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Comparative of Growth Parameters and Antioxidant Potentials of Alpinia speciosa Treated with Vermicompost, Farm Yard Manure and Bioinoculants

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Abstract: Due to continuous agriculture, the soil fertility of farmland is depleted. To increase soil fertility, humans use inorganic fertilizers. Although they promote crop growth, susceptibility is a negative effect of excessive use of chemical fertilizers. Chemicals are widely used in modern agriculture to control plant diseases and pathogen mutants are also provided. To overcome all these factors, it is recommended to use organic fertilizer. This study aims to compare the effects vermicompost, farmyard manure and bioinoculants on growth parameters, namely, branch length, branch number, leaf number, fresh weight and dry weight, and antioxidant potentials of *Alpinia speciosa*. The manural potential of three manures: vermicompost, farmyard Manure (FYM) and bioinoculants was evaluated at different intervals of time. Our research shows that when applied to *Alpinia speciosa* plants, growth parameters, antioxidants and enzyme antioxidant activity rates in FYM and vermicompost treatments are fast and high compared with other treatments and controls.

***Index Terms - Alpinia speciosa*, Farm yard manure, Bioinoculants, Vermicompost**

I. INTRODUCTION

In recent years, much progress has been made in improving the quality and quantity of agriculture. Soil is the natural energy body for plant growth on the earth's surface and is composed of minerals, organic matter, air, water, and soil microfauna and flora. Clean disposal of organic waste through composting is an environmentally sound and economically viable technology that can be used to produce organic fertilizers, which is a basic and valuable input for organic agriculture. In India, around 350 million tons of agricultural waste is generated each year, most of which is agricultural waste [1].

Traditional plant-based medicines have played a key role in our country's health system. Medicinal plants play an important role in the development of powerful therapeutic agents. Herbs are the backbone of precious traditional medical practice. Recently, interest in medicinal plant research has increased worldwide. According to reports, medicinal plants used in various traditional systems have immunity potential against various diseases. Oxidative damage is one of the main causes of many diseases. Free radicals are molecules with unpaired electrons. A molecule that loses an electron becomes a free radical, thus forming a self-perpetuating chain system [2]. Antioxidants are substances that delay or inhibit oxidative damage to target molecules. Antioxidants prevent the formation of ROS, remove reactive metabolites and make them less reactive molecules to intercept ROS attacks, and improve the resistance of sensitive biological targets to ROS attacks, and promote the repair of ROS damage, thus protecting cells from ROS toxicity Finally, it provides a favorable environment for the effective functioning of other antioxidants. Many chemotherapeutics are used to treat various diseases, but they face the problem of side effects and the costs of treatment are quite high. Ethnobotanical studies have shown that many traditional herbs are used to treat diseases that generally have little or no side effects [3].

The modern inorganic farming system using synthetic fertilizers leads to rapid destruction of soil structure, decreased response to continuous fertilizer use, loss of beneficial microorganisms and subsequent microbial nutrient circulation systems,

especially pesticides and pesticides that are harmful to human health. Pesticide residues in food. To overcome these problems, organic farming is now adopted to avoid or basically eliminate the use of chemical compounds, such as fertilizers / pesticides and herbicides, and instead use natural sources such as organic fertilizers [4]. Organic farming is a natural agricultural system that does not consume basic natural resources such as agriculture, water, soil fertility and diversified biological reserves, and meets the food, nutrition and medicine needs of society.

Vermicomposting (VC) is a simple and effective technique to reprocess of agricultural waste, city garbage and kitchen waste along with bioconversion of organic waste materials into nutritious compost by earthworm action [5]. Consequence of these VC on plant growth is well reported but mostly it used as a main source of 'N' and 'P' is a significant nutrient as a part of some key plant structural components and worked as catalysis in the change of numerous keys of 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 *Alpinia speciosa*.

II. MATERIALS AND METHODS

Collection of Medicinal Plants

Alpinia speciosa 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.
Farm yard 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 theyield of numerous plant species.

Study Site and Procedure

Plot for *Alpinia speciosa* 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; T_4 - Farm yard manure; T5- Combined (*Azospirillum brasileins* + *Bacillus megaterium* + VC+FYM) and C-Control (without VC / FYM / Bioinoculants). 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, root length, number of branches, numberof leaves, were recorded at 30, 60 and 90 days. Fresh weight and dry weightwere 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,

$$\frac{0.01 \times Y}{X10} \times \frac{30}{} \text{ g of ascorbic acid}$$

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 20 ºC 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 bufferand 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 betweengroups 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 *Alpinia speciosa* is represented in Table 1. Among the different treatment, the highest shoot length (inches) was recorded in T4-FYM (11.6), which is statistically significant followed by T4-FYM (11.5), T1-*Azospirillum brasilense* (11.5), *Bacillus megaterium* (11.5), T5-comined treatment (10.5), T3-VC (10.4)) and the lowest was observed in C-Control (9). The highest number of branches was recorded (inches) in T4-FYM (44) followed by, T3-VC (43.4) which is statistically significant, followed by T2-*Bacillus megaterium* (39.8), *Azospirillum brasilense* (38.2), T5-comined treatment (35.2) and the least number of branches was observed in and C-Control (31.4) respectively. While the highest number of leaves was observed in T4-FYM (308) followed by T3-VC (303.8) which is statistically significant, and T2-*Bacillus megaterium* (278.6) was the third to observe the greater number of leaves followed by, T1- *Azospirillum brasilense* (267.4), T5-comined treatment (246.4) and C-Control (219.8). And also, the highest fresh weight (g) and dry weight (g) was observed in T4-FYM which is statistically significant followed by T4 (FYM) *i.e.*, fresh weight is 905g and dry weight 190g.

Table 1: Influence of biofertilizers, vermicompost, FYM and combined treatment on growth parameters of *Alpinia speciosa*

Treatment		Shootlength (inch)	No. of branches	No. of Leaves	Fresh weight (g)	Dry weight (g)
T1	*Azospirillum brasilense*	11.5	38.2	267.4*	870*	185*
T2	*Bacillus megaterium*	11.5	39.8	278.6*	820*	180*
T3	Vermicompost	10.4	43.4*	303.8*	715*	155
T4	Farm yard manure (FYM)	11.6	44*	308*	905*	190*
T5	T1+T2+T3+T4	10.5	35.2	246.4	710*	160*
C	Control	9	31.4	219.8	465	95

*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 *Alpinia speciosa* at different time intervals were quantified and are depicted in Table 2. The highest quantity of carotenoids (mg/g) was recorded in T4-FYM (2.934) followed by T3-VC (2.921), T2-*Bacillus megaterium* (2.816), T5 combined treatment (2.816), T1-*Azospirillum brasilens* (2.728) and C-control (2.719). Similarly, the highest quantity of ascorbic acid (mg/g) was observed in T4-FYM (0.264) which is statistically significant, followed by T5 combined treatment (0.0528), T3-VC (0.165), T2-*Bacillus megaterium* (0.165), T1-*Azospirillum brasilens* (0.132) and C-Control (0.122). The highest total phenols (mg/g) were found in both T4-FYM and T5 combined treatment (5.4), followed by, T3-VC (5.3), T2-*Bacillus megaterium* (5.2), T1-*Azospirillum brasilens* and C-control (4.6). The catalase activity (units/min/mg) was highest in T4-FYM followed by, T1-*Azospirillum brasilens* (1), T3-VC and T5 combined treatment (0.9), T2-*Bacillus megaterium* (0.8) and C-control (0.7). Whereas the highest peroxidase activity (mmol) was observed in T5 combined treatment (30.1) which is statistically significant followed by T4-FYM (29.1), T2-*Bacillus megaterium* (26.8), T3-VC (24.9), T1-*Azospirillum brasilens* (21.2) and the lowest was found in C-control (19.98).

Table 2: Influence of biofertilizers, vermicompost, FYM and combined treatment on antioxidant content of *Alpinia speciosa*

Treatment		Carotenoids (mg/g)	Ascorbicacid (mg/g)	Total phenols (mg/g)	Catalase (units/min/ mg of the sample)	Peroxidase (mmol)
T1	*Azospirillum brasilense*	2.728	0.132	4.6	1	21.2
T2	*Bacillus megaterium*	2.816	0.165*	5.2	0.8	26.8
T3	Vermicompost	2.921	0.165*	5.3	0.9	24.9
T4	*Farm yard manure (FYM)*	2.934	0.264*	5.4	1.2	29.1*
T5	T1+T2+T3+T4	2.816	0.234*	5.4	0.9	30.1*
C	Control	2.719	0.122	4.6	0.7	19.98

*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. Achieving maximum yield with high quality produce is the ultimate goal of any crop management program. The organic, bio-fertilizer and NPK provides additional nutrients and increases availability of native soil nutrients with increased microbial activities. Organic and bio-fertilizer improves the humic substances in soil which improves the yield of the crops [22]. El-Sayed et al also reported that application of Phosphate Solubilizing Bacteria (PSB) has positive effect on plant height [23]. Bio-fertilizers boost the activity of micro-organisms in the soil and make the unavailable micronutrients available for the plant growth [24]. 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 [25]. 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, nutrientuptake, 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 [26]. 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 [27]. 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 [28].

In the present study, for *Alpinia speciosa*, T4 (FYM) treated plants recorded with all the growth parameters, antioxidants content and antioxidant enzymatic activity and peroxidase recorded significantly higher activity compared to other treatments and control. The increase in the growth parameters of *Alpinia speciosa* may due to the effects of farm yard manure which increases soil aggregation, soil aeration and increasing water holding capacity and offers good environmental conditions for the root system of *Alpinia speciosa*. In addition, farmyard manures are slow release nutrients all over the growth seasons. The results of the present study show that biomass yield of *Alpinia speciosa* increased by the treatment of farmyard manure and this is attributed to enhanced nutrient uptake and in addition, farmyard manure fertilizer being an excellent source of macro- and micro-nutrients, could have contributed to enhanced biomass production. Similar findings were observed by Van Averbeke et al who reported an increased total fresh and oven dry above ground biomass of Solanum retroflexum with increased nitrogen application rates [29]. Ngetich et al also reported the increase in the growth and yield of spider plant by the application farmyard manure [30].

In the present study *Alpinia speciosa*, the T4 (FYM) treated recorded maximum shoot length, number of branches and number of leaves. Wherein in T3 (VC) was second best treatment when compared to other treatments and control. Non enzymic antioxidants like total carotenoids and ascorbic acid was highest in T4 (FYM) plants followed by T3 (VC) treated plants. Hence a farm yard manure is the best suitable growth enhancing manure followed by vermicompost for *Alpinia speciosa* as they have enhanced the qualitative and quantitative properties of the plant. This may also enhance the therapeutic value of the plant.

V. CONCLUSION

The results of study delineated that the application of FYM can significantly improve plant growth, antioxidants and antioxidant enzyme activities of Alpinia speciosa and vermicompost second only to FYM. This organic farming or farming technique can be extended to other medicinal plants because it is inexpensive.

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