Contribution of Arbuscular Mycorrhizal Fungi and Phosphate Solubilizer on the Growth of Marigold (*Tagetes erecta* L.) as Influenced by Insecticide and Biopesticidee

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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 of marigold (Athur orange) as influenced by chemical and bio pesticide. The insecticide Metasystox 25 E C was applied at different concentrations viz., 100 ml, 200 ml and 300 ml ha-1 and biopesticide Neem oil was applied @ 500 ml, 750 ml and 1000 ml ha-1. All the three different concentration of biopesticide significantly increased the root colonization percentage, spore numbers, phosphobacterial population, plant height, plant dry weight and flower yield when compared to the control. When insecticide was applied, it drastically suppressed all above growth and yield attributes of marigold. The study revealed that foliar application of Neem oil @ 1000 ml ha-1is safer to Mycorrhizal development, phosphobacterial population, growth and yield of Marigold than the use of chemical insecticide.

***Keywords*—**AM fungi, Glomus fasciculatum, Bacillus megaterium, Marigold and Insecticides, Bio pesticide.

I. 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 . 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. Marigolds is one of the famous flowers and are used in most of the festivals. The pigments in the marigold are sometimes extracted and used as the food coloring for humans and live stocks.

AM 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. Phosphate solublizing biofertilizers are used to increase crop productivity by way of helping in solubilization of insoluble phosphorus, stimulating plant growth by providing hormones, vitamins and other promoting substances.

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II. Materials and Methods

A. Isolation and screening of AM fungi and Phosphobacteria

The rhizosphere soils of marigold samples were collected from twenty five different locations in Cuddalore District, Tamilnadu. Four different AM fungal species *viz., Glomus fasciculatum, Glomus mosseae, Gigaspora margarita* and *Acaulospora laevis* were isolated, characterized and identified under stereozoom microscope according to Gerdemann and Trappe (1974).

Isolated AM fungi were 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 marigold. However, the degree of root infection and colonization varied considerably between them. The response of marigold in terms of root colonization by AM fungi was the highest with *Glomus fasciculatum* followed by *Glomus mosseae,Gigaspora margarita* and *Acaulospora laevis.* Acid phosphatase and alkaline phosphatase activities were also the highest in *Glomus fasciculatum.*

Twenty five phosphobacterial isolates were screened for phosphate solubilizing efficiency, producing potential of Indole Acetic Acid (IAA) and Gibberellic acid (GA_3) and Siderophore. Among twenty five 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.

Pot culture experiment

A pot culture experiment was conducted to find out the compatibility of chemical insecticide and biopesticide on the growth and yield of marigold inoculated with *Glomus fasciculatum* and *Bacillus megaterium* by spraying chemical insecticide and biopesticide *viz.,* Metasystox 25 E C and Neem oil on 40 DAT at various concentrations. Earthen pots of 30 cm diameter were filled with clay loam soil @ 8 kg pot^{-1} collected from garden land, Annamalai nagar (soil pH 7.5, available N 210 kg ha^{-1} and available phosphorus 16.71 kg ha^{-1}). The recommended dose of inorganic fertilizer 40:50:30 kg/ha respectively. Seven treatments were maintained with inoculation in which unsprayed served as control and three replications were maintained for each treatment under Randomized Block Design.

Treatment details 1

T_1	: control
T_2	: Metasystox 25 E C 100 ml ha^{-1}+*G.fasciculatum+B.megaterium*
T_3	: Metasystox 25 E C 200 ml ha^{-1}+*G.fasciculatum+B.megaterium*
T_4	: Metasystox 25 E C 300 ml ha^{-1}+*G.fasciculatum+B.megaterium*
T_5	: Neem oil 500 ml ha^{-1}+*G.fasciculatum+B.megaterium*
T_6	: Neem oil 750 ml ha^{-1}+*G.fasciculatum+B.megaterium*
T_7	: Neem oil 1000 ml ha^{-1}+*G.fasciculatum+B.megaterium*

Per cent root colonization of AM fungi in marigold

The percent root colonization of the marigold was determined by the method of Phillips and Hayman (1970).

Survey for the occurrence of AM fungal spores

AM fungal spore population was estimated by wet sieving and decanting method of Gerdemann and Nicolson (1963).

Phosphobacterial population

Enumeration of Phosphobacteria from the rhizosphere soils of marigold grown fields by serial dilution Sperber (1958).

Plant height

The plant height was recorded at 30, 60 and 90 DAT. 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.

Numbers of flowers plant-1 and flower yield plant-1

The total numbers of flowers harvested from every plant in the first harvest to last harvest were added and expressed as total number of flower per plant.

Statistical analysis

The data recorded on various characters were statistically analysed as per the method by Gomez and Gomez (1984).

III. Results and Discussion

Effect of AM fungi and phospobacteria on marigold influenced by chemical and biopesticide.

The per cent root infection by inoculated marigold, increased with the advancement age of the plants. The maximum root infection AM fungal spore and PSB population were observed at 90 DAT**.**Neem oil (1000 ml) sprayed marigold plants were observed to have the maximum per cent root infection (93.56%) followed by 750 ml and 500 ml neem oil application. The least colonization was recorded (58.36%) at Metasystox 25 EC @ 300 ml ha^{-1}. Neem oil increased the spore number also in marigold plants. 188, 187 and 186 spore numbers were recorded at 1000 ml, 750 ml and 500 ml of neem oil respectively. The minimum spore numbers were recorded at Metasystox 25 E C @ 300 ml ha^{-1} (125).Spraying of chemical insecticides (Metasystox 25 E C @ 300 ml ha^{-1}.) affect the PSB population at 4.69 10^6cfu g^{-1}.The maximum PSB populations were observed in neem oil (1000 ml) applied plants(9.69 x 10^6 cfu g^{-1}).

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 (34.69 g $plant^{-1}$) and plant height (66.35cm) 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 Metasystox 25 E C @ 300 ml ha^{-1} sprayed plants. The flowers yield were recorded on 90 DAT in neem oil (1000 ml) sprayed plants (175.16) followed by 750 ml and 500 ml neem oil sprayed plants. The numbers of flowers 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 flowers than the neem oil. The insecticides show very high degree of variation towards plant height, plant dry weight and flower yield. However, Metasystox 25 E C 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 recommended dose is safer to mycorrhizal development, phosphobacterial population plant height, plant dry weight and flower yield than the chemical insecticides.

Schmidt Brigitta et al. (2015) reported the AM fungi inoculation had the maximum plant height and biomass, also determining some physiological indexes, as transpiration, leaf area, chlorophyll content and dry matter of marigold. Leila Tabrizi et al (2015) ; Bharathiraja and Tholkappian (2011) also reported AM fungi inoculation showed superior plant growth and yield of marigold.

IV. Conclusion

In the present study, the maximum root colonization percentage, spore numbers, phosphobacterial population, growth and yield parameters were recorded the maximum by the use of biopesticide, when compared with insecticide. 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 the above organisms are strictly not significant at chemical insecticide. When insecticide was 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.

The future concerns about phosphorous depletion, cost of fertilizers, environment protection and thus sustainable agricultural practices will assure the development of nature-friendly technologies, including biotechnologies, which use diverse living organisms in humans benefit. While the arbuscular mycorrhizal fungi and phosphate solubilizing bacteria have great potential to become one of the tools of organic agriculture, very little known in the present by horticulturists and agriculturists, it is important to demonstrate its potentials and look out for further applications, which could be used and accepted by the producers and consumers as well.

References

[1] Van Hamme JD, Singh A, Ward OP. Physiological aspects. part 1 in a series of papers devoted to surfactants in microbiology and biotechnology. Biotechnol Adv. 2006;24:604–620. https://doi.org/10.1016/j.biotechadv.2006.08.001

[2] Das,P.,Mukherjee,S.,Sen,R.,2008.Antimicrobial potential of a lipopeptide biosurfactant derived from a marine Bacillus circulans. J.Appl.Microbiol.104, 1675–1684. https://doi.org/10.1111/j.1365-2672.2007.03701.x

[3] Nalini, S., Parthasarathi, R., 2014. Production and characterization of rhamnolipids produced by Serratia rubidaea SNAU02 under solid-state fermentation and its application as biocontrol agent.Bioresour.Technol.173,231–238. https://doi.org/10.1016/j.biortech.2014.09.051

[4] Nalini, S., Parthasarathi, R., 2013.Biosurfactant production by Serratia rubidaea SNAU02 isolated from hydrocarbon contaminated soil and its physico-chemical characterization. Bioresour.Technol.147, 619–622. https://doi.org/10.1016/j.biortech.2013.08.041

[5] S.J. Kalil, F. Maugeri, M.I. Rodrigues, Response surface analysis and simulation as a tool for bioprocess design and optimization, Process Biochem. 2002. Volume 35(6),539–550. https://doi.org/10.1016/S0032-9592(99)00101-6

[6] Velioglu, Z., R. O. Urek.,2015.Optimization of cultural conditions for biosurfactant production by Pleurotus djamor in solid state fermentation. Jof Bioscience and Bioengineering. 120 (5): 526-531.. https://doi.org/10.1016/j.jbiosc.2015.03.007

[7] Tian, X.L.,Cao,L.X.,Tan,H.M.,Zeng,Q.G.,Jia,Y.Y.,Han,W.Q.,Zhou,S.N.,2003 . Study on the communities of endophytic fungi and endophytic actinomycetes from rice and their antipathogenic activities in vitro. WorldJ.Microbiol.Biotechnol. 20, 303–309. https://doi.org/10.1023/B:WIBI.0000023843.83692.3f

[8] Prasad, B., Dutt, B.L., Nagaich, B.B., 1973.Inducing sporulation in Alternariasolani I. Effect of water treatment. Mycopathol.Mycol.Appl.49, 141–146. https://doi.org/10.1007/BF02050856