THEORETICAL BIOLOGY FORUM

TBF: 52-59 (2024)
Research Article

# Biostimulants- Advanced technique for enhancing yield and L-DOPA content in velvet bean (*Mucuna pruriens* L.)

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## ABSTRACT

*Velvet bean (Mucuna pruriens L.) is a perennial leguminous medicinal crop that has a restorative effect which makes it as a valuable ingredient in pharmacological and therapeutic applications. The seed contains non-protein amino acid L-DOPA having pharmaceutical properties viz.., anti-parkinson, aphrodisiac and neuro-protective. The impact of four bio-stimulants on seed yield and L-DOPA content in velvet bean was studied at ICAR-KVK, Bengaluru Rural District, Karnataka, India. Seed treatment with Sinnorhizobium mililoti and application of RDF along with a foliar spray of humic acid based bio-stimulant at 3ml/l resulted in maximum vine length (383.60 cm), number of branches plant$^{-1}$ (9.87), number of leaves plant$^{-1}$ (117.73), leaf area (20930 cm$^2$), LAI (5.81), pod weight plant$^{-1}$(270.64 g), seed yield (46.71 q ha$^{-1}$), protein(31.94 %), L-DOPA content (5.52%) and L-DOPA yield (256.98 kg ha$^{-1}$). The same treatment enhanced the number of days taken to 50 % flowering and maturity their by extending the vegetative and reproductive phase which in turn increase the yield. Therefore, humic acid based bio-stimulant can be employed in the commercial cultivation of velvet beanalong with seed treatment with S. mililoti and application of RDF to meet the required demand of L-DOPA by phyto-pharmaceutical industries.*

***Keywords-*** *Velvet bean, RDF,Humic acid,Foliar application, L-DOPA*

**ARTICLE HISTORY**
20 January 2024: Received
09 February 2024: Revised
16 March 2024: Accepted
12 April 2024: Available Online

DOI:
https://doi.org/10.61739/TBF.2024.13.1.52

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## 1. Introduction

Velvet bean (*Mucuna pruriens*), often known as cowhage, belongs to fabaceae family and is also called as Atmagupta in Sanskrit, mucuna, cowitch, kawaanch, kapikachhu or Alkushi in other regional dialects. It is indigenous to tropical regions, mainly in Africa, India, and the West Indies. It is an annual herbaceous climber with 22 pairs of chromosomes in its diploid genome (2n = 2x = 22). In India, 14 species of mucuna are noticed in the foothills of the Himalayas, the plains of West Bengal, Madhya Pradesh, Uttar Pradesh, Karnataka, Kerala, Andhra Pradesh and the Andaman & Nicobar Islands. Among 14 species, *M. pruriens*, *M. utilis* and *M. pachylobia* are cultivated for medicine, fodder and vegetable purposes.

The flowering plant has slender branches, alternate lanceolate leaves, and white flowers with a bluish-purple, butterfly-shaped corolla that can reach a length of 15 meters. When the plant is young, it is nearly or entirely covered with fuzzy hairs (trichomes), which on contact with skin, cause an excruciating itch. It has been determined that the presence of 5-hydroxytryptamine (5- HT) in the hairs accounts for this unusual feature [19]. Protein, mucunainand serotonin are the additional chemicals that cause the itch, however after maturity, it almost completely goes away [39]. It's about 10 centimeter long pods can contain up to seven seeds, the seeds measure 1 to 1.9 cm long, 0.8 to 1.3 cm wide and 4 to 6.5 cm thick. They are lustrous black or brown in colour, occasionally speckled and flattened homogeneous ellipsoids. Although the majority of mucuna species are resistant of abiotic conditions including drought, low soil fertility, and high soil acidity, they are vulnerable to frost and do not do well in cold, damp soils, [11]. The genus thrives in conditions that are warm, humid and have a lot of rainfall up to 1500 meters above MSL.

Like most legumes, the velvet bean works symbiotically with soil microorganisms to fix atmospheric nitrogen.

Seeds contain L-3, 4 dihydroxy phenylalanine (L-DOPA), a non-protein amino acid that is frequently used as an anti-Parkinson's, anti-hypertensive and aphrodisiac medication. About 4% of this chemical is present in crude weight, while between 2% and 2.5% of it is present in crystalline form. It is also used as a nervine tonic, anti-diabetic and for the treatment of leucorrhoea, spermatorrhoea other menstrual diseases and also used to cure scorpion stings. By virtue of its high protein content of 23–35 % and digestibility, which is equivalent to rice bean, soybean and lima bean [13], so it is regarded as a potential source of dietary proteins [16, 34].

In addition, the seeds have phytic acid, oligosaccharides, glutathione, mulhingallic acid, and several alkaloids, including mucunine, mucunadine, nicotine, prurienine and prurinidine. Lecithin and sitosterol are found in seed kernel oil. It is also cultivated for its food, decorative, live mulch, green manure, smothering and nematicidal properties in addition to its remarkable medical benefits [9].

In comparison to the most popular pulses, velvet bean has greater levels of ash content of 2.9 to 5.5%, 4.1 to 14.39% of crude lipid, 5.3 to 11.5% of crude fibre and 42.79 to 64.88% of crude carbohydrate. It contains 806-2790 mg/100 g of potassium, 85-477 mg/100 g of magnesium, 104-900 mg/100 g of calcium, 1.3-15 mg/100 g of iron, 4-70 mg/100 g of sodium, 98-498 mg/100 g of phosphorus, 0.33-4.34 mg/100 g of copper, 1-15 mg/100 g of zinc and 0.56-9.26 mg/100 g manganese [34].

The positive effects of productive symbiosis between legumes and nitrogen-fixing bacteria in obtaining yield are well known and these benefits result from the bacteria's ability to fix significant amounts of atmospheric nitrogen, which allows them to survive in soils depleted in nitrogen without the need for nitrogen fertilizers while also increasing the nitrogen content of the soil [5]. Native legumes might not only be genetically predisposed to flourishing in the field, but also due to the development of advantageous interactions between bacteria with other microorganisms in the soil [4]. Since endophytic bacteria have the potential for biotechnological and agronomic uses, natural legumes are anticipated to offer a valuable reservoir of these organisms.

A desirable goal for minimizing the hunger caused by micronutrient deficiencies in the globe is to enhance the nutritional profile of legumes. For many years, using inorganic or synthetic fertilizers has been a standard procedure and acknowledged solution to these issues but they lead to ill effects on the eco system [24]. The usage of natural bio-stimulants is gaining popularity across the globe due to growing knowledge of the bad effects of these chemicals and the current paradigm shift towards "green farming" [44].

One of the most effective and cutting-edge ways to increase plant quality and yield, especially when both abiotic and biotic stressors are present, is to apply bio-stimulants. Regardless of the nutrient content, a substance or microbe is considered to be a plant bio-stimulant if it is administered to plants with the goal of enhancing nutritional effectiveness, abiotic stress tolerance, and/or crop quality features. Humic compounds (HA), seaweed extract (SE), Protein hydrolysateandamino acid formulations are four major kinds of bio-stimulants that have been shown to influence on root growth and uptake of nutrients, including bacteria that promote plant growth.

Due to their positive effects, plant bio-stimulants are now widely employed in farming. The usage of bio-stimulants is safe for both human and the environment, especially for minimizing the use of plant protection chemicals in agriculture. Numerous metabolic processes, including photosynthesis, respiration,ion uptake and nucleic acid synthesis are effectedby bio-stimulants. Various plant bio-stimulants used in different quantities and with distinct modes of action have produced positive impacts on yield and quality [35].

L-DOPA has recently been in high demand from the phyto-pharmaceutical industry in order to provide effective medications for Parkinson disease and hypertension. By increasing seed yield and chemical potency with regard to L-DOPA, bio-stimulants play a crucial role in helping to meet the essential demand for the substance by the phyto pharmaceutical industries.

## 2. Material and Methods

### 2.1 Experimental site

The study was conducted during the *kharif,* 2022 at ICAR –KrishiVigyan Kendra, Hadonahalli, Doddaballapur Taluk, Bengaluru Rural District, Karnataka State, India, which is situated at an elevation of 896 m MSL with 12058 North latitude and 77035 East longitude. The research area's soil was a red soil with a consistent fertility status, a pH of 5.68, an electrical conductivity of 0.13 dS/m, and a medium organic carbon content of 0.38. The soil has low available nitrogen (201.64 kg/ha), medium availablephosphorous(36.80 kg/ha), and high available potassium (150.36 kg/ha).

### 2.2 Variety and Spacing

Velvet Bean Var. Arka Dhanwantari seeds were obtained from ICAR-Indian Institute of Horticultural Research, Hesaragatta, Bengaluruand sown at a rate of 30 kilogrammes per hectare while maintaining a 60 cm x 60 cm spacing.

This variety is a long duration (180-190 days) and high-yielding cultivar (staked; 4-4.5 and unstaked; 2-2.5 t $ha^{-1}$) that bears clusters of purple flowers and medium-sized, shiny-black seeds. L-DOPA is produced in roughly 190 kg/ha by this variety.

### 2.3 Treatment and experimental design

The experiment was conducted during in RCB design *Kharif*, 2022. Seed treatment of velvet bean was done with native root nodulating bacteria *Sinnorhizobium mililoti.* Humic acid, amino acid, sea weed extract, and microbial consortia based bio-stimulants were applied along with the recommended fertilizer dose (100 (N):80 (P):40 (K) kg and FYM15t $ha^{-1}$). At 30 & 60 days after sowing(DAS), amino acid (3ml/l),humic acid (3ml/l), seaweed extract (1ml/l)and Arka microbial consortia (10ml/l) were applied to the crop as soil (1500 l/ha) and foliar (750 l/ha) application.

### 2.4 Observations on growth parameters

Vine length, number of branches $plant^{-1}$, number of leaves $plant^{-1}$, leaf area and leaf area index of velvet bean plants were recorded at harvest of the pods.

### 2.5 Days taken to 50% flowering and pod maturity

By keeping track of the days that passed between the date of sowing and the time that 50% of the plants in a plot began to bloom, the number of days required for 50% flowering was calculated. Whereas length of time needed for pod maturity is measured in days, starting from the day the seeds were sown until the pods were finally harvested.

### 2.6 Observations on yield parameters

Inflorescence number$plant^{-1}$was recorded at full bloom stage, while, number of pods per inflorescence, number of pods$plant^{-1}$, length, width, weight of pod, pod weight $plant^{-1}$, number of seeds $pod^{-1}$, seed weight $pod^{-1}$, seed yield $plant^{-1}$and seed yield per hectare was recorded after harvesting the crop.

### 2.7 Bio-chemical observations

#### 2.7.1 L-DOPA content in seeds

UHPLC (Ultra High-Performance Liquid Chromatographic) analysis was done at IIHR, Bengaluru to determine the content of L-DOPA from nine treatments of the experiment [45].

#### 2.7.2 Crude protein content (%) in seeds

By multiplying the total nitrogen content of the seed with the 6.25 factor and expressing the result in percentage, the crude protein content of the seed was calculated.

#### 2.7.3 Chlorophyll content of leaf (SPAD units)

SPAD-502 chlorophyll meter was used to assess the chlorophyll content of mature, healthy leaves from the plant's centre at its peak growth stage. This tool makes it possible to measure the chlorophyll content without endangering the plants. The readings are presented in SPAD units.

## 3. Results and Discussion

### 3.1 Growth parameters

The treatment, comprising seed treatment with *S.mililoti* and application of RDF along with foliar spray ofhumic acid-based bio-stimulant @ 3ml resulted in significantly higher vine length (383.60 cm), number of branches $plant^{-1}$(9.87), number of leaves $plant^{-1}$(117.73), leaf area $plant^{-1}$(20930 cm2) and leaf area index (5.81).

In contrast, control plants exhibited lower vine length (261.27 cm), number of branches plant$^{-1}$(6.13), number of leaves plant$^{-1}$ (81.06), leaf area plant$^{-1}$(12241 cm2) and leaf area index of 3.40 (Table 1.).

Increased vine length may be due to the balanced nutrient utilization made possible by RDF and bio-stimulants, which may have fostered root growth and proliferation as well as improved soil nutrient absorption.Humic acid, which gives nitrogen-fixing bacteria carbon and other nutritional minerals as an energy source and increases their biological activity, may also have an impact on the increased plant height [2, 27, 51, 18, 7]. The right quantity of humic acid boosted the effectiveness of photosynthesis, which resulted in the accumulation of nutrients in the plant and encouraged it to grow more branches [14, 27, 26, 33, 15] .

Humic acid can contribute immensely to cell division, elongation, and the production of additional carbohydrates when paired with enhanced enzymatic activity, which may have led to an increase in the number of leaves, leaf area and eventually provided a better leaf area index [10, 27, 12, 18, 30, 40].

*Plate 1. General view of the experimental plot at 75 days after sowing*

*Plate2. Inflorescence and pods of velvet bean*

*Table 1: Effect of bio-stimulants on growth of velvet bean (Mucuna pruriens L.)*

| Treatments | Vine length (cm) | Number of branches plant$^{-1}$ | Number of leaves plant$^{-1}$ | Leaf area plant$^{-1}$(cm$^2$) | Leaf area index |
|---|---|---|---|---|---|
| $T_1$ – Control | 261.27$^{f}$ | 6.13$^{g}$ | 81.06$^{g}$ | 12241$^{g}$ | 3.40$^{g}$ |
| $T_2$ – *S. meliloti* + RDF + HA (SA) | 329.43$^{c}$ | 7.94$^{c}$ | 105.59$^{bc}$ | 17887$^{c}$ | 4.97$^{c}$ |
| $T_3$– *S. meliloti* + RDF + HA (FA) | 383.60$^{a}$ | 9.87$^{a}$ | 117.73$^{a}$ | 20930$^{a}$ | 5.81$^{a}$ |
| $T_4$– *S. meliloti* + RDF + AA (SA) | 297.07$^{de}$ | 7.18$^{de}$ | 95.42$^{ef}$ | 15316$^{d}$ | 4.25$^{de}$ |
| $T_5$– *S. meliloti* + RDF + AA (FA) | 311.33$^{cd}$ | 7.52$^{cd}$ | 102.83$^{cd}$ | 17316$^{c}$ | 4.81$^{c}$ |
| $T_6$ – *S. meliloti* + RDF + SWE (SA) | 304.88$^{d}$ | 7.33$^{de}$ | 97.56$^{de}$ | 15885$^{d}$ | 4.41$^{d}$ |
| $T_7$– *S. meliloti* + RDF + SWE (FA) | 358.58$^{b}$ | 9.11$^{b}$ | 112.03$^{ab}$ | 19228$^{b}$ | 5.34$^{b}$ |
| $T_8$– *S. meliloti* + RDF + MC (SA) | 279.32$^{ef}$ | 6.52$^{fg}$ | 88.61$^{fg}$ | 13686$^{f}$ | 3.80$^{f}$ |
| $T_9$- *S. meliloti* + RDF + MC (FA) | 290.1$^{de}$ | 6.79$^{ef}$ | 92.55$^{ef}$ | 14461$^{e}$ | 4.02$^{ef}$ |
| **S.Em. ±** | 7.78 | 0.13 | 2.33 | 216.73 | 0.1 |
| **CD @ 5%** | 23.32 | 0.4 | 6.98 | 649.74 | 0.31 |

**Note : RDF: Recommended dose of fertilizers, HA: Humic acid, AA: Amino acid, SWE: Sea weed extract, MC: Microbial consortia, SA: Soil application, FA: Foliar application*

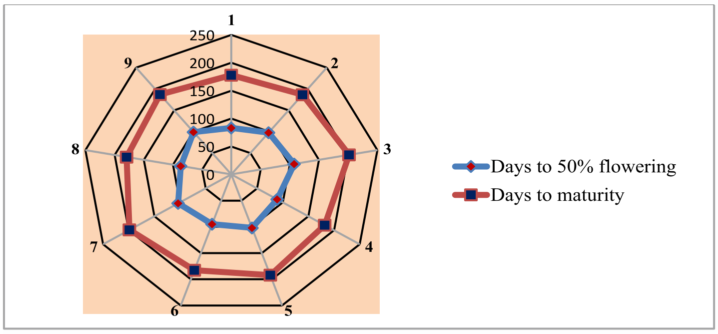



*Figure 1.Days taken to 50% flowering and days to maturity in velvet bean (Mucuna pruriens L.)as influenced bybio-stimulants*

### 3.2. Flowering and maturity

Maximum number of days to 50% flowering (107.57 days) and maturity (201.62 days) was noticed with seed treatment with *S.mililoti* and application of RDF along with foliar spray of humic acid-based bio-stimulant @ 3ml/l (Figure. 1).

Humic acid has been shown to affect the systems responsible for physiological respiration, which in turn promotes plant growth and flowering. It promotes the production of proteins, nutrients, cation exchange capacity, antioxidant metabolism and enzyme activity.When bio-stimulants were sprayed on plants, they quickly increased cell division and elongation in the region of meristem, which accelerated vegetative development and postponed flowering and maturation [37, 30, 8] .

### 3.3 Number of inflorescence and pods

Seed treatment with *S.mililoti* and application of RDF along with foliar spray ofhumic acid-based bio-stimulant @ 3ml/l resulted in maximum number of inflorescences plant$^{-1}$(11.01), number of pods per inflorescence (14.02) and number of pods plant$^{-1}$(40.82) which is *on par* with RDF and foliar application of sea weed extract based bio-stimulant @1ml/l and RDF and foliar application of amino acid based bio-stimulant @ 3ml/l.Whereas, lesser number of inflorescences, number of pods per inflorescence and number of pods plant$^{-1}$was observed in untreated plants (Figure 2.). This could be due to increase the availability of nutrients, boosting photosynthetic activity, and increasing assimilate partitioning for inflorescence development by humic acid and other bio-stimulants. Significant extension of vegetative phase and the presence of gibberellins, such as $GA_1$, $GA_3$, $GA_4$, $GA_5$, $GA_6$, $GA_7$and $GA_{13}$, which affect leaf expansion and produce higher amounts of photosynthates, more flowers were produced in plants [46, 8, 20,43].

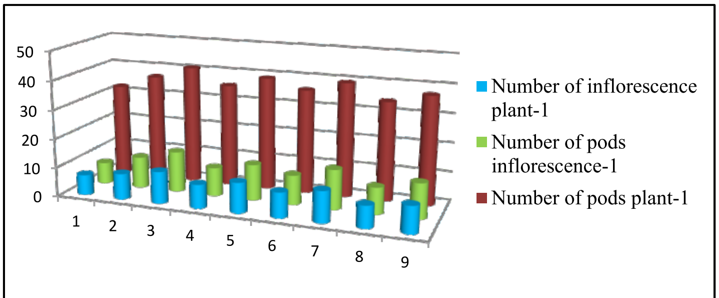



***Figure 2.Number of inflorescence plant-1, number of pods per inflorescence and number of pods plant-1in velvet bean (Mucuna pruriens L.) as influenced by bio-stimulants***

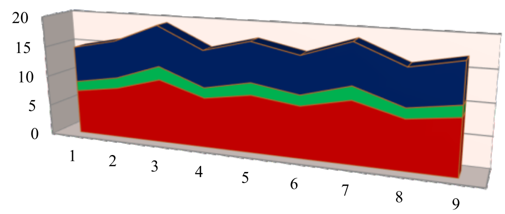

***Plate 3. Number of pods per inflorescence as effected by different bio-stimulants***

### 3.3 Pod characteristics

Maximum pod length (10.49 cm), pod width (2.19 cm) and pod weight (6.63 g) in velvet bean was observed with seed treatment of *S.mililoti* and application of RDF along with foliar spray ofhumic acid-based bio-stimulant @ 3ml/l, which is *on par* with RDF along with foliar application of sea weed extract based bio-stimulant @ 1ml/l and also with RDF and foliar application of amino acid based bio-stimulant @ 3ml/l (Figure.3).

The administration of humic acid, which affects the respiration process, the amount of amino acids, sugars and nitrate accumulation, is likely what caused this large increase in pod length and pod breadth. A greater amount of carbohydrates and auxins are produced and accumulated as a result of increased photosynthetic activity, which also increases the number of reproductive components per plant by favouring the preservation of more flowers. Humic acid also contributes significantly to the formation and development of new cells in the plant meristem, which enhances pod set [50, 36, 1, 41, 29].

The enhancement in pod weight may be due to humic acid's role in cellular respiration, photosynthesis, protein synthesis and other enzymatic processes [31, 22, 21].




| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|---|
| Pod weight (g) | 5.86 | 6.13 | 6.63 | 5.98 | 6.41 | 6.05 | 6.54 | 5.95 | 6.29 |
| Pod width (cm) | 1.61 | 1.75 | 2.19 | 1.68 | 1.97 | 1.71 | 2.15 | 1.63 | 1.78 |
| Pod length (cm) | 7.32 | 8.43 | 10.49 | 8.17 | 9.36 | 8.38 | 10.02 | 7.99 | 9.01 |

**RDF and Bio-stimulant treatments**

***Figure 3. Influence of bio-stimulants on pod length,pod width and pod weight in velvet Bean (Mucuna pruriens L.)***

### 3.5 Yield parameters

Seed treatment with *S.mililoti* and application of RDF along with foliar spray of humic acid-based bio-stimulant @ 3ml/l, resulted in higher pod weight plant-$^{1}$(270.64 g), number of seeds pod$^{-1}$ (6.52), seed weight pod$^{-1}$(4.12 g), seed yield plant$^{-1}$ (168.18 g) and seed yield per hectare (46.71 q)

The favorable physiological effects of humic acid include impact on plant cell metabolism, photosynthesis and raising leaf chlorophyll concentration, which may have improved pod and seed weight [48, 38, 42] .

Increased yield may also be the result of more nutrients, like nitrogen and phosphorus, being available from the bio-stimulants, which leads to the development of more branches, plant spread, leaf area and maximum photosynthesis that enhances the food accumulation, as well as the diversion of photosynthates towards sinks resulting in better growth and subsequently, a greater number of pods and increased yield [49, 17, 3].

*Table 2: Influence of bio-stimulants on pod and seed yield in velvet bean (Mucuna pruriens L.)*

| Treatments | Pod weight per plant (g) | Number of seeds per pod | Seed weight per pod (g) | Seed yield per plant(g) | Seed yield ha$^{-1}$ (q) |
|---|---|---|---|---|---|
| $T_1$ – Control | $188.40^{e}$ | $4.49^{e}$ | $3.52^{d}$ | $113.17^{f}$ | $31.43^{e}$ |
| $T_2$ – *S. meliloti* + RDF + HA (SA) | $224.17^{cd}$ | $5.54^{abcde}$ | $3.76^{bcd}$ | $137.5^{cd}$ | $38.19^{bcde}$ |
| $T_3$– *S. meliloti* + RDF + HA (FA) | $270.64^{a}$ | $6.52^{a}$ | $4.12^{a}$ | $168.18^{a}$ | $46.71^{a}$ |
| $T_4$– *S. meliloti* + RDF + AA (SA) | $211.93^{d}$ | $5.25^{cde}$ | $3.61^{cd}$ | $127.94^{de}$ | $35.53^{de}$ |
| $T_5$– *S. meliloti* + RDF + AA (FA) | $250.05^{ab}$ | $6.21^{abc}$ | $3.92^{abc}$ | $152.92^{b}$ | $42.47^{abc}$ |
| $T_6$ – *S. meliloti* + RDF + SWE (SA) | $217.32^{cd}$ | $5.36^{bcde}$ | $3.64^{cd}$ | $130.75^{cde}$ | $36.31^{cde}$ |
| $T_7$– *S. meliloti* + RDF + SWE (FA) | $257.74^{a}$ | $6.36^{ab}$ | $4.01^{ab}$ | $158.03^{ab}$ | $43.90^{ab}$ |
| $T_8$– *S. meliloti* + RDF + MB (SA) | $203.01^{de}$ | $5.07^{de}$ | $3.59^{cd}$ | $122.49^{ef}$ | $34.02^{de}$ |
| $T_9$- *S. meliloti* + RDF + MB (FA) | $234.55^{bc}$ | $5.97^{abcd}$ | $3.79^{bcd}$ | $141.33^{c}$ | $39.25^{bcd}$ |
| **S.Em. ±** | 6.74 | 0.29 | 0.1 | 3.43 | 2.09 |
| CD @ 5% | 20.2 | 0.87 | 0.31 | 10.29 | 6.25 |

**Note : RDF: Recommended dose of fertilizers, HA: Humic acid, AA: Amino acid, SWE: Sea weed extract, MC: Microbial consortia, SA: Soil application, FA: Foliar application*

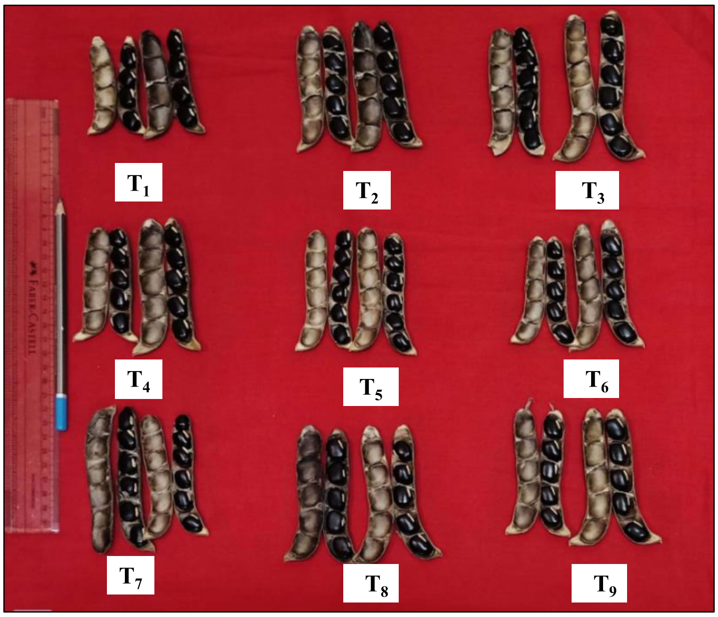



***Plate 4.Number of seeds per pod as influenced by different bio-stimulants***

## 3.6 Chlorophyll and crude protein

Bio-stimulants treatment significantly elevated the chlorophyll and protein content. The higher chlorophyll content (53.90) and protein (31.94 %) was noticed with seed treatment of *S.mililoti* and application of RDF along with foliar spray ofhumic acid-based bio-stimulant @ 3ml/l which is *on par* with RDF in combination with foliar application of sea weed extract based bio-stimulant @ 1ml/l and RDF along with foliar application of amino acid based bio-stimulant @ 3ml/l (Figure 3).The nitrogen in bio-stimulants may have assisted the plant in producing more chlorophyll, which is especially important for glossy, healthy, and darker-colored leaves [47, 6, 25, 28]. Humic acid increases uptake of nutrients, cell permeability and hormonal effects on respiratory catalytic activity, which may have assisted in enhancing N uptake and resulting in a higher protein production and protein content. In addition, humic acid has a high nitrogen intake, which promotes the development of dense vegetative growth. Higher nitrogen absorption also results in higher photosynthesis and protein content in the plant. Additionally, it plays a crucial part in the enzyme nitrate reductase's function in reducing nitrate to ammonia, which forms the building blocks for the amino acids required for protein synthesis [28, 32, 23].

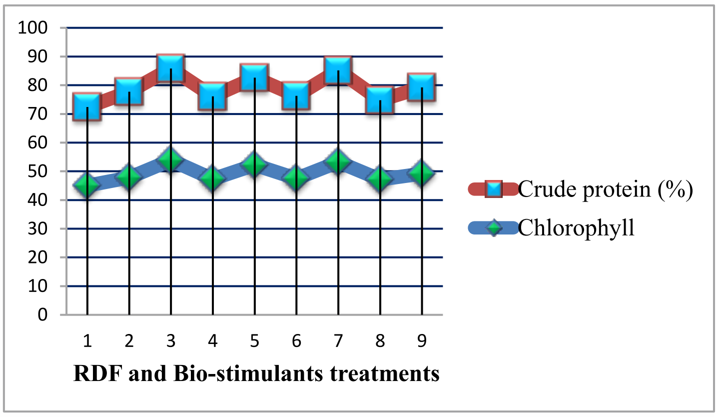



***Figure 3. Effect of bio-stimulants on chlorophyll and crude protein (%) of velvet bean (Mucuna pruriensL.)***

## 3.3 L-DOPA content and yield

The higher L-DOPA content (5.52 %) and L-DOPA yield (256.98 kg ha$^{-1}$) was noticed in seed treatment with *S.mililoti* and application of RDF along with foliar spray ofhumic acid-based bio-stimulant @ 3ml/l, which is *on par* with RDF in combination with foliar application of sea weed extract based bio-stimulant @ 1ml/l and RDF along with foliar application of amino acid based bio-stimulant 3 ml/l (Figure 4, 5 and 6). This may be because humic acid plays a role in promoting several biological functions in plants, particularly photosynthesis and cell division, which serve as the biocenter for the synthesis of secondary substances like glycosides. Additionally, humic acid has well-balanced hormonal components that promote the synthesis and storage of carbohydrates, which in turn speeds up the metabolic processes and produces more secondary metabolites [6, 28].

*Table 3: Effect of bio-stimulants on L-DOPA content and yield in velvet bean (Mucuna pruriens L.)*

| Treatments | L-DOPA content (%) | L-DOPA yield (kg/ha) |
|---|---|---|
| $T_1$ – Control | 3.86$^{b}$ | 121.42$^{g}$ |
| $T_2$ – *S. meliloti* + RDF + HA (SA) | 4.49$^{ab}$ | 170.56$^{d}$ |
| $T_3$– *S. meliloti* + RDF + HA (FA) | 5.52$^{a}$ | 256.98$^{a}$ |
| $T_4$– *S. meliloti* + RDF + AA (SA) | 4.18$^{b}$ | 149.25$^{ef}$ |
| $T_5$– *S. meliloti* + RDF + AA (FA) | 4.65$^{ab}$ | 198.02$^{c}$ |
| $T_6$ – *S. meliloti* + RDF + SWE (SA) | 4.24$^{b}$ | 154.98$^{e}$ |
| $T_7$– *S. meliloti* + RDF + SWE (FA) | 4.82$^{ab}$ | 212.38$^{b}$ |
| $T_8$– *S. meliloti* + RDF + MB (SA) | 4.12$^{b}$ | 136.22$^{fg}$ |
| $T_9$- *S. meliloti* + RDF + MB (FA) | 4.6$^{ab}$ | 179.83$^{d}$ |
| **S.Em. ±** | 0.32 | 4.4 |
| CD @ 5% | 0.95 | 13.19 |

**Note : RDF: Recommended dose of fertilizers, HA: Humic acid, AA: Amino acid, SWE: Sea weed extract, MC: Microbial consortia, SA: Soil application, FA: Foliar application*

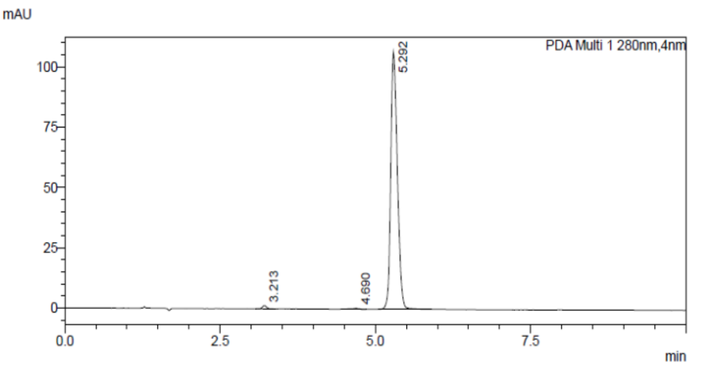



***Figure 4.HPLC chromatogram of L-DOPA standard***

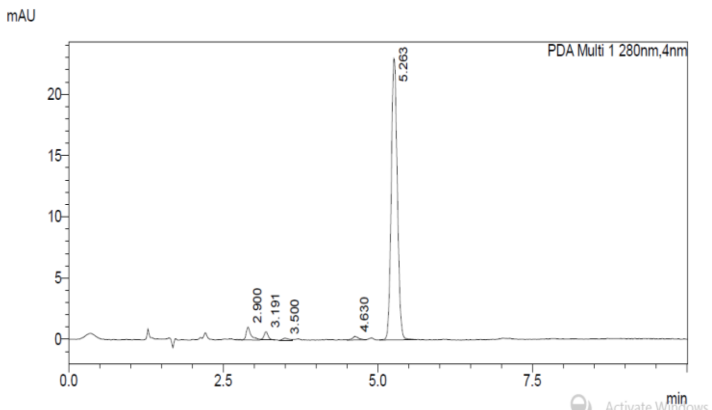



***Figure 5.HPLC chromatogram of L-DOPA in control treatment during kharif, 2022***

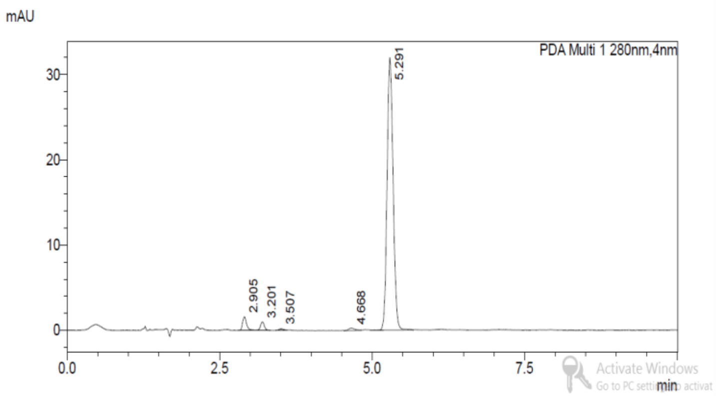



***Figure 6.HPLC chromatogram of L-DOPA in treatment comprises of RDF and Foliar application of humic acid based bio-stimulant***

## Conclusion

Seed treatment with *Sinnorhizobium mililoti* and application of recommended dose of fertilizer along with foliar spray of humic acid based bio-stimulant @ 3ml/l has resulted in better growth, maximum seed yield and L-DOPA content in velvet bean.

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