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Effect of Biofertilizers and Vesicular Arbuscular Mycorrhizae on Holy Basil (*Ocimum sanctum*)

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A B S T R A C T

Ocimum sanctum is an important medicinal and aromatic plant widely grown tropical and subtropical climate in India. The present investigation was conducted to study the effect of biofertilizers and Vesicular Arbuscular Mycorrhizae (VAM) on Basil (*Ocimum sanctum*). In this biofertilizers *viz*., *Azospirillum*, *Azotobacter*, Phosphate solublizing bacteria (PSB), VAM and in various combinations were applied and observed the plant height, girth of plant, number of branches, number of leaves per plant, leaf area index, nitrogen and phosphorous content. Results revealed that the combined soil application of *Azotobacter* (25%), *Azospirillum* (25%), PSB (25%) and VAM (25%) showed better results than the individual applications. The combined effect of biofertilizers and VAM improves the plant growth and productivity.

Introduction

The herb, Holy basil (*Ocimum sanctum* L.) has been known for its curative properties and has been utilized as antimycotoxic, analgestic, antibacterial, antihaemorrhagic, antioxidant properties and it is considered as a good rejuvenator (Ghosh, 1995). A wide range of chemical compounds including coumestans, triterpenes and their glycosides have been known to possess the medicinial uses composition and the pharmacological profile as a medicinal plant. It bear major essential oils like camphor, citral, linalool, eugenol, thymol, geraniol and other constituents are known to use in perfumery and cosmetic industries (Gupta *et al.*, 2000). Biofertilizers are organic products containing specific beneficial microorganisms in concentrated forms derived from the rhizosphere (Mishra and Dadhich, 2010).

In recent years biofertilizers have emerged as promising component of integrating nutrient supply system in sustainable agriculture. Biofertilizers provide plant with their nutritional requirements without undesirable impact on the environment. Biofertilizers are organic products containing specific microorganisms in concentrated forms

derived from the soil root zone (Mishra and Dadhich, 2010). In recent years biofertilizers have emerged as promising component of integrating nutrient supply system in agriculture. Microbial fertilizers are considered as an important part of environment friendly sustainable agricultural practices with low cost inputs mainly including nitrogen fixing, phosphate solubilizing and plant promoting microorganisms. Biofertilizers are important for medicinal and aromatic plants to produce the best product in both quantity and quality and it is also safe for human, animal and the environment.

The VAM fungi and soil microorganisms develop special characteristic structures called as arbuscles and vesicles. The arbuscules help in the transfer of nutrients from the soil into the root system (Divya, 2015). In view of this effect of VAM and soil microorganisms is important thrust area in plant growth and development especially in medicinal plants. Vesicular Arbuscular Mycorrhizal fungi improve plant growth through phosphorous nutrition. In addition to phosphorous they also help in the uptake of other nutrient element. Nutrient absorption by fungal symbionts is due to external hyphae of the fungus proliferating beyond the nutrient depletion zone and reaching the source of nutrient. The improved plant growth promoting substances, tolerance to drought, salinity and transplantation shock, resistance to soil-borne pathogen and synergetic interaction with other beneficial microorganism (Sandhya *et al.*, 1989).

VAM fungi inoculation is one of the promising tools for the conservation and sustainable maintenance of medicinal herbs. The objective of this work was to study the effect of the application of VAM fungi and biofertilizers on the vegetative growth and chemical composition of basil.

Materials and Methods

The investigation on effect of biofertilizers and VAM on Basil was carried out in the Department of Plant Physiology and Microbiology, College of Agricultural Technology, Theni during the year 2018-2019. The pot culture experiment was conducted in the nursery located at the College of Agricultural Technology, Kullapuram, Theni.

The experimental site is situated in the Southern Agro climatic zone of Tamil Nadu at 10°5’ North latitude and 77°5’ East longitude. The bio-inoculants *Azospirillum* and *Azotobacter* were collected from Biofertilizer production unit, Karukodai, Uthamapalayam, Theni district, Tamil Nadu. PSB and VAM were collected from ADA Office, Andipatti, Theni district, Tamil Nadu.

Pot culture experiment

The experiment was laid out in completely randomized design with 11 treatments and 3 replications. The experimental set up was homogenous, which was carried out in pots. The nutrient status of the experimental set up was unique.

So we select the Completely Randomized Block Design (CRD) as the experimental design. After seedlings of *Ocimum sanctum* were transplanted in each pots. The pots were provided with water facilities. There were 11 treatments resulting from Biofertilizers like *Azospirillum*, *Azotobacter*, *Phosphobacteria*, VAM and control. The pots were maintained in the open shade at the temperature of 27 °C - 30° C. After 15^{th}, 30^{th} and 45^{th} days of growth we observed Plant Height, Number of Leaves, Number of Branches, Girth of the plant and Leaf Area Index. The biofertilizers dose were calculated as per the recommendations per ha.

Assessing the plant growth parameters

Plant height

Plant height (cm) was measured by following the procedure of Lindarman (1983), the plant height of all the plants and their mean height was calculated.

Number of branches per plant

According to Bolan (1991), the total number branches per plant was recorded at 30^{th}, 45^{th} days and expressed in numbers.

Number of leaves

(Indicates a plant's physiological age): the number of leaves per plant was recorded at 15^{th}, 30^{th}, 45^{th} days and expressed in numbers.

Leaf area index

Leaf area index is calculated by dividing the leaf area per plant to the ground area.

$$LAI = \frac{\text{Leaf area per plant}}{\text{Ground area occupied}}$$

Leaf Area = LBK × Number of leaves per plant

Girth of the plant

The value is estimated by surrounding the thread around the girth and the thread length is measured using scale.

Assessment of VAM fungal association in roots

The VAM associations with the inoculated roots of the individual plants were assessed. In this method, roots infected with VAM biofertilizers were collected. They are chopped into pieces and they were bleached with H_2O_2 solution. Then staining was performed with Typhan Blue solution. Following this, destaining was performed and we kept it for overnight. We observed VAM infection under microscope.

Nitrogen and Phosphorus estimation

Nitrogen and Phosphorus content in the leaves was estimated by the Macrokjeldhal and Colorimetric method.

Statistical analysis

Data were subjected to analysis of variance (Gomez and Gomez, 1984). Duncan's Multiple Range Tests was used to separate means. Percent values were transformed by arcsine or square root.

Results and Discussion

In the present study the maximum girth (1.8 cm) was observed in the treatment which involve integrated application of biofertilizers (*Azotobacter* 25% + PSB 25% + VAM 50%) T8 on par with (*Azotobacter* 100%) T2 followed by (VAM 100%) T3 followed by (*Azotobacter* 50% + VAM 50% T5). The minimum girth (1.6 cm) was observed in control (T11) (Table 1).

The seedling rootstocks with VAM fungi shows increased stem diameter (Ikram *et al.*, 1992). The maximum leaf area index was observed in the treatment T6 which involves the application of *Azospirillum* 50% + VAM 50% on par with T7 (PSB 50% + VAM 50%) records LAI-1.2 in plants. The minimum LAI was observed in T11 - control which gives LAI - 0.7 (Table 2).

The combined application of VAM and *Azotobacter* increases leaf area per plant (Mahantesh Sajan, 2002). The treatment T10

recorded more number (17) of branches which involves the integrated application of biofertilizers (T10 - *Azospirillum* 25% + *Azotobacter* 25% + PSB 25% + VAM 25%) followed by (T7- PSB 50% + VAM 50%) which gives 16 number of branches.

The less number of branches (5) was observed in treatment (T5 - *Azotobacter* 25% + VAM 25%) (Table 3).

The treatment T8 recorded more number of leaves (95) which involves application of biofertilizers (T8 - *Azotobacter* 25% + PSB 25% + VAM 50%). The less number of leaves (66) was observed in control (T11) (Table 4).

In earlier results of several field experiments indicated that crops inoculated with *Azotobacter* shows increase in yield from 7 to 12% over the uninoculated crops (Mishustin and Shilnikova, 1968).

Azotobacter pose ability to produce vitamins and growth substances which enhances the seed germination (Shende *et al.,* 1977).

The free living, gram negative, motile and mesophilic *Azotobacter* spp are capable of fixing an average of 20 kg of N/ha for a year (Rawia *et al.,* 2009).

Yield increased ranges from 2 to 45 per cent in vegetables, 9 to 24 per cent in sugarcane, 0 to 31 per cent in maize, sorghum, mustard etc., on *Azotobacter* inoculation (Pandey and Kumar, 1989). Besides N_2 fixation, *Azotobacter* synthesizes and secretes considerable amounts of biologically active substances like B vitamins, nicotinic acid, pantothenic acid, biotin, heteroxins, gibberellins etc. which enhance root growth of plants (Rao, 1986). VAM may be an alternative to rising agricultural energy and to increase crop yield and fertilizer cost. Mycorrhizal interactions in sustainable agriculture (Paul Schiener and Bethlenfalvay, 1995) and he explained about the enhancement of soil structure by applying VAM to sustainable farming. Possible role of soil microorganisms in aggregation in soils (Tisdall, 1994) and here the stabilization of soil fertility by some soil microbes like VAM is explained. Growth of VAM mycelium through bulk soil (Caml *et al.,* 1991) Soil mycelia of VAM fungi not only extended the range of plant roots for nutrient uptake but also may connect roots, allowing the transfer of small amounts of nutrients between plants.

The role of the external mycelial network of VAM fungi, a study of C transfer between plants interconnected by common mycelium. i.e., the transfer of C_{14} from *Lolium perenne* donor to *Plantago lanceolata* receiver mediated by VAM when the sp grown together or separately nutrient transfer between the root zones of soybean and maize plants connected by a common mycorrhizal mycelium (Bethlenfalvay *et al.,* 1991), VAM as a symbiotic fungus root association can greatly increase the utilization of nutrients. VAM affect lowland tropical rain forest plant growth.

Dual symbiosis between VAM and *Azotobacter* enhances the leaf yield and also the biomasses and nutrient uptake (Arora *et al.,* 2016).

In the present study, the treatment T10 gives maximum plant height (46 cm) which involves the integrated application of biofertilizers (T10-*Azospirillum* 25% + *Azotobacter* 25% + PSB 25% + VAM 25%).

The minimum plant height (30.8 cm) was observed in control (T11) (Table 5). The growth of plants could be enhanced effectively by the combined inoculation of VAM and *Azospirillum* (Pacovsky, 1988 and Subba Rao *et al.,* 2001).

Table.1 Effect of biofertilizers on Stem girth (cm)

Treatment	15th day	30th day	45th day
T1 - *Azospirillum* (100%)	0.7	1.3	1.7
T2 - *Azotobacter* (100%)	0.8	1.2	1.8
T3 - VAM (100%)	0.8	1.4	1.8
T4 - PSB (100%)	0.7	1.1	1.7
T5 - *Azotobacter* (50%) + VAM (50%)	0.8	1.4	1.8
T6 - *Azospirillum* (50%) + VAM (50%)	0.8	1.3	1.6
T7- PSB (50%) + VAM (50%)	0.7	1.3	1.7
T8 - *Azotobacter* (25%) + PSB (25%) + VAM (25%)	0.9	1.5	1.8
T9 - *Azospirillum* (25%) + PSB (25%) + VAM (50%)	0.8	1.4	1.7
T10 - *Azotobacter* (25%) + *Azospirillum* (25%) + PSB (25%) + VAM (25%)	0.7	1.3	1.7
T11 – Control	0.8	1.2	1.6
	SEd=0.016 CD (0.01)=0.04 CD (0.05)=0.03	SEd=0.027 CD (0.01)=0.07 CD (0.05)=0.05	SEd=0.035 CD (0.01)=0.09 CD (0.05)=0.07

Table.2 Effect of biofertilizers on Leaf Area Index

Treatment	15th day	30th day	45th day
T1 - *Azospirillum* (100%)	0.16	0.6	1.1
T2 - *Azotobacter* (100%)	0.11	0.3	1.0
T3 - VAM (100%)	0.15	0.4	0.9
T4 - PSB (100%)	0.09	0.1	0.8
T5 - *Azotobacter* (50%) + VAM (50%)	0.1	0.2	1.0
T6 - *Azospirillum* (50%) + VAM (50%)	0.13	0.5	1.2
T7- PSB (50%) + VAM (50%)	0.11	0.5	1.2
T8 - *Azotobacter* (25%) + PSB (25%) + VAM (25%)	0.17	0.3	0.9
T9 - *Azospirillum* (25%) + PSB (25%) + VAM (50%)	0.16	0.6	0.8
T10 - *Azotobacter* (25%) + *Azospirillum* (25%) + PSB (25%) + VAM (25%)	0.18	0.6	0.9
T11 – Control	0.14	0.2	0.7
	SEd=0.003 CD (0.01)=0.008 CD (0.05)=0.006	SEd=0.08 CD (0.01)=0.17 CD (0.05)=0.23	SEd=0.017 CD (0.01) = 0.05 CD (0.05) = 0.03

Table.3 Effect of biofertilizers on number of branches

Treatment	15th day	30th day	45th day
T1 - *Azospirillum* (100%)	-	8	16
T2 - *Azotobacter* (100%)	-	6	12
T3 - VAM (100%)	-	7	10
T4 - PSB (100%)	-	4	5
T5 - *Azotobacter* (50%) + VAM (50%)	-	7	9
T6 - *Azospirillum* (50%) + VAM (50%)	-	10	12
T7- PSB (50%) + VAM (50%)	-	13	16
T8 - *Azotobacter* (25%) + PSB (25%) + VAM (25%)	-	12	16
T9 - *Azospirillum* (25%) + PSB (25%) + VAM (50%)	-	7	12
T10 - *Azotobacter* (25%) + *Azospirillum* (25%) + PSB (25%) + VAM (25%)	-	12	17
T11 –Control	-	8	11
	-	SEd=0.47 CD (0.01)=1.32 CD (0.05)=0.97	SEd=0.22 CD (0.01)=2.32 CD (0.05)=0.62

Table.4 Effect of biofertilizers on number of leaves

Treatment	15th day	30th day	45th day
T1 - *Azospirillum* (100%)	13	40	92
T2 - *Azotobacter* (100%)	11	24	79
T3 - VAM (100%)	15	38	82
T4 - PSB (100%)	12	22	68
T5 - *Azotobacter* (50%) + VAM (50%)	13	24	72
T6 - *Azospirillum* (50%) + VAM (50%)	15	43	90
T7- PSB (50%) + VAM (50%)	13	47	87
T8 - *Azotobacter* (25%) + PSB (25%) + VAM (25%)	17	52	95
T9 - *Azospirillum* (25%) + PSB (25%) + VAM (50%)	14	40	73
T10 - *Azotobacter* (25%) + *Azospirillum* (25%) + PSB (25%) + VAM (25%)	17	47	92
T11 –Control	14	28	66
	SEd=0.29 CD (0.01)=0.84 CD (0.05)=0.62	SEd=0.47 CD (0.01)=1.34 CD (0.05)=0.98	SEd=1.41 CD(0.01)=3.98 CD(0.05)=2.93

Table.5 Effect of biofertilizers on plant height (cm)

Treatment	15th day	30th day	45th day
T1 - *Azospirillum* (100%)	15.5	35.5	42
T2 - *Azotobacter* (100%)	14.1	18.1	32
T3 - VAM (100%)	16.4	29.5	44.5
T4 - PSB (100%)	12.9	19.8	35
T5 - *Azotobacter* (50%) + VAM (50%)	14.7	19.7	36.5
T6 - *Azospirillum* (50%) + VAM (50%)	17.4	33.8	43.5
T7- PSB (50%) + VAM (50%)	12.9	27	43
T8 - *Azotobacter* (25%) + PSB (25%) +VAM (25%)	15.8	32.1	43.5
T9 - *Azospirillum* (25%) + PSB(25%) + VAM (50%)	14	34	39
T10 - *Azotobacter* (25%) + *Azospirillum* (25%) + PSB (25%) + VAM (25%)	19.1	39	46
T11 – Control	8.9	16.6	30.8
	SEd=0.35 CD (0.01)=1.01 CD (0.05)=0.74	SEd=0.61 CD (0.01)=1.74 CD (0.05)=1.28	SEd=0.68 CD (0.01)=1.92 CD (0.05)=1.41

Table.6 Effect of biofertilizers on phosphorus content

Treatment	P content (%)
T1 - *Azospirillum* (100%)	0.002
T2 - *Azotobacter* (100%)	0.003
T3 - VAM (100%)	0.002
T4 - PSB (100%)	0.002
T5 - *Azotobacter* (50%) + VAM (50%)	0.002
T6 - *Azospirillum* (50%) + VAM (50%)	0.004
T7- PSB (50%) + VAM (50%)	0.003
T8 - *Azotobacter* (25%) + PSB (25%) + VAM (25%)	0.003
T9 - *Azospirillum* (25%) + PSB(25%) + VAM (50%)	0.003
T10 - *Azotobacter* (25%) + *Azospirillum* (25%) + PSB (25%) + VAM (25%)	0.003
T11 –Control	0.001
SEd **CD (0.01)** **CD (0.05)**	0.0014 0.002 0.004

Table.7 Effect of biofertilizers on nitrogen content

Treatment	N content (%)
T1 - *Azospirillum* (100%)	0.952
T2 - *Azotobacter* (100%)	1.148
T3 - VAM (100%)	1.036
T4 - PSB (100%)	1.232
T5 - *Azotobacter* (50%) + VAM (50%)	1.568
T6 - *Azospirillum* (50%) + VAM (50%)	0.896
T7- PSB (50%) + VAM (50%)	0.868
T8 - *Azotobacter* (25%) + PSB (25%) + VAM (25%)	1.008
T9 - *Azospirillum* (25%) + PSB (25%) + VAM (50%)	1.652
T10 - *Azotobacter* (25%) + *Azospirillum* (25%) + PSB (25%) + VAM (25%)	0.952
T11 –Control	0.644
SEd **CD (0.01)** **CD (0.05)**	0.05 0.03 0.018

Treatments

T1	***Azospirillum* (100%)**
T2	*Azotobacter* (100%)
T3	VAM (100%)
T4	PSB (100%)
T5	*Azotobacter* (50%) + VAM (50%)
T6	*Azospirillum* (50%) + VAM (50%)
T7	PSB (50%) + VAM (50%)
T8	*Azotobacter* (25%) + PSB (25%) + VAM (50%)
T9	*Azospirillum* (25%) + PSB (25%) + VAM (50%)
T10	*Azotobacter* (25%) + *Azospirillum* (25%) + PSB (25%) + VAM (25%)
T11	Control

Interaction between VAM and *Azotobacter* and their effects on rhizosphere microflora and plant growth (Bagyaraj, 1978) and he concluded that the association of VAM and *Azotobacter* gives more number of bacteria and actinomycetes population than inoculated with alone. The interactions of *Azotobacter chrococcum* and *Piriformospora indica* give beneficial effects on shoot length, Root length, Fresh root and shoot weight and panicle number (Kamil prajapati, 2008). Combined effects of *Piriformospora indica* and *Azotobacter chrococcum* enhance plant growth, antioxidant potential and steriol glycoside content in *Steria rebaudiana.* It enhances the plant growth parameters like plant height, Total dry weight, leaf yield and also the biomass was associated with chlorophyll content and nutrient uptake. The treatment T10 recorded more phosphorus percent which involves the application of (T10 - *Azospirillum* 25% + *Azotobacter* 25%

+ PSB 25% + VAM 25% (0.003%) followed by (T8 - *Azotobacter* 25% + PSB 25% + VAM 50% - 0.003%) it on par with (T9 - *Azospirillum* 25% + PSB 25% + VAM 50% - 0.003%) and the minimum percent in T11 - control (0.001%) (Table 6).

Effect of dual inoculation of *Azotobacter* and Mycorrhiza with N and P fertilizer rates on grain yield and some characteristics of spring sunflower (Mirzakhani *et al.,* 2009) These interactions gives increased grain yield, harvest index, hectolite weight, root dry weight, seed yield, mycorrhizal colonization on root in peach. VAM and *Azospirillum* greatly enhance the mobilization of P and N in the crop plants (Baldani *et al.,* 1983; Harley, 1989). The treatment T10 recorded more phosphorus percent which involves the application of T9 - *Azospirillum* (25%) + PSB (25%) + VAM (50%) (1.652%) followed by (T5 - *Azotobacter* (50%) + VAM (50%) – 1.568 and the minimum percent in T11 - control (0.644%) (Table 7).

Early reports on growth and yield of crop plants could be enhanced effectively by increasing the uptake of P and N from soil by combined inoculation of VAM and *Azospirillum* (Pacovsky, 1988). VAM and *Azospirillum* sp. together provide a means by which cereal plants lacking symbiotic N fixers (*Rhizobium*) could compensate the N deficiency and he also suggested that combined inoculation of *Azospirillum* and VAM increase the uptake of N greater than the estimated needs (Barea *et al.,* 1983). Increase in plant dry weight, shoot to root ratios and the N content of dually infected plants could be accounted for by summing VAM and *Azospirillum* (Pacovsky *et al.,* 1985). The use of phosphate solubilizing bacteria as inoculants simultaneously increases Phosphorous uptake by the plant and crop yield. PSB not only increased P availability in the soil but also performed as plant growth promoting bacteria. PSB improve N, P and K nutrition and may function as biocontrol agents of photopathogenic fungi, synthesizing phytohormones in the rhizophere, and as a result may promote plant growth and development. PSB play a vital role in P availability from both organic and mineral sources (Iyer and Rajkumar, 2017). This role in attributed to the ability of PSB to produce low molecular weight acids (Al-Enazy *et al.,* 2017) such as formic, acetic, propionic, lactic, glycoliuc, fumaric and succinic acid (Rashid *et al.,* 2004) which use their carboxyl and hydroxyl groups to chelate cation such as Ca^{+2} and Mg^{+2}. This chelation solubilizes insoluble soil phosphorous (Sharma *et al.,* 2013).

In conclusion, the chemical fertilizers possess threat to the environment so the use of biofertilizers is both economic and environmental friendly. In this study we highlighted use of VAM and N and P biofertilizers for the development of growth parameters on Basil. Treatment of biofertilizers in combination with *Azospirillum*, *Azotobacter*, PSB and VAM has significantly enhanced the growth parameters which include plant height, girth of the plant, number of leaves per plant, number of branches per plant, leaf area index and N and P content. This study has revealed that there is a huge potential for the use of biofertilizer and VAM in holy basil.

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