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ORIGINAL RESEARCH ARTICLE

Co-Inoculation Effect of Am Fungi and Phosphobacteria on the Growth and Yield of Rhizosphere of Bhendi (*Abelmoschus esculentus* L.) as Influenced by Chemicals and Biopesticides

K. Sivakumar* and P. Tholkappian

Department of Agricultural Microbiology, Faculty of Agriculture, Annamalai University, Annamalai Nagar – 608 002, Tamilnadu, India

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ABSTRACT

A pot culture experiment was conducted to study the Co-inoculation effect of AM fungi (*Glomus fasciculatum*) and phosphobacteria (*Bacillus megaterium*) on the growth and yield of rhizosphere of bhendi (var. Arka anamika) as influenced by chemical and bio pesticides. The insecticide Tracer 240SC was applied at different concentration *viz.*, 100 ml, 200 ml and 300 ml ha^{-1} and biopesticides Neem oil was applied @ 500 ml, 750 ml and 1000 ml ha^{-1}. All the three different concentration of biopesticides significantly increased the root colonization percentage, spore numbers, phosphobacterial population, plant height, plant dry weight and fruit yield when compared to the control. When insecticides were applied, it drastically suppressed all above growth and yield parameters. The study revealed that foliar application of Neem oil @ 1000 ml ha^{-1} is safer to Mycorrhizal development, phosphobacterial population, growth and yield of bhendi than the use of chemical insecticides.

Key words: *Glomus fasciculatum*, *Bacillus megaterium*, Arka anamika and Insecticides.

1. INTRODUCTION

In India, the farmers regularly use more amounts of chemical fertilizers and pesticides for crop production especially for vegetable crops, thus the soil which leads to soil pollution and ground water contamination, ultimately causing health hazards [1]. In order to avoid the environmental pollution especially soil pollution, most of the scientists are recommending the use of biofertilizers along with biopesticides in a sustainable manner to maintain the soil health and also the productivity.

Bhendi (*Abelmoschus esculentus* L.) or ladies finger is an important vegetable of the tropical countries and most popular in India, Nigeria, Pakistan, Cameroon, Iraq and Ghana. Though, it is virtually not grown in Europe and North America, yet, lot of people in these countries have started liking this vegetable because of good amount of vitamin A and folic acid, besides carbohydrates, phosphorus, magnesium and potassium.

AM fungi is the most abundant kind of mycorrhizae found in association with every taxonomic group of plants and the list of species not infected is probably far shorter than the infected ones. These fungal associations are beneficial to crop plants in many ways, including enhancing the nutrient availability especially phosphorus, enhancing water uptake, inducing resistant against diseases and increasing the yield [2].

Phosphorus solublizing biofertilizers are carrier based preparations containing living cells of microbes like bacteria, fungi and actinomycetes which may help in increasing crop productivity by way of helping in solubilization of insoluble phosphorus, stimulating plant growth by providing hormones, vitamins and other promoting substances.

2. MATERIALS AND METHODS

Isolation and screening of AM fungi and Phosphobacteria

Bhendi rhizosphere soil sample were collected from twenty different locations in Cuddalore District of Tamilnadu. Four different AM fungal species *viz.*, *Glomus fasciculatum*, *Glomus mosseae*, *Gigaspora margarita* and *Acaulospora*

***Corresponding Author:** K. Sivakumar, **E. mail:** ksivamicro@gmail.com

laevis were isolated, characterized and identified under stereozoom microscope according to Gerdemann and Trappe [3].

Isolated AM fungi are screened for the efficiency by root colonization percentage, AM fungal spore numbers, acid phosphatase and alkaline phosphatase activity in soil. All the four AM fungal species colonized the roots of bhendi. However, the degree of root infection and colonization varied considerably between them. The response of bhendi in terms of root colonization by AM fungi was the highest with *Glomus fasciculatum* followed by *Glomus mosseae*, *Gigaspora margarita* and *Acaulospora laevis* in soils. Acid phosphatase and alkaline phosphatase activities were also the highest in *Glomus fasciculatum.*

Twenty phosphobacterial isolates were screened for Phosphate solubilizing efficiency, producing potential of Indole acetic acid (IAA) and Gibberellic acid (GA_3) and Siderophore. Among twenty isolates, *Bacillus megatherium* was found to be the most efficient isolate in solubilizing various insoluble phosphates. Based on the above screening tests, the isolates *Glomus fasciculatum* and *Bacillus megaterium* were found to be most efficient strains and selected for further studies.

A pot culture experiment was conducted to find out the compatibility and toxicity of chemical insecticide and biopesticide on the growth and yield of bhendi inoculated with *Glomus fasciculatum* and *Bacillus megaterium* by spraying chemical insecticide and biopesticide *viz.,* Tracer 240 SC and Neem oil on 40 DAS at various concentrations. Earthen pots of 30 cm diameter were filled with garden land soil @ 8 kg pot^{-1} collected from experimental farm, Annamalai University (soil pH 7.3, available N 195 kg ha^{-1} and available phosphorus 16.5 kg ha^{-1}). The recommended dose of nitrogen, phosphorus and potash were applied as urea (100 kg N kg ha^{-1}), single super phosphate (50 kg P kg ha^{-1}) and muriate of potash (50 kg K kg ha^{-1}) respectively. Seven treatments were tried with inoculation in which unsprayed served as control and three replications were maintained for each treatment under randomized block design.

Treatment details

T_1 : Control
T_2 : Tracer 240SC 100 ml ha^{-1} + *G. fasciculatum* + *B. megatherium*
T_3 : Tracer 240SC 200 ml ha^{-1} + *G. fasciculatum* + *B. megatherium*
T_4 : Tracer 240SC 300 ml ha^{-1} + *G. fasciculatum* + *B. megatherium*
T_5 : Neem oil 500 ml + *G. fasciculatum* + *B. megatherium*
T_6 : Neem oil 750 ml + *G. fasciculatum* + *B. megatherium*
T_7 : Neem oil 1000 ml + *G. fasciculatum* + *B. megatherium*

AM fungal colonization in bhendi roots

The percentage mycorrhizal colonization of the roots was determined by the method of Phillips and Hayman [4].

The roots were washed gently in tap water. The washed roots were cut into one cm size and then immersed in 10 per cent KOH solution for clearing the host cytoplasm and nuclei for stain penetration. Then it was autoclaved at 15 lbs/sq. inch pressure for about 20 minutes. Then, the root bits were taken out and washed with tap water for about three times or until no brown colour appeared in the rinsed water. The roots were acidified with two per cent hydrochloric acid (3 - 4 minutes) for proper staining. The acid was poured off without rinsing with water and root bits were stained with 0.05 per cent tryphan blue in lactophenol solution and boiled for 10 minutes.

These root bits were examined under compound microscope. Fifty root segments in each replication were used to determine AM fungal colonization per cent.

$$\text{Per cent root colonization} = \frac{\text{Number of root bits with infection}}{\text{Total number of root bits examined}} \times 100$$

Survey for the occurrence of AM fungal spores

AM fungal spore population was estimated by wet sieving and decanting method of Gerdemann and Nicolson [5].

One hundred gram of rhizosphere soil samples were taken from Bhendi mixed thoroughly in one litre of tap water to settle down the heavier particles for few seconds. The suspension was decanted through a coarse soil-sieve (500 - 800 µm sieve) to remove large pieces of organic matter.

The liquid which passed through the sieve was collected separately and stirred to resuspend all particles. The suspension was decanted through a sieve fine enough to retain desired spores (38 - 250 µm sieves). The material retained on the sieve

was washed with a stream of water to ensure that all colloidal materials were passed through the sieve. The small amount of remaining debris were transferred to a shallow layer of water in a petridish and examined under a Stereo zoom microscope. The spore numbers from each soil sample were counted and expressed per 100 g of soil.

Phosphobacterial population

Phosphobacteria enumerated from the rhizosphere soils of different bhendi grown fields by serial dilution [6]. The soil samples were serially diluted upto 10^{-4} dilution. One ml of aliquots of last dilution was plated in using Sperber's hydroxy apatite medium. The plates were incubated upto two weeks at 28±2°C. The bacterial colonies showing clear zone were enumerated and expressed as cfu g^{-1} of oven dry soil.

Plant height

The plant height was recorded at 30, 60 and 90 DAS. The height was measured from the ground level to the tip of the growing point. The mean values were calculated and recorded in cm.

Determination of plant dry weight

The plant samples were collected from each treatment and their dry weight was determined by drying the samples in hot air oven at 60°C till a constant weight was obtained.

Determination of fruit yield

The total numbers of matured fruits were counted at 30, 60 and 90 DAS were recorded.

3. RESULTS

The per cent root infection by inoculated bhendi, increased with the advancement age of the plants. The maximum root infection AM fungal spore and PSB population were observed at 90 DAS (**Table 1**).

Neem oil (1000 ml) sprayed bhendi plants were observed to have the maximum per cent root infection (95.56%) followed by 750 ml and 500 ml neem oil application. The least colonization was recorded (60.33%) at Tracer 240SC @ 300 ml $ha^{-1.}$ Neem oil increased the spore number also in bhendi plants. 190, 189 and 188 spore numbers were recorded at 1000 ml, 750 ml and 500 ml of neem oil respectively. The minimum spore numbers were recorded at Tracer 240SC @ 300 ml $ha^{-1.}$ (129).

Spraying of chemical insecticides (Tracer 240SC @ 300 ml $ha^{-1.}$) affect the PSB population at 3.66 10^6cfu g^{-1}. The maximum PSB populations were observed in neem oil (1000 ml) applied plants (8.66 x 10^6 cfu g^{-1}).

(**Table 2**) shows more variation were observed in root colonization, AM fungal spore number and Phosphobacterial population in all treatments when insecticides were applied at a dose higher than the recommended quantity, it drastically suppressed the root colonization per cent, AM fungal spore number and phosphobacterial population as the result of toxic effect to *Glomus fasciculatum* and *Bacillus megatherium.* Neem oil increase the AM colonization and AM fungal spore number and phosphobacterial population.

The maximum plant dry weight (35.68 g $plant^{-1}$) and plant height (76.30 cm) were registered in neem oil 1000 ml followed by 750 ml and 500 ml of neem oil sprayed plants. The least plant dry weight and plant height were recorded in Tracer 240SC @ 300 ml ha^{-1} sprayed plants. The numbers of fruits were recorded on 90 DAS in neem oil (1000 ml) sprayed plants (18.00) followed by 750 ml and 500 ml neem oil sprayed plants. The numbers of fruits were increased with decrease in the dose of insecticides application above the recommended dose. Among the two different applications, chemical insecticide significantly reduced the number of fruits than the neem oil. The insecticides show very high degree of variation towards plant height, plant dry weight and fruit yield. However, Tracer 240SC exhibited more reduction on the above characters. The least reduction was noticed in Neem oil at recommended dose @ 1000 ml ha^{-1}. The study revealed that foliar application of Neem oil at

Table 1: Effect of combined inoculation of *G. fasciculatum* and *B. megaterium* on the per cent root colonization, spore and PSB population and fruit yield in the rhizosphere of bhendi as influenced by chemical and bio-pesticides

Treatments	AMF root colonization (%)			AMF spore number (100 g^{-1} soil)			Phosphate solubilizing bacterial population (×10^6cfu g^{-1} oven dry soil)		
	30DAS	60DAS	90DAS	30DAS	60DAS	90DAS	30DAS	60DAS	90DAS
$T_{1.}$ Control	69.78	83.29	91.70	109	167	185	4.33	6.00	7.66
$T_{2.}$ Tracer 240SC @ 100ml ha^{-1} + *G. fasciculatum*+ *B. megaterium*	67.00	80.15	88.75	101	158	175	2.33	3.00	5.33
$T_{3.}$ Tracer 240SC @ 200ml ha^{-1} + *G. fasciculatum*+ *B. megaterium*	54.84	72.00	75.34	98	131	157	2.00	3.00	4.66
$T_{4.}$ Tracer 240SC @ 300ml ha^{-1} + *G. fasciculatum*+ *B. megaterium*	38.67	55.76	60.33	68	89	129	1.66	2.33	3.66

T_{5}- Neem oil 500 ml ha^{-1} + *G. fasciculatum*+ *B. megaterium*	72.56	84.54	93.65	110	169	188	4.66	6.00	8.00
T_{6}- Neem oil 750 ml ha^{-1} + *G. fasciculatum*+ *B. megaterium*	74.14	85.36	94.02	112	170	189	5.33	6.66	8.33
T_{7}- Neem oil 1000 ml ha^{-1} + *G. fasciculatum*+ *B. megaterium*	78.73	85.92	95.56	113	172	190	5.66	7.00	8.66
S.Ed	0.08	0.14	0.38	0.13	0.48	1.09	0.13	0.24	0.58
CD(p=0.05)	0.15	0.29	0.75	0.26	0.57	2.17	0.25	0.47	1.15

Table 2: Effect of combined inoculation of *G. fasciculatum* and *B. megaterium* on the plant dry weight, plant height and plant phosphorus content of bhendi as influenced by chemical and biopesticides

Treatments	Plant dry weight (g plant $^{-1}$)			Plant height (cm)			Fruit yield (plant^{-1})		
	30DAS	60DAS	90DAS	30DAS	60DAS	90DAS	30DAS	60DAS	90DAS
T_{1}- Control	19.56	28.40	31.70	23.40	43.70	62.80	07	09	11
T_{2}- Tracer 240SC @ 100ml ha^{-1} + *G. fasciculatum*+ *B. megaterium*	19.34	28.10	30.20	19.25	36.25	60.12	05	08	10
T_{3}- Tracer 240SC @ 200ml ha^{-1} + *G. fasciculatum*+ *B. megaterium*	18.48	21.62	28.12	18.56	31.59	59.36	05	08	10
T_{4}- Tracer 240 SC@ 300ml ha^{-1} + *G. fasciculatum*+ *B. megaterium*	12.31	16.41	20.18	15.23	28.36	56.69	03	07	09
T_{5}- Neem oil 500 ml ha^{-1} + *G. fasciculatum*+ *B. megaterium*	21.85	31.34	34.45	24.10	44.30	63.20	07	09	11
T_{6}- Neem oil 750 ml ha^{-1} + *G. fasciculatum*+ *B. megaterium*	22.34	31.98	35.09	30.00	52.80	73.40	09	11	15
T_{7}- Neem oil 1000 ml ha^{-1} + *G. fasciculatum*+ *B. megaterium*	23.65	32.05	35.68	30.20	58.30	76.30	09	12	18
S.Ed	0.14	0.23	0.59	0.38	1.22	1.73	0.32	0.49	0.59
CD(p=0.05)	0.27	0.45	1.08	1.11	2.41	3.68	0.95	1.28	1.36

recommended dose is safer to mycorrhizal development, phosphobacterial population plant height, plant dry weight and fruit yield than the chemical insecticides.

4. CONCLUSIONS

In the present study, the maximum root colonization percentage, spore numbers, phosphobacterial population, growth and yield parameters were recorded maximum by the use of biopesticides, when compared with insecticides. But there was no significant difference noticed between neem oil at recommended dose @ 500 ml ha^{-1} and the control (unsprayed). The populations of all above organisms are strictly not significant at chemical insecticides. When insecticides were applied at a dose higher than the recommended quantity, it drastically suppressed all above parameters. The study revealed that foliar application of Neem oil at recommended dose @ 1000 ml ha^{-1} at recommended dose is safer to Mycorrhizal development than the chemical insecticides.

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