phenolic contents and other defense related proteins in plants treated with *Trichoderma harzianum* & *Pseudomonas fluorescens* at 30 DAS indicated that seed treatment triggered the plants to synthesize defense related compounds, this results confirms the finding of M'Piga et al. [20].





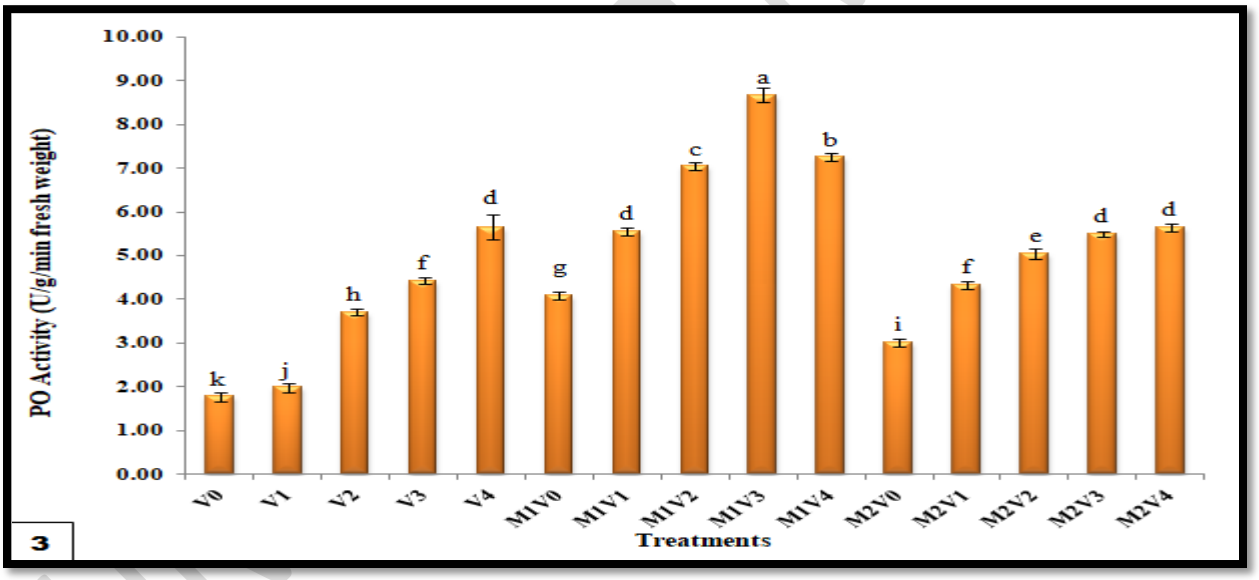


Figure. B. Effects on production of defense related compounds in pea plant raised in soil substituted with different doses of vermicompost singly and in combination with seed treatment with *Trichoderma harzianum* and *Pseudomonas fluorescence* at 30 days after sowing (DAS) in different treatments. **1).** Total phenol content (TPC), **2.** Phenylalanine ammonia-lyase (PAL), **3.** Peroxidase assay (PO). Results are expressed as means of three replicates, and vertical bars indicate standard deviations of the means. Different letters indicate significant differences among treatment within the results at the same time interval according to Duncan's multiple range test at $P \leq 0.05$.

3.2.1. Total phenol content (TPC)

The activity of TPC is the process to find out the amount of phenol in the plant samples. Phenol content in the plant strengthens the cell wall [21] and due to their redox properties also act as

antioxidants [22, 23]. Plants treated with microbial agents were shown significantly higher accumulation of phenol as compared to untreated plants. The results showed that the levels of phenol were 3.3 times higher in M1V2 than V2 (1144.00). In general, TPC was found to be maximum in plants that were grown from seed treated with *T. harzianum* followed by *P. fluorescens* and control, however it was detected highest in M1V2 (2207.33) [Fig. B. (1)]. The results are confirmed by the finding of Surekha et al. [24] where induced resistance against wilt and blight in legumes was due to synthesis of high amount of phenols by *Trichoderma viride*.

3.2.2. Phenylalanine ammonia-lyase (PAL)

PAL is a very important enzyme in the phenylpropanoid biosynthesis pathway that leads to synthesis of phytoalexins or phenolic compounds, which act as defensive substances in plants such as antimicrobial activity, synthesis of various signaling compounds such as salicylic acid [25]. In this study PAL level significantly increased in plants grown from seed treatment with *T. harzianum* and *P. fluorescens* and small amount of PAL activity was also recorded in control. Maximum PAL activity was detected in plants from M1V1 (1033) followed by M2V1 (996.55) and minimum (400.10) in control [Fig. B. (2)]. Similarly, a significantly increased phenolic content was also observed in the plants of M2V1.

3.2.3. Peroxidase assay (PO)

PO is the key enzyme that plays a very crucial role in the synthesis of lignin [26]. In this study, analysis of plants indicated that pea seeds treated with the beneficial microbe i.e. *T. harzianum* and vermicompost @ 3.0% exhibited maximum (8.68) activities of PO compared with the plants treated with *P. fluorescens* and untreated control [Fig. B. (3)]. Similar results were also obtained in a previous study, where microbial mixture of *Bacillus* strains IN937a and IN937b was found to induce maximum SOD and PO activity compared with untreated control [27]. Zdor and Anderson [28] also reported that rhizosphere colonization of bean plants by various bacteria induced the peroxidase activity.

As per the findings of the present study *T. harzianum* treated seeds in soil substituted with different doses of vermicompost performed better as compared to vermicompost alone and *P. fluorescens* treated seeds grown in soil substituted with different doses of vermicompost. *T. harzianum* produces some proteins that show strong antifungal activities when applied in vitro, alone and /or combined, against plant pathogens as reported by Harman [29] confirm the role of the given beneficial microbial species in triggering activities of PO in plants when applied as seed treatment. A proteomic approach to identify *Trichoderma* - induced enzymes also showed increased levels of antioxidant and other detoxifying enzymes in maize leaves [30]. These reports confirm the role of the given beneficial microbial species in triggering activities of PO in plants when applied as seed treatment and strengthen the results obtained in the present study.

Colonization by the beneficial microorganisms leading to significantly increase in the activities of defense- related enzymes especially to improvement of physio - chemical properties of the soil

tal phenols, PAL and PO in the present study suggests that these parameters are regulated by the signals released by the microbes. Moreover, induction of phenols is also linked with induced PAL activity, which catalyses the first step in synthesis of phenols. Increased PAL activity and phenol accumulation in the present study may, thus, be correlated with enhanced defense response by the beneficial microbes.

CONCLUSION

On the basis of results obtained it may be concluded that among the various treatments, V4 was found very effective in all growth parameters as well as in plant defense - related compounds. Whereas, in case of treatment combinations M1V3 has given best results among all the treatments. Therefore, vermicompost along with beneficial microbes like *T. harzianum* and *P. fluorescens* are preferably the best in growth and development of plants as they produce growth hormones, improvement of physio - chemical properties of the soil by releasing micronutrients in available form as well as helping in protection from diseases through production of several essential defense-related compounds.

Vermicompost improved the soil nutrients status by supporting growth and development of microbial biomass by providing suitable habitat that results better crop growth and development. *T. harzianum* is not only a good biocontrol agent, but also play a major role as promoter of soil fertility. Furthermore, the use of organic soil amendment has prolonged effects on soil physical, chemical and biological properties as compared to various commercial chemical fertilizers and pesticides. The integrated application of vermicompost and beneficial microbes has the potential to substitute the chemical fertilizer uses in agriculture. By using this integrated approach we would be able to develop environmentally sound sustainable agricultural practices.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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