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Application studies of Bio-inoculants and organic fertilizers on the growth and yield of *Vigna aconitifolia* (Jacq.) Marechal

Varsha M. Chaudhari

Department of Microbiology, PSGVPM's S.I. Patil Arts, G.B.Patel Science and STCO-OP KVS Commerce College, Tal-Shahada, Dist- Nandurbar - 425 409 (India)
Author for Correspondence email : varsharaj2913@gmail.com

Abstract

An increase in World's population causes an increase in the demand for food. To fulfill the increasing demand, use of synthetic fertilizers and pesticides in agricultural sectors causes rapid and healthy growth of plants with soil pollution and environmental toxicity. Synthetic fertilizers mainly nitrogen, potassium, and phosphorous are important micronutrients for plant growth but they cause severe health hazards. Applications of Bio-inoculants are the best alternatives for today's agronomy. In the present study, correlative applications of chemical fertilizers with bio-inoculants were studied on the growth and yield of *Vigna aconitifolia* (Jacq.) Marechal. The seeds of *Vigna aconitifolia* were treated with bio-inoculants separately and in combination along with chemical fertilizer urea. Data obtained revealed that seeds treated with bio-inoculants showed a significant increase in the morphological properties of plants as compared to seeds coated with chemical fertilizer and untreated seeds.

Key words : *Vigna aconitifolia* (Jacq.) Maréchal, *Rhizobium, Azotobacter*, Synthetic fertilizer, Growth, ANOVA.

Modern agriculture continuously applies chemical fertilizers and pesticides to increase food production, as these serve as a fast-food for plants causing them to grow more efficiently and rapidly[12]. But the constant application of chemical fertilizers causes poor soil quality, degradation of soil structure, and pollution of the soil, generally, these are not absorbed and can interfere with both underground and surface water[1] causing eutrophication, high cost[2], and might lead to heavy metals collection in plant tissues, which affect the fruit edibility and nutritional value[7]. Bioinoculants are defined as preparations containing living microorganisms that when applied to the soil or seeds, forms the association with roots or the inner part of the plant and stimulate growth by increasing important nutrients supply to the host plant[8,10,16]. Bio inoculant use in modern agriculture is increasing continuously nowadays as it offers an effective tool to replace the use of pesticides, chemical fertilizers, and other harmful supplements[3-5,17]. The microorganisms generally applied as biofertilizers are nitrogen-fixing bacteria in soil, algae, cyanobacteria, and phosphate-solubilizing bacteria, in combination with some molds and fungi[11]. The use of biofertilizers is eco-friendly, productive, more efficient[15], cost-effective[9], non-toxic, and easy to apply; they help to maintain the soil structure and biodiversity of the agricultural land. Thus, they serve as an excellent alternative to chemical fertilizers.

Moth bean (Vigna aconitifolia (Jacq.) Maréchal) is one of the important proteinaceous legume crops belonging to the family *Fabaceae*[14], It is an exceptionally hardy legume and known by various other names including Mat bean, Matki, Turkish gram, or dew bean. The pods, sprouts, and protein-rich seeds of this crop are commonly consumed in India. The main aim of this study was to evaluate the growth and yield of Moth bean with bioinoculants as eco-friendly fertilizers with the synthetic fertilizer urea.

The healthy seeds of Moth beans (*Vigna aconitifolia* (Jacq.) Marechal) and chemical fertilizer urea were purchased from the local market of Shahada, Dist- Nandurbar, and used in the present research study. Two organisms *Azotobacter* and *Rhizobium* were isolated from rhizospheric soil samples and used for biofetilillizer production.

Isolation of Azotobacter and Rhizobium :

Isolation and identification of *Azotobacter and Rhizobium* were carried out as the method described by Dubey and Maheshwari. *Azotobacter* was isolated on the sterile nitrogen-free Ashby's mannitol salt agar plate after incubation at RT for more than 3 days. Mucoid, transparent colony of *Azotobacter* on Ashby's mannitol salt agar plate was selected, purified further, and confirmed by morphological and biochemical tests.

The culture of *Rhizobium* was isolated from freshly collected healthy nodules of the nodulated plant as the method[6] described in the book. The sample was inoculated on congo red yeast extract mannitol salt agar plate. Plates were incubated for 48-72 h. Pink-colored, large, gummy isolated colonies were purified further and confirmed by morphological and biochemical tests.

Preparation of liquid biofertilizer and slurry:

Pure cultures of both isolates were inoculated in the respective selected liquid medium in an Erlenmeyer flask containing 100 ml sterile nitrogen-free Ashby's mannitol salt broth and in 100 ml sterile yeast extract mannitol broth respectively and cell biomass obtained after growth was mixed with 0.5% sugary solution to form a thick paste for coating the seeds of Moth beans and the remaining medium with organism was stored in the refrigerator for use as liquid biofertilizer preparation.

Seeds treatment :

The seed treatment for the present investigation was used as a direct seed coating method[13] with sugary syrup applied in seed treatment. 20 healthy seeds of moth bean (approx. 50 g) were coated with the thick paste of *Azotobacter* and *Rhizobium* biofertilizers separately, and 5gm paste of each with the combination. All the seeds were dried under the shade in the laboratory and sown the next day. Seed treatments were carried out in the manner like T1 Control, T2 *Rhizobium,* T3 *Azotobacter* T4 *Rhizobium + Azotobacter,* T5 *Rhizobium* + Urea (0.5g), T6 *Azotobacter* + Urea (0.5g), T7 Only Urea (0.5g). Control was maintained by one set of seeds in sterile distilled water. All the experimental pots were allowed to grow up to 20-30 days at room temperature and watered daily. After incubation, the number of seeds germinated in each fertilizer treated and a control pot was observed. Morphological properties like shoot length, root length, the number of leaves per plant, and plant height along with chlorophyll content were measured in seeds treated with bio-inoculants, chemical fertilizer, and in control. Data were analyzed statistically by the ANOVA method.

Bioinoculants *Azotobacter* and *Rhizobium* were prepared in the present investigation and applied separately and in combination to healthy seeds for evaluation of their effect on morphological properties and yield of moth bean plants.

Experimental data (Table-1) revealed that the coating of seeds with *Azotobacter* and *Rhizobium* bioinoculants in combination had a great influence on the yield of moth beans The inoculation of inoculants had a significant effect on plant yield, shoot length, root length, number of leaves, plant height, and chlorophyll content of plants. All characters observed differed significantly under the treatments of seeds coated with bioinoculants as compared to chemical fertilizer and control. Bioinoculant plants absorbed more nutrients from the soil, the increase in the growth parameters may be due to the favorable actions of bioinoculants which resulted in more availability of nitrogen, certain growth-promoting hormones like auxins, gibberellins, vitamins, and organic acid secreted by bio-inoculants.

Root length and shoot length :

When root length and shoot lengths were measured in centimetres results (Table 1) revealed that elongated roots and shoots were observed in seeds treated with Treatment T2, *i. e.* with the treatment of *Rhizobium* separately. Maximum root length 12.5± 0.3cm and shoot length 10.2 ± 0.14 cm were observed in seeds treated with treatment T2 as compared to control or treatment T5. While minimum root length, as well as shoot length, was observed in control and in seeds treated with only chemical fertilizers.

Table-1. Growth and yield of Moth bean seeds with Boinoculants,

Treatment	Root length (cm)	Shoot length (cm)	Number of leaves	Plant height(cm)	Chlorophyll
T_1 (Control)	1.2 ± 0.2	5.4 ± 0.4	05	6.6 ± 0.2	0.30 ± 0.2
T_2 (*Rhizobium*)	12.5 ± 0.3	10.2 ± 0.14	11	21.9± 0.4	0.110 ± 2.2
T_3 (*Azotobacter*)	8.3 ± 0.1	7.2 ± 0.3	10	15.5 ± 0.5	0.83 ± 2.3
T_4 (*Rhizobium + Azotobacter*)	10.2 ± 1.2	8.4 ± 0.2	13	20.5 ± 2.1	0.105 ± 0.2
T_5 (*Rhizobium* + Urea)	4.4 ± 0.5	6.2 ± 0.2	02	10.6 ± 0.23	0.63 ± 0.31
T_6 (*Azotobacter*+Urea)	2.4 ± 0.2	4.8 ± 1.1	01	7.2 ± 0.5	0.61 ± 0.4
T_7 Urea	1.8 ± 0.1	0.4 ± 0.2	00	2.2 ± 0.2	0.53 ± 0.3

Plant height :

Large variations were observed in the height of the plants due to the treatment of bioinoculants separately and in combination. The maximum height recorded in seeds treated with *Rhizobium* biofertilizer was 21.9± 0.4 cm. and seeds treated with combined treatment of *Azo-Rhizo* biofertilizer was 20.5 ± 2.1 cm. As well seeds treated with *Azotobacter* bioinoculants were 20.5± 2.1cm. Similarly, a maximum number of leaves 11 and chlorophyll content of 0.110 ±2.2 were observed in plants treated with treatment T2 *Rhizobium* biofertilizer and T4, *Azo-Rhizo* biofertilizers as compared to control. Results in Table-1 reveals that significant variation in the number of leaves as well as all other morphological parameters of plants were observed due to the treatment of

Figure 1 Pot assay of different treatments of bioinoculants to Moth bean seeds.

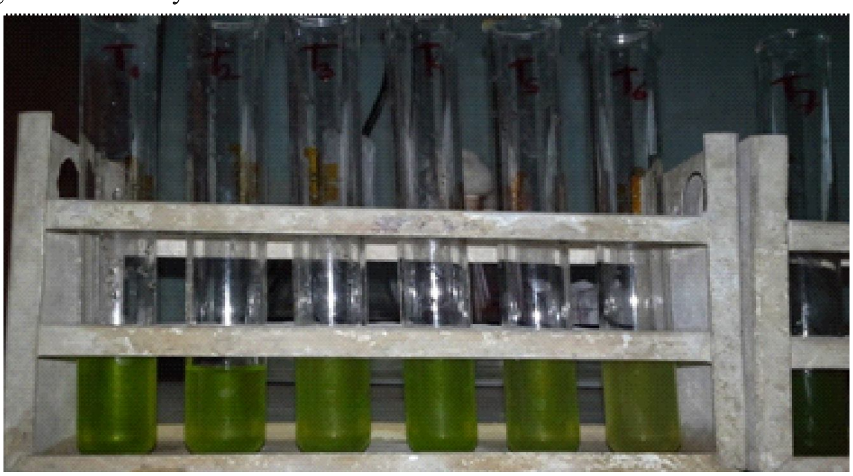

Figure 2 Chlorophyll estimation from each pot.

biofertilizers. Microbial cells present in biofertilizers through their metabolic activities supply essential nutrients as well as growth substances for enhanced growth and morphological properties of plants.

Data obtained in the present study revealed that bioinoculants have significant importance in the growth of the plant *Vigna aconitifolia* (Jacq.) Marechal. Seeds coated with bioinoculants *Azotobacter* and R*hizobium* showed an enhanced increase in the shoot length, root length, plant height as well as chlorophyll content of the plant as compared to chemical fertilizer. Bioinoculants are eco-friendly fertilizers for the growth of plants.

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