© August 2021| IJIRT | Volume 8 Issue 3 | ISSN: 2349-6002

Comparative study of Growth Parameters and Antioxidant Potential of Vermicompost, Farmyard Manure and Bioinoculants Treated Adathoda vasica

Shubha[1], Prathibha K.Y.[2], Manjula A. C.[3], Manjula K. R.[4], Keshamma. E[5]*

[1]*Department of Botany, Government First Grade College, Vijayanagara, Bengaluru, Karnataka, India*

[2]*Department of Botany, Maharani's Science College for Women, Bengaluru, Karnataka, India*

[3]*Department of Sericulture, Maharani's Science College for Women, Bengaluru, Karnataka, India*

[4]*Department of Biotechnology, REVA University, Bengaluru, Karnataka, India*

[5]*Department of Biochemistry, Maharani's Science College for Women, Bengaluru, Karnataka, India*

***Abstract* - The agricultural lands are depleted of soil fertility due to continuous cultivation. In order to increase the soil fertility, man uses inorganic fertilizers. Though they promote the growth of crops, susceptibility is the negative impact by over utilization of fertilizers. Application of chemicals is widely practiced to control plant disease in modern agriculture and it also provides mutants of pathogens. To overcome all these factors application of organic manure is suggested. The present study was aimed to compare the effect of vermicompost, farmyard manure and bioinoculants on growth parameters namely shoot length, number of branches, number of leaves, fresh weight and dry weight and also the antioxidant potentials of Adathoda vasica. The manural potential of three manures: vermicompost, farmyard Manure (FYM) and bioinoculants was evaluated at different intervals of time. Our research showed the growth parameters, antioxidants and enzymatic antioxidant activity rate was high in FYM and in combination of all the treatment when applied to Adathoda vasica plants when compared to other treatments.**

***Index Terms* - Adathoda vasica, Vermicompost, Farmyard manure, Bioinoculants.**

I.INTRODUCTION

In recent years, numerous advances have been achieved towards enhancement on the quality and quantity in agriculture. Soil is a natural dynamic body on the surface of earth in which plants grow and is composed of minerals, organic matter, air, water, soil micro flora and fauna. The clean discarding of organic wastes by composting is an environmentally sound and economically viable technology resulting in the production of organic fertilizer which is a basic and valuable input in organic farming. In India, about 350 million tonnes of agricultural wastes are generated annually is in major portion [1].

Traditional plant-based medicines have played a key role in the health care system of our country. Medicinal plants play an important role in the development of potent therapeutic agents. Herbal drugs form the backbone of the invaluable traditional medical practices. Recently interest in medicinal plant research has increased all over the world. It has been reported that medicinal plants used in various traditional systems have immune potential against various diseases. Oxidative damage is one of the major causes of many diseases. A free radical is a molecule with an unpaired electron. The molecule, which losses an electron, becomes free radicals giving rise to a self-perpetuating chain system [2]. Antioxidants are any substances that delays or inhibits oxidative damage to a target molecule. The antioxidants protect the cells against ROS toxicity by preventing ROS formation, interception of ROS attack by scavenging the reactive metabolites, converting them to less reactive molecules, by enhancing the resistivity of sensitive biological targets to ROS attack, facilitating the repair of damage caused by ROS and finally by providing a favourable environment for the effective functioning of other antioxidants. Many chemotherapeutics agents are used in the treatment of various diseases but are facing problems of side effects and therapy is quite costly. Ethnobotanical search reveals use of many traditional herbs in treatment of diseases which are usually free from or having minimum side effects [3].

The modern inorganic farming system with the input of synthetic fertilizer has resulted in the rapid destruction of soil structure, fall of fertilizer response on continuous usage, loss of beneficial microorganisms with subsequent microbial nutrient recycling system and above all the human health hazardous substance as pesticide and insecticide residue in the food produce. In order to overcome these problems faced, organic farming is adopted now- a-days which avoids or largely excludes the use of compound chemicals such as chemical fertilizers/pesticides and herbicides, instead natural sources such as organic manures are used [4]. Organic farming is a system of natural farming which fulfils the food, nutrition and pharmaceutical needs of society without depleting essential natural resources of agriculture, water, soil fertility and diverse biological reserves.

Vermicomposting (VC) is a simple and effective technique to reprocess the agricultural waste, city garbage and kitchen waste along with bioconversion of organic waste materials into nutritious compost by earthworm action [5]. Consequence of VC on plant growth is well reported but mostly it is used as a main source of 'N' 'P' and is a significant nutrient or as a part of some key plant structural components and worked as catalysis in various biochemical reactions in plants. VC biotechnology will bring in 'economic prosperity' for the farmers, 'ecological security' for the farms and 'food security' for the people. Farmyard manure refers to the decomposed mixture of dung and urine of farm animals along with litter and left-over material from roughages or fodder fed to the cattle. On an average well decomposed farmyard manure contains 0.5 per cent N, 0.2 per cent P2O5 and 0.5 percent K2O. Farmyard manure (FYM), also provides growth-regulating substances [6] and improves the physical [7], chemical [8], and microbial [9] properties of the soil. Farmyard manure is readily available in the crop-livestock farming systems. FYM application has been reported to improve crop growth by supplying plant nutrients including micronutrients as well as improving soil physical, chemical, and biological properties [10]. FYM provides a better environment for root development by improving the soil structure. Ibrahim et al. (2010) reported a significant increase in rice root length and root volume with FYM application which indicates that the better root development would allow the plant to exploit more water under water stress conditions [10].

Various strategies, such as the production of genetically modified organisms (mainly plants), the generation of crosses that are naturally resistant to pests, and the use of natural compounds and plant beneficial microorganisms have been proposed [11]. The beneficial microorganisms that may be part of bioinoculants, whether these are biofertilizers, biocides, or biostimulants, may be beneficial fungi such as Trichoderma spp., arbuscular mycorrhizal fungi, and rhizospheric or endophytic bacteria [12-14]. A long list of commercialized bacterial inoculants, based mainly in plant growth-promoting bacteria (PGPB), has been reviewed by Glick [15] and includes Agrobacterium radiobacter, Azospirillum brasilense, Azospirillum lipoferum, Azotobacter chroococcum, Bacillus firmus, Bacillus licheniformis, Bacillus megaterium, Bacillus mucilaginous, Bacillus pumilus, Bacillus spp., Bacillus subtilis, Bacillus subtilis var. amyloliquefaciens, Burkholderia cepacia, Delftia acidovorans, Paenibacillus macerans, Pantoea agglomerans, Pseudomonas aureofaciens, Pseudomonas chlororaphis, Pseudomonas fluorescens, Pseudomonas solanacearum, Pseudomonas spp., Pseudomonas syringae, Serratia entomophila, Streptomyces griseoviridis, Streptomyces spp., Streptomyces lydicus, and Rhizobium spp. However, the list is still growing, and new bacterial species with beneficial properties for sustainable agriculture are being described [16].

Since medicinal plants play a vital role in maintaining health to control and to cure certain diseases, an attempt was made in this study to compare the effect of vermicompost, farmyard manure, bioinoculants and combined application of all on the growth and antioxidant potentials of Adathoda vasica.

II. METHODS AND MATERIALS COLLECTION OF MEDICINAL PLANTS

Adathoda vasica medicinal plant was collected from Dhanvanthri vana, Jnanabharathi Campus, Bangalore University, Bangalore and Biotechnology Centre, Hulimavu, Bangalore and grown at Vedic Biofarm, Kanakapura Road, Bangalore.

COMPOSTS AND BIOINOCULANTS USED

Vermicompost: Vermicompost was procured from Biotechnology Centre, Hulimavu, Bengaluru for our experiments.

Farmyard manure: For the present study FYM used contained cow dung, urine and plant material that is straw mixed with soil.

Bacillus megaterium: Bacillus megaterium is a rod-shaped, Gram-positive, endospore forming, aerotolerant species of bacteria used as a soil inoculant in agriculture and horticulture. Bacterium is arranged into the streptobacillus form. It is a very efficient phosphate solubilizer for crop plants but its efficacy on medicinal plants has not been tried.

Azospirillum brasilens: Motile, free-living, gram-negative bacteria that occur in the soil. They are aerobic or microaerophilic and are sometimes capable of nitrogen fixation. Azospirillum has been found to colonize, promote growth and increase the yield of numerous plant species.

STUDY SITE AND PROCEDURE

Plot for Adathoda vasica was made at the farm with the help of local farmers. The experiments were carried out on land with 10 replicates for each treatment and treatments were assigned to each plot randomly. The treatment consisted of 4 groups viz T1-Bioinoculant (Azospirillum brasilens); T2 - Bioinoculant (Bacillus megaterium); T3 – Vermicompost; T4 - Farm yard manure; T5-Combined (Azospirillum brasileins + Bacillus megaterium + VC+FYM) and C-Control. The plant was treated after two days of planting at intervals of 30, 60 and 90 days respectively. Then at each interval at 30, 60 and 90 days, the growth parameters were recorded and at 90th day the plant samples were analysed for enzymatic and nonenzymic antioxidants.

Growth Parameters:

Growth parameters viz., root length, number of branches, number of leaves, were recorded at 30, 60 and 90 days. Fresh weight and dry weight were recorded at 90 days after planting.

ANALYSIS OF ANTIOXIDANTS

Estimation of Vitamin A (Total carotenoids)

Fresh leaf tissue was homogenized using acetone adding a pinch of clean, fine sand. The extract was centrifuged and supernatant was collected. Finally, absorbance was read at 440nm and total carotenoids content was calculated by the method of lkan R [17].

Estimation of Vitamin C (Ascorbic acid)

Vitamin C was determined by the method of Varley et al [18]. Required quantity of tissue was homogenized in distilled water and filtered. Glacial acetic acid was added to the filtrate and titrated against DCPIP (Dichlorophenol indophenol). Standard titration was done by using ascorbic acid and distilled water as blank. The quantity of ascorbic acid was determined by using the formula,

$$\frac{0.01 \times Y}{X} \times \frac{30}{10} \text{ g of ascorbic acid}$$

where X = Standard titre value, Y = Blank titre value.

Estimation of Phenolics:

Five hundred milligram (500mg) of dried powdered plant sample was mixed with methanolic HCI and boiled in water bath for few minutes. This was allowed to stand for 2 hrs and filtered through Whatman no.1 filter paper. 35% of sodium carbonate, distilled water and Folin's Denis reagent were added to 0.1ml of phenolic extract and incubated at room temperature for one minute. Absorbance (OD) values were read at 640nm. The standard graph was made using caffeic acid as standard using a standard solution of 100 g /ml caffeic acid. Dilutions are made at the range of 10 g/ml [19].

ENZYMATIC ANTIOXIDANT ACTIVITY

Catalase activity: Fresh leaf sample was ground in 0.1M phosphate buffer, pH 7.0 in a prechilled mortar and pestle. The homogenate was centrifuged at 15,000g at 4°C. and supernatant was collected. To this supernatant mixture, hydrogen peroxide enzyme extract was added and incubated at 20oC for 1 minute.

Then the reaction was stopped by adding 0.7N sulphuric acid. The reaction mixture was titrated against 0.01N potassium permanganate [20].

Peroxidase Activity: Peroxidase activity was assayed using o-dianisidine as hydrogen donor and hydrogen peroxide as electron acceptor. In a prechilled mortar and pestle the fresh leaf sample was homogenized in 0.1M phosphate buffer, pH 6.0. with a pinch of clean white sand. Homogenate was filtered and centrifuged at 60,000g for 20min at 4°C and supernatant was used as enzyme source. To a mixture of o-dianisidine, hydrogen peroxide, phosphate buffer and distilled water enzyme source was added. After 5 minutes' reaction was stopped by adding sulphuric acid. Then the absorbance was read at 430nm [21].

Statistical Analysis: Data obtained were analysed by two-way ANOVA. Significant F ratios between groups were further subjected to least significant difference (LSD) probability, p value < 0.05 were considered significant using Graphpad prism software.

III. RESULTS

Effect of biofertilizers, vermicompost, FYM and combined treatment on morphological characters such as shoot height, number of branches and leaves, fresh weight and dry weight of Adathoda vasica is represented in Table 1. Among the different treatment, the highest shoot length (inches) was recorded in T5-comined treatment (15.11), which is statistically significant followed by T3-VC (14.11), T4-FYM (13.22), C-Control (12.7), T1- Azospirillum brasilense (10.83) and the lowest was observed in T2-Bacillus megaterium (9.65). The highest number of branches was recorded (inches) in T5-comined treatment (14.2), which is statistically significant followed by T3-VC (9.8), T2-Bacillus megaterium (8.4), T4-FYM (8.4), C-Control (7), and the least number of branches was observed in T1- Azospirillum brasilense (5) respectively. While the highest number of leaves was observed in T5-comined treatment (109), which is statistically significant, T4-FYM (106) was the second to observe the greater number of leaves followed by T3-VC (84), T1- Azospirillum brasilense (81), C-Control 7(3), and T2-Bacillus megaterium (18). But the highest fresh weight (260.34g) and dry weight (80.98g) was observed in T4-FYM which is statistically significant followed by T5 (combined treatment) i.e., fresh weight is 230.34g and dry weight 70.12g.

Table 1: Influence of biofertilizers, vermicompost, FYM and combined treatment on growth parameters of Adathoda vasica

Treatment		Shoot length (inch)	No. of branches	No. of Leaves	Fresh weight (g)	Dry weight (g)
T1	*Azospirillum brasilense*	10.83	5	81	190.28	65.22
T2	*Bacillus megaterium*	9.65	8.4	18	190.23	55.63
T3	Vermicompost	14.11	9.8	84	180.66	55.28
T4	Farmyard manure (FYM)	13.22	8.4	106*	260.57*	80.98*
T5	T1+T2+T3+T4	15.11*	14.2*	109*	230.34	70.12
C	Control	12.7	7	73	225.56	75.44

*Significant at P< 0.05

The efficiency of biofertilizer, vermicompost, farmyard manure and combined treatment on the levels of antioxidants, catalase and peroxidase which involved in the detoxication of reactive oxygen species in Adathoda vasica at different time intervals were quantified and are depicted in Table 2. The highest quantity of carotenoids (mg/g) was recorded in T5 combined treatment (2.988) followed by T4-FYM (2.941), T1-Azospirillum brasilens (2.786), T2-Bacillus megaterium (2.734), T3-VC (2.412) and C-control (2.151). Similarly, the highest quantity of ascorbic acid (mg/g) was observed in C-control (0.029), followed by T5 combined treatment (0.0528) which is statistically significant, T4-FYM (0.0462), T1-Azospirillum brasilens (0.0429), T3-VC (0.0429) and T2-Bacillus megaterium (0.0396). The highest total phenols (mg/g) were found in T4-FYM (4.4), followed by T5 combined treatment (3.9), T3-VC (3.8), T1-Azospirillum brasilens (3.7), T2-Bacillus megaterium (3.7) and C-control (3.6). The catalase activity (units/min/mg) was highest in T4-FYM followed by, T1-Azospirillum brasilens (0.9), T3-VC (0.9), T5 combined treatment (0.9), T2-Bacillus megaterium (0.8) and C-control (0.8). Whereas the highest peroxidase activity (mmol) was observed in T4-FYM (8.04) followed by C-control (6.77), T1-Azospirillum brasilens (6.19), T2-Bacillus megaterium (4.54), T3-VC (3.94) and T5 combined treatment (3.09).

Table 2: Influence of biofertilizers, vermicompost, FYM and combined treatment on antioxidant content of Adathoda vasica

Treatment		Carotenoids (mg/g)	Ascorbic acid (mg/g)	Total phenols (mg/g)	Catalase (units/min/ mg of the sample)	Peroxidase (mmol)
T1	*Azospirillum brasilense*	2.786	0.0429	3.7	0.9	6.19
T2	*Bacillus megaterium*	2.734	0.0396	3.7	0.8	4.54
T3	Vermicompost	2.412	0.0429	3.8	0.9	3.94
T4	*Farmyard manure (FYM)*	2.941	0.0462*	4.4	1.2	8.04
T5	T1+T2+T3 +T4	2.988	0.0528*	3.9	0.9	3.09
C	Control	2.151	0.029	3.6	0.8	6.77

*Significant at P< 0.05

IV. DISCUSSION

There is an ever-increasing demand for herbal medicines in recent years due to the side effects of synthetic drugs and antibiotics. Hence there is a need for conservation and cultivation of medicinal plants so that the medicinal plants are not extracted from the wild and become endangered. The traditional Indian diet, spices and medicinal plants are rich sources of natural antioxidants. There is an inverse relationship between the dietary intake of antioxidant rich foods and the incidence of human diseases [22]. There are several advantages in commercial cultivation of medicinal plants by using composts and biofertilizers since it enhances quality and quantity of medicinal plants and maintaining the soil sustainability as the use of commercial chemical fertilizers are reduced. Azospirillum inoculation significantly increased the growth, yield, nutrient uptake, dry matter and Vitamin C content in chili. (Balakrishnan, 1988). According to Rajasekharan and Ganeshan (1999) about 400 plants were used for regular production of Ayurvedic, Unani, Siddha and tribal medicine [23]. Rural population of our subcontinent has much faith in the efficacy and healing power of the age-old system of herbal medicine. It is important because the natural plant products are biologically more compatible with human system and comparatively less toxic than the synthesis [24]. There is a need to evaluate the local herbs for estimating the mineral and nutrient compositions so as to determine the potential of indigenous source of medicine [25].

In the present study, for Adathoda vasica T5 (combinations of all) treated plants recorded with maximum shoot length, number of branches and number of leaves followed by T3 (vermicompost) except for number of leaves. This might be due to excellent supply of better nutrients and plant growth promoters to the plants which is present in the worm casts. It was reported that the combined inoculation of bioinoculants increased the number of panicles per plant [26]. The vermicompost with a relatively high content of humus-like compounds, active microorganisms and enzymes, greatly contribute to the enhancement of the biochemical fertility of soils degraded by intensive – cultivation, pollution or natural causes [27]. The casts of earthworms are one of the most useful and active agents in introducing suitable chemical, physical and microbiological changes in the soil and, thereby, directly increasing the fertility and crop producing power in the soil [28]. A study by Tomati et al revealed that positive effect of vermicompost on the growth of Begonias and Coleus, especially a stimulation of rooting, time of flowering, lengthening of internode [29]. In the present study Adathoda vasica, the T5 treated recorded maximum shoot length, number of branches and number of leaves followed by T3 except for number of leaves, wherein in T4 (FYM) was second best treatment when compared to other treatments and control. Non enzymic antioxidants like total carotenoids and ascorbic acid was highest in T5 plants followed by T4 plants. But Total phenols, Catalase and peroxidase activity was maximum in T4 (FYM) treated plants compared to other treatments and control. Hence a consortium of all is the best suitable growth enhancing manure followed by FYM for Adathoda vasica as they have enhanced the qualitative and quantitative properties of the plant. This may also enhance the therapeutic value of the plant.

V. CONCLUSION

In conclusion the results revealed that application of farmyard mannure and in combination with microbial inoculums and vermicompost has significant effect on the plant growth, antioxidants and antioxidant enzyme activity of Adathoda vasica. This kind of cultivation technology or organic farming can be extended to other medicinal plants as this is economical.

REFERENCES

[1] Weblink1:https://icar.org.in/sites/default/files/Creating-Wealth-From-Agricultural-Waste.pdf. Last accessed on August 18, 2021.

[2] Shackelford RE, Kaufmann WK, Paules RS. Oxidative stress and cell cycle checkpoint function. Free Radical Biology and Medicine. 2000;28(9):1387-404.

[3] Indurwade NH, Biyani KR, Kosalge SB, Redasani VV, Khade RV. Drug Lines. 2005; 7: 13-14.

[4] Dhawan AS and Deshmukh MS. Kisan World. 2005; 32:27-28.

[5] Jadia CD, Fulekar MH. Vermicomposting of vegetable waste: A biophysicochemical process based on hydro-operating bioreactor. African journal of Biotechnology. 2008;7(20).

[6] Sharma AR, Mittra BN. Effect of green manuring and mineral fertilizer on growth and yield of crops in rice-based cropping on acid lateritic soil. The Journal of Agricultural Science. 1988;110(3):605-8.

[7] El-Shakweer MH, El-Sayad EA, Ewees MS. Soil and plant analysis as a guide for interpretation of the improvement efficiency of organic conditioners added to different soils in Egypt. Communications in Soil Science and Plant Analysis. 1998;29(11-14):2067-88.

[8] Schjønning P, Christensen BT, Carstensen B. Physical and chemical properties of a sandy loam receiving animal manure, mineral fertilizer or no fertilizer for 90 years. European Journal of Soil Science. 1994;45(3):257-68.

[9] Belay A, Claassens AS, Wehner FC, De Beer JM. Influence of residual manure on selected nutrient elements and microbial composition of soil under long-term crop rotation. South African Journal of Plant and Soil. 2001;18(1):1-6.

[10] Mengistu DK, Mekonnen LS. Integrated agronomic crop managements to improve tef productivity under terminal drought. Water Stress. Vienna Tech. 2011; 1:235-54.

[11] Santoyo G, Sánchez-Yáñez JM, de los Santos-Villalobos S. Methods for detecting biocontrol and plant growth-promoting traits in Rhizobacteria. InMethods in rhizosphere biology research 2019:133-149.

[12] Santoyo G, Moreno-Hagelsieb G, del Carmen Orozco-Mosqueda M, Glick BR. Plant growth-promoting bacterial endophytes. Microbiological research. 2016; 183:92-9.

[13] Begum N, Qin C, Ahanger MA, Raza S, Khan MI, Ashraf M, Ahmed N, Zhang L. Role of arbuscular mycorrhizal fungi in plant growth regulation: implications in abiotic stress tolerance. Frontiers in plant science. 2019; 10:1068.

[14] Ojuederie OB, Olanrewaju OS, Babalola OO. Plant growth promoting rhizobacterial mitigation of drought stress in crop plants: Implications for sustainable agriculture. Agronomy. 2019;9(11):712.

[15] Glick BR. Plant growth-promoting bacteria: mechanisms and applications. Scientifica. 2012:963041.

[16] Glick BR. Issues regarding the use of PGPB. In Beneficial plant-bacterial interactions 2020:361-383.

[17] Ikan R. Natural products: a laboratory guide. Academic Press; 1991.

[18] Varley H, Gowenlock AH, McMurray JR, McLauchlan DM. Varley's practical clinical biochemistry. Heinemann Medical; 1988.

[19] Nitsch JP, Nitsch C. Haploid plants from pollen grains. Science. 1969;163(3862):85-7.

[20] Braber JM. Catalase and Peroxidase in Primary Bean Leaves during Development and Senescence. Zeitschrift für Pflanzenphysiologie. 1980;97(2):135-44.

[21] Sumner JB, Gjessing EC. A method for the determination of peroxidase activity. Arch. Biochem. 1943;2(3):291.

[22] Halliwell B. Antioxidants in diseases mechanisms and therapy. Advances in pharmacology. Ed Sier. 1999;38: Academic press.3-17.

[23] Rajesekharan PE. Conservation of medicinal plant biodiversity-an Indian perspective. J Med Arom Plant Sci. 2002; 24:132-47.

[24] Pal DC. Ethno-medicine-Its importance and role in modern medicine. Journal of Economic and Taxonomic Botany. 2003;27(1):68-74.

[25] Rahila T, Rukhsandra N, Zaidi AA, Shamshila R. Phytochemical screening of medicinal plants belonging to family Euphorbiaceae. Pakistan Veterinary Journal. 1994;14(3):160-2.

[26] Shanmugam PM, Veeraputhran R. Effect of organic manure, biofertilizers, inorganic nitrogen and zinc on growth and yield of rabi rice (Oryza sativa L.). Madras Agricultural Journal. 2000;87(1/3):90-3.
[27] Perucci P. Enzyme activity and microbial biomass in a field soil amended with municipal refuse. Biology and Fertility of soils. 1992;14(1):54-60.
[28] Joshi, N.V. and Kelkar, B.V. The role of earthworms in soil fertility. Indian Journal of Agriculture Sciences. 1951;21 (4):189-196.
[29] Tomati U, Grappelli A, Galli E. Fertility factors in earthworm humus. InProceedings of the International Symposium on Agricultural Environment. Prospects in Earthworm Farming. Publication Ministero della Ricerca Scientifica e Technologia, Rome. 198:49-56.