Original Research Article

Evaluation of Synergistic Effects of Vermicompost and Beneficial Microbes on Pea

ABSTRACT

The present manuscript was to evaluate the synergistic effects of vermicompost and beneficial microbes on Pea plants. The result showed significant increase in plant growth parameters like shoot length, root length, total fresh and dry weight of shoots and roots along with enhanced production of defense–related compounds. Among all the treatments vermicompost @ 40% was found very effective in all growth parameters as well as in plant defense related compounds. Whereas, in case of treatment combinations application of vermicompost @ 30% along with seed treatment with *Trichoderma harzianum* has given best results followed by treatment combination application of vermicompost @ 20% and seed treatment with *Pseudomonas fluorescens*.

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 major (NPK) and 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 METHODS

To access the synergistic effects of different doses of vermicompost amended with soil and along with the combination of beneficial microbes (*T. harzianum* & *P. fluorescens*) on pea plants was done under net house condition. The seeds of Pea variety "Azad P-3" were procured from local market of Varanasi, Utter Pradesh (UP).

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

Soil was collected from the BHU farm and sterilized in autoclave at 121℃ for 20 minutes. Then according to the treatments different amounts of vermicompost was mixed with soil and filled in plastic pot.

The experiment was laid out in a completely randomized design with seed treatment by two beneficial microbes with three replication and fifteen treatments *viz*.,T1= Control (V0), T2= Vermicompost @ 10% (V1), T3=Vermicompost @ 20% (V2), T4=Vermicompost @ 30% (V3), T5=Vermicompost @ 40% (V4), T6= Seed treatment with *T. harzianum* (M1), T7=Seed treatment with *T. harzianum* (M1)+ Vermicompost @ 10% (V1), T8=Seed treatment with *T. harzianum* (M1)+ Vermicompost @ 20% (V2), T9=Seed treatment with *T. harzianum* (M1)+Vermicompost @ 30% (V3), T10 = Seed treatment with *T. harzianum* (M1)+Vermicompost @ 40% (V4), T11 = Seed treatment with *P. fluorescens* (M2), T12 = Seed treatment with *P. fluorescens (*M2)+Vermicompost @ 10% (V1), T13 = Seed treatment with *P. fluorescens* (M2)+Vermicompost @ 20% (V2), T14 = Seed treatment with *P. fluorescens* (M2)+Vermicompost @ 30% (V3), T15 = Seed treatment with *P. fluorescens* (M2)+Vermicompost @ 40% (V4).

The observations were recorded for plant growth parameter *viz*. Shoot length (cm), root length (cm), fresh shoot and root weight (g), dry root and shoot weight (g).

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

2.2.1. 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 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. Standard curves were made for each assay using various concentration of Gallic acid in 95% methanol. Absorbance values were converted to GA equivalents (GAE) per g fresh weight (FW).

2.2.2. 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).

2.2.3. Peroxidase (PO) assay

With slight modification, of Hammerschmidt et al. [8] method, peroxidase activity was examined. 0.1 g of leaf sample was taken and crushed in 2 ml of 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. The reaction mixture consisted of 0.05 ml enzyme of extract, 1.5 ml of 0.05 M pyrogallol and 0.5 ml of H_2O_2 (1% v/v). The changes in absorbance were recorded at 420 nm after 30 seconds interval for 3 minutes.

2.3. Statistical analysis

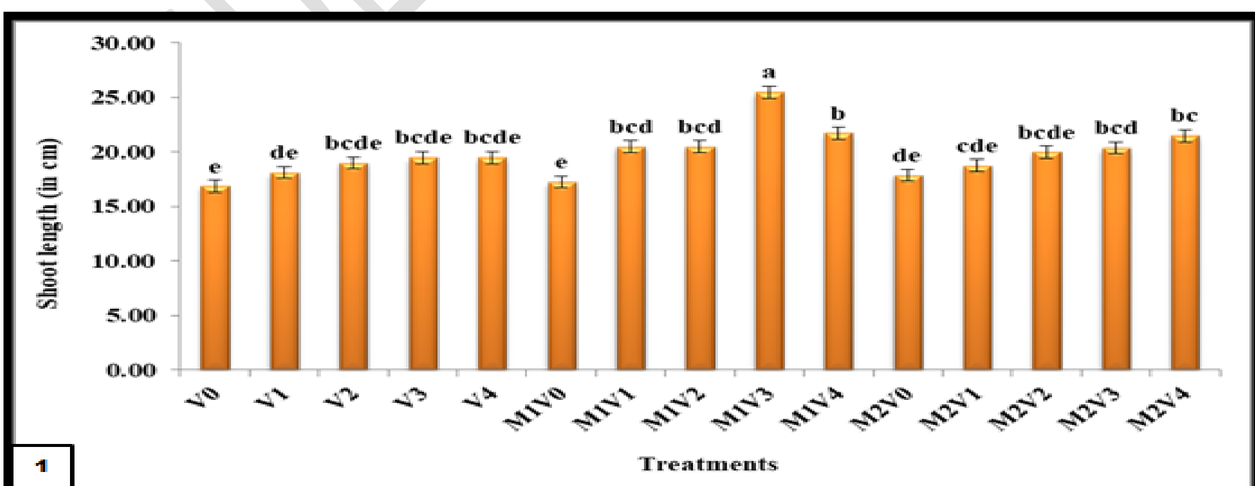
The results of the experiment were analysed statistically by using computer software Statistical Package for the Social Sciences (SPSS) version 16.0 and subjected to one - way ANOVA (analysis of variance) to find the differences in growth and plant defense related compounds for different treatments. The level of significance applied for analysis of different treatment is $p \leq 0.05$.

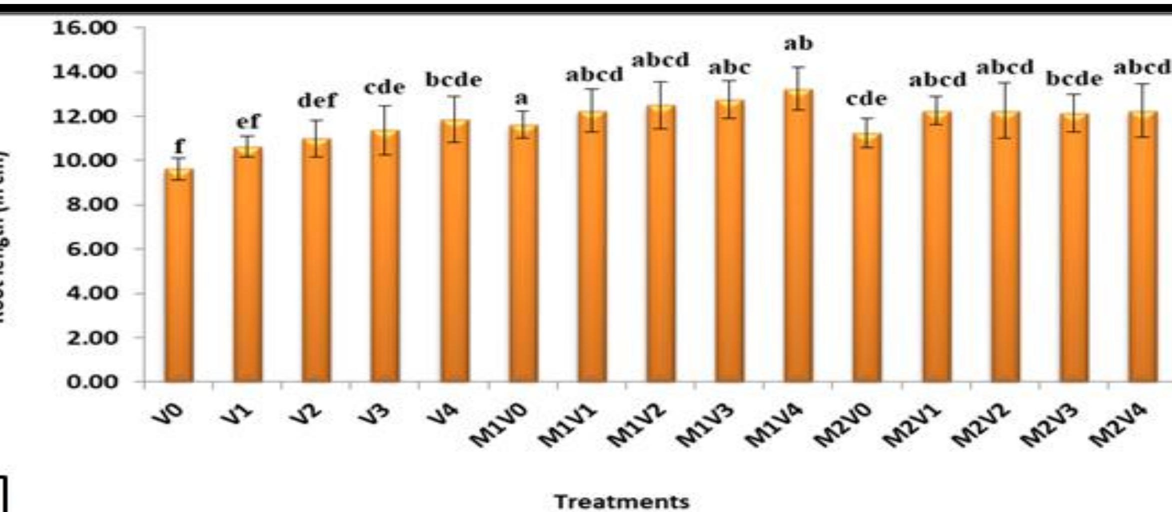
3. RESULTS AND DISCUSSION

3.1. Growth parameters

In the present study the results demonstrated the synergistic effects of vermicompost and beneficial microbes significantly resulted in increased shoot length, root length, total fresh and dry weight of shoot and roots. The best result was confirmed in the treatment combination with the application of vermicompost @ 30% and seed treatment with *Trichoderma harzianum* recorded maximum values of these parameters. The data are presented in Figure. A. (1-6). 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. This results is showing conformity with the findings of [9-18].

Apart from vermicompost and *T. harzianum* addition of *P. fluorescens* also have been significantly increased the shoot length, root length, fresh and dry root and shoot weight. Growth promotion activities might be duo 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 solubilization of inorganic phosphates and mineralisation of organic phosphates, which makes phosphorus available to the plants as the finding confirmed by Rodriguez and Fraga. [19].




Root length (in cm)
16.00
14.00
12.00
10.00
8.00
6.00
4.00
2.00
0.00
f
ef
def
cde
bcde
a
abcd
abcd
abc
ab
cde
abcd
abcd
bcde
abcd
V0
V1
V2
V3
V4
M1V0
M1V1
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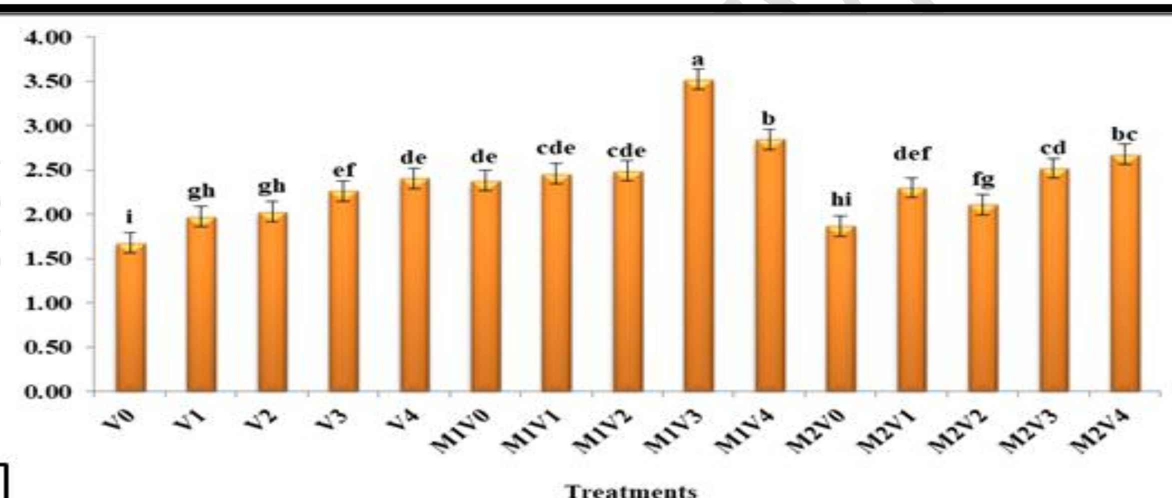
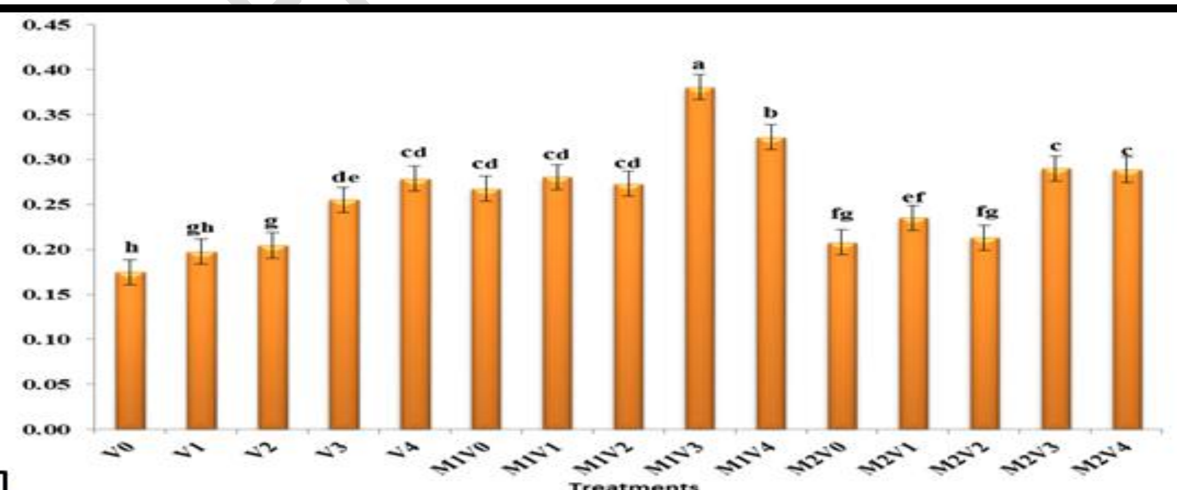
M1V3
M1V4
M2V0
M2V1
M2V2
M2V3
M2V4
Treatments

2


Fresh shoot weight (in gram)
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
i
gh
gh
ef
de
de
cde
cde
a
b
hi
def
fg
cd
bc
V0
V1
V2
V3
V4
M1V0
M1V1
M1V2
M1V3
M1V4
M2V0
M2V1
M2V2
M2V3
M2V4
Treatments

3


Dry shoot weight (in gram)
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
h
gh
g
de
cd
cd
cd
cd
a
b
fg
ef
fg
c
c
V0
V1
V2
V3
V4
M1V0
M1V1
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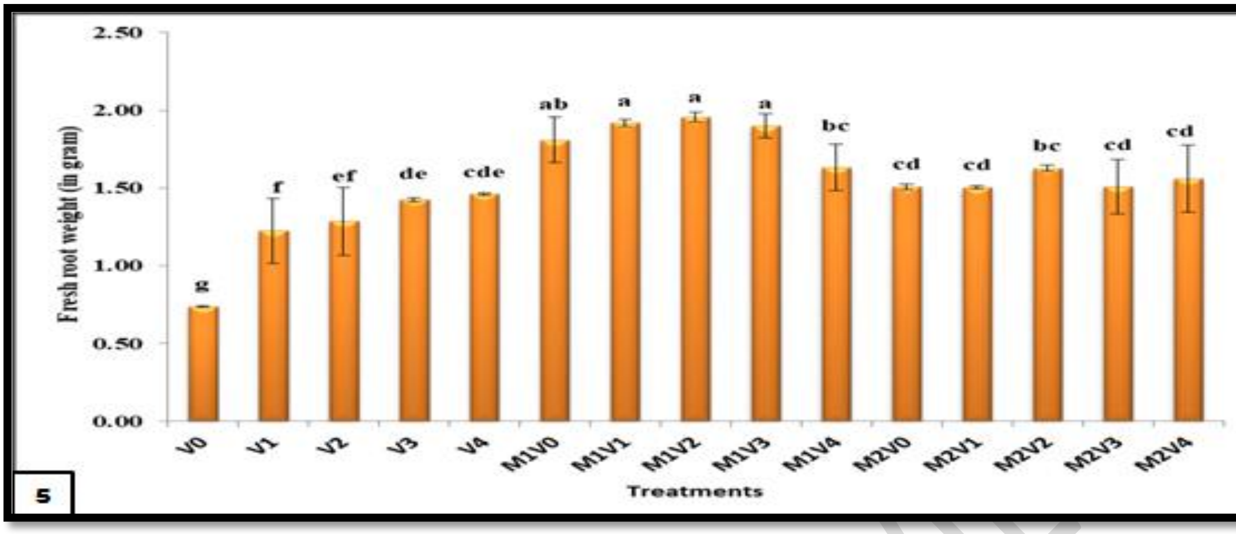
M1V3
M1V4
M2V0
M2V1
M2V2
M2V3
M2V4
Treatments

4



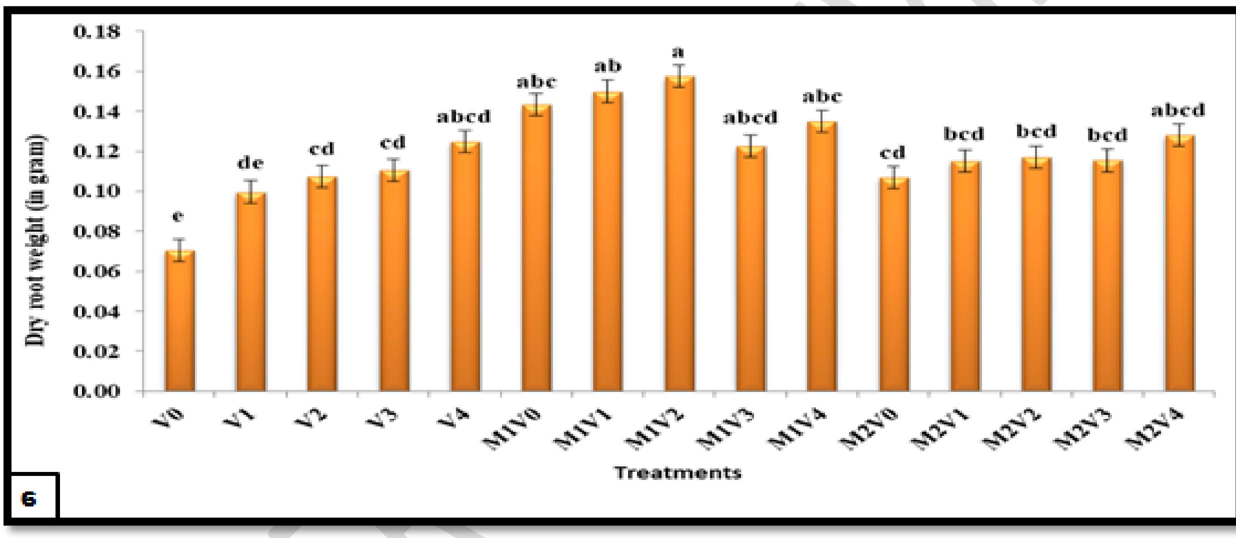


Figure. A. Effects on plant growth parameters, raised in soil substituted with different doses of vermicompost singly and in combination with seed treatment with *Trichoderma harzianum* and *Pseudomonas fluorescence* in different treatments at 30 days after sowing (DAS): **1**. Shoot length; **2**. Root 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

Results from present 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 present 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].

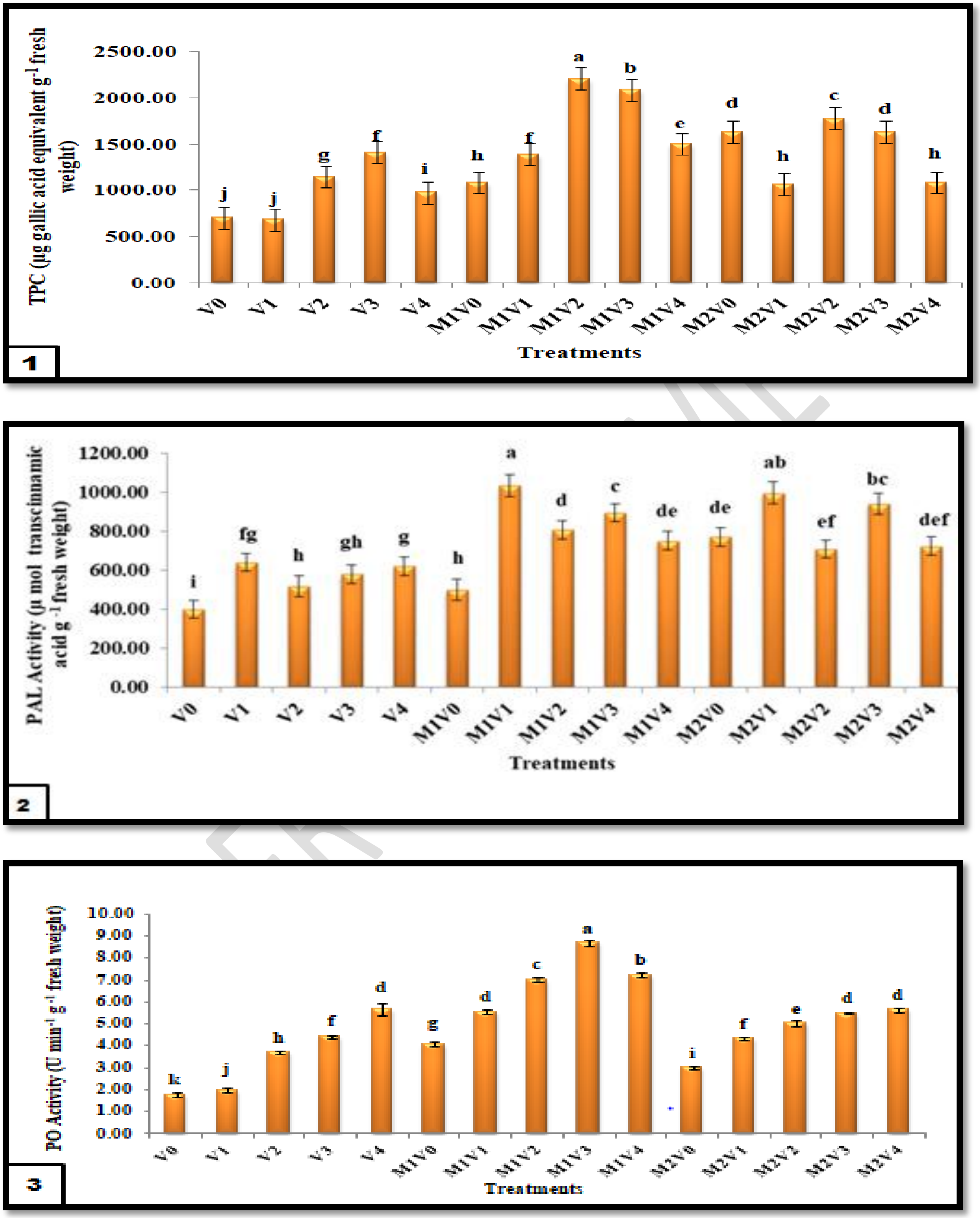


Figure. B. Effects on production of defense related compounds in pea plant raised in soil substituted with different doses of vermicompost singly and in combination with seed treatment with *Trichoderma harzianum* and *Pseudomonas fluorescence* at 30 days after sowing (DAS) in different treatments.1.Total 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 plants samples. The results showed higher TPC in the plants where seed treated with *T. harzianum* along with vermicompost @ 20% followed by the seed treatment with *T. harzianum* and Vermicompost @ 30% at 30 DAS. The results are confirmed by the finding of Surekha et al. [21] 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 **[22]**. In the present study maximum PAL activity was detected in plants from treatment combination with vermicompost @ 10% and seed treatment with *T. harzianum*. Similarly, a significantly increase in phenolic content was also observed in the plants of treatment combination with vermicompost @ 10% and seed treatment with *P. fluorescens.*

3.2.3. Peroxidase assay (PO)

PO is the key enzyme that plays a very crucial role in the synthesis of lignin [23]. In the present study, analysis of plants indicated that pea seeds treated with the beneficial microbe i.e. *T. harzianum* with vermicompost @ 30% exhibited maximum activities of PO compared with the plants treated with *P. fluoresence. T. harzianum* produces some proteins that show strong antifungal activities when applied in vitro, alone and /or combined, against plant pathogens [24]. These reports confirm the role of the given beneficial microbial species in triggering activities of PO in plants when applied as seed treatment.

CONCLUSIONS

Among all the treatments vermicompost alone @ 40% was found very effective in all growth parameters as well as in plant defense–related compounds. Whereas, in case of treatment combinations application of vermicompost @ 30% along with seed treatment with *T. harzianum* has given best results among all the treatments. Therefore, vermicompost along with beneficial microbes like *T. harzianum* and *P. fluorescens* preferably best in growth and development of plants as they produce growth hormones, releasing micronutrients in available form as well as helps in protection from diseases by producing several defenses - related compounds.

FUTURE LINE OF WORK

Sustainable farming is getting popularity among the farmer's and hence studies on the possibilities of using green manure crops and other locally available organic sources such as vermicompost, household wastes, urban wastes etc. which can be converted into beneficial soil amendments and lightening the landfills load may be tested and studied.

- ❖ There is an urgent need to screen and test the large numbers of organic sources of fertilizers which are having profound influence on yield and crop quality.
- ❖ Organic manures may be tested with and without bio-fertilizers to know the exact role of the latter in enhancing crop yield and productivity.
- ❖ Research must be taken up to reduce the cost of producing organic fertilizers thereby reducing the cost of cultivation.

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