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Research Article

Influence of vermicompost on the activity of the plant growth regulators in the leaves of the Indian butter bean plant, *Dolichos lab lab* L.

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Abstract

Different percentages of vermicompost (25, 50, 75 and 100%) derived from the earthworm, *Eudrilus eugeniae* was made with red soil. The plants were grown in these vermicompost-red soil mixtures for maximum 90 days period of exposure and the activity of the plant hormones such as Indole acetic acid (IAA), Indole butyric acid (IBA), Naphthalene acetic acid (NAA) and Gibberellic acid (GA) were noticed at 30, 60 and 90 days period of exposures. The maximum Indole acetic acid, Indole butyric acid and Naphthalene acetic acid activity were noticed in the leaves of the plant grown in 75% vermicompost concentration whereas the maximum activity of gibberellic acid was observed in 25% vermicompost concentration at 30 days period of exposures but at the same time at 60 days period of exposure the similar trend of result was also noticed in the Indole acetic acid and Indole butyric acid activity whereas Naphthalene acetic acid and Gibberellic acid activity were maximum in the leaves of the plant cultivated in 50% vermicompost concentration. However, at 90 days period of exposure all the hormones activity was maximum in 50% vermicompost concentration. In the present study it has been proved that the irregular trend of results was noticed in the activity of the plant growth regulators in the leaves of the Indian butter bean plant exposed to different percentages of vermicompost. The difference was found to be statistically significant at 1% level.

Keywords: *Eudrilus eugeniae*, vermicompost, Indian butter bean plant, Indole acetic acid, Indole butyric acid, Naphthalene acetic acid and Gibberellic acid.

Introduction

The continuous use of cultivable land has led to depletion in the fertility status of soil. In order to fertilize the land as well as to increase the agricultural production to face the food scarcity in accordance with the increasing population modern agricultural operation known as green revolution was introduced. In this operation the use of chemical fertilizers and pesticides was recommended. The indiscriminate use of the chemicals has modified the physical, chemical and biological activity of the soil. It also caused an important impact on the health of crops, animals and human beings that derive their food from it. In order to overcome all these unwanted factors the application of organic manure especially vermicompost is recommended. Vermicomposts are organic materials, broken down by interactions between earthworms and microorganism, in a mesophilic process (up to 25ºC), to produce fully stabilized organic soil amendments with low C: N ratios. They have fine particulate structure, good moisture-holding capacity, and contain nutrients such as N, P, K, Ca and Mg in forms readily taken up by plants (Lavelle and Martin, 1992; Prabha *et al.,* 2005; Arancon and Edwards, 2009). Vermicompost also consists of plant enzymes such as Peroxidase, Polyphenol oxidase and Catalase (Prabha, 2006), plant growth hormones (Indole acetic acid, Indole butyric acid, Naphthalene acetic acid and Gibberellic acid)(Ramasamy, 2009) and many beneficial microbes such as Nitrogen fixation bacteria and plant growth hormones synthesizing microbes(*Azospirillum brasilence*) (Kolb and Martin, 1985; Kucey, 1988; Molla *et al*., 2001) *Azospirillum lipoferum* (Lee *et al*., 1988), *Azotobacter paspali* (Barea and Brown, 1974) and *Pseudomonas putida* (Glick *et al*., 1986; Caron *et al*., 1995; Xie *et al*., 1996).Vermicompost is a good substitute for commercial fertilizers and has more N, P and K than the normal heap manure (Srivastava and Beohar, 2004). Besides that, earthworms release vitamins such as vitamins A, B_1, B_2, B_3, C and E in the vermicompost (Prabha, 2007; Ramasamy, 2009), B group, some provitamin D, vitamin B_{12} and free amino acids in the soil.

Though numerous studies on the influence of vermicompost on the growth and yield status of different plant varieties have been carried out by many authors, the literature survey revealed that the scientific information on the influence of vermicompost on the biochemical constituents of the plants especially plant growth regulators' activity is scanty. So, the present investigation has been studied to examine the activity of the plant growth regulators in the leaves of the Indian butter bean plant.

Materials and Methods

Collection and culturing of earthworms

The earthworm, *Eudrilus eugeniae* was collected from worm farm, Kondegoundampalayam village, Pollachi Taluk, Coimbatore District, Tamil Nadu, India. The collected earthworms were acclimatized under the laboratory condition for a period of three months by providing predecomposed cow dung as feeding material in the cement tank. The water was sprinkled on alternate days to maintain the optimum (60-70%) moisture content and temperature ranges between 25ºC to 30ºC by using hygrometer and thermometer respectively. Care was taken to avoid the entry of natural enemies. At the end of 75 days vermicompost was collected and stored in the shadow place. Different percentages of vermicompost (25%, 50%, 75% and 100%) were prepared by mixing red soil (w/w) (collected from Kanuvai village situated 10 km north of Coimbatore where intensive cultivation is going on) in flats (1.5m length x 1m breadth x 60cm height) for the Indian butter bean plant (*Dolichos lab lab* L.). 10 plants were grown in each flat with 20cm inter-plant distance in two rows (40cm row to row distance) in the vermicompost-red soil mixtures for 30, 60 and 90 days period of exposures. The control plant was also grown in red soil alone separately for a period of 90 days. The plants were watered daily. At the end of 30, 60 and 90 days periods of exposures the samples of leaves were collected from the plants grown in the above mentioned mixtures for the estimation of the activity of the plant growth regulators.

Estimation of Plant hormones

Isolation of Indole acetic acid, Indole butyric acid, Naphthalene acetic acid and Gibberellic acid in the vermicompost and leaves were carried out by the method of Miezah and Pudu (2008).

Procedure

Eighty percent (80%) methanol extraction

Fifteen grams of leaf was weighed into an extraction bottle and 150 ml of chilled methanol was added. This process was repeated six times to obtain a final/total volume of 900 ml of 80% methanol. 150 ml of 80% methanolic slurry of leaf alone was placed in a mechanical stirrer for approximately 24 hour. The slurry was allowed to stand and partitioned/ separated out into the liquid and solid phase prior to decanting the supernatant. The filtrate was then centrifuged for 6 minutes at 4000rpm, filtered and stored at 5º C in a freezer. The same volume of 80% was added to the residue in the extraction bottle and shaken for another 24 hour after which the solution was again decanted, centrifuged for 6 minutes at 4000 rpm and filtered.

The extraction process was repeated a third time following which all the extracts were bulked and reduced to aqueous phase(48ml) using a rotary evaporator prior to storage in a refrigerator.

Co-Chromatography of extracts and their standards

Dried extracts from the solvent partitioning were dissolved in 1 ml of methanol standard for plant growth hormone. Benzyl amino purine (BAP), Indole acetic acid (IAA) and Gibberellic acids (GA3) for each extract were also prepared. Each extract and its prepared standard were spot loaded onto thin layer chromatographic plates of dimensions 20 x 20 cm and silica gel (60 254) of thickness 0.25mm and developed in isopropanol: ammonium hydroxide: water (84:4:4v/v/v) to about 18 cm in a vertical direction. The distance moved by the solvent system (solvent front) and the spots were measured, after which the relative fluidity, the Rf values were calculated by dividing the distance moved by the solvent system by the distance moved by the spots. The Rf value of the extract plant growth hormones were compared to the Rf value of the standard hormones. The results were expressed in mg/g. The result of the influence of different percentages of vermicompost on the activity of the plant growth hormones was analyzed by employing Duncan's multiple range test (DMRT).

$$\text{Rf (Retention factor)} = \frac{\text{Distance traveled by sample}}{\text{Distance traveled by mobile phase}}$$

$$\text{Calculation} = \frac{\text{Sample value}}{\text{Standard value}} \text{X} \frac{\text{Standard concentration}}{\text{Sample concentration}} \text{X } 100$$

Results

The study on the influence of different concentration of vermicompost on the activity of the plant growth regulators (Indole acetic acid, Indole butyric acid, Naphthalene acetic acid and Gibberellic acid) in the leaves of the Indian butter bean plant for different periods of exposures (30, 60 and 90 days) revealed that the maximum activity of Indole acetic acid 0.70 ± 0.03%, Indole butyric acid 0.77 ± 0.02% and Naphthalene acetic acid 0.64 ± 0.02% was noticed in the leaves of the plant grown in 75% vermicompost concentration at 30 days period of exposure whereas Gibberellic acid (0.08 ± 0.01%) showed maximum activity in 25% vermicompost concentration. The similar trend of results was also noticed in the activity of Indole acetic acid (0.75 ± 0.01%) and Indole butyric acid (0.66 ± 0.01%) at 60 days period of exposure but at the same time Naphthalene acetic acid (0.72 ± 0.03%) and Gibberellic acid (0.12 ± 0.03%) exhibited the maximum activity in 50% vermicompost concentration. However, at 90 days period of exposure all the hormones showed maximum activity in the leaves of the plant grown in 50% vermicompost concentration. But in the same breathe the minimum activity of all the hormones were noticed in the control plants tested at all periods of exposure (Table 1).

Table1. Influence of vermicompost on the activity of the Plant growth regulators in the leaves of the Indian butter bean plant, *Dolichos lab lab* L.

Period (in days)	% of Vermicompost	Name of the plant growth regulators			
		IAA (%)	IBA (%)	NAA (%)	GA (%)
30	Control	0.24 ± 0.02	0.17 ± 0.02	0.26 ± 0.02	0.04 ± 0.01
	25	0.44 ± 0.02	0.23 ± 0.01	0.50 ± 0.03	0.08 ± 0.01
	50	0.57 ± 0.02	0.56 ± 0.02	0.62 ± 0.03	0.06 ± 0.02
	75	0.70 ± .03	0.77 ± 0.02	0.64 ± 0.02	0.07 ± 0.02
	100	0.39 ± 0.03	0.20 ± 0.02	0.42 ± 0.01	0.03 ± 0.03
60	Control	0.30 ± 0.03	0.22 ± 0.02	0.27 ± 0.01	0.03 ± 0.02
	25	0.50 ± 0.02	0.38 ± 0.02	0.46 ± 0.03	0.07 ± 0.02
	50	0.71 ± 0.02	0.61 ± 0.03	0.72 ± 0.03	0.12 ± 0.03
	75	0.75 ± 0.01	0.66 ± 0.01	0.58 ± 0.02	0.06 ± 0.02
	100	0.43 ± 0.02	0.13 ± 0.01	0.44 ± 0.02	0.09 ± 0.02
90	Control	0.22 ± 0.02	0.10 ± 0.01	0.19 ± 0.02	0.01 ± 0.03
	25	0.39 ± 0.03	0.20 ± 0.02	0.44 ± 0.02	0.05 ± 0.03
	50	0.61 ± 0.02	0.55 ± 0.03	0.51 ± 0.02	0.08 ± 0.02
	75	0.59 ± 0.03	0.50 ± 0.03	0.49 ± 0.02	0.05 ± 0.01
	100	0.36 ± 0.02	0.11 ± 0.03	0.28 ± 0.01	0.07 ± 0.01
CD ($p<0.01$)		0.0482	0.0046	0.037	0.0356

Values are expressed by mean ± SD of six samples; IAA- Indole acetic acid; IBA- Indole butyric acid; NAA-Naphthalene acetic acid; GA- Gibberellic acid

Discussion

The present investigation proved that the different percentages of vermicompost showed the irregular trend of results in the activity of all the plant growth regulators of leaves of the Indian butter bean plant. It has also been revealed that a particular concentration of vermicompost highly influenced the activity of a particular plant growth regulator at particular period of exposure. The present result also expressed that when the plant was exposed to one particular concentration of vermicompost the level of the plant growth regulators was found to be maximum whereas in the remaining vermicompost concentration tested the level of plant growth regulators was significantly lesser. This may due to the availability of nutrients in the above mentioned vermicompost concentration. This level of nutrients might have highly influenced the favourable physiological condition for the maximum synthesize of the plant growth regulators whereas in the remaining concentration tested the nutrient level was lesser or higher that might not have supported to the better activity of the plant growth hormones favourably. The present research also showed that when the concentration of vermicompost increased by degrees the level of hormones decreased in the leaves of the plant. Particularly in 100% vermicompost concentration the marked decrease was noticed when compared to other concentrations tested. This also proved that the plant might have needed optimum nutrients for the better synthesize of the hormones. It has also been noticed that the level of the four growth hormones increased upto 60 days period of exposure. Afterwards the activity decreased gradually at 90 days period of exposures.

The plant exposed to different exposure periods namely 30, 60 and 90 days revealed that the plant synthesized and accumulated higher levels of hormone mainly at 60 days period of exposure and secondly at 30 days period of exposure. The maximum synthesize of all the four growth hormones during 60 days period of exposure may be due to the milk stage of the plant. But at the same time the gradual decrease in the hormone synthesize during 90 days period of exposure may be due to the age of the plant. Hormones are chemical substances produced by one part of a plant and transported to another part of the plant where they cause a reaction to occur. Hormones are stored within the plant until they are needed. They are transported via phloem and other tissues. Hormones are released in small concentrations. They are released to all cells but only specific cells will react because they have special receptors for each hormone and they perform specific functions (Hartmann and Kester, 1968; Ross *et al*., 1992; Mauseth, 1998; Raven *et al*., 1999). They regulate most of life cycle events in plant such as cell division, seed and bud dormancy, seed germination, flowering, fruit shed and ripening and cutting rooting etc. The plant growth hormones such as auxins and gibberellin promote shoot growth, inhibit root growth, stimulate enlargement of cell by increasing plasticity of cell walls, stem growth, initiation of roots, and differentiation of cell types, delay the development of fruit ripening and inhibit lateral branches. In high concentration it kill all most any living plant tissues. Gibberellin increases stem growth, flowering, brokening of dormancy of buds and seeds, increase the size of the fruits, delaying the aging process and increases delaying age process and increases seed production (Salisbury and Ross, 1992; Davies, 1995; Ross *et al*., 2002; Taiz and Zeiger, 2002).

Weig-Xing *et al*. (2000) have suggested that the level of endogenous hormones in leaves varied with the stages of floret development and the types of the plant hormone and genotypes. They have also reported that the changes in the concentrations of endogenous phytohormones in strawberry plants could be responsible for the morphological changes of roots due to fertilization. The endogenous level of plant growth regulators also affects all the uptake and utilization of minerals. The active principles or precursors are synthesized in the leaves, translocated, biosynthesised, and stored in root tubers. Growth of leaves and development of root tuber depend on several factors such as nutrition. They also reported that the IAA level in leaves of the wheat plant (*Triticum aestivum* L.) exhibited slight changes during floret development, yet there were some genotypic difference. The IAA level in the leaves decreased from anther lobe formation to meiosis, but doubled at heading. The fluctuation of GA_{1+3} levels in spikes of the wheat plant exhibited a decrease trend and significant genotypic difference during floret development. They also revealed that the lower levels of GA_{1+3} and IAA in leaves might be favorable for the development of fertile florets.

Large proportion of leaf photosynthates are required for the growth and development of tuberous root. The rate and amount of photosynthate produced by the leaves and the proportion of photosynthate that is translocated greatly influence size, yield, development and growth of tuber as well as secondary metabolite accumulation. This transport and partitioning of leaf assimilate to the sink tuber is one of the important factors controlling productivity. However from the present study, we concluded that treatment with N P K in the vermicompost could improve the biochemical status of the plant. Biological activities of the plants are markedly enhanced by microbial interactions in the rhizosphere of plants (Tilak and Reddy, 2006). Such syntrophic associations are of ecological importance with implied agricultural significance. The plant growth promoting rhizobacteria (PGPRs) can influence plant growth directly through the production of phytohormones and indirectly through nitrogen fixation and production of bio-control agents against soil-borne phytopathogens (Glick, 2003).

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