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Indole Acetic Acid and Radish Biomass Productive Influence of Rhizobacteria, *Pseudomonas trivialias* (L) and Earthworm, *Eudrilus eugenae* (L).

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Abstract

The IAA production is widespread among environmental bacteria that inhabit soils, waters, plant and animal hosts. The specific density of soil microorganisms and earthworms in soil serve to orchestrate the crop growth progression. There is fruitful interactions among soil microorganisms with earthworms and crop in the field that can change the response of growth and development. The rhizobacteria, *Pseudomonas trivialis* (L) is a deleterious rhizobacteria (DRB) or a plant growth promoting rhi-zobacteria (PGPR) depending on the plant species. It is well known to produce Indole Acetic Acid (IAA), a hormone that can affect plant growth. In the present attempt, rhizobacteria, *Pseudomonas trivialis* (L) in different concentrations (CFU/ml) (0; 10^6; 10^7 and 10^8) tested on radish, *Raphanus sativus* (L) to characterize its effect in presence and in absence of earthworms. Presence of rhizobacteria, *Pseudomonas trivialis* (L), earthworms were found enhancing growth of the radish, *Rhaphanus sativus* (L) with reference to biomass aboveground and total. This response was found significant over the control. The biomass of radish, *Rhaphanus sativus* (L) below ground was also found improved up to some extent, but not significant. Radish biomass promoting influence in vermin-field was observed directly proportional to the titer of inoculums of rhizobacteria, *Pseudomonas trivialis* (L). The rhizobacterial inoculum of about 10^8 CFU/ml titer was found most suitable for excellent biomass production of radish, *Rhaphanus sativus* (L). Vermi-field with rhizobacterial population can be utilized for crop yield.

Keywords: Rhizobacterial population; Vermi-field; Indole Acetic Acid (IAA).

INTRODUCTION

Indole-3-acetic acid (IAA, 3-IAA) is the most common, naturally occurring, plant hormone of the auxin class. Indole-3-acetic acid (IAA, 3-IAA) is the best known of the auxins, and has been the subject of extensive studies by plant physiologists. IAA is predominantly produced in cells of the apex (bud) and very young leaves of a plant. Plants can synthesize IAA by several independent biosynthetic pathways. Four of them start from tryptophan, but there is also a biosynthetic pathway independent of tryptophan. Plants mainly produce IAA from tryptophan through indole-3-pyruvic acid. IAA is also produced from tryptophan through indole-3-acetaldoxime in *Arabidopsis thaliana* (L). In rats, IAA is a product of both endogenous and colonic microbial metabolism from dietary tryptophan along with tryptophol. This was first observed in rats infected by *Trypanosoma brucei gambiense* (L) and correlation with protein intake has been confirmed in 2015. Humans did not excrete IAA. It is a derivative of indole, containing a carboxymethyl substituent. It is a colorless solid that is soluble in polar organic solvents. Radish, *Raphanus sativus* L. is important root vegetable of economic importance. Its Leaves and roots have been used in various parts of the world (Gutiérrez and Perez, 2004; Nishio, 2017). This species is used in traditional medicine to treat respiratory and liver diseases (Paredes, 1984). Radish vegetable crop is grown and consumed throughout the world. It is mostly eaten raw as a crunchy salad vegetable. It have many varieties, varying in size, flavor, color, and length of time they take to mature. Radishes owe their sharp flavor to the various chemical compounds produced by the plants, including glucosinolate, myrosinase, and isothiocyanate. They are sometimes grown as companion plants and suffer from few pests and diseases. They germinate quickly and grow rapidly, smaller varieties being ready for consumption within a month, while larger daikon varieties take several months. Another use of radish is as cover or catch crop in winter (*Price, et al, 2013)* or as a forage crop (*Fitzgerald, et al, 1984).* Some radishes are grown for their seeds; daikon, for instance, may be grown for oil production. Others are used for sprouting. The root's extracts have antibacterial activity against several bacterial species (Caceres, 1987). Radish has been cultivated in ancient history and was popularly used in Egypt. In Japan, the long white type is the most favorable vegetable (Yamagishi, 2017). Increasing the economic value of radish required exploration new methods of utilization (Nishio, 2017).

Soil microbiota can be closely associated with soil fauna such as earthworms which have an important in ecosystem functions like decomposition, mineralization organic matter and nutrient cycling (Brown *et al*., 1999 and 2004; Lavelle and Spain, 2001; Lavelle, 2002). Earthworms to phylum Annelida, subclass Oligochaeta and consider the large members of soil invertebrates (Julka, 1993; Ismail, 1997; Kooch *et al*., 2008). Brown *et al*., (2004) and Scheu, (2003), studied the effect of earthworms on plant development. Brown *et al*.,(1999), Scheu ,(2003), Arthur *et al*.,(2017) and Zhenggao *et al*.,(2017) reported flourishing the crops in presence of earthworms. The activity of soil dwelling earthworms changes soil properties (biotic and abiotic) and increase plant growth (Groenigen *et al*., 2014; Dai *et al*., 2004). Earthworm casts (their faeces) are also very important in soils and are responsible for some of the fine crumb structure of soils (Ojha and Devkota, 2014).

The dual interaction between organisms has important role on plant development due to the liberation of nutrients (Ingham *et al*., 1985; Blouin *et al*., 2005; Jana *et al*., 2010). Plant growth-promoting rhizobacteria (PGPR) may have a positive effect on plant development by different mechanisms in a direct or indirect manner. PGPR release signal molecules similar to phytohormones, solubilize nutrients, fix atmospheric nitrogen, or produce ethylene; they can effect indirectly on plant development by suppression several phytopathogens (Nehl *et al*., 1996; Persello-Cartieaux *et al*., 2003). Indole acetic acid concentration can have positive or negative effects according to plant sensitivity (Barazani and Friedman, 1999). The auxins have a vital role in stimulation of celldivision and cell elongation (Apine and Jadhav, 2011). Auxin in low concentration stimulate root elongation, whereas higher concentration inhibits the root elongation (Madhaiyan *et al*.,2007). Auxin-like compounds have also been detected in earthworm dejections (Canellas, *et al*., 2002; Muscolo *et al*., 1998) and could be a reason of

stimulatory effect of earthworms (Brownet al., 2004) and vermicompost (Canellas, *et al*., 2002) on plant growth. The activity of earthworms affects both biotic and abiotic soil characterizations, in turn affecting plant flourishing (Groenigen, *et al*., 2014).

In the other hand, other free living rhizospheric bacteria described as deleterious rhizobacteria (DRB) (Suslow and Schroth, 1982) can inhibit root and shoot growth. DRB can live as endophyte intercellularly and in intracellular spaces without any disease symptoms. Suppression of plant growth mechanisms includes production of toxic substances, phytohormones, or as a result of interactions with other microorganisms (Kremer, 2007). The plant growth inhibition by DRB depends on interaction of biotic and abiotic factors such as the host plant, environmental factors of soil, time, and, in agroecosystems, management (Kremer, 2007). The organism may act as a DRB or PGPR according to different factors, i.e. plant type, conditions of soil (biotic and abiotic factors) or the presence or absence of mycorrhizae (Nehlet al.,1996).

It is thus of import to study the effect of gradient concentrations of inoculum when studying the effect of a rhizobacteria (Suarezet al., 2014). Pseudomonas trivialis recorded in a few studies as stress-tolerant, and plant growth-promoting rhizobacterium. There are also few published data which consider jointly the impact of microorganisms and earthworms on plant development. In current research, we tested the effects of the earthworm (Allolobophora caliginos) and the rhizospheric bacteria P. trivialis on the growth of R. sativus var. aegyptiacus. Our study aimed to study the effect of different concentrations of P. trivialison the growth of R. sativus var. aegyptiacus. Also, study the synergistic effects of P. trivialis with earthworms A. caliginos on radish plants growth.

MATERIAL AND METHOD

The study was carried out through the steps like: Preparation of Inoculum of *Pseudomonas trivialias* (L); Determination of Indoleacetic Acid (IAA); Maintenance of Vermiculture Unit; Seeds and Seedlings of Radish, *Raphanus sativus* (L); Study Design; Parameters of Analysis and Statistical Analysis.

(I). Preparation of Inoculum of *Pseudomonas trivialias* (L) (strain X33d):

Inoculum of *Pseudomonas trivialias* (L) (strain X33d) was procured from Someshwar Science College, Someshwarnagar Baramati. Rhizobacteria were grown in B-King (glycerol 10 ml L^{-1}, peptone 15 g L^{-1}, magnesium sulfate 1.0 M [1 ml L^{-1}] and dibasic potassium phosphate 1.5 g L^{-1}) liquid medium at 28 ± 2 ℃ and 160 rpm for 48 h. The concentration was adjusted to 10^9 cells ml^{-1} with a spectrophotometer at a wavelength of 660 nm and absorbance of one. The colony forming unit (CFU) is a unit used to estimate the number of viable bacteria in a sample. The visual appearance of a colony in a cell culture requires significant growth, and when counting colonies it is uncertain if the colony arose from one cell or a group of cells. Expressing results as colony-forming units reflects this uncertainty (Juliana Mayz1, *et al*, 2013).

Table 1. Selected Parameters of *Pseudomonas trivialis* (L).

Serial No.	Parameters	Yes / No
1.	Fluorescent on king's -B	Yes
2.	Growth on cetrimide medium	Yes
3.	Gram's Staining	No
4.	KOH solubility	Yes
5.	Cytochrome C Oxodase	Yes
6.	Nitrate Reductase	No
7.	Catalase Test	Yes
8.	Gelatin Hydrolysis	Yes
9	Casein Hydrolysis	Yes
10	Starch Hydrolysis	No
11.	Indole Production	No

12.	Arginine Dehydrogenase	No
13.	Lavan Formation	No
14.	Tween 80 Hydrolysis	Yes
15.	H_2S production	No
16.	Voges- Proskauer	No
17.	Carbon source utilization:	--
18.	L-Arabinose	No
19.	D-Cellobiose	No
20.	D-Fructose	No
21.	Citrate	Yes
22.	D- Alanine	Yes
23.	D-Sorbitol	No
24.	D-Galactose	No
25.	Glycerol	No
26.	Glucose	Yes
27.	Lactose	No
28.	Maltose	No
29.	Manitol	No
30.	Proline	Yes
31.	Sucrose	No
32	D-Trehalose	Yes

(II). Determination of Indoleacetic Acid (IAA):

Rhizobacteria were grown in 20 ml of B-King liquid medium supplemented with 0.5 g L^{-1} of L-tryptophan and incubated at 28 ± 2 ℃ and 160 rpm for 72 h. Rhizobacterial cultures were centrifuged at 5000 rpm for 15 min and one ml of the supernatant was mixed in two ml of Salkowski's reagent ($FeCl_3$ 12 g L^{-1} in H_2SO_4 7.9 M), letting it stand at room temperature and complete darkness for 30 min (Glickmann and Dessaux 1995). The indoleacetic acid (IAA) of each sample was quantified with a spectrophotometer at a wavelength of 530 nm. The production of IAA of each rhizobacterial strain was determined by a standard curve of 0, 5, 10, 15, 20, 25, 30, 35, 40 µg ml^{-1} of pure IAA, considering B-King medium without inoculating as control. Three replicates per treatment were performed, and the experiment was conducted twice.

(III). Maintenance of Vermiculture Unit:

Earthworms need bedding in addition to food. The newspaper, coir (coconut husk fiber), and shredded cardboard were selected for bedding materials for earthworm composting. The bedding materials were processed for soaking in clean water and then squeezed it to remove excess liquid. The bedding should be damp, like a wrung-out sponge. Two inch layer of damp bedding was spread at the bottom of the top bin (the middle bin is empty). Addition of cocoons of earthworms, *Eudrilus eugenae* (L) procured from Department of Zoology, Savitribai Phule Pune University, to the bedding was carried. It need not need to spread them out. Addition of a small amount of food scraps (about 1–2 cups) to the bin was made. The scraps were covered with another layer of damp bedding. Care about keeping the side air vents free (not to block) was taken. the side air vents. All the food scraps are covered with bedding material and it was confirmed. The lid of top bin was replaced. Excess moisture was allowed to drain to the lower most bin. The excess liquid from the lower most bin was removed (this is because, it generally accumulates). The worms were found to get settle in their new home, worm bin. Earthorm eggs (cocoons) in the worm "starter" (inoculant) were observed hatching. The were fed with a small amount (1–2 cups of kitchen fruit and vegetable scraps) and the bins were kept for two weeks. This helped the worm population to begin to

grow without initially overwhelming the system. As the mature worms eat and grow, they start to lay eggs. Juvenile worms appear, and the population increase. In about 6 weeks, the immature worms become mature and lay eggs of their own. This method of Maintenance of Vermiculture Unit belong to Mansi Ramesh das, *et al*, 2018.

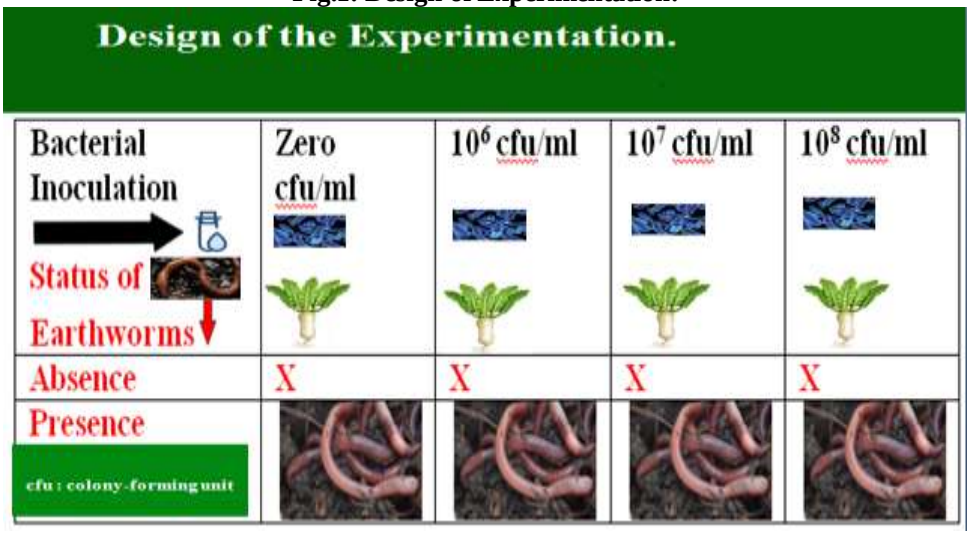
(IV). Seeds and Seedlings of Radish, *Raphanus sativus* (L):

White Long Radish Seeds [Cherriette (F1)] were obtained from Jairon Agrotech, Indore (India) through local dealer. Seeds were surface sterilized with 1.25% sodium hypochlorite solution for 10 min and then rinsed with deionized water three times. Rich, loamy soil amended with composted manure was collected from the Gosavi Agriculture farm (just near the "Dr. APIS" shrikrupa niwas, Malegaon Colony Baramati). For seedling production, a 200-cavity polystyrene germination tray was used, previously disinfected with 3% sodium hypochlorite solution for 5 min and washed-rinsed with sterile distilled water. The pre-sterilized "germination tray" was filled with a mixture based on vermicompost, soil, and sand (2:1:1 v/v). One seed per cavity was placed in the germination tray and maintained in greenhouse at 26 ± 5 ℃ and 60 ± 5% RH for five days. A typical spring *radish* germinates in about 5 days. The seedlings were processed further plantation according the study design.

(V). Study Design:

The four inoculation concentrations of rhizobacteria, *Pseudomonas trivialias* (L) (strain X33d) in the unit of CFU/ml were selected. The four inoculation concentrations of rhizobacteria, *Pseudomonas trivialias* (L) (strain X33d) in the unit of CFU include: Zero CFU/ml; 10^6 CFU/ml; 10^7 CFU/ml and 10^8 CFU/ml. Further, for each CFU/ml; two groups (with earthworms and without earthworms) were considered. Thus, total eight groups for experimentation were designed as shown in the table-1. For each group, the low tunnel raised bed was prepared. The standard composition of bedding along with soil was followed. In each group twenty five healthy earthworms of *Eudrilus eugenae* (L) species were transferred. The four groups were with earthworms and remaining four groups were without earthworms. The system was allowed for acclimatization for a week. Twenty five fresh five day old seedlings of radish, *Raphanus sativus* (L) were planted in each of the individuals group of tunnel bed. The plants were maintained for three weeks (21 days).

Fig.1: Design of Experimentation:



(VI). Parameters of Analysis:
The analysis was carried out on twenty second day after plantation of the seedlings on tunnel bed. The parameters of analysis include:
1. Above ground height (cm) of radish, *Raphnus sativus* (L) plant grown in the soil with of different inoculation dosages (cfu/ml) of *Pseudomonas trivialis* (L) in the absence (C) and in presence (E) of earthworm, *Eudrilus eugenae* (L).
2.Below ground height (cm) of radish, *Raphnus sativus* (L) plant grown in the soil with of different inoculation dosages (cfu/ml) of *Pseudomonas trivialis* (L) in the absence (C) and in presence (E) of earthworm, *Eudrilus eugenae* (L).
3.Above Ground Dry Biomass (grams) of radish, *Raphnus sativus* (L) plant grown in the soil with of different inoculation dosages (cfu/ml) of *Pseudomonas trivialis* (L) in the absence (C) and in presence (E) of earthworm, *Eudrilus eugenae* (L).
4.Below Ground Dry Biomass (grams) of radish, *Raphnus sativus* (L) plant grown in the soil with of different inoculation dosages (cfu/ml) of *Pseudomonas trivialis* (L) in the absence (C) and in presence (E) of earthworm, *Eudrilus eugenae* (L).
5.Total Biomass Production (grams) of radish, *Raphnus sativus* (L) plant grown in the soil with of different inoculation dosages (cfu/ml) of *Pseudomonas trivialis* (L) in the absence (C) and in presence (E) of earthworm, *Eudrilus eugenae* (L).
6. IAA Strength (microgram per milliliter) produced by different inoculation dosages (cfu/ml) of *Pseudomonas trivialis* (L).

(VII). Statistical Analysis:
The whole attempt was repeated for three times for consistency in the results. The collected data was subjected for statistical analysis. The results were expressed as mean ± S.E.M. (standard error). Comparisons were made using one-way ANOVA followed by Duncan's and Bonferroni's *post-hoc* tests. In all comparisons, values of $p < 0.05$ were considered statistically significant. Statistical tests were performed using the SPSS program (Statistical Package for Social Sciences, version 10.0, for Windows https://spss.en.softonic.com/).

RESULTS AND DISCUSSION
The results on the attempt on utilization of rhizobacteria, *Pseudomonas trivialis* (L) and earthworm, *Eudrilus eugenae* (L) for the qualitative and quantitative yield in the crop of radish, *Raphanus sativus*) (L) are presented in table – 2 to 8 ; figure- 2 to 8 and explained away according to the parameters considered in the studies.

(I). Above ground height (cm) of radish, *Raphnus sativus* (L) plant grown in the soil with of different inoculation dosages (cfu/ml) of *Pseudomonas trivialis* (L) in the absence (C) and in presence (E) of earthworm, *Eudrilus eugenae* (L) (Table- 2 and Fig.2):

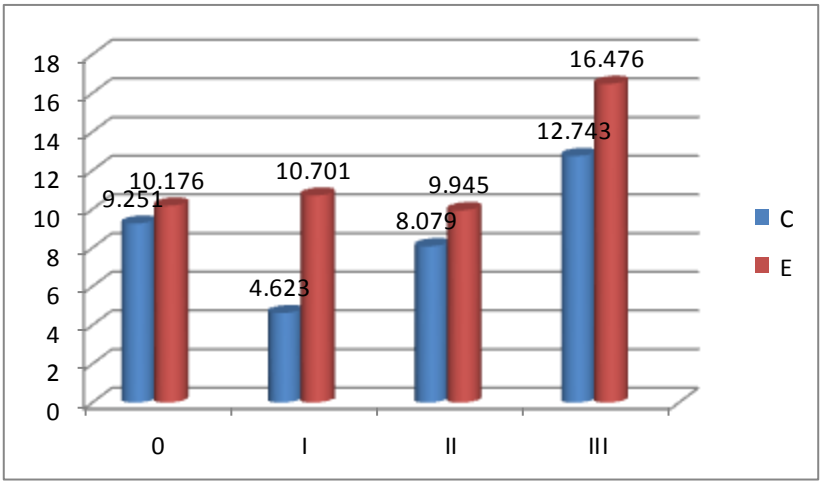
The above ground height (cm) of radish, *Raphnus sativus* (L) plant grown in the soil without the rhizobial bacteria and without earthworm in the present reported 9.251 (±0.729). The above ground height (cm) of radish, *Raphnus sativus* (L) plant grown in the soil without the rhizobial bacteria and with earthworm in the present reported 10.176 (±0.981).

Table – 2: Above ground height (cm) of radish, *Raphnus sativus* (L) plant grown in the soil with of different inoculation dosages (cfu/ml) of *Pseudomonas trivialis* (L) in the absence (C) and in presence (E) of earthworm, *Eudrilus eugenae* (L).

Serial No.	*P. trivilias* Inoculum (cfu/ml)	Group (C / E)	Above Ground Height (cm)
1.	0	C	9.251 (±0.729)
2.	0	E	10.176 (±0.981)
3.	10^6	C	4.623 (±0.663)
4.	10^6	E	10.701 (±0.736)
5.	10^7	C	8.079 (±0.432)
6.	10^7	E	9.945 (±0.786)
7.	10^8	C	12.743 (±0.943)
8.	10^8	E	16.476 (±1.084)

C: In Absence of Earthworm in soil.
E: In Presence of Earthworm in soil.

Fig. – 2: Above ground height (cm) of radish, *Raphnus sativus* (L) plant grown in the soil with of different inoculation dosages (cfu/ml) [I: 10^6; II: 10^7; III: 10^8] of *Pseudomonas trivialis* (L) in the absence (C) and in presence (E) of earthworm, *Eudrilus eugenae* (L).



C: In Absence of Earthworm in soil; E: In Presence of Earthworm in soil; [I: 10^6; II: 10^7; III: 10^8].

The above ground height (cm) of radish, *Raphnus sativus* (L) plant grown in the soil with 10^6 CFU rhizobial bacteria and without earthworm in the present reported 4.623 (±0.663). The above ground height (cm) of radish, *Raphnus sativus* (L) plant grown in the soil with 10^6 CFU rhizobial bacteria and with earthworm in the present reported 10.701 (±0.736).
The above ground height (cm) of radish, *Raphnus sativus* (L) plant grown in the soil with 10^7 CFU rhizobial bacteria and without earthworm in the present reported 8.079 (±0.432). The above ground height (cm) of radish, *Raphnus sativus* (L) plant grown in the soil with 10^7 CFU rhizobial bacteria and with earthworm in the present reported 9.945 (±0.786).
The above ground height (cm) of radish, *Raphnus sativus* (L) plant grown in the soil with 10^8 CFU rhizobial bacteria and without earthworm in the present reported 12.743 (±0.943). The above ground

height (cm) of radish, *Raphnus sativus* (L) plant grown in the soil with 10^8 CFU rhizobial bacteria and with earthworm in the present reported 16.476 (±1.084). Presence of rhizobial bacteria and earthworm in soil was found significantly improved the above ground height of radish, *Raphnus sativus* (L).

(II). Below ground height (cm) of radish, *Raphnus sativus* (L) plant grown in the soil with of different inoculation dosages (cfu/ml) of *Pseudomonas trivialis* (L) in the absence (C) and in presence (E) of earthworm, *Eudrilus eugenae* (L) (Table- 3 and Fig.3):

The below ground height (cm) of radish, *Raphnus sativus* (L) plant grown in the soil without the rhizobial bacteria and without earthworm in the present reported 11.786 (±0.928). The below ground height (cm) of radish, *Raphnus sativus* (L) plant grown in the soil without the rhizobial bacteria and with earthworm in the present reported 13.395 (±1.077).

The below ground height (cm) of radish, *Raphnus sativus* (L) plant grown in the soil with 10^6 CFU rhizobial bacteria and without earthworm in the present reported 4.356 (±0.991). The below ground height (cm) of radish, *Raphnus sativus* (L) plant grown in the soil with 10^6 CFU rhizobial bacteria and with earthworm in the present reported 10.337 (±1.727).

The below ground height (cm) of radish, *Raphnus sativus* (L) plant grown in the soil with 10^7 CFU rhizobial bacteria and without earthworm in the present reported 12.571 (±1.786). The below ground height (cm) of radish, *Raphnus sativus* (L) plant grown in the soil with 10^7 CFU rhizobial bacteria and with earthworm in the present reported 11.328 (±0.933).

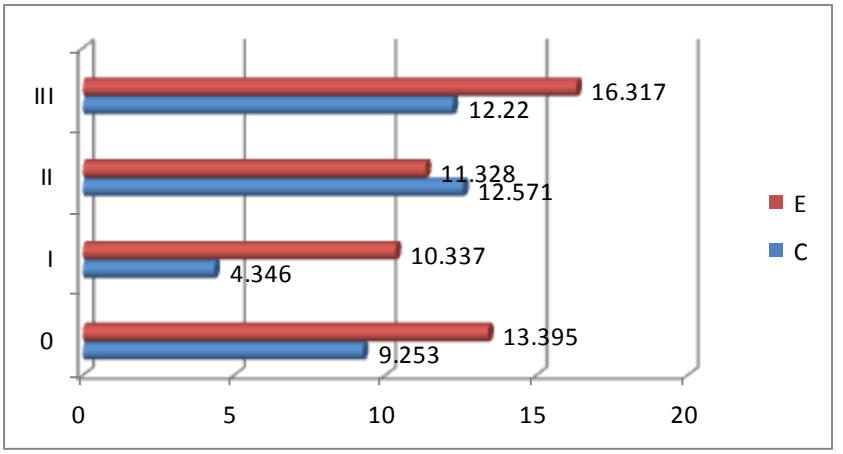
The below ground height (cm) of radish, *Raphnus sativus* (L) plant grown in the soil with 10^8 CFU rhizobial bacteria and without earthworm in the present reported 12.022 (±2.023). The below ground height (cm) of radish, *Raphnus sativus* (L) plant grown in the soil with 10^8 CFU rhizobial bacteria and with earthworm in the present reported 16.317 (±1.324). Presence of rhizobial bacteria and earthworm in soil was found significantly improved the below ground height of radish, *Raphnus sativus* (L).

Table – 3: Below ground length (cm) of radish, *Raphnus sativus* (L) plant grown in the soil with of different inoculation dosages (cfu/ml) of *Pseudomonas trivialis* (L) in the absence (C) and in presence (E) of earthworm, *Eudrilus eugenae* (L).

Serial No.	*P. trivilias* Inoculum (cfu/ml)	Group (C / E)	Below Ground Height (cm)
1.	0	C	11.786 (±0.928)
2.	0	E	13.395 (±1.077)
3.	10^6	C	4.356 (±0.991)
4.	10^6	E	10.337 (±1.727)
5.	10^7	C	12.571 (±1.786)
6.	10^7	E	11.328 (±0.933)
7.	10^8	C	12.022 (±2.023)
8.	10^8	E	16.317 (±1.324)

C: In Absence of Earthworm in soil.
E: In Presence of Earthworm in soil.

Fig. – 3: Below ground length (cm) of radish, *Raphnus sativus* (L) plant grown in the soil with of different inoculation dosages (cfu/ml) [I: 10^6; II: 10^7; III: 10^8] of *Pseudomonas trivialis* (L) in the absence (C) and in presence (E) of earthworm, *Eudrilus eugenae* (L).



C: In Absence of Earthworm in soil; E: In Presence of Earthworm in soil; [I: 10^6; II: 10^7; III: 10^8].

(III). Above Ground Dry Biomass (grams) of radish, *Raphnus sativus* (L) plant grown in the soil with of different inoculation dosages (cfu/ml) of *Pseudomonas trivialis* (L) in the absence (C) and in presence (E) of earthworm, *Eudrilus eugenae* (L) (Table- 4 and Fig.4):

The above ground dry Biomass (grams) of radish, *Raphnus sativus* (L) plant grown in the soil without the rhizobial bacteria and without earthworm in the present reported 00.031 (±0.002). The above ground dry Biomass (grams) of radish, *Raphnus sativus* (L) plant grown in the soil without the rhizobial bacteria and with earthworm in the present reported 00.065 (±0.002).

The above dry Biomass (grams) of radish, *Raphnus sativus* (L) plant grown in the soil with 10^6 CFU rhizobial bacteria and without earthworm in the present reported 00.022 (±0.001). The above dry Biomass (grams) of radish, *Raphnus sativus* (L) plant grown in the soil with 10^6 CFU rhizobial bacteria and with earthworm in the present reported 00.046 (±0.003).

The above dry Biomass (grams) of radish, *Raphnus sativus* (L) plant grown in the soil with 10^7 CFU rhizobial bacteria and without earthworm in the present reported 00.028 (±0.002). The above dry Biomass (grams) of radish, *Raphnus sativus* (L) plant grown in the soil with 10^7 CFU rhizobial bacteria and with earthworm in the present reported 00.055 (±0.006).

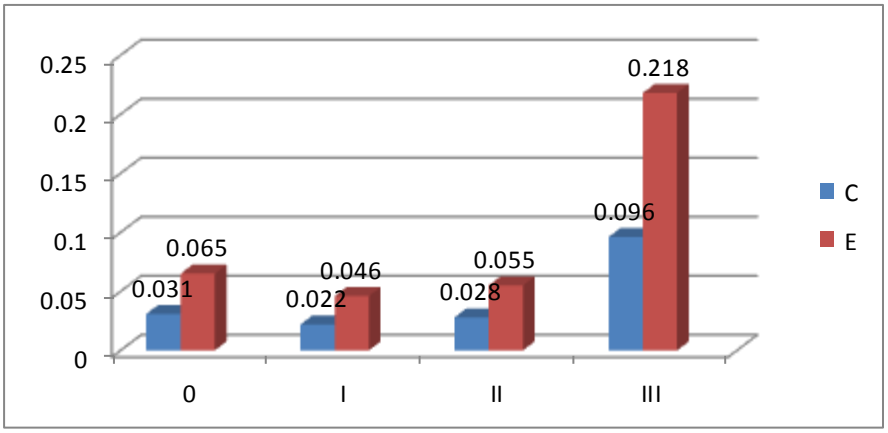
The above dry Biomass (grams) of radish, *Raphnus sativus* (L) plant grown in the soil with 10^8 CFU rhizobial bacteria and without earthworm in the present reported 00.096 (±0.007). The above ground dry Biomass (grams) of radish, *Raphnus sativus* (L) plant grown in the soil with 10^8 CFU rhizobial bacteria and with earthworm in the present reported 00.218 (±0.014). Presence of rhizobial bacteria and earthworm in soil was found significantly improved the above ground dry Biomass (grams) of radish, *Raphnus sativus* (L).

Table – 4: Above Ground Dry Biomass (grams) of radish, *Raphnus sativus* (L) plant grown in the soil with of different inoculation dosages (cfu/ml) of *Pseudomonas trivialis* (L) in the absence (C) and in presence (E) of earthworm, *Eudrilus eugenae* (L).

Serial No.	*P. trivilias* Inoculum (cfu/ml)	Group (C / E)	Above Ground Dry Biomass (grams)
1.	0	C	00.031 (±0.002)
2.	0	E	00.065 (±0.002)
3.	10^6	C	00.022 (±0.001)
4.	10^6	E	00.046 (±0.003)
5.	10^7	C	00.028 (±0.002)
6.	10^7	E	00.055 (±0.006)
7.	10^8	C	00.096 (±0.007)
8.	10^8	E	00.218 (±0.014)

C: In Absence of Earthworm in soil.
E: In Presence of Earthworm in soil.

Fig. – 4: Above Ground Dry Biomass (grams) of radish, *Raphnus sativus* (L) plant grown in the soil with of different inoculation dosages (cfu/ml) of *Pseudomonas trivialis* (L) in the absence (C) and in presence (E) of earthworm, *Eudrilus eugenae* (L).



C: In Absence of Earthworm in soil; E: In Presence of Earthworm in soil; [I: 10^6; II: 10^7; III: 10^8].

(IV). Below Ground Dry Biomass (grams) of radish, *Raphnus sativus* (L) plant grown in the soil with of different inoculation dosages (cfu/ml) of *Pseudomonas trivialis* (L) in the absence (C) and in presence (E) of earthworm, *Eudrilus eugenae* (L) (Table- 5 and Fig.5):

The below ground dry Biomass (grams) of radish, *Raphnus sativus* (L) plant grown in the soil without the rhizobial bacteria and without earthworm in the present reported 00.009 (±0.001). The below ground dry Biomass (grams) of radish, *Raphnus sativus* (L) plant grown in the soil without the rhizobial bacteria and with earthworm in the present reported 00.013 (±0.002).

The below dry Biomass (grams) of radish, *Raphnus sativus* (L) plant grown in the soil with 10^6 CFU rhizobial bacteria and without earthworm in the present reported 00.015 (±0.001). The below dry

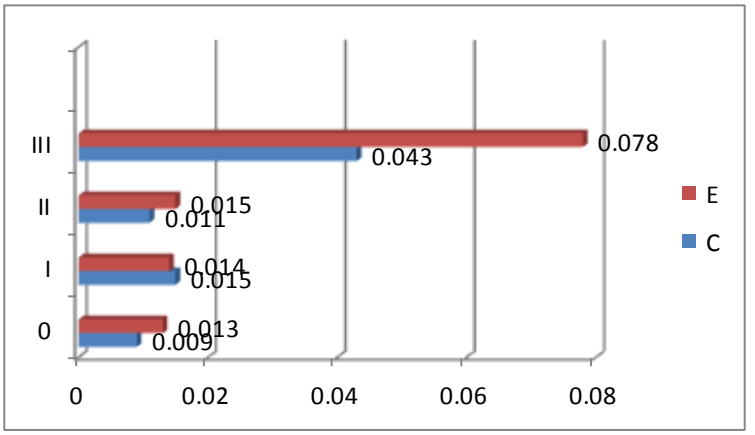
Biomass (grams) of radish, *Raphnus sativus* (L) plant grown in the soil with 10^6 CFU rhizobial bacteria and with earthworm in the present reported 00.014 (±0.003).
The below dry Biomass (grams) of radish, *Raphnus sativus* (L) plant grown in the soil with 10^7 CFU rhizobial bacteria and without earthworm in the present reported 00.011 (±0.001). The below dry Biomass (grams) of radish, *Raphnus sativus* (L) plant grown in the soil with 10^7 CFU rhizobial bacteria and with earthworm in the present reported 00.015 (±0.002).

Table – 5: Below Ground Dry Biomass (grams) of radish, *Raphnus sativus* (L) plant grown in the soil with of different inoculation dosages (cfu/ml) of *Pseudomonas trivialis* (L) in the absence (C) and in presence (E) of earthworm, *Eudrilus eugenae* (L).

Serial No.	*P. trivilias* Inoculum (cfu/ml)	Group (C / E)	Below Ground Dry Biomass (grams)
1.	0	C	00.009 (±0.001)
2.	0	E	00.013 (±0.002)
3.	10^6	C	00.015 (±0.001)
4.	10^6	E	00.014 (±0.003)
5.	10^7	C	00.011 (±0.001)
6.	10^7	E	00.015 (±0.002)
7.	10^8	C	00.043 (±0.008)
8.	10^8	E	00.078 (±0.003)

C: In Absence of Earthworm in soil.
E: In Presence of Earthworm in soil.

Fig. – 5: Below Ground Dry Biomass (grams) of radish, *Raphnus sativus* (L) plant grown in the soil with of different inoculation dosages (cfu/ml) of *Pseudomonas trivialis* (L) in the absence (C) and in presence (E) of earthworm, *Eudrilus eugenae* (L).



C: In Absence of Earthworm in soil; E: In Presence of Earthworm in soil; [I: 10^6; II: 10^7; III: 10^8].

The below dry Biomass (grams) of radish, *Raphnus sativus* (L) plant grown in the soil with 10^8 CFU rhizobial bacteria and without earthworm in the present reported 00.043 (±0.008). The below ground dry Biomass (grams) of radish, *Raphnus sativus* (L) plant grown in the soil with 10^8 CFU rhizobial bacteria and with earthworm in the present reported 00.078 (±0.003). Presence of rhizobial bacteria and

earthworm in soil was found significantly improved the below ground dry Biomass (grams) of radish, *Raphnus sativus* (L).

(V). Total Biomass Production (grams) of radish, *Raphnus sativus* (L) plant grown in the soil with of different inoculation dosages (cfu/ml) of *Pseudomonas trivialis* (L) in the absence (C) and in presence (E) of earthworm, *Eudrilus eugenae* (L) (Table- 6 and Fig.6):

The total biomass production (grams) of radish, *Raphnus sativus* (L) plant grown in the soil without the rhizobial bacteria and without earthworm in the present reported 00.041 (±0.001). The total biomass production (grams) of radish, *Raphnus sativus* (L) plant grown in the soil without the rhizobial bacteria and with earthworm in the present reported 00.078 (±0.003).

The total biomass production (grams) of radish, *Raphnus sativus* (L) plant grown in the soil with 10^6 CFU rhizobial bacteria and without earthworm in the present reported 00.037 (±0.001). The total biomass production (grams) of radish, *Raphnus sativus* (L) plant grown in the soil with 10^6 CFU rhizobial bacteria and with earthworm in the present reported 00.061 (±0.003). The total biomass production (grams) of radish, *Raphnus sativus* (L) plant grown in the soil with 10^7 CFU rhizobial bacteria and without earthworm in the present reported 00.039 (±0.003). The total biomass production (grams) of radish, *Raphnus sativus* (L) plant grown in the soil with 10^7 CFU rhizobial bacteria and with earthworm in the present reported 00.071 (±0.004).

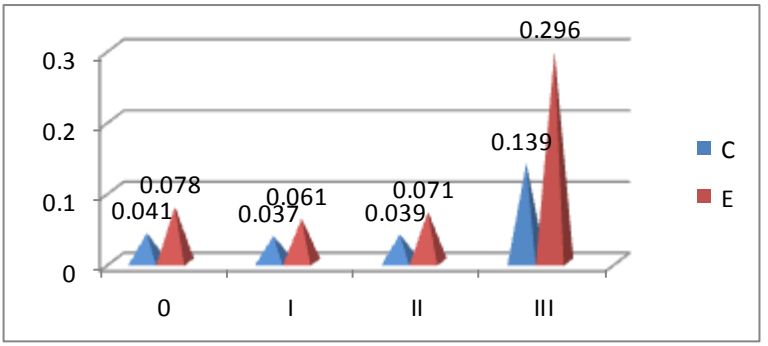
The total biomass production (grams) of radish, *Raphnus sativus* (L) plant grown in the soil with 10^8 CFU rhizobial bacteria and without earthworm in the present reported 00.139 (±0.011). The total biomass production below ground dry Biomass (grams) of radish, *Raphnus sativus* (L) plant grown in the soil with 10^8 CFU rhizobial bacteria and with earthworm in the present reported 00.296 (±0.003). Presence of rhizobial bacteria and earthworm in soil was found significantly improved the total biomass production (grams) of radish, *Raphnus sativus* (L).

Table – 6: Total Biomass Production (grams) of radish, *Raphnus sativus* (L) plant grown in the soil with of different inoculation dosages (cfu/ml) of *Pseudomonas trivialis* (L) in the absence (C) and in presence (E) of earthworm, *Eudrilus eugenae* (L).

Serial No.	*P. trivilias* Inoculum (cfu/ml)	Group (C / E)	Total Biomass Production (grams)
1.	0	C	00.041 (±0.001)
2.	0	E	00.078 (±0.003)
3.	10^6	C	00.037 (±0.001)
4.	10^6	E	00.061 (±0.003)
5.	10^7	C	00.039 (±0.003)
6.	10^7	E	00.071 (±0.004)
7.	10^8	C	00.139 (±0.011)
8.	10^8	E	00.296 (±0.003)

C: In Absence of Earthworm in soil.
E: In Presence of Earthworm in soil.

Fig. – 6: Total Biomass Production (grams) of radish, *Raphnus sativus* (L) plant grown in the soil with of different inoculation dosages (cfu/ml) of *Pseudomonas trivialis* (L) in the absence (C) and in presence (E) of earthworm, *Eudrilus eugenae* (L).



C: In Absence of Earthworm in soil; E: In Presence of Earthworm in soil; [I: 10^6; II: 10^7; III: 10^8].

(VI). IAA Strength (microgram per milliliter) of Soil With Biotic Components [Bacteria, *P. trivialis* (L); Earthworm and Radish Plant] (table-7 and fig.7):

The soil without bacteria, *P. trivialis* (L); Earthworm and Radish Plant [Group: 0] was found reported the production of 00.786 (±0.013) microgram per milliliter Indole Acetic Acid.

The soil with Earthworm, *Eudrilus eugenae* (L) [Group: I] was found reported the production of 1.775 (±0.013) microgram per milliliter Indole Acetic Acid.

The soil with radish, *Raphnus sativus* (L) [Group: II] was found reported the production of 1.382 (±0.013) microgram per milliliter Indole Acetic Acid.

The with bacteria, *Pseudomonas trivialis* (L) [Group: III] was found reported the production of 10.845 (±0.286) microgram per milliliter Indole Acetic Acid.

The soil with Earthworm, *Eudrilus eugenae* (L) and radish, *Raphnus sativus* (L) [Group: IV] was found reported the production of 3.352 (±0.299) microgram per milliliter Indole Acetic Acid.

The soil with bacteria, *Pseudomonas trivialis* (L) and Earthworm, *Eudrilus eugenae* (L) [Group: V] was found reported the production of 1.776 (±0.124) microgram per milliliter Indole Acetic Acid.

The soil with bacteria, *Pseudomonas trivialis* (L) and radish, *Raphnus sativus* (L) [Group: VI] was found reported the production of 9.861 (±0.754) microgram per milliliter Indole Acetic Acid.

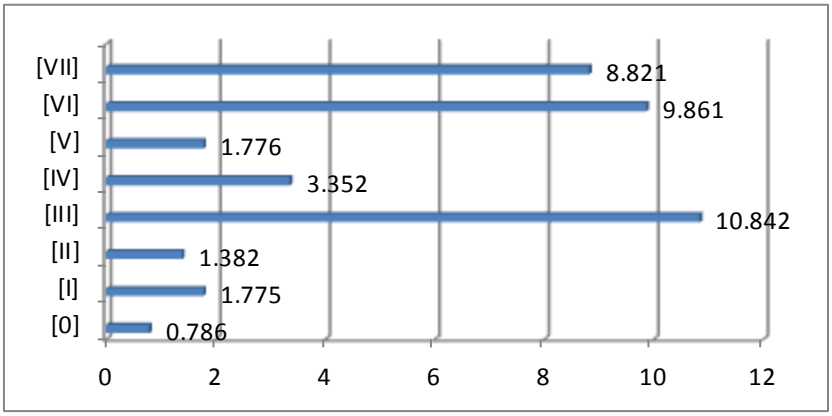
The soil with bacteria, *Pseudomonas trivialis* (L) Earthworm, *Eudrilus eugenae* (L) and radish, *Raphnus sativus* (L) [Group: VII] was found reported the production of 8.821(±0.987) microgram per milliliter Indole Acetic Acid.

The Indole Acetic Acid production was found most significant in the soil with all the three biotic components (Rhizobacteria; Earthworm and Radish plant).

Table – 7: IAA Strength (microgram per milliliter) of Soil Without Bacteria, *P. trivialis* (L); Earthworm and Radish [0]; With Earthworm, *Eudrilus eugenae* (L) [I]; With radish, *Raphnus sativus* (L) [II]; With Bacteria, *Pseudomonas trivialis* (L) [III]; With Plant and Earthworm (radish, *Raphnus sativus* L and Earthworm, *Eudrilus eugenae* L)[IV]; With Bacteria and Earthworm (Bacteria, *Pseudomonas trivialis* L and Earthworm, *Eudrilus eugenae* L) [V]; With Plant and Bacteria (radish, *Raphnus sativus* L and Bacteria, *Pseudomonas trivialis* L) [VI] ; With Plant, Bacteria and Earthworm ((radish, *Raphnus sativus* L; Bacteria, *Pseudomonas trivialis* L and Earthworm, , *Eudrilus eugenae* L) [VII].

Serial No.	Group Specification	IAA (microgram per milliliter)
1.	0. [Soil Without Bacteria, *P. trivialis* (L); Earthworm and Radish].	00.786 (±0.013)
2.	I. [Soil With Earthworm, *Eudrilus eugenae* (L)].	1.775 (±0.013)
3.	II. [Soil With radish, *Raphnus sativus* (L)].	1.382 (±0.013)
4.	III. [Soil With Bacteria, *Pseudomonas trivialis* (L)].	10.845 (±0.286)
5.	IV. [Soil With Plant and Earthworm (radish, *Raphnus sativus* (L) and Earthworm, *Eudrilus eugenae* (L)].	3.352 (±.789)
6.	V. [Soil With Bacteria and Earthworm (Bacteria, *Pseudomonas trivialis* (L) and Earthworm, *Eudrilus eugenae* (L)].	1.776 (±0.124)
7.	VI. [Soil With Plant and Bacteria (radish, *Raphnus sativus* (L) and Bacteria, *Pseudomonas trivialis* (L)].	9.861 (±0.754)
8.	VII. [Soil With Plant, Bacteria and Earthworm ((radish, *Raphnus sativus* (L); Bacteria, *Pseudomonas trivialis* (L) and Earthworm, *Eudrilus eugenae* (L)].	8.821 (±0.987)

Fig.– 7: IAA Strength (microgram per milliliter) of Soil Without Soil Without Bacteria, *P. trivialis* (L); Earthworm and Radish Plant[0]; With Earthworm, *Eudrilus eugenae* (L) [I]; With radish, *Raphnus sativus* (L) [II]; With Bacteria, *Pseudomonas trivialis* (L) [III]; With Plant and Earthworm (radish, *Raphnus sativus* L and Earthworm, *Eudrilus eugenae* L)[IV]; With Bacteria and Earthworm (Bacteria, *Pseudomonas trivialis* L and Earthworm, *Eudrilus eugenae* L) [V]; With Plant and Bacteria (radish, *Raphnus sativus* L and Bacteria, *Pseudomonas trivialis* L) [VI] ; With Plant, Bacteria and Earthworm ((radish, *Raphnus sativus* L; Bacteria, *Pseudomonas trivialis* L and Earthworm, , *Eudrilus eugenae* L) [VII].



(VII). IAA Production (microgram per milliliter) by Rhizobacteria, *Pseudomonas trivialis* (L) in the absence (C) and in presence (E) of earthworm, *Eudrilus eugenae* (L) at different inoculation dosages (cfu/ml) (table-8 and fig.8):

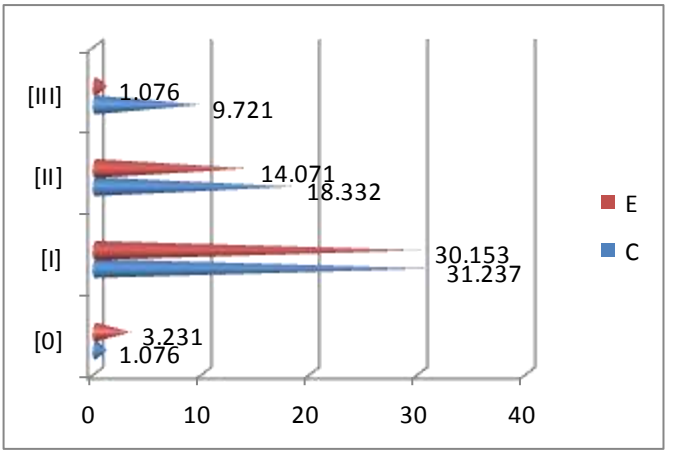
With Biotic Components [Bacteria, *P. trivialis* (L); Earthworm and Radish Plant] (table-7 and fig.7):
The Indole Acetic Acid production (microgram per milliliter) by the soil with zero CFU inoculum of rhizobacteria, *Pseudomonas trivialias* (L), in absence of earthworm was found recorded 01.076 (±0.031) units. The Indole Acetic Acid production (microgram per milliliter) by the soil with zero CFU inoculum of rhizobacteria, *Pseudomonas trivialias* (L), in presence of earthworm was found recorded 03.231 (±0.336) units.

Table – 8: IAA Production of *Pseudomonas trivialis* (L) in the absence (C) and in presence (E) of earthworm, *Eudrilus eugenae* (L) by the bacteria, Pseudomonas trivialis (L) at different inoculation dosages (cfu/ml).

Serial No.	*P. trivilias* Inoculum (cfu/ml)	Group (C / E)	Above Ground Height (cm)
1.	0	C	01.076 (±0.031)
2.	0	E	03.231 (±0.336)
3.	10^6	C	31.237 (±4.773)
4.	10^6	E	30.153 (±7.786)
5.	10^7	C	18.332 (±4.866)
6.	10^7	E	14.071 (±3.735)
7.	10^8	C	09.721 (±1.211)
8.	10^8	E	01.076 (±0.044)

C: In Absence of Earthworm in soil.
E: In Presence of Earthworm in soil.

Fig. – 8: IAA Production of *Pseudomonas trivialis* (L) in the absence (C) and in presence (E) of earthworm, *Eudrilus eugenae* (L) by the bacteria, Pseudomonas trivialis (L) at different inoculation dosages (cfu/ml).



The Indole Acetic Acid production (microgram per milliliter) by the soil with 10^6 CFU inoculum of rhizobacteria, *Pseudomonas trivialias* (L), in absence of earthworm was found recorded 31.237 (±4.773) units. The Indole Acetic Acid production (microgram per milliliter) by the soil with 10^6 CFU inoculum of rhizobacteria, *Pseudomonas trivialias* (L), in presence of earthworm was found recorded 30.153 (±7.786) units.

The Indole Acetic Acid production (microgram per milliliter) by the soil with 10^7 CFU inoculum of rhizobacteria, *Pseudomonas trivialias* (L), in absence of earthworm was found recorded 18.332 (±4.866) units. The Indole Acetic Acid production (microgram per milliliter) by the soil with 10^7 CFU inoculum of rhizobacteria, *Pseudomonas trivialias* (L), in presence of earthworm was found recorded 14.071 (±3.735) units.

The Indole Acetic Acid production (microgram per milliliter) by the soil with 10^8 CFU inoculum of rhizobacteria, *Pseudomonas trivialias* (L), in absence of earthworm was found recorded 09.721 (±1.211) units. The Indole Acetic Acid production (microgram per milliliter) by the soil with 10^8 CFU inoculum of rhizobacteria, *Pseudomonas trivialias* (L), in presence of earthworm was found recorded 01.076 (±0.044) units.

The hypothesis to explain the negative effect on plantgrowth concerns the amount of IAA perceived by the plant, which is a combination of the amount of IAA produced by the bacteria and the life time and diffusion of IAA in the soil. According to Loper and Schroth (1986), up to 80% of rhizobacteria can synthesize indole-3-acetic acid (IAA). But the dose response curve of plants to exoge-nous auxin application is bell shaped (Taiz and Zeiger, 2010). Root growth promotion by free living PGPR, e.g., Alcaligenes faecalis, Enterobacter cloacae, Acetobacter diazotrophicus, species of Azospir-illum, Pseudomonas, and Xanthomonas, as well as by symbionts, such as Bradyrhizobium japonicum and Rhizobium spp., has been related to low levels of IAA production (Patten and Click, 1996). In contrast, the inhibitory effect of some deleterious rhizobacteria (DRB) has been related to high amounts of IAA

production, e.g., *Enterobacter taylorae* (Sarwar and Kremer, 1995) and *Pseudomonas putida* (Xie *et al*., 1996).

Possible mechanism of exerting influence of earthworms in soil on the plant growth promotion may be explained away through the steps like: mineralization; modifications of soil porosity; biocontrol of pests and parasites in soil; production of plant growth regulators; provision of symbiosis (Brown *et al*.,2004; Scheu, 2003). Presence of earthworm in soil affects on mineralization of soil organic matter. This mineralization in it's turn helps for significant improvement of availability of nutrients from soil to the plants.

Endogeic earthworms significantly modify soil aggregation and porosity, which in turn control water flow in soil. The earthworm produce the castings approximately five times it's own weight. The castings of earthworm are more easily disaggregated by water than the bulk soil. Although casts initially led to larger soil porosity (on average 50%), their structure remain unstable. As a consequence, water inputs led to a faster decrease in soil porosity in the presence of casts.

Microbial activity in worm castings is 10 to 20 times higher than in the soil and organic matter that the worm ingests. Large pores in between casts were rapidly replaced by small elongated and rounded pores (N. Bottinelli, *et al*, 2010). Earthworm cast lifespan and associated porosity are of primary importance in the regulation of soil porosity turnover and the ecological functions that are under its control. Soil structural porosity thus depends on the balance between the production and degradation of casts. Improvements to the soil structure will occur when the former predominates. Thus presence of earthworm in soil leads into modification of soil porosity and aggregation, which induces changes in water and oxygen availability to plants.

Vermiwash a collection of excretory products and mucus secretion of earthworms along with micronutrients from the soil organic molecules. The vermiwash is supposed to work for killing the soil pests and parasites of the plant crops.

The production of plant growth hormones, such as indole acetic acid (IAA), gibberellins and cytokinins, as well as other PGRs such as humates are some of the byproducts of the increased microbial activities promoted by earthworms which probably directly influence plant growth and yields. Several workers have reported the presence of humates and plant growth hormones such as auxins, gibberrillic acids and cytokinins in vermicomposts. Almost all Lumbricid earthworms (Oligochaeta: Lumbricidae) harbor extracellular species-specific bacterial symbionts of the genus *Verminephrobacter* (*Betaproteobacteria*) in their nephridia (Lund, *et al*, 2010). The symbionts have a beneficial effect on host reproduction and likely live on their host's waste products. They are vertically transmitted and presumably associated with earthworms already at the origin of Lumbricidae 62–136 million years ago. The *Verminephrobacter* genomes carry signs of bottleneck-induced genetic drift, such as accelerated evolutionary rates, low codon usage bias, and extensive genome shuffling, which are characteristic of vertically transmitted intracellular symbionts. The production of plant growth hormones, such as indole acetic acid (IAA), gibberellins and cytokinins, as well as other PGRs such as humates are some of the byproducts of the increased microbial activities promoted by earthworms which probably directly influence plant growth and yields. Several workers have reported the presence of humates and plant growth hormones such as auxins, gibberrillic acids and cytokinins in vermicomposts.

CONCLUSION

The symbionts have a beneficial effect on host reproduction and likely live on their host's waste products. The results of the attempt on utilization of rhizobacteria, *Pseudomonas trivialis* (L) and earthworm, *Eudrilus eugenae* (L) for the qualitative and quantitative yield in the crop of radish, *Raphanus sativus*) (L) enabled to demonstrate a correlation between responses of different inoculation treatment of rhizobacteria and the absence or presence of earthworms on growth and development of the plants. Increasing of plant

growth parameters (especially shoots) at different concentrations of rhizobacteria, *Pseudomonas trivialis* (L) especially in presence of earthworms suggests the synergistic interaction of rhizobacteria and earthworm for plant growth promoting effects. It may also be possible for promotion of growth in earthworms in presence of rhizobacterial population. The process of mineralization; modifications of soil porosity; biocontrol of pests and parasites in soil; production of plant growth regulators and provision of symbionts are the possible reasons for the earthworms to exert positive influence on plant growth. The attempt may open a new avenue in the field of green revolution.

ACKNOWLEDGEMENTS
12 December is the Birthday of Hon. Sharad Govindrao Pawar (Birth: 12 December, 1940) renowned international social personality. His kind self previously served as the Chief Minister of Maharashtra on three separate occasions and held the posts of Minister of Defence and Minister of Agriculture in the Government of India. Through the best compliments from Science Research Center, the present research work is wishing Hon. Sharad Govindrao Pawar "Happy Birthday".

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