GROWTH, YIELD AND SECONDARY METABOLITE PRODUCTION RESPONSES TO MICROBIAL ELICITATION IN *Withania somnifera* (L.) DUNAL

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by

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(2019-12-020)

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2021

DECLARATION

I, hereby declare that this thesis entitled "**GROWTH, YIELD AND SECONDARY METABOLITE PRODUCTION RESPONSES TO MICROBIAL ELICITATION IN *Withania somnifera* (L.) DUNAL**" is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

Vellayani

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CERTIFICATE

Certified that this thesis entitled **"GROWTH, YIELD AND SECONDARY METABOLITE PRODUCTION RESPONSES TO MICROBIAL ELICITATION IN *Withania somnifera* (L.) DUNAL"** is a record of research work done independently by **Ms. Ragin Shaji (2019-12-020)** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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Date: 17.12.2021

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We, the undersigned members of the advisory committee of **Ms. Ragin Shaji (2019-12-020)**, a candidate for the degree of **Master of Science in Horticulture** with major in **Plantation Crops and Spices**, agree that the thesis entitled **"GROWTH, YIELD AND SECONDARY METABOLITE PRODUCTION RESPONSES TO MICROBIAL ELICITATION IN *Withania somnifera* (L.) DUNAL"** may be submitted by **Ms. Ragin Shaji (2019-12-020)**, in partial fulfilment of the requirements for the degree.

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RAGIN SHAJI

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LIST OF ABBREVIATIONS

ha	Hectare
PGPR	Plan Growth Promoting Rhizobacteria
cm	Centimetere
et al.	And others
ACC	1-aminocyclopropane-1-carboxylic acid
IAA	Indole-3 acetic acid
cfu mL^{-1}	Colony Forming Unit per milliliter
mL	Millilitere
mM	Millimolar
NaCl	Sodium Chloride
kg	Kilograms
Cd	Cadmium
DAT	Days after transplanting
g $plant^{-1}$	Gram per plant
mg g^{-1}	Milligram per gram
µg	Micro gram
µM	Micro molar
^{o}C	Degree Celsius
VOC	Volatile organic compounds

mm	Millimetre
Nos.	Numbers
WSS	Water soluble sugar
DW	Dry weight
C	Carbon
N	Nitrogen
mg B kg^{-1}	Milligram boron per kilogram
lb $plot^{-1}$	Pound per plot
m^2	Metre square
GA	Gibberillic acid
GAE	Gallic acid equivalent
QE	Quercetin equivalent
IC50	Half-maximal inhibitory concentration
min	Minutes
Fe	Iron
CRD	Completely Randomized Design
L	Litre
h	Hours
OD	Optical density
nm	Nanometere
$HgCl_2$	Mercuric Chloride

Bam	*Bacillus amyloliquefaciens*
Bp	*Bacillus pumilus*
Bv	*Bacillus velezensis*
UV	Ultraviolet
cm^3	Centimeter cube
DMSO	Dimethyl sulphoxide
HCl	Hydrochloic acid
DAS	Days after sowing
ppm	Parts per million
SE	Standard Error
CD	Critical difference
KAU GRAPES	Kerala Agricultural University- General R-shiny based Analysis Platform Empowered by Statistics

INTRODUCTION

1. INTRODUCTION

The cultivation of medicinal plants has assumed greater importance due to their tremendous applications in traditional and modern systems of medicine. India being a rich repository of medicinal plants, more than 960 species of medicinal plants is annually traded; of which 178 species have yearly consumption levels of more than 100 metric tons (IBEF, 2021). Among these, ashwagandha (*Withania somnifera* L. Dunal) holds most prominent position as one of the ancient and highly priced herbs. The crop has been identified by National Medicinal Plant Board of India as one among the 32 priority medicinal plants selected for cultivation in India (CIMAP, 2004).

Withania is one of the medicinally important genera of the family, Solanaceae comprising of 23 species (Shah *et al*., 2013). Among these, only two species namely *Withania somnifera L.* Dunal and *Withania coagulans* Dunal. are considered to be of economic importance. In India, the only cultivated species is *W. somnifera*. It is popularly known as Indian Ginseng due to its similarity in adaptogenic properties of Chinese and Siberian Ginseng (Singh and Gilca, 2010).

The roots of ashwagandha are rich in alkaloids, steroidal lactones and saponins. It is recognized as a folk remedy for leucoderma, insomnia, nervous breakdown, goiter, inflammation, flatulence, anxiety, memory loss etc. They also possess aphrodisiac, diuretic, anthelminthic, astringent, and thermogenic properties. The adaptogenic and rejuvenating properties of the plant classifies it as a 'rasayana' herb. Ashwagandha can also be used to treat cancer and neurodegenerative diseases including Alzheimer's, Parkinson's, Huntington's diseases (Mishra *et al*., 2000; Dickson and Vickers, 2001; Sing *et al.,* 2011; Mukherjee *et al.,* 2020). The inhibitory effect of several compounds isolated from *W. somnifera* against Corona virus disease 2019 (COVID-19), a pandemic disease spread around the world has been reported (Balkrishna *et al*., 2020; Alharbi, 2021; Patil *et al*., 2021).This plant also has immense application in cosmetic and food industries.

In India, ashwagandha is cultivated over an area of 10780 ha. The cultivation is mainly concentrated in the northern states of Madhya Pradesh (more than 5000 ha) Rajasthan, Gujarat, Uttar Pradesh and Punjab (Abhishek, 2018). The annual production of ashwagandha in India is 8429t but its actual requirement is 12,500 tones (Shalini *et al.*, 2017).

This warrants enhanced production and its distribution to non-traditional areas. The urge for organically grown medicinal plants in national and international markets and need for conservation of the crop and environment without compromising production necessitates the evolution of eco-friendly techniques that has the potential to increase productivity and quality of the plant produce.

The application of plant growth promoting rhizobacteria (PGPR) is an eco-friendly approach to boost up the crop productivity, quality and plant defense mechanisms against biotic and abiotic stresses (Backer *et al.,* 2018). Seed biopriming or application of PGPRs is reported to enhance productivity of several horticultural and agricultural crops. Plant growth promoting rhizobacteria, *Bacillus* species with their spore forming characteristics allow for easy cultivation and are good colonizers of soil and plants (Zheng *et al*., 2012). The *Bacillus* species also produce spores under unfavorable environmental conditions as well (Kaki *et al.,* 2013). *Bacillus* spp. take part in improving the plant physiology by activating defense linked enzymes and regulating nutrient uptake and water transport (Radhakrishnan *et al.,* 2017). The effect of *Bacillus* sp. on yield and quality of *W. somnifera* has not yet been determined.

Hence the study entitled "Growth, yield and secondary metabolite production responses to microbial elicitation in *Withania somnifera* L. Dunal" has been undertaken with the objective to evaluate the effect of bacterial inoculants on seed germination, seedling vigour, growth, yield and secondary metabolite production in *Withania somnifera* (L.) Dunal.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Ashwagandha (*Withania somnifera* L.) Dunal is a highly revered herb in the traditional and modern systems of medicine in India. Its use extends back over 2000 to 3000 years due to its wide ranging health benefits. Ashwagandha is an evergreen small woody shrub or herb that reaches a height of 50- 150 cm. The crop is having whitish brown fleshy tap roots with alkaloid content ranging from 0.13 to 0.31 per cent. The leaves are simple, ovate and alternate. The greenish yellow colored inconspicuous flowers are borne in axillary umbellate cyme. The berries are orange- red when mature and enclosed in a persistent calyx. Seeds are reniform and yellow (Nigam and Kandalkar, 1985; Sreerekha *et al.,* 2004).

The therapeutic and nutraceutical properties of this plant are attributed to the presence of steroidal lactones and alkaloids which are primarily present in the roots and to some extent in leaves and seeds. To cop up with the market demands of ashwagandha it is essential to increase extensive cultivation of this crop without compromising quality. The medicinal plant cultivation calls in for ecofriendly and sustainable technologies. The possibilities of productivity and quality enhancement using PGPR (Plant Growth Promoting Rhizobacteria) in medicinal plants needed to be unveiled (Hall *et al*., 2012). In this chapter an attempt has been made to review the relevant research works pertaining to the topic "Growth, yield and secondary metabolite production responses to microbial elicitation in *Withania somnifera* (L.) Dunal."

2.1 Biopriming

Seed pretreatment or priming is an effective seed invigoration technique to hasten and synchronize seedling emergence, rooting and crop establishment (Nawaz *et al*., 2013). It is a process wherein seeds are soaked in different solutions for the initiation of certain metabolic processes to enhance seed germination (Paparella *et al*., 2015). Seed priming is useful for increasing the rate and uniformity of seed germination especially under adverse conditions like elevated temperature, moisture imbalance, salinity etc. Primed seeds show

reduced photo and thermo dormancy that facilitates germination at wide range of temperatures with better weed and pathogen competing capacity. Seed priming was observed to contribute to the improvement in the quality of herbs such as *Rosmarinus officinalis* L. and *Salvia splendens* L. (Hill *et al*., 2008; Girolamo and Barbanti, 2012; Jegadeeswari and Ushamalini, 2019).

Seed priming techniques include hydropriming, osmo priming, solid matrix priming, chemopriming, thermo priming, halo priming, hormonal priming and bio priming. Among these, biopriming is an ecofriendly approach that promotes seed germination, seedling vigour, biotic and abiotic resistance and consequently improving plant health (Reddy, 2012; Nawaz *et al*., 2013; Paparella *et al*., 2015).

Biopriming of seeds integrates the benefits of biological and physiological aspects of priming. In this technique, seeds are treated with beneficial microorganisms followed by seed hydration (Reddy, 2012; Singh *et al*., 2016a; 2016b). Biopriming of seeds trigger colonization of beneficial microbes in the rhizospheric zone of the plant and modifies the physiological, transcriptional, metabolic and epigenetic behavior of the plant (Yadav *et al*., 2013; Meena *et al*., 2017).

Biopriming enhances the germination rate and uniformity, resulting in higher crop establishment, improved harvest, quality and yield (Reddy, 2012; Bisen *et al*., 2014; Mahmood *et al*., 2016). It is also effective for increased uptake of primary and secondary nutrients, enhanced nutritional qualities, better accumulation of carbohydrates and higher rate of primary and secondary metabolite production and high recovery of fresh and dry biomass (Revillas *et al*., 2000; Sharif, 2012; Yadav *et al*., 2017; Singh *et al*., 2018). Biopriming boosts up the abiotic stress resistance and reduce the ingression of soil and seed borne diseases in plants through mycoparasitism, antibiosis, induced phenolic production, antioxidant production, increased nutrient uptake and expression of defense linked enzymes (Jensen *et al*., 2004; Pandey *et al*., 2012; Patel *et al*., 2016).

2.2 *Bacillus* as Plant Growth Promoting Rhizobacteria

Microbial interaction with plants has great implications in the cultivation of agricultural and horticultural crops. PGPRs are naturally occurring soil bacteria that colonize in the rhizosphere around the roots, root surface or within the root that boost plant growth through multiple mechanisms (Podile and Kishore, 2007; Kumari *et al*., 2016). PGPRs include *Acetobacter* spp., *Azospirillum* spp., *Enterobacter* spp., *Rhizobium* spp., *Burkholdaria* spp., *Erwinia* spp., *Flavobacterium* spp., *Pseudomonas* spp., and *Bacillus* spp. (Saharan and Nehra, 2011; Solanki *et al*., 2017).

PGPRs may influence plant growth directly by interacting with host plant through various mechanisms including production of ACC deaminase and plant growth regulators, symbiotic nitrogen fixation and mineral solubilization that result in increased availability of plant nutrients. It also causes indirect stimulation through antagonistic activity against pathogens that contribute to the plant resistance (Ahmed *et al*., 2008; Desai *et al*., 2011; Dilnashin *et al*., 2020). They actively participate in the processes like decomposition, mobilization, mineralization and storage of nutrients, nitrogen fixation and denitrification. They enhance plant growth, both root and shoot system (Mantelin and Touraine, 2004; Huang *et al*., 2015), yield and quality (Bharti *et al*., 2014), and improves biotic resistance of plants. PGPRs also take part in betterment of soil structure and bioremediation of metal contaminated soils (Spaepen *et al*., 2007; Verma *et al*., 2010).

Among PGPRs, *Bacillus* and *Pseudomonas* are predominantly being exploited in agriculture (Piggot and Hilbert, 2004; Tiago *et al*., 2004; Podile and Kishore, 2007). *Bacillus* is a universal bacterium with wider adaptability and is able to produce endospores which help them to survive under varying stress conditions (Chowdhury *et al*., 2013). Ubiquitous and saprophytic nature, spore forming ability and multiple mode of action of the members of *Bacilli* make them well adapted to less conductive environment (Cano and Borucki, 1995; Piggot and Hilbert, 2004; Mendizabal *et al*., 2012).

Bacillus spp. is recognized as one of the most efficient phosphate solubilizing bacteria. They have key role in plant growth promotion especially root system development and induction of disease and pest resistance by means of production of phytohormone precursors, siderophores and phosphate solubilization. *Bacillus* spp. are observed to enhance the production of phytohormrones, stress related metabolites and stress response genes, thereby improving stress tolerance in their host plants (Probanza *et al*., 1996; Deepa *et al*., 2010; Kundan and Pant, 2015; Hashem *et* al., 2019; Kashyap *et al*., 2019).

2.2.1 *Bacillus amyloliquefaciens*

B. amyloliquefaciens is a gram positive soil bacterium that are facultatively associated with hosts and found in vicinity of root zone of the plants (Idriss *et al*., 2002; Campisano *et al.*, 2014; Hossain *et al*., 2015). Similar to other *Bacillus* spp., endospore forming ability of this bacterium enable them to adapt with different ecological conditions for longer time periods. The proven capabilities of *B. amyloliquefaciens* on plant growth promotion by the production of siderophores, hydrolytic enzymes and growth regulators like IAA, phosphate solubilization, disease suppression and stress tolerance, widen the scope for utilizing them in crop production systems (Danielsson *et al*., 2007; Tan *et al*., 2013; Daim *et al*., 2014).

2.2.1.1 Effect of Application of *Bacillus amyloliquefaciens* on Seed Germination and Seedling Growth

In a study conducted by Islam *et al*. (2016) cucumber seeds treated with *B. amyloliquefaciens* PPB12 at $1x10^8$ cfu mL^{-1} displayed 66.02 and 65.63 per cent increased shoot length and root length, respectively at three weeks after planting over uninoculated control.

Biopriming of chilli seeds with *B. amyloliquefaciens* at $1x10^9$ cfu mL^{-1} recorded an enhanced germination of 95 per cent in comparison with uninoculated control which recorded only 83 per cent germination (Hernandez *et al*., 2018).

In a study by Gowtham *et al.,* (2018) it was observed that chilli seeds treated with *B. amyloliquefaciens* at $1x10^8$ cfu mL^{-1} resulted in maximum enhancement of seed germination (84.75 per cent) and seedling vigor (1423.8) compared to uninoculated control.

Jiao *et al*. (2020) reported that biopriming of tobacco seeds with *B. amyloliquefaciens* YN201732 at $1x10^6$ cfu mL^{-1} resulted in 32.27 per cent enhancement of seed germination over uninoculated control.

Tomato seeds treated with *B. amyloliquefaciens* INR937 at $1x10^8$ cfu mL^{-1} exhibited enhanced germination of 77- 83 per cent. This treatment recorded maximum vigor of 789 over untreated control which recorded 678 (Girish and Umesha, 2005).

2.2.1.2 Effect of Application of *Bacillus amyloliquefaciens* on Plant Growth

Cucumber seedlings treated with 10 ml of *B. amyloliquefaciens at* 10^8 cfu mL^{-1} showed significant increase in the plant growth promoting attributes such as shoot height, root length, root surface area and yield by 71.60, 56.30, 65.60 and 90.00 per cent respectively over untreated control (Shao *et al.,* 2014).

The seedlings of *Codonopsis pilosula* (Franch.) Nannf, a medicinal herb used by Chinese in folk medicine, when treated with *B. amyloliquefaciens* GB03 at $1x10^9$ cfu mL^{-1}under elevated salt stress conditions exhibited significant increase in all the observed growth parameters in all treatments over corresponding control and maximum enhancement of shoot fresh and dry weight and root fresh and dry weight by 55.10, 38.20, 72.70 and 117.00 per cent were observed at salt concentrations of 50, 150, 100 and 0 mM NaCl treatments respectively (Han *et al.,* 2016).

In a study conducted by Islam *et al*. (2016), cucumber seeds treated with *B. amyloliquefaciens* PPB12 at $1x10^8$ cfu mL^{-1} displayed 66.02 and 65.63 per cent increased shoot length and root length, respectively over uninoculated control.

Wang *et al*. (2016) reported that banana seedlings treated with bio organic fertilizer formulated from *B. amyloliquefaciens* at 2 per cent weight of potting media showed significant increase in pseudo stem diameter, plant height and fresh and dry weight of banana plants by 21.53, 12.33, 22.57 and 34.63 per cent respectively. Field application of bio organic fertilizer at 6 kg seedlings^{-1} showed enhancement in plant height by 11.96 per cent and pseudo stem diameter by 10.4 per cent in field 1 and that of by 14.48 per cent and 12.51 per cent, respectively in field 2.

Codonopsis pilosula seedlings on inoculation with *B. amyloliquefaciens* GBO3 at 1 mL seedling^{-1} showed an increase of shoot length, root volume, root diameter, shoot fresh weight, shoot dry weight, root fresh weight and root dry weight by 26, 25, 37, 43, 45, 51 and 38 per cent respectively and double fold enhancement in the number of branches over the control plants (Zhao *et al*., 2016).

Plant growth features such as shoot height, stem diameter, leaf area and fresh weight of cucumber seedlings were enhanced by 59.60, 12.90, 54.80 and 41.00 per cent respectively when treated with *B. amyloliquefaciens* L-S60 at 1x10^{7} cfu mL^{-1} over the control (Qin *et al.*, 2017).

Seed priming of *Phaseolus vulgaris* L. with *B. amyloliquefaciens* HLA at 1x10^{8} cfu mL^{-1} for 25 min showed significant increase in stem length (34.08 per cent), leaf area (96.5 per cent), root fresh weight (46.15 per cent) and root dry weight (70.41 per cent) with respect to the control (Mokrani *et al*., 2018).

Rahman *et al*. (2018) reported that bacterial cultures of B. *amyloliquefaciens* (UQ154), *B. velezensis* (UQ156) and *Acinetobacter* sp. (UQ202) applied as seed treatments followed by soil drench at transplanting in tomato plants significantly promoted plant growth features and vigor of the plants and *B. amyloliquefaciens* exhibited a higher, 45.80 per cent total fresh weight, 10.90 per cent shoot length and the highest seedling vigor compared with other microbes and uninoculated control.

Kazerooni *et al*. (2021) reported that seed treatment with *B. amyloliquefaciens I* B11 at $1x10^8$ cfu mL^{-1} on *Capsicum annuum* cv. Geumsugangsan significantly increased plant height by 14.77, 18.03 and 31.76 per cent and plant fresh weight by 54.26, 35.94 and 33.26 per cent in the salt, drought, and Cd stressed conditions, respectively, compared with the corresponding control.

2.2.1.3 Effect of Application of *Bacillus amyloliquefaciens* on Yield

In a study conducted by Meng *et al*. (2012) it was reported that seedling treatment followed by soil drench of *B. amyloliquefaciens* at 10^5 and 10^6 cfu mL^{-1} at 20 DAT enhanced the tuber yield and plant height of potato. Higher concentration of bacterial inoculum yielded much better result than the lower concentration.

Shen *et al*. (2015) found that two years of continuous application of bioorganic fertilizer containing *B. amyloliquefaciens* NJN-6 significantly increased the mean banana fruit weight by 2±0.09, 3.7±0.19 and 3.5±0.17 kg $plant^{-1}$ compared to cattle manure compost, pig manure compost and chemical fertilizer, respectively.

Among the seven PGPRs evaluated for enhancement of agro morphological traits of black cumin under elevating water deficit conditions, *B. amyloliquefaciens* showed the best results. The bacterial inoculation under 100 per cent water requirement treatment recorded 38.70, 17.13, 24.80 and 35.20 per cent increase in seed number per capsule, 1000 seed weight, biological yield and grain yield respectively, over unninoculated control (Bosh *et al*., 2018).

Capsicum annuum var. *aviculare* subjected to seed priming with *B. amyloliquefaciens* at $1x10^9$ cfu mL^{-1} exhibited significantly higher seed yield (270.5 g $plant^{-1}$) compared to that of control (190.8 g $plant^{-1}$). It also increased oil yield and promoted plant growth parameters including germination per cent, plant height, root length and fresh and dry weight of plant (Hernandez *et al.*, 2018).

2.2.1.4 Effect of Application of *Bacillus amyloliquefaciens* on Biochemical Parameters

According to Girish and Umesha (2005), tomato seeds treated with *B. amyloliquefaciens* INR937 at $1x10^8$ cfu mL^{-1} exhibited the highest total phenol content, 0.1883 mg g^{-1} compared to that (0.0541 mg g^{-1}) of uninoculated control.

Zhao *et al*. (2016) reported a double fold enhancement in secondary metabolite production of lobetyolin in *C. pilosula* by the inoculation of *B. amyloliquefaciens* GBO3 at 1mL on three week old seedlings.

In a study by Rahman *et al*. (2018), overnight dipping of roots of strawberry plug plants in *B. amyloliquefaciens* BChi1 at 1 x 10^9 cfu mL^{-1} significantly enhanced the total anthocyanin, total carotenoids, total flavonoids, total phenolics and antioxidant activity in addition to 48 per cent higher fruit yield.

According to Chiappero *et al*. (2019), *Mentha piperita* plants treated with 1 x 10^9 cfu mL^{-1} of bacterial suspension of *B. amyloliquefaciens* WCS417 at one week after planting of micropropagated shoots in pots under varying drought induced conditions showed 30 per cent and 60 per cent enhanced antioxidative enzyme activity, respectively under medium stressed and severe stressed conditions and 30-40 per cent higher phenolic accumulation under severe stressed conditions in bacteria inoculated plants with respect to the corresponding uninoculated control plants.

Micropropagated *Mentha piperita* treated with microbial volatile organic compounds emitted by *B. amyloliquefaciens* GB03 under elevated salt stress conditions exhibited enhanced jasmonic acid levels by 25 per cent under non stressed conditions and 2.6 fold enhancements in salicylic acid content under NaCl 75mM salt stressed condition compared to the corresponding control plants (Cappellari and Banchio, 2020).

Jamet *et al*. (2020) reported that inoculation of bell pepper with *B. amyloliquefaciens* at 10^7 cfu mL^{-1} modulated earliness and enhanced nutritional quality

by exhibiting significant increase in concentration of calcium, iron and vitamin C of 561 mg kg^{-1}, 182 mg kg^{-1} and 561 μg 100 g^{-1} dried mass respectively over control.

Peppermint seedlings cultivated under salt stress and treated with volatile organic compounds (VOC) emitted by *B. amyloliquefaciens* GB03 showed 3.3, 5.6 and 6.5 fold increase in essential oil content in plants grown under 0, 75 and 100 mM salt stressed condition over uninoculated control. Similarly the concentrations of menthone, menthol and pulegone were approximately 6.7, 5.8 and 3.4 fold higher in VOC treated, 75 mM salt stressed plants over corresponding control (Cappellari *et al*., 2020)

Biopriming of chilli cultivar Geumsugangsan with *B. amyloliquefaciens* B11 at $1x10^8$ cfu mL^{-1}significantly enhanced the total chlorophyll content by 28.46, 28.85, and 36.04 per cent and salicylic acid content by 46.55, 50.70, and 70.61 per cent under salt, drought and heavy metal stress conditions respectively and protein content were enhanced by 21.45 per cent over control (Kazerooni *et al*., 2021).

2.2.2 *Bacillus pumilus*

B. pumilus are gram positive, endospore forming, rod shaped and predominant soil inhabitants (Priest, 1993). They functions as plant growth promoting rhizobacteria by residing within the root zones of plants and take part in plant growth promotion, nitrogen fixation, stress alleviation and antimicrobial activities through the production of several bioactive substances (Huag *et al*., 2003; Myresiotis *et al*., 2014; Yuan and Gao, 2015; Ansari *et al*., 2019).

2.2.2.1 Effect of Application of *Bacillus pumilus* on Seed Germination and Seedling Growth

The tomato seeds when treated with fresh suspension of *B. pumilus* SE34, enhanced germination of tomato seeds over the untreated control. The treatment had no significant effect on root length but showed a significant increase in shoot length and seedling vigour (Girish and Umesha, 2005).

In a study conducted by Kumar *et al*. (2013) biopriming of coriander and fennel with *B. pumilus* B3 and *B. pumilus* B13 at 10^{6-7} cfu mL^{-1} resulted in 83.33 and 58.33 per cent increase in germination rate respectively over control.

2.2.2.2 Effect of Application of *Bacillus pumilus* on Plant Growth

Red pepper plug seedlings were treated with *B. cereus* MJ-1, *B. macroides* CJ-29, and *B. pumilus* CJ-69 at 10^8 cfu mL^{-1} and *B. pumilus* inoculated seedlings showed the greatest increase in the plant height and root fresh weight of the plants by 12 and 20 per cent respectively compared to other microbial treatments (Joo *et al*., 2004).

Ren *et al*. (2013) reported that *B. pumilus* JK-SX001 treated *Poplar deltoides* seedlings at 10^5 cfu mL^{-1} under green house condition exhibited high increase in biomass production, shoot length and stem diameter with average values 8.68 g, 132.33 cm and 6.80 mm in comparison with that of control with average values 6.25 g, 113.33 cm and 5.24 mm, respectively.

Heidarzadeh and Ravari (2015) reported 60 per cent higher root length and 84 per cent higher shoot length in tomato plants when seedlings were treated with *B. pumilus* strain ToIrMA-KC806242 at 1.5×10^7 cfu mL^{-1} over the untreated control.

A significant increase of 22 per cent were observed in shoot fresh weight of tomato seedlings inoculated with 1 ml of *B. pumilus* at 1 m cc^{-1} 100 units over the uninoculated control plants (Sirajuddin *et al*., 2016).

Xie *et al*. (2019) stated that *Glycyrrhiza uralensis* seedlings treated with 100 mL of bacterial suspension of *B. pumilus* at 10^6 cfu mL^{-1} significantly increased the total biomass by 34.90 per cent over the control.

2.2.2.3 Effect of Application of *Bacillus pumilus* on Yield

Seeds of Snowball variety of cauliflower were inoculated with *B. pumilus* at 10^8 cfu mL^{-1} showed an increase in curd diameter by 78.86 per cent, curd depth by 80.34 per

cent, curd weight by 72.24 per cent and curd yield by 30.85 per cent over unioculated control (Dipta *et al*., 2017).

Seed priming followed by soil drenching of *B. pumilus* YSPMK11 at 9 x 10^8 cfu mL^{-1} at 30 and 50 per cent bloom stage on cauliflower plants infected with *Sclerotinia* showed 36 per cent higher yield over uninoculated control and 24–27 per cent higher yield when compared to commercial fungicides used by the famers in the mid hills of Himachal Pradesh (Kaushal *et al*., 2017).

Seedlings of strawberry cultivar 'Festival' dipped in cell suspension of *B. pumilus* Nos. 2 and 3 at 1x 10^8 cfu ml^{-1} for 30 min significantly raised the yield by 66.70 and 73.30 per cent respectively over uninoculated control (Kareem *et al*., 2021).

2.2.2.4 Effect of Application of *Bacillus pumilus* on Biochemical Parameters

Among three bacterial strains (*B. pumilus* WP8, *Pseudomonas putida* RBP1 and *Erwinia persicinus* RA2) used for treating seedlings of tomato cultivar 'jingdan No. 1' at 10^8 cfu mL^{-1}under induced salt stressed conditions, *B. pumilus* WP8 exhibited significant effects on improving tomato fruit quality by enhancing the water soluble sugar (WSS) and vitamin-C content with a maximum of 6.22±0.28 and 7.73±0.25 per cent WSS and 26.76±1.05 and 27.62±1.95 mg 100 g^{-1} DW of vitamin-C at high salt concentrations respectively in first and second seasons (Shen *et al*., 2012).

The roots of rose cultivar 'Black prince' treated with *B. pumilus* at 10^6 cells ml^{-1} for one hour positively modified the chemical composition of the extracted essential oil and exhibited 26 per cent enhanced essential oil yield (Araujo *et al*., 2020).

The inoculation of *Glycyrrhiza uralensis* seedlings with *B. pumilus* suspension of 10^8 cfu mL^{-1} significantly increased primary metabolites such as soluble sugar, soluble protein, and free amino acids by regulating the C and N metabolic processes and one of the important secondary metabolites, glycyrrhizic acid (Zhang *et al*., 2020).

Tomato seedlings inoculated with 1 ml of *B. pumilus* at 1 m cc^{-1} 100 units under elevated boron concentrations (10, 20 and 50 mg B kg^{-1}) significantly enhanced the leaf chlorophyll contents by 42, 44, and 36 per cent as compared to the respective uninoculated control (Sirajuddin *et al*., 2016).

2.2.3 Bacillus velezensis

Among *Bacillus sp.*, numerous strains of *B. velezensis* are preferred in agriculture as a plant growth promoter, biocontrol agent and stress alleviator. It is a gram positive, endospore forming aerobic bacterium. Bio-active molecules like cyclic lipopeptides, polyketides etc., contribute for its pathogen suppression and plant growth promotion activities. Induced systemic resistance in plants are triggered by the secondary metabolites produced by *B. velezensis* (Chen *et al*., 2007; Rabbee *et al*., 2019)

2.2.3.1 Effect of Application of *Bacillus velezensis* on Seed Germination and Seedling Growth

Devi *et al*. (2020) evaluated twelve endophytic *Bacillus* isolates on germination and vigour of tomato seeds *in vitro*. Among the isolates, *B. velezensis* is recorded the highest germination per cent (95 per cent) and vigour index (1073.50 and 1472.5) at 7^{th} and 14^{th} day, respectively.

Chen *et al*. (2019) reported that pregerminated seeds of peanuts inoculated with *B. velezensis* NDO-2 at 10^9 cfu mL^{-1} concentration displayed significant increase in peanut seedling height, root length, seedling dry weight and root dry weight (40.3 cm, 15.2 cm, 2.59 g and 0.51 g, respectively) compared to control (35.7 cm, 12.1 cm, 2.23 g and 0.43 g, respectively).

B. velezensis NKG-2 primed tomato seeds at concentrations of 6×10^6 to 6×10^9 cfu mL^{-1} under pathogen challenged conditions showed a significant increase in shoot length and vigor index when compared to the control seedlings. (Myo *et al*., 2019).

B. velezensis B006 at 1×10^7 cfu mL^{-1} when applied on brinjal seedlings at 30 DAT, recorded increase in plant height, stem diameter, root length and root dry weight of seedlings by 16.70, 20.70, 28.70 and 67.00 per cent, respectively over uninoculated control (Yan *et al*., 2020).

2.2.3.2 Effect of Application of *Bacillus velezensis* on Plant Growth

Torres *et al*. (2019) reported that tomato, chilli, pumpkin and cucumber plants inoculated with *B. velezensis* XT1 showed an increase in aerial fresh weight by 53.00, 63.60, 129.20 and 100.80 per cent, respectively over the control plants.

Chilli seedlings treated with 20 ml of bacterial suspension of *B. velezensis* BS1 at 1 x 10^7 cfu mL^{-1} at weekly intervals exhibited the average values of plant height (107.8 cm), leaf length (66.7cm), leaf width (37.1 cm), root length (190.8 cm) and root fresh weight (114.3 mg) which were higher than that of untreated plants (76.3 cm, 46.5 cm, 25.5 cm, 115.6 cm, and 30.1 mg, respectively) Shin *et al*. (2021).

2.2.3.3 Effect of Application of *Bacillus velezensis* on Yield

Cabbage seedlings drenched with spore suspensions of different strains of *Bacillus* alone and in combination with 100 ml of 1×10^6 cfu mL^{-1} at the time of transplanting showed significant enhancement in marketable head yield compared to nonbacterized control. Among the various strains, *B. velezensis* AP305 recorded the highest marketable head yield (25.86 lb $plot^{-1}$) (Liu *et al*., 2016).

The scab disease induced potato seeds inoculated with *B. velezensis* 8-4 at 325ml per $667m^2$ recorded 19.91 per cent higher tuber yield over all other treatments including fungicidal treatments (Cui *et al*., 2019).

Chilly seedlings grown in media amended with *B. velezensis* NJAU-Z9 at 1×10^7 cfu g^{-1} dry weight, showed increased fruit yield by 11 and 24 per cent respectively in two crop seasons compared to uninoculated control (Zhang *et al*., 2019).

Rahma *et al*. (2020) reported that soaking of onion seeds in *B. velezensis* B-27 at 10^8 cfu mL^{-1} suspension for 30 min followed by foliar sprays at weekly intervals showed significant increase in the plant height (27.12 cm), number of leaves (23), tillers and bulbs (8) and tuber weight (33.64 kg) compared to control.

Among three bacterial isolates tested against fusarium wilt disease of tomato, seeds that were inoculated with *B. velezensis* PGA106 at 10^7 cfu mL^{-1} suspensions were significantly superior over the others in enhancing plant growth features and they recorded 194.60 per cent increased fruit yield over uninoculated control (Siripornvisal *et al*., 2021).

2.2.3.4 Effect of Application of *Bacillus velezensis* on Biochemical Parameters

Root dipping of *Buglossoides arvensis* plants in *B. velezensis* LBUM288 at 2×10^8 cfu mL^{-1} resulted in significantly higher total lipid and stearidonic acid production by 26.80 and 30.50 per cent respectively, over uninoculated control (Novinscak and Filion, 2018).

Bayisa *et al*. (2020) noticed double fold enhancement of proline content in sesame plants exposed to *B. velezensis* AR1 at a concentration of 10^5 cfu mL^{-1} spore as seed treatment followed by foliar spray at weekly intervals, over the untreated control. Also, the concentration of GA, IAA, leaf chlorophyll, total nitrogen and phosphorus contents were also significantly increased by 203.86 mg g^{-1} fresh weight, 7.79 mg g^{-1} fresh weight, 0.31 mg g^{-1} fresh weight, 0.15 per cent and 0.13 per cent, respectively compared to control.

Apple seedlings, when treated with *B. velezensis* at 2×10^9 cfu mL^{-1} at transplanting followed by application at 70 DAT showed an increase of 29.26 per cent in iron content; 7.22, 11.63, 12.86 per cent, respectively in total nitrogen content of root, stem and leaves; 17.30 and 48.07 percent in total phosphorus content of root and stem; 15.31 and 6.53 per cent potassium content of roots and leaves, over the control (Wang *et al*., 2020).

Anoectochilus roxburghii and *A. formosanus* inoculated with bacterial suspension of *B. velezensis* D2WM and ZJ-11 at 1×10^8 cfu mL^{-1} significantly enhanced the kinsenoside content by 9.33 and 21.65 per cent and flavonoid content by 44.70 and 21.07 per cent respectively, over uninoculated control (Wei *et al*., 2020).

In a study, by Ilmiah *et al*. (2021), snake fruit (*Salacca zalacca*) treated with 1×10^9 cfu mL^{-1} of *B. velezensis* B-27 combined with 10 kg goat manure $plant^{-1}$ exhibited the highest antioxidant (IC50 of 37.83 µg ml^{-1}), flavonoid (5.35 mg GAE 100 g^{-1}) and total phenolic contents (44 mg QE 100 g^{-1}). The average of flavonoid content and total phenol content by *B. velezensis* inoculated plants (5.24 and 4.33 mg QE 100 g^{-1}) was higher than that of uninoculated treatments (5.04 and 4.16 mg QE 100 g^{-1}).

2.3 Plant Growth Promoting Rhizobacteria Consortium

In the rhizospheric zone, PGPRs reside as a community with diverse range of microorganisms and their interaction make the soil ecosystem more dynamic for crop growth (Beneduzi *et al*., 2012; Bhuyan *et al*., 2015). Microbial application in combination is more in line with the environmental sustainability and it might be more beneficial to below-ground biomass of the plant (Bharti *et al*., 2014; He *et al*., 2019). Growth promoting effects of PGPRs on crops are more pronounced in co-inoculation than individual application (Rolli *et al*., 2015; Berg and Koskella, 2018; Vrieze *et al*., 2018). Sometimes bacteria that show no or little effect on single application exhibit better results as consortium (Berendsen *et al*., 2018). *Bacillus* spp. is biocompatible with other microorganism including *Azospirillum* and *Acetobacter* and hence can be used as a component in consortia for crop improvement and soil fertility maintenance (Kashyap *et al*., 2019). A combination of different strains of the same species can also be considered as a consortium and they have been reported to boost the beneficial traits in crops over their single application (Ju *et al*., 2019).

2.3.1 Effect of Application of PGPR Consortium on Plant Growth

Raupach and Kloepper (2000) observed that application of mixture of *B. pumilus* INR7, *Curtobacterium flaccumfaciens* ME1, and *B. subtilis* GB03 at 10^9-10^{10} cfu mL^{-1} in cucumber in season-1 and season-2 recorded the highest average main runner length (85.60 cm and 48.2 cm) over their individual application (83.10, 75.9 and 67.70 cm; 42.2, 39.7 and 40.9 cm) and control (50.5 and 31.9 cm).

Application of mixture of *Bacillus pumilus* and *Pseudomonas alcaligenes* at 1.5 x 10^7 cfu mL^{-1} and 10 ml of *Rhizobium* sp. (1 g fungal mycelium) in lentil yielded the highest plant length and plant dry weight (78.4 cm and 2.18 g $plant^{-1}$) over their individual (73.4 - 73.4 cm and 2.06-2.12 g $plant^{-1}$) and control (68.6 cm and 1.90 g $plant^{-1}$) (Akthar *et al.* 2010).

According to Chakraborty *et al*. (2011), co-application of *B. pumilus* and *Glomus mosseae* in mandarin plants at 175 spores 100 g^{-1}soil enhanced the growth of seedlings in terms of increase in height and number of leaves.

In a study conducted by Anith *et al*. (2015), tomato seedlings treated with co-cultivated *B. pumilus* and *Piriformospora indica* followed by their mixed inoculation exhibited better growth promotion than their individual application. The combination treatments, cocultivation and mixed inoculation recorded significantly higher average values of fresh shoot weight (0.876 and 815 g $plant^{-1}$), dry shoot weight (67.15 and 61.6 mg $plant^{-1}$), fresh root weight (0.138 and 0.128 g $plant^{-1}$), dry root weight (8.43 and 7.19 mg $plant^{-1}$) plant height (9.16 and 9.12 cm) and number of leaves (4.73 and 4.7) over untreated control which recorded 0.401 g $plant^{-1}$, 30.77 mg $plant^{-1}$, 0.065 g $plant^{-1}$, 3.61 mg $plant^{-1}$, 6.80 cm and 3.67 respectively for the said parameters.

Seedling treatment of *Ocimum basilicum* grown under salinity stress with consortium of *B. pumilus* strain NBRC 12092 and *Glomus mossae* for 30 min resulted in 24 per cent increase in leaf fresh weight than their individual application (21.5 and 18 per cent, respectively) compared to uninoculated control (Yadav, 2017).

Co-inoculation of *B. amyloliquefaciens, B. pumilus, B.mojavensis* and *P. putida* in tomato seedlings at a volume of 1 ml each recorded 34.17, 176.32, 27.63 and 34.34 per cent higher total biomass, root fresh and dry weight and root:shoot ratio respectively over uninoculated control (He *et al*., 2019).

Kaushal *et al*. (2019) found that seed priming of bell pepper with rhizobacterial consortia of *B. pumilus* and *B. subtilis* at 9 x 10^8cfu mL^{-1} followed by soil application at 50 per cent bloom stage at 10 mL $plant^{-1}$ significantly enhanced shoot biomass (241.20 per cent), root biomass (119.6 per cent), shoot length (113.2 per cent), root length (92.8 per cent) and pepper yield (397.36 per cent) over untreated control.

Co-inoculation of *B. velezensis* S141 with *Bradyrhizobium diazoefficiens* USDA110 at 1:1 ratio (1 mL of 10^6 cfu mL^{-1}) showed the highest root, total plant dry weight, nitrogen-fixing efficiency and nodulation in soybean plants which were higher than single inoculation by 40.50, 22.90, 55.80 and 29.40 per cent, respectively (Sibponkrung *et al*., 2020).

2.3.2 Effect of Application of PGPR Consortium on Yield

Bell pepper c.v. 'Camelot' showed significantly higher mean fruit yield and mean fruit weight (130.8 and 18.4 kg $plot^{-1}$) compared to control (94 and 12.5 kg $plot^{-1}$) when treated with bio formulation of *B. amyloliquefaciens* IN937a and *B. subtilis* GB03 at 1.2 kg m^{-3} (Herman *et al*., 2008).

Akthar *et al.* (2010) described the growth promotion and disease suppression in lentil plants by application of *Bacillus pumilus* and *Pseudomonas alcaligenes* at 1.5 x 10^7cfu mL^{-1} and 10 ml of *Rhizobium* sp. (1 g fungal mycelium) together. This treatment recorded significantly higher pod yield (44 and 34 pods $plant^{-1}$) over untreated control (20 and 14 pods $plant^{-1}$) under normal and *Fusarium oxysporum* stressed conditions, respectively.

Among different formulations of bacterial strains with varying combinations applied as soil drench around six year old pepper vines, a combination containing the strains *B.velezensis* KN12 and DS29, *B. amyloliquefaciens* DL1, *B. subtilis* BH15 and V1.21 and *B. cereus* CS30 at 10^9 cfu g^{-1} recorded increased chlorophyll a and b content and 4.5 per cent increase in yield compared to uninoculated control (Nguyen *et al*., 2021).

2.3.3 Effect of Application of PGPR Consortium on Biochemical Parameters

The application of PGPR consortia 1 comprising of *B. pumilus* SE34, T4 and INR 7 and consortia 2 comprising of *B. pumilus* SE34, T4 and *P. fluorescens* UOM14 in Moringa, showed 405.70 and 105.83 per cent enhancement in leaf Fe content, respectively over uninoculated control (Sonbarse *et al*., 2017).

Amaranth seeds bacterized with talc formulation of *B. pumilus* and *B. subtilis* at 8 g kg^{-1} of seeds showed enhanced nutritional quality by improving crude protein, dry matter, fat and essential amino acids including methionine, lysine and tryptophan respectively by 22.13, 32.25, 30.77, 47.68, 59.41 and 38.05 per cent (Panday *et al*.,2018).

Guo *et al*. (2020) observed that quality of chilli fruits were enhanced on inoculation with PGPR formulation of *B. amyloliquefaciens* GB03 with *Bacillus* sp. WM13-24, *Pseudomonas* sp. M30 and *Sinorhizobium meliloti* ACCC17578 at 1:1:1:1 ratio. The contents of soluble protein, ascorbic acid and total organic acids of chilli were significantly increased by 3.60, 17.76 and 18.00 per cent under PGPR treatment compared to the control.

MATERIALS AND METHODS

3 MATERIALS AND METHODS

The study entitled “Growth, yield and secondary metabolite production responses to microbial elicitation in *Withania somnifera* (L.) Dunal.” was conducted at the Department of Plantation Crops and Spices, College of Agriculture, Vellayani, Thiruvananthapuram during 2019-2021. The study aimed at evaluating the effect of bacterial inoculants on seed germination, seedling vigour, growth, yield and secondary metabolite production in *W. somnifera*. The study was conducted in two phases. Phase 1-Effect of bioprimimg of seeds on seed germination and seedling growth; Phase 2-Effect of bacterial inoculants on plant growth, yield and secondary metabolite production.

The details of the materials used and methods employed during the course of the study are elaborated in this chapter.

3.1 PHASE 1: EFFECT OF BIOPRIMING ON SEED GERMINATION AND SEEDLING GROWTH

3.1.1 Experimental Material

The seeds of *W. somnifera* for the study were procured from Anand Agricultural University, Gujarat and the microbial cultures were obtained from the Department of Agricultural Microbiology, College of Agriculture, Vellayani.

3.1.2 Design of the Experiment

The experiment was conducted in Completely Randomized Design (CRD) with nine treatments replicated thrice with 50 seeds per replication.

3.1.3 Biopriming of the Seeds with *Bacillus* spp.

The priming of seeds was done using the *Bacillus* spp**.,** *Bacillus amyloliquefaciens* VLY-24, *Bacillus pumilus* VLY- 17 and *Bacillus velezensis* PCSE-10 and their combinations.

3.1.3.1 Media Preparation

Nutrient agar medium was used to grow the bacterial inoculants. The media comprised of peptone (0.5 per cent), beef extract (0.3 per cent), agar (1 per cent), NaCl (0.5 per cent) and distilled water. It was prepared by suspending 28 g of nutrient agar powder (Hi Media Laboratories, Mumbai) in 1 L of distilled water. The completely dissolved mixture was autoclaved at 121°C for 15 min. After cooling, the medium was poured into sterilized Petri plates @ 25 mL $plate^{-1}$ and kept undisturbed until the agar got solidified.

3.1.3.2 Preparation of Bacterial Suspension for Seed Soaking

The pure cultures of *B. amyloliquefaciens* VLY-24, *B. pumilus* VLY- 17 and *B. velezensis* PCSE-10 (Plate 1A, 1B and 1C respectively) were heavily cross streaked on nutrient agar medium and incubated at room temperature for 48 h. After incubation, 10 mL of sterile water was poured into all the plates and bacterial inoculants were scraped out and collected in centrifugal tubes. The tubes were centrifuged to get uniformly distributed bacterial suspension. After centrifugation, OD values of the suspension cultures were read at 600 nm and the OD values were adjusted to 0.8 by adding sterile water as required to bring bacterial concentration in the suspension to 10^8 cfu mL^{-1}.

3.1.3.3 Biopriming of Seed

Uniform sized, healthy seeds of *W. somnifera* were surface sterilized with 0.1 per cent of $HgCl_2$ for one min and soaked in suspension of the following bacterial inoculants for 24 h (Plate 2A). The *Bacillus* spp. and their combinations used for priming the seeds are depicted in Table 1.

Plate 1: Pure cultures of *Bacillus* spp: (A): *Bacillus amyloliquefaciens* VLY-24; (B): *Bacillus pumilus* VLY- 17; (C): *Bacillus velezensis* PCSE-10

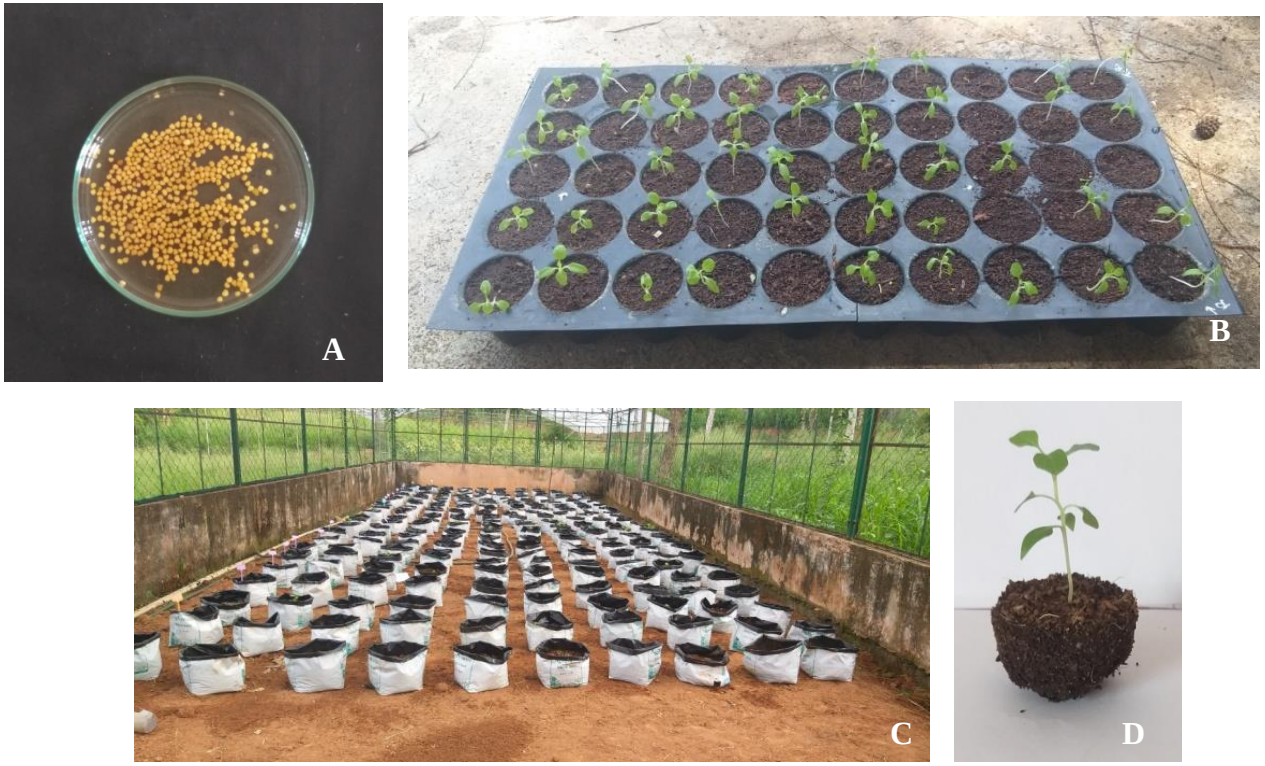

Plate 2: Seed biopriming and seedling growth in *W. Somnifera* (A): Bioprimed seeds of *W. somnifera*; (B): 30 days old seedlings in portrays (C): Polybags filled with the potting media (D): 45 day old seedling of *W. somnifera*

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Nutrient agar medium was used to grow the bacterial inoculants. The media comprised of peptone (0.5 per cent), beef extract (0.3 per cent), agar (1 per cent), NaCl (0.5 per cent) and distilled water. It was prepared by suspending 28 g of nutrient agar powder (Hi Media Laboratories, Mumbai) in 1 L of distilled water. The completely dissolved mixture was autoclaved at 121°C for 15 min. After cooling, the medium was poured into sterilized Petri plates @ 25 mL $plate^{-1}$ and kept undisturbed until the agar got solidified.

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Table 1. Seed priming using *Bacillus* spp. to evaluate seed germination and seedling growth

Treatment No.	Priming treatments
T1	*B. amyloliquefaciens* VLY-24 (*Bam*)
T2	*B. pumilus* VLY- 17 (*Bp*)
T3	*B. velezensis* PCSE-10 (*Bv*)
T4	*B. amyloliquefaciens* VLY-24 + *B. pumilus* VLY- 17 (*Bam+ Bp*)
T5	*B. amyloliquefaciens* VLY-24 + *B. velezensis* PCSE-10 (*Bam+Bv*)
T6	*B. pumilus* VLY- 17 + *B. velezensis* PCSE-10 (*Bp+Bv*)
T7	*B. amyloliquefaciens* VLY-24 + *B. pumilus* VLY- 17+ *B. velezensis* PCSE-10 (*Bam+ Bp+Bv*)
T8	Hydropriming
T9 (control)	Untreated

3.1.4 Raising of Seedlings

Seeds pretreated with respective bacterial suspension cultures were sown in 50 celled polythene protrays containing soil and vermicompost in the ratio of 1:1. Each treatment was replicated thrice in such a way that one protray represented one replication. The seeds were sown at the rate of one seed per cell by making a small depression (0.5 cm) in the centre of the cell and covered with thin layer of medium. Protrays were irrigated daily with tap water using a rose can to maintain optimum moisture for better germination and growth. The seedlings were maintained for 45 days in protrays. The seedlings (30 days old) in portrays is presented in Plate 2 B.

3.2 **Phase 2: Effect of *Bacillus* spp. on Growth, Yield and Secondary Metabolite Production.**

The variation in growth, yield and secondary metabolite production of *W. somnifera* in response to different bacterization treatments (biopriming followed by seedling dip with respective bacterial inoculants) were analyzed in the second experiment.

3.2.1 Experimental Material

Uniform sized seedlings of 45 days old with 3-4 leaves from the Phase-1 (Plate 2 D) study were selected and transplanted after root dip in the respective bacterial suspension for 30 min to UV stabilized grow bags of size of 40 cm × 24 cm × 24 cm with 600 gauge thickness and 15 kg capacity. Each grow bag was filled with 13 kg of the potting medium comprised of soil, sand and well decomposed farmyard manure at 2:1:1 ratio (Plate 2 C). Transplanting was done at the rate of one seedling per bag.

3.2.2 Design of the Experiment

The experiment was conducted in Completely Randomized Design (CRD) with eight treatments replicated thrice, with 10 plants per replication. The treatments of the Phase 2 study are presented in Table 2.

Table 2. Seedling inoculation using *Bacillus* spp. to evaluate plant growth, yield and secondary metabolite production

Treatment No.	Priming treatments
T1	*B. amyloliquefaciens* VLY-24 (*Bam*)
T2	*B. pumilus* VLY- 17 (*Bp*)
T3	*B. velezensis* PCSE-10 (*Bv*)
T4	*B. amyloliquefaciens* VLY-24 + *B. pumilus* VLY- 17 (*Bam+ Bp*)
T5	*B. amyloliquefaciens* VLY-24 + *B. velezensis* PCSE-10 (*Bam+Bv*)
T6	*B. pumilus* VLY- 17 + *B.velezensis* PCSE-10 (*Bp+Bv*)
T7	*B. amyloliquefaciens* VLY-24 + *B. pumilus* VLY- 17+ *B. velezensis* PCSE-10 (*Bam+ Bp+Bv*)
T8	Untreated (Control)

3.2.3 Aftercare

Necessary cultural practices for crop growth and establishment were carried out regularly. Plants were irrigated on alternate days. Weeding was done manually when needed. White flies were observed during the initial and flowering stages of crop growth

and were effectively controlled using biocontrol agent *Lecanicillium lecanii*. The crop was maintained in polybags for 180 days. Staking of the crop at 60 DAS and pest incidence in *W. somnifera* is depicted in Plate 3 and 4 respectively.

3.2.4 Harvest

W. somnifera were harvested at 180 days after sowing. The maturity for harvest was determined based on the yellowing of berries (Plate 5). A light irrigation one day prior to harvest was given to facilitate easy uprooting of plants. Roots and shoots were separated by cutting transversely at the base of the shoot. They were washed separately with clean water to remove adhering soil particles.

3.2.5 Drying and Sample Preparation

Drying was carried out in hot air oven at 50°C separately for each plant parts until constant weight has been obtained. The harvested roots after cleaning were dried for 72 h. Stem portions, after removing the leaves were chopped into small pieces and dried for 48 h. Leaves were dried for 36 h after keeping in shade for a day. Flowers along with unopened buds were dried for 24 h, while fruits were dried for 48 h. The dried leaves and roots were ground and sieved and taken for chemical analysis.

3.3 Observations on Seed Germination and Seedling Vigour of Bioprimed Seeds of *W. somnifera*.

Three seedlings from each replication were randomly selected and tagged for recording observations on seedling parameters,

3.3.1 Days to Initial Sprouting

Bioprimed seeds were sown in 50 celled protrays at the rate of one seed per cell. Days to first sprouting from the date of sowing were counted for each replication of each treatment and the mean was calculated and expressed in days.

3.3.2 Germination Per Cent

Influence of biopriming on seed viability was studied by observing germination per cent. It was calculated based on the number of seeds germinated till the end of experiment-1 and expressed in per cent.

Germination per cent (G) = N/T x 100

where,

N = number of seeds germinated until the last day of the experiment

T = number of seeds sown

3.3.3 Survival Per Cent

Survival per cent was calculated by counting the number of seedling remained alive at the end of first experiment in relation to total number of seeds sown and expressed in percentage.

Survival per cent (S) = N/T x 100

where N- Number of surviving plants at end of the study

T - Number of seeds sown

3.3.4 Germination Index

Germination index was calculated as described by the Association of Official Seed Analysis (AOSA, 1983) using the following formula.

Germination index (GI) = $(X_1/Y_1) + (X_1-X_2/Y_2) +\ldots\ldots\ldots\ldots\ldots\ldots+ (X_n-X_{n+1}/Y_n)$

where,

X_1 - Number of seeds germinated on first day

X_2 - Number of seeds germinated on second day

X_n - Number of seeds germinated on n^{th} day

Y_1 - Number of days from sowing to first count

Y_2 - Number of days from sowing to second count

Y_n - Number of days from sowing to n^{th} count


Plate 3: Staked plants of *W. somnifera* 60 DAS


Plate 4: Whiteflies observed during the initial stages of crop growth


Plate 5: *W. somnifera* plants ready for harvest (180 DAS)

3.3.5 Mean Germination Time

The measure of rate and time spread of germination or the length of lag period from initiation of imbibition to radical emergence is called mean germination time (MGT). It was calculated using the formula given by Mudaris (1998) and expressed in days.

$$MGT = \Sigma F_i n_i / \Sigma N$$

Where,

Fi - Number of seeds germinated at time t

ni - Days from sowing

N - Total number of germinated seeds

3.3.6 Seedling Vigor Index

Seedling vigour index was estimated following the methodology suggested by Baki and Anderson (1973).

Seedling vigor index (I) = germination per cent x seedling length (cm)

3.3.7 Basal Shoot Girth

Basal shoot girth of the tagged seedlings was taken by measuring the circumference at the collar region using a twine and scale on 45 days after sowing. The mean was calculated and expressed in centimetre (cm).

3.3.8 Number of Leaves

The total number of leaves of the 45 day old tagged seedlings was counted and the mean was worked out and expressed in numbers.

3.3.9 Shoot Length

At 45 DAS, shoot length of the observational seedlings was measured from collar region to apex of the shoot using a ruler. The mean was calculated` and expressed in centimetre (cm).

3.3.10 Root Length

Root length of 45 day old tagged seedlings was measured from collar region to tip of the primary root using a measuring tape and observations were recorded. The mean was calculated and expressed in centimetre (cm)

3.3.11 Seedling Length

Seedling length of 45 day old tagged seedlings was taken by measuring the length of seedlings from to tip of the primary root to apex of the shoot using a ruler. The mean was expressed in centimetre (cm)

3.3.12 Root Volume

Water displacement technique was used to calculate the root volume. The roots of tagged plants were collected and washed to remove adhering soil particles. The roots were then carefully wiped using a soft cloth and immersed in a graduated cylinder containing known volume of water (10 ml). Rise in water level after root dipping was noted down and volume of the root was calculated using the following formula and expressed in centimetre cube (cm^3).

Root volume = V_1-V_2

where; V_1 - volume of the water after immersing the roots into the cylinder

V_2 - volume of the water taken

3.3.13 Allometric Index

Allometric index is the ratio of length of the root to length of the shoot of a plant. It was calculated as follows.

Allometric Index = Root length / Shoot length

3.4 Effect of *Bacillus* spp. on Growth, Yield and Secondary Metabolite Production

The observations were recorded from randomly selected and tagged three plants from each replication. The plant growth parameters were recorded at 30 days interval from transplanting to harvest (30DAT, 60DAT, 90DAT, 120DAT and at harvest). The yield and biochemical parameters were recorded at harvest. The methodologies adopted for the study are described below.

3.4.1 Plant Growth Parameters

3.4.1.1 Days to First Flowering

Tagged plants were inspected regularly and days to first flowering were recorded by counting the number of days taken for first flowering from the date of sowing. The mean was calculated and expressed in days.

3.4.1.2 Days to Fruit Set

Tagged plants were inspected regularly and days to fruit set were recorded by counting the number of days taken for fruiting from the date of flowering. The mean was worked out and expressed in days.

3.4.1.3 Shoot Length

Shoot length was recorded at 30 days interval by measuring the length of the primary shoot from ground level to the tip of the young fully opened leaf of the tagged plants. The average was worked out and recorded in centimetre (cm).

3.4.1.4 Number of Branches

Number of branches arising from the primary shoot of the observational plants at 30 days interval was counted. The mean was calculated and recorded in number.

3.4.1.5 Number of Leaves

Number of leaves of the tagged sample plants at 30 days interval was counted and average was worked out and expressed in number.

3.4.1.6 Collar Girth

Collar girth of the tagged plants was taken by measuring the circumference at the collar region using a twine and scale at 30 days interval. The mean was worked out and expressed in centimeters (cm).

3.4.1.7 Leaf Area

Leaf area of the tagged plants was calculated by measuring the maximum length and width of fifth leaf from the tip of labeled plant. This was multiplied by a constant value k (0.7028) and further multiplied with number of leaves per plant. The average value was recorded in centimetre square (cm^2) (Patidar *et al*., 1990). The process was repeated every 30 days from transplanting to harvest.

3.4.1.8 Number of Flowering Branches

Number of flowering branches arising from the primary shoot of the observational plants was counted at 30 days interval. The mean was worked out and recorded in number.

3.4.2 Yield and Yield Components

3.4.2.1 Stem Weight (Fresh and Dry)

The stem portions of the tagged plants were separated from leaves and berries. It was then cut into small pieces of 5 cm length and the fresh weight was taken. Drying was carried out as mentioned in 3.2.5. Dry weight of the stem was taken and average values were expressed in g plant^{-1}.

3.4.2.2 Leaf Weight (Fresh and Dry)

The fresh leaves were separated from the shoots and weighed. The fresh leaves were dried as mentioned in 3.2.5 after keeping them in shade for a day and weighed. The mean fresh and dry weight of the leaves were calculated and expressed in g plant^{-1}.

3.4.2.3 Berry Weight (Fresh and Dry)

The fully matured fruits of labeled plants were collected and the fresh weight was taken. After drying as mentioned in 3.2.5, dry weight of the berries was obtained. The mean fresh and dry weight were worked out and expressed in g plant^{-1}.

3.4.2.4 Shoot weight (Fresh and Dry)

Tagged plants were uprooted and shoots were separated from roots by cutting from the collar region at harvest. The shoots were weighed fresh. The shoots (leaf, stem, flowers, unopened buds and berries) were then dried as mentioned in 3.2.5 and weighed to obtain the dry weight. The average values were determined and expressed in gram (g).

3.4.2.5 Number of Berries Plant^{-1}

Fully matured fruits of labeled plants were collected and counted. Mean value was worked out and expressed in numbers.

3.4.2.6 Seed Yield Plant^{-1}

Fruits from the observational plants were collected and dried as mentioned in 3.2.5. The dried fruits were crushed and winnowed to separate the seeds and weight was taken. The average value was calculated and expressed in grams per plant (g plant^{-1}).

3.4.2.7 100 Seed Weight

A number of 100 seeds were counted from each sample lot and weighed. The average was worked out and expressed in gram (g).

3.4.2.8 Root Length

The length of primary root of the tagged plants was measured using a measuring tape at harvest. The mean value was calculated and expressed in centimetre (cm).

3.4.2.9 Root Diameter

The root diameter at the thickest portion of the tagged plants of each replication was measured using Vernier calipers. The average was calculated and expressed in centimetre (cm).

3.4.2.10 Root Volume

Root volume for the observational plants were calculated as mentioned in 3.3.11 and expressed in cm^3.

3.4.2.11 Root Yield per Plant (Fresh and Dry)

Labeled plants were uprooted without damaging and washed with clean water. After draining the water the roots were weighed. The mean of the fresh weight was worked out. Dry weight was estimated after drying as mentioned in 3.2.5. The fresh and dry weights were expressed in g plant^{-1}.

3.4.2.12 Total Dry Matter Production

The plants were dried as mentioned in 3.2.5 and total dry matter production was estimated after weighing. The average was worked out and expressed in g plant^{-1}.

3.4.2.13 Harvest Index

The ratio of economic product of a plant to its total biomass is called harvest index. It was calculated using the following formula. The harvest index was calculated separately, in terms of leaves and roots.

Harvest Index (HI) = Dry weight of the economic part / Dry weight of the whole plant

3.4.3 Biochemical Estimation

3.4.3.1 Chlorophyll Content

Chlorophyll extraction and estimation from the leaf samples were done according the procedure given by Arnon (1949). Leaf samples were collected at harvest and finely cut into small bits. Leaf bits (0.5 g) were ground with 5ml of DMSO (Dimethyl sulphoxide) and kept overnight. The volume of the mixture was made up to 25 mL with DMSO. Absorbance was read at 645 and 663 nm against DMSO as blank. Chlorophyll content present in the sample was calculated as below.

Chlorophyll a (mg/g fresh wt.) = $(12.7 \times A_{663} - 2.69 \times A_{645}) \times V/1000 \times W$

Chlorophyll a (mg/g fresh wt.) = $(22.9 \times A_{645} - 4.68 \times A_{663}) \times V/1000 \times W$

Total Chlorophyll (mg/g fresh wt.) = $(20.2 \times A_{645} + 8.02 \times A_{663}) \times V/1000 \times W$

Where;

A = Absorbance at respective wavelengths (645 and 663 nm)

V = Volume of the chlorophyll extract (mL)

W = Fresh weight of the sample (g).

3.4.3.2 Total Alkaloid Content in Roots

The total alkaloid content was determined by the procedure described by Shamsa *et al*. (2008). The dried root powder (1mg) was dissolved in 1ml dimethyl sulphoxide and 1mL of 2N HCl was added. The extract were filtered and transferred to a separating funnel. Into this 5 ml of bromocresol green solution followed by 5 mL of phosphate buffer were added. The mixture was shaken with chloroform and collected in a 10 ml volumetric flask. Working standard solutions of atropine (20, 40, 60, 80 and 100 µg) were prepared in the same manner and absorbance was determined against reagent blank at 430 nm. The concentration of alkaloid was expressed in µg mg^{-1} of the sample. The yield of total alkaloid on per plant basis was calculated by multiplying the concentration of total alkaloids in leaf in mg with total dry leaf yield per plant and average was expressed in µg $plant^{-1}$.

3.4.3.3 Total Carbohydrate Content in Roots

Total carbohydrate content in roots was estimated using anthrone reagent method suggested by Sadasivam and Manickam (2008). The dry root powder of 100 mg was hydrolyzed by keeping in water bath after adding 5mL of 2.5 N- HCl for 3 h. After neutralizing with solid sodium carbonate the volume was made to 100 mL. The sample was centrifuged at 8000 rpm for three min and the supernatant was collected. From this, 1mL of the supernatant taken in test tubes served as test samples. Working standard was prepared from stock solution (100 mg glucose in 100 mL distilled water) by diluting 10 ml of stock solution with 100 mL distilled water. Then 0.2, 0.4, 0.6, 0.8 and 1 mL of working standard were pippetted out into a series of test tubes and made upto 1 mL by adding distilled water. Blank were made by taking 1mL of distilled water in a test tube. Each tube was added with 4 mL of anthrone reagent (200 mg of anthrone in 100 mL of sulphuric acid). They were heated over water bath for eight min and cooled to room temperature. Absorbance was read at 630 nm in spectrophotometer. Standard graph were plotted with concentration of the standard on the X- axis and absorbance on the Y- axis. Amount of carbohydrate present in the sample tube were calculated from the graph as

Amount of carbohydrate present in 100 mg of the sample = mg of glucose × 100/ volume of test sample

3.4.3.4 Total Protein Content in Roots

Total protein content in roots was estimated according to Lowry's method of protein estimation (Sadasivam and Manickam, 2008). The root sample was ground using 10 ml of tris buffer. The extract was centrifuged and supernatant was collected. Working standards of 0.2, 0.4, 0.6, 0.8 and 1.0 ml and 0.5 mL of sample extract were pippeted out into a series of test tubes. The volume was made upto 1 mL by adding distilled water in each tube. A 5 mL of alkaline copper solution was added to each sample and allowed to cool for ten min. 0.5 mL of Folin Ciocalteu reagent was added in all the tubes. After thorough mixing, the complex was incubated at room temperature for 30 min. Absorbance was recorded at 660 nm against distilled water blank containing 5 mL of alkaline copper solution. Standard graph was plotted and the amount of protein was expressed in mg g^{-1}.

3.4.3.5 Total Withanolide Content in Roots

Quantitative estimation of total withanolide content in roots was determined from modified spectrophotometer method proposed by Mishra (1994). Dried roots of the plant were ground and sieved. 500g of 60 mesh dried root powder was soaked in 25 mL of ethyl alcohol overnight at room temperature with occasional stirring. The extract was then filtered through Whatman No. 1 filter paper using ethyl alcohol and the residue was washed twice. The final volume was made upto 50 mL and an aliquot of 1ml taken in a test tube served as test sample. Working standard was prepared from stock solution (by diluting 10 mL of stock solution to 100 mL with conc. H_2SO_4. Glacial acetic acid (AR grade) 2 mL followed by colour reagent 2 mL of (8 ml of stock solution - ferric chloride hexahydrate 21.5 g dissolved in 100 mL of orthophosphoric acid and diluted to 100 ml with conc. H_2SO_4) was added and kept over ice bath for 5 min. The absorbance was recorded at 540 nm and the concentration of withanolides was calculated using standard

graph for cholesterol and expressed in µg mg^{-1}. The yield of total withanolide on per plant basis was calculated by multiplying the concentration of total withanolides in root with total dry root yield per plant and the average was expressed in µg $plant^{-1}$.

3.4.4 Statistical Analysis

Analysis of variance was calculated based on Completely Randomized Design using the web application KAU GRAPES (Gopinath *et al.*, 2020).

RESULTS

4. RESULTS

The study entitled “Growth, yield and secondary metabolite production responses to microbial elicitation in *Withania somnifera* (L.) Dunal.” was conducted at the Department of Plantation Crops and Spices, College of Agriculture, Vellayani, Thiruvananthapuram during 2019-2021. The plants subjected to the different microbial treatments were observed for various morphological, yield and biochemical parameters. The collected data were statistically analyzed and the results obtained are presented in this chapter.

4.1 PHASE 1- EFFECT OF BIOPRIMING ON SEED GERMINATION AND SEEDLING GROWTH

The observations pertaining to the effect of biopriming of seeds of *W. somnifera* with bacterial inoculants either individually or in combination, on seed germination and seedling growth parameters were recorded in the first phase of the study.

4.1.1 Days to Initial Sprouting

A significant variation in days to initial sprouting of *W. somnifera* has been observed among various microbial treatments (Table 3). The earliest germination was recorded in T7 (Mixture of *B. amyloliquefaciens, B. pumilus* and *B. velezensis*) (5.33 days) and it was on par with T1 (*B. amyloliquefaciens*), T2 (*B. pumilus*), T3 (*B. velezensis*), T4 (combination of *B. amyloliquefaciens* and *B. pumilus*) and T5 (combination of *B. amyloliquefaciens* and *B. velezensis*). T6 (combination of *B. pumilus* and *B. velezensis*) took 6.67 days to germinate. The control treatment (T9) took the longest time to germinate (9.00 days) and it was on par with the T8 (hydropriming) which recorded 8.00 days to germinate.

4.1.2 Germination Per Cent

The germination per cent significantly varied among the different treatments (Table 3). T7 recorded the highest germination of 96.67 per cent and it was on par with T1, T2, T4 and T5 (92.00, 90.6, 94.00 and 92.67 per cent respectively). The lowest germination was observed in control T9 (70.67 per cent). Plate 6 depicts the germination of trio combination (T7) treated *W. somnifera* and control plants

4.1.3 Survival Per Cent

The data on survival per cent of *W. somnifera* is presented in Table 3. The highest survival per cent of 92.67 was recorded in T7. It was observed to be on par with T1, T2, T3, T4 and T5 (83.33, 84.67, 88.00, 89.33 and 82.00 per cent respectively). Control treatment (T9) recorded the lowest survival of 48.67 per cent.

4.1.4 Germination Index

The germination index significantly varied among all the treatments tried (Table 3). The highest germination index (6.15) was recorded in T6, which was on par with T1, T2, T4, T5 and T7 (4.86, 4.85, 5.30, 5.33 and 5.64, respectively). T9 (control) showed the lowest germination index (2.91) which was on par with T8 (3.89).

4.1.5 Mean Germination Time

The different treatments tried varied significantly with respect to mean germination time. The data is presented in Table 3. The minimum mean germination time was recorded in T6 (8.67) and it was on par withT2, T4, T5 and T7 (10.39, 10.34, 10.01 and 9.77 respectively). T9 exhibited the highest mean germination time of 12.91which was on par with T1, T3 and T8 (11.16, 11.04 and 12.40, respectively).

4.1.6 Leaf Area

The data on leaf area of the seedlings at 45 DAS is presented in Table 4. Significantly higher values (13.38 cm^2) of leaf area were observed in T7. T7 was on par with T5 (12.04 cm^2). T9 recorded the least leaf area (2.40 cm^2). The hydropriming treatment (T8) recorded 3.94 cm^2 which was on par with T1 and T4 (4.64 and 5.30 cm^2 respectively).

4.1.7 Basal Shoot Girth

The data on basal shoot girth of the seedlings of *W. somnifera* at 45 DAS is presented in Table 4. There was significant variation among all the treatments tried. The highest basal shoot girth was observed in T7 (0.81 cm) which was on par with T3, T5 and T6 (0.68, 0.66 and 0.77 cm, respectively). T9 recorded the lowest basal shoot girth (0.48 cm) and that was on par with T4 and T8 (0.63 and 0.53 cm, respectively).

4.1.8 Number of Leaves

The data on the total number of leaves in the seedlings has been depicted in Table 4. T7 recorded significantly higher (6.07) number of leaves which were on par with T2, T4 and T5 (5.87, 5.67 and 5.80, respectively). The lowest (3.67) number of leaves was observed in T9.

4.1.9 Shoot length

The shoot length of the seedlings varied significantly among the treatments (Table 4). T7 recorded the highest shoot length (5.77 cm) whereas T9 recorded the lowest (3.20 cm).

4.1.10 Root Length

Significant variation was observed in root length among the different treatments tried. The data on root length of the seedlings is presented in Table 4. The roots were

significantly longer in T7 (4.16 cm) and was on par with T6 (3.34 cm). T9 exhibited the lowest value of 1.52 cm, which was observed to be on par with T8 (2.27 cm).

4.1.11 Seedling Length

The data on seedling length of *W. somnifera* at 45 DAS are presented in Table 4. The seedling length significantly differed with respect to the treatments tried. The highest seedling length (9.92 cm) was observed in T7.The lowest seedling length (5.14 cm) was recorded in the control treatment, T9. The effect of biopriming on seedling length of *W. somnifera* at 45 DAS is presented in Plate 7.

4.1.12 Root Volume

Root volume also recorded significant variation among the treatments tried. The root volume was significantly higher in T7 (0.54 cm^3), which was on par with T4 and T6 (0.36 cm^3). T9 recorded the lowest (0.13 cm^3) root volume. It was on par with T1, T2, T3, T5 and T8 (0.27, 0.24, 0.27, 0.27 and 0.16 cm^3, respectively) (Table 4).

4.1.13 Allometric Index

Allometric index differed significantly among the priming treatments tried. The data on allometric index is revealed in Table 4. T6 recorded the highest value (0.76), which was observed to be on par with T1, T2, T3, T4, T5 and T7 (0.59, 0.60, 0.67, 0.65, 0.70 and 0.72. respectively). T9 recorded the least (0.46) allometric index and was on par with T1, T2 and T8 (0.59, 0.60 and 0.52 respectively).

4.1.14 Seedling Vigour Index

A significant variation in seedling vigour index was observed in all the treatments applied (Table 4). The significantly highest and lowest seedling vigour index was recorded in T7 (958.93) and T9 (341.96), respectively.

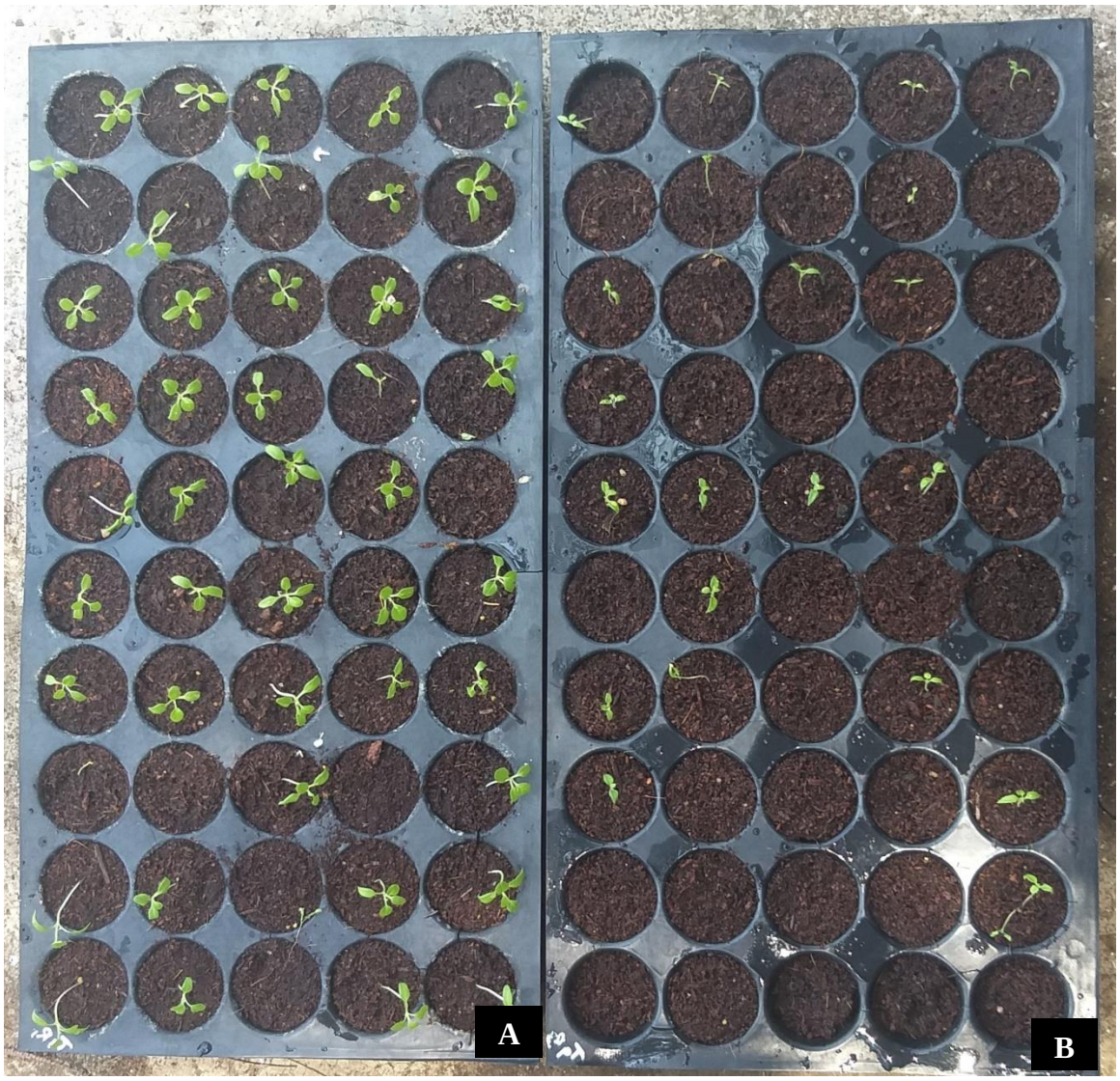

Plate 6: Effect of biopriming on germination of *W. somnifera;* (A): *B. amyloliquefaciens* + *B. pumilus* +*B. velezensis*; (B): control

Table 3. Effect of biopriming on seed germination parameters of *W. somnifera*

T. No.	SPT	DIS	GP	SP	GI	MGT
T1	*Bam*	6.33±0.58^{bc}	92.00±2.00^{abc}	83.33±8.33^{abc}	4.86±0.27^{abc}	11.16±0.93^{abc}
T2	*Bp*	6.00±1.00^{bc}	90.67±4.16^{abc}	84.67±8.08^{abc}	4.85±0.22^{abc}	10.39±1.02^{bcd}
T3	*Bv*	6.33±0.58^{bc}	89.33±4.62^{bc}	88.00±6.93^{ab}	4.77±0.47^{bc}	11.04±1.86^{abc}
T4	*Bam+Bp*	6.00±0.58^{bc}	94.00±3.46^{ab}	89.33±8.08^{ab}	5.30±0.58^{ab}	10.34±0.52^{bcd}
T5	*Bam+Bv*	5.67±0.58^{bc}	92.67±3.06^{abc}	82.00±4.00^{abc}	5.33±0.11^{ab}	10.01±0.81^{cd}
T6	*Bp+Bv*	6.67±0.58^{b}	86.67±7.57^{cd}	78.67±9.02^{bc}	6.15±2.16^{a}	8.67±2.03^{d}
T7	*Bam+Bp+Bv*	5.33±0.58^{c}	96.67±4.16^{a}	92.67±1.16^{a}	5.64±0.24^{ab}	9.77±0.35^{cd}
T8	Hydropriming	8.00±1.00^{a}	82.00±4.00^{d}	72.67±3.06^{c}	3.89±0.46^{cd}	12.40±2.03^{ab}
T9	Control	9.00±0.00^{a}	70.67±3.06^{e}	48.67±9.87^{d}	2.91±0.27^{d}	12.91±0.97^{a}
SE m (±)		0.37	2.47	4.10	0.5	0.76
C.D. (0.05)		1.10	7.32	12.18	1.34	2.26

T.No: Treatment number; SPT: Seed priming treatment; DIS: Days to initial sprouting; GP: Germination percent; SP: Survival per cent; GI: Germination index; MGT: Mean germination time. Each figure represents mean (± SD) of three replications. Figures followed by same letter in a column do not differ significantly (P>0.05)

Table 4. Effect of seed biopriming on seedling growth parameters of *W. somnifera* at 45 DAS

T. No.	SPT	LA (cm^2)	BSG (cm)	NoL	ShL (cm)	RL (cm)	SL (cm)	RV (cm^3)	AI	SVI
T1	*Bam*	4.64±0.53^{cd}	0.64±0.07^{bc}	5.07±0.31^{cd}	4.29±0.55^{bc}	2.90±0.19^{bc}	7.89±0.10^{b}	0.27±0.03^{bc}	0.59±0.06^{abc}	724.76±18.17^{b}
T2	*Bp*	8.09±0.46^{b}	0.64±0.07^{bc}	5.87±0.12^{a}	4.84±0.28^{bc}	2.83±0.20^{bc}	7.68±0.37^{bc}	0.24±0.07^{bc}	0.60±0.05^{abc}	695.16±10.64^{b}
T3	*Bv*	5.35±1.18^{c}	0.68±0.14^{abc}	5.33±0.31^{bc}	4.40±0.20^{bc}	2.92±0.36^{bc}	7.32±0.37^{bc}	0.27±0.12^{bc}	0.67±0.09^{ab}	654.44±53.72^{bc}
T4	*Bam+Bp*	5.30±0.61^{cd}	0.63±0.00^{bcd}	5.67±0.23^{ab}	4.92±0.43^{b}	3.11±0.18^{b}	8.03±0.34^{b}	0.36±0.05^{ab}	0.65±0.10^{ab}	755.00±39.13^{b}
T5	*Bam+Bv*	12.04±0.09^{a}	0.66±0.10^{abc}	5.80±0.40^{a}	4.98±0.13^{b}	2.91±0.85^{bc}	7.04±1.31^{bc}	0.27±0.20^{bc}	0.70±0.14^{ab}	655.40±143.96^{bc}
T6	*Bp+Bv*	6.84±0.49^{b}	0.77±0.07^{ab}	4.80±0.20^{d}	4.39±0.39^{bc}	3.34±0.59^{ab}	7.73±0.29^{bc}	0.36±0.13^{ab}	0.76±0.20^{a}	670.93±73.40^{b}
T7	*Bam+Bp+Bv*	13.38±1.66^{a}	0.81±0.13^{a}	6.07±0.12^{a}	5.77±0.56^{a}	4.16±0.49^{a}	9.92±1.05^{a}	0.54±0.19^{a}	0.72±0.01^{a}	958.93±109.18^{a}
T8	Hydropriming	3.94±0.28^{d}	0.53±0.12^{cd}	4.93±0.23^{cd}	4.13±0.50^{c}	2.27±0.59^{cd}	6.58±0.47^{c}	0.16±0.04^{c}	0.52±0.15^{bc}	538.67±31.40^{c}
T9	Control	2.40±0.64^{d}	0.48±0.05^{d}	3.67±0.23^{e}	3.20±0.50^{d}	1.52±0.32^{d}	5.14±1.28^{d}	0.13±0.05^{c}	0.46±0.04^{c}	341.96±50.90^{d}
SE m (±)		0.462	0.05	0.15	0.24	0.27	0.44	0.07	0.06	41.49
C.D. (0.05)		1.372	0.16	0.43	0.72	0.81	1.30	0.20	0.19	123.26

T.No: Treatment number; SPT: Seed priming treatment; BSG: Basal shoot girth; NoL: Number of leaves; ShL: Shoot length; RL: Root length; SL: seedling length; RV: Root volume; AI: Allometric index: SVI: Seedling vigour index Each figure represents mean (± SD) of three replications. Figures followed by same letter in a column do not differ significantly (P>0.05).

Plate 7: Effect of biopriming on seedling length of *W.somnifera;* (A): *Bam+ Bp+Bv*; (B): *Bam+ Bp;* (C): *Bam*; (D): *Bp+Bv*; (E): *Bp*;(F): *Bv*; (G): *Bam+Bv*; (H): Hydropriming; (I): control

4.2 PHASE II- EFFECT OF *BACILLUS* SPP. ON GROWTH, YIELD AND SECONDARY METABOLITE PRODUCTION.

The data pertaining to the effect of seedling treatments with bacterial inoculants either individually or in combination on growth, yield and secondary metabolite production were recorded in the second phase of the study. The data has been statistically analyzed and results obtained are detailed below.

4.2.1 Plant Growth Parameters

The plant growth parameters, shoot length, number of branches, number of leaves, collar girth, leaf area and number of flowering branches were recorded from transplanting (45 DAS) to harvest (180 DAS) at 30 days interval. Growth of *Bam+Bp+Bv* treated *W. somnifera* vs control treatment is depicted in Plate 8.

4.2.1.1 Days to First Flowering

The data on days to first flowering of *W. somnifera* has been depicted in Table 5. Significant difference was observed among the treatments with respect to days to first flowering. T5 (*Bam +Bv)* exhibited significant earliness in flowering in 83.22 days compared to other treatments. The control (T8) took longest time (106.33 days) to flower, which was observed to be on par with T2, T3, T4, T6, and T7 (99.22, 97.44, 102.33, 101.56 and 100.44 days, respectively).

4.2.1.2 Days to Fruit Set

Data collected on days to fruit set in *W. Somnifera* from the day of flowering has been shown in Table 5. The data did not show any significant variation in days to fruit set among the treatments applied.

4.2.1.3 Shoot Length

The shoot length of *W. somnifera* as influenced by different treatments is depicted in Table 6. A significant variation in shoot length was observed in all the stages of observation.

At transplanting, the shoot length (5.77 cm) was significantly higher in T7. T7 followed by T5 and T4 recorded 4.98 and 4.92 cm of shoot length respectively. The lowest shoot length (4.13 cm) was observed in T8 and it was on par with T1, T3 and T6 (4.29, 4.40 and 4.39 cm respectively).

At 30 DAT, T7 recorded the highest shoot length (13.07cm) followed by T5 with a shoot length of 9.87 cm. The least value was observed in T8 (5.63cm) which was on par with T1, T2 and T3 (6.56, 6.01 and 5.93 cm, respectively).

At 60 DAT, T7 recorded maximum shoot length of 47.51 cm. The shoot length was minimum in T8 (20.00 cm) and it was on par with T2 and T3 (27.47 and 26.92 cm, respectively).

At 90 DAT, the shoot length (67.26 cm) was maximum in T7 and was on par with T5 (66.97 cm) and T6 (62.98 cm). The least value (46.33 cm) was recorded in T8 and was on par with T1, T2, T3 and T4 (49.52, 47.77, 47.74 and 53.06 cm, respectively). The shoot length of *W. somnifera* at 90 DAS is depicted Plate 9.

At 120 DAT, T7 recorded the highest shoot length (73.50 cm) which was found to be on par with T5 (72.94 cm) and T6 (70.68 cm). The lowest shoot length (53.12 cm) was recorded by T8 (control) and was on par with T1, T2 and T3 (55.90, 55.34 and 54.10 cm, respectively).

At harvest, the shoot length (78.99 cm) was significantly higher in T7 which was observed to be on par with T5 (78.20 cm) and T6 (76.48 cm). The least value (57.41cm)

was observed in T8 and was on par with T1, T2 and T3 (61.51, 60.51 and 60.12 cm, respectively). The shoot length of *W. somnifera* at harvest is depicted Plate 10.

4.2.1.4 Number of Branches

The data on number of branches of *W. somnifera* recorded at 30 days interval from transplanting to harvest is presented in Table 7. Significant variation was observed among the various treatments with respect to the number of branches at all stages of observation. No branches were observed at transplanting stage.

At 30 DAT, significantly higher number of branches (2.44) was observed in T7 which was on par with T2 and T5. The least value (0.33) was observed in T6 and T8 and was found to be on par with T1, T3 and T4 (1.00, 1.11 and 0.56 respectively).

At 60 DAT, both T5 and T7 exhibited the highest number of branches (3.67). These were found to be on par with T1, T2, T4 and T6 (3.00, 2.78, 2.33 and 2.56).The least value (1.11) was recorded in T8 and was found to be on par with T3, T4 and T6 (2.00, 2.33 and 2.56, respectively)

At 90 DAT, T5 recorded more number of branches (4.89) which was on par with T1 and T7 (4.33 and 4.56 respectively). The number of branches (2.11) was lowest in T8 and it was on par with T3 (3.33).

At 120 DAT, T7 recorded the highest number of branches (6.22) which was on par with T5 (5.44). T8 recorded a minimum of 3.00 and was on par with T3 (4.22).

At harvest, the number of branches (8.78) was the highest in T7 and it was on par with T5 (7.33). The least value was observed in T8 (3.89) and it was on par with T2, T3, T4 and T6 (5.11, 5.56, 5.00 and 5.33 respectively)

4.2.1.5 Number of Leaves

Table 8 represents the number of leaves of *W. somnifera* at different stages of observation as influenced by different microbial treatments. There was significant difference in the number of leaves among all the treatments tried at different stages of observation.

At the time of transplanting, the highest number of leaves (6.07) was recorded in T7. T7 was on par with T2, T4 and T5 (5.87, 5.67 and 4.80, respectively). The lowest (4.80) number of leaves was observed in T6 which was on par with T1 and T8 (5.07 and 4.93, respectively).

At 30 DAT, T7 recorded significantly higher of leaves (23.22) and was on par with T5 (21.90). The lowest value was recorded in T8 (9.00).

At 60 DAT, T5 recorded a maximum of 77.33 numbers of leaves which was the highest among all the treatments. T5 was on par with T7 (76.44). The lowest number of leaves (33.67) was recorded in T8.

At 90 DAT, significantly higher number of leaves (95.00) was noticed in T7. T8 recorded the least number of leaves (44.56).

At 120 DAT, the highest number of leaves (85.89) was recorded in T7. T7 followed by T5 recorded 72.78 numbers of leaves. The least significant value (48.78) was observed in T8 and was on par with T4 (51.11).

At harvest, the plants from T7 recorded significantly higher average number of leaves (71.00) and were on par with T5 (65.33).The lowest number of leaves (38.00) was noticed in T4.

Plate 8: Growth of *Bacillus amyloliquefaciens* + *Bacillus pumilus*+ *Bacillus velezensis* treated plants vs control; (A): 30 DAT; (B): 90 DAT; (C): 120 DAT

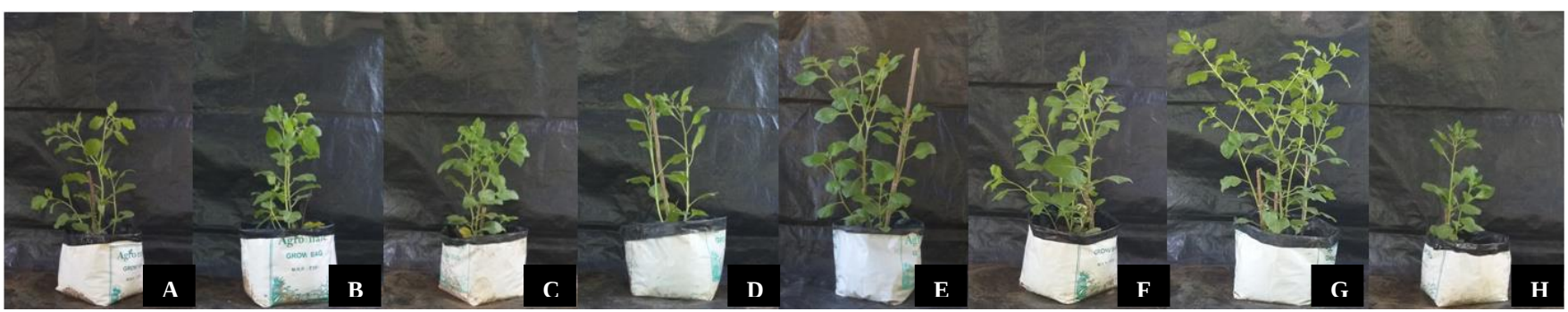

Plate 9: Effect of bacterization with *Bacillus* spp. on shoot length of *W. somnifera;* (A): *Bam*; (B): *Bp;* (C): *Bv*; (D): *Bam*+*Bp*; (E): *Bam*+*Bv*; (F): *Bp*+*Bv*; (G): *Bam*+ *Bp*+*Bv*; (H): control

4.2.1.6 Collar Girth

The data on collar girth of *W. somnifera* in response to different microbial treatments recorded at 30 days interval is shown in Table 9. Significant variation in collar girth was observed only at 30 DAT and at harvest.

At 30 DAT, significantly higher collar girth was noticed in T5 (1.50 cm) which was on par with T7 (1.43 cm). T3 recorded the least value of 1.10 cm and that was on par with T1, T2, T4, T6 and T8 (1.13, 1.12, 1.10, 1.24 and 1.14 cm respectively).

There was no significant difference among the treatments with regard to the collar girth of observational plants at 60, 90 and 120 DAT.

At harvest, the average of collar girth was the highest in T7 (3.91 cm) that was on par with T5 (3.30 cm). The least average value (2.30 cm) was observed in T8 and it was on par with T1, T2, T3, T4 and T6 (2.74, 2.77, 3.02, 2.46 and 3.04 cm, respectively).

4.2.1.7 Leaf Area

Table 10. represents the data on the leaf area of *W. somnifera* influenced by the different treatments tried. A significant variation has been observed in each stages of observation.

At the time of transplanting, significantly higher values (13.38 cm^2) of leaf area were observed in T7 and were on par with T5 (12.04 cm^2). The hydropriming treatment (T8) recorded 3.94 cm^2 of leaf area which was on par with T1, T3 and T4 (12.04, 5.35 and 5.30 cm^2, respectively).

At 30 DAT, T5 recorded significantly higher leaf area (463.55 cm^2) and was on par with T7 (462.62 cm^2). The least value (66.96 cm^2) was observed in T8. T8 was on par with T4 (76.80 cm^2).

At 60 DAT, the highest leaf area (4758.31 cm^2) was recorded in T7. T7 followed by T5 recorded leaf area of 4147.40cm^2. T4 recorded 1292.11cm^2 which was the lowest among all treatments. The leaf area of control treatment was 1706.22 cm^2 which in turn was on par with T1 (1798.14 cm^2).

At 90 DAT, significantly higher leaf area (7324.60 cm^2) was noticed in T7. T7 followed by T5 recorded leaf area of 5328.63 cm^2. T8 recorded the lowest value (2359.44 cm^2) and was observed to be on par with T4 (1292.11 cm^2).

At 120 DAT, T7 recorded maximum leaf area of 4538.95 cm^2. The least value (1670.85 cm^2) was observed in T4and was on par with T8 (2359.44 cm^2).

At harvest, T7 recorded the most significant leaf area of 5146.81 cm^2. It was followed by T5 (3857.85 cm^2). T8 recorded the lowest leaf area (1478.31 cm^2) and was on par with T4 (1584.76 cm^2).

4.2.1.8 Number of Flowering Branches

The data on the number of flowering branches is tabulated in Table 11. At transplanting and at 30 DAT, no flowering branches were noticed in any of the treatments. From 60 DAT to harvest, significant variation was noticed among all the treatments tried.

At 60 DAT, T7 recorded maximum number of flowering branches, 0.89 and it was on par with T1 and T5 (0.44 and 0.56, respectively). The lowest number of flowering branches (0.11) was recorded in T3, T6 and T8.

AT 90 DAT, the number of flowering branches was the highest (3.33) in T5. It was on par with T1 and T7 (2.44). The least number of flowering branches were noticed in T8 (0.78) that was on par with T2, T3, T4 and T6 (2.00, 1.44, 2.00 and 1.78, respectively).

Plate 10: Effect of bacterization with *Bacillus* spp. on shoot length of *W.somnifera;* (A): *Bam +Bv*; (B): *Bam+Bp+Bv;* (C): *Bv*; (D): *Bam*; (E): control;(F): *Bp+ Bv*; (G): *Bp*; (H): *Bam+Bp*

At 120 DAT, significantly higher number of flowering branches (4.67) was observed in T5 and T7. They were on par with T1, T2, T4 and T6 (4.00, 3.56, 3.56 and 3.22, respectively). T8 showed the least number of flowering branches (1.78) which was on par with T3 and T6 (2.78 and 3.22, respectively).

At harvest, the number of flowering branches (7.89) was observed to be significantly higher in T7 followed by T5 (6.78). The lowest average number of flowering branches was recorded in T8 (3.56) and that was on par with T1, T2, T3 and T4 (4.89, 4.67, 4.56 and 4.78, respectively).

4.2.2 Yield and Yield Components

The data on yield parameters recorded at harvest were statistically analyzed and the results obtained are presented below. The shoot yield parameters in terms of fresh and dry weight of leaf, stem, berries and shoot are presented in Table 12.

4.2.2.1 Leaf Fresh Weight

A significant variation in leaf fresh weight was observed among the treatments. The highest (45.89 g plant $^{-1}$) leaf fresh weight was recorded in T7. The control treatment recorded the lowest (16.66 g plant $^{-1}$) leaf fresh weight which was on par with T4 (18.68 g plant $^{-1}$).

4.2.2.2 Leaf Dry Weight

A significantly higher (5.07 g plant $^{-1}$) leaf dry weight was observed in T7 and was on par with T3 and T5 (3.57 and 4.97 g plant $^{-1}$). The control treatment recorded the least leaf dry weight of 1.64 g plant $^{-1}$. It was on par with T1, T2 and T4 (3.10, 3.21 and 2.67 g plant $^{-1}$, respectively).

4.2.2.3 Stem Fresh Weight

Among the treatments, T7 recorded a significantly higher stem fresh weight, 61.85 g plant $^{-1}$. This was followed by T6, which recorded 51.20 g plant $^{-1}$ of stem fresh weight. The lowest (22.58 g plant $^{-1}$) was observed in the control plants. T8 was on par with T2 and T3 (25.92 and 25.75 g plant $^{-1}$, respectively).

4.2.2.4 Stem Dry Weight

A significant variation in stem dry weight was noticed among the treatments tried in *W. somnifera*. The stem dry weight was observed to be the highest (9.78 g plant $^{-1}$) in T7 which was on par with T5 (9.28 g plant $^{-1}$) and T6 (8.70 g plant $^{-1}$). The least dry weight (6.94 g plant $^{-1}$) was observed in T8. It was on par with T1, T2, T3 and T4 (7.27, 7.08, 6.96 and 7.79 g plant $^{-1}$, respectively).

4.2.2.5 Berry Fresh Weight

The berry fresh weight varied significantly among the treatments. It was observed to be the highest (8.85 g plant $^{-1}$) in T5. T5 was on par with T7 (7.85 g plant $^{-1}$). T3 recorded the least (3.34 g plant $^{-1}$) fresh berry weight which was on par with T1, T2, T4 and T8 (3.56, 3.75, 4.28 and 3.60 g plant $^{-1}$, respectively).

4.2.2.6 Berry Dry Weight

The dry weight of berries varied significantly among the treatments. It was observed to be the highest (5.33 g plant $^{-1}$) in T5. It was observed to be on par with T7 (3.96 g plant $^{-1}$). T1 recorded the lowest (1.77 g plant $^{-1}$) dry weight of berries and was on par with T2, T3, T4, T6 and T8 (1.83, 1.83, 2.31, 2.70 and 2.59 g plant $^{-1}$, respectively).

4.2.2.7 Shoot Fresh Weight

The shoot fresh weight of *W. somnifera* exhibited significant variation among the treatments.T5 recorded the highest shoot fresh weight (97.48 g plant $^{-1}$) which was

observed to be on par with T7 (90.39 g plant $^{-1}$). T8 (60.58 g plant $^{-1}$) recorded the lowest shoot fresh weight and was on par with T1, T2, T3 and T4 (65.30, 67.27, 64.20 and 73.26 g plant $^{-1}$, respectively).

4.2.2.8 Shoot Dry Weight

A significant variation in shoot dry weight existed among the treatments applied. The highest shoot dry weight (17.52 g plant $^{-1}$) was observed in T5 which was on par with T6 (14.86 g plant $^{-1}$) and T7 (16.84 g plant $^{-1}$). T3 recorded the least shoot dry weight (10.68 g plant $^{-1}$) and was on par with T1, T2, T4 and T8 (11.35, 12.62, 13.23 and 11.22 g plant $^{-1}$, respectively).

4.2.2.9 Number of Berries Plant^{-1}

The data on number of berries recorded per plant in *W. somnifera* have been tabulated in Table 13. The average number of berries per plant differed significantly among the treatments. The highest values was recorded in T5 (90.56) and it was on par with T7 (87.56). T3 recorded the least (26.00) and it was on par with T1, T2, T4 and T8 (31.56, 29.11, 38.33 and 38.67 respectively). Effect of bacterization with *Bacillus* spp. on berry yield of *W. somnifera* is depicted in Plate 11.

4.2.2.10 Seed Yield Plant^{-1}

The seed yield per plant varied significantly among the treatments (Table 13). T5 with an average seed yield of 7.35 g plant^{-1} followed by T7 with 7.34 g plant^{-1} recorded the most significant results. The average seed yield was the lowest in T3 (1.59 g plant^{-1}) and it was on par with T1, T2, T4 and T8 (1.88, 1.70, 2.71 and 2.04 g plant^{-1}, respectively).

4.2.2.11 100 Seed Weight

A significant difference among the treatments was noticed with regard to the average of 100 seed weight of *W. somnifera* (Table 13). The highest value was recorded

in T7 (0.26 g) which was on par with T5 (0.25 g). The lowest of 100 seed weight was observed in T8 (0.18 g) and it was on par with T1, T2 and T3 (0.20, 0.19 and 0.18).

4.2.2.12 Root Length

The mean root length varied significantly among the various treatments tried. The data is presented in Table 14. T7 recorded maximum root length, 21.27 cm. T7 was on par with T5 which recorded 20.31 cm root length. The lowest root length (13.43 cm) was recorded in T1 which was observed to be on par with T2, T3, T4 and T8 (14.90, 15.62, 13.96 and 13.81 cm, respectively). The Effect of bacterization with *Bacillus* spp. on root length of *W.somnifera* is presented in plate 12.

4.2.2.13 Root Diameter

The data on root diameter of *W. somnifera* recorded at harvest is presented in Table 14. The most significant value (1.33 cm) was recorded in T7 which was on par with T5. The lowest root diameter, 0.81 cm was observed in T4 was on par with T1, T2, T3 and T8 (0.93, 0.92 and 0.82 cm).

4.2.2.14 Root Volume

The root volume differed significantly among the various treatments tried (Table 14). The highest root volume was observed in T7 (5.39 cm^3). T7 was observed to be on par with T5 (1.29 cm^3). T4 was the least significant treatment which recorded 1.61 cm^3 and it was on par with T1 and T8 (2.50 and 2.39 cm^3 respectively)

4.2.2.15 Root Fresh Yield Plant^{-1}

The data on fresh root yield of *W. somnifera* has been presented in Table 14. It varied significantly among the treatments and the highest root fresh yield was recorded in T7 (5.47 g $plant^{-1}$) and was on par with T5 (5.37 g $plant^{-1}$). The least value was noticed in T4 (1.82 g $plant^{-1}$) and it was on par with T1 and T8 (2.64 and 2.65 g $plant^{-1}$, respectively).

Plate 11: Effect of bacterization with *Bacillus* spp. on berry yield of *W.somnifera;* (A): *Bam+ Bv*; (B): *Bam+Bp+Bv;* (C): *Bp+Bv*; (D): *Bam+Bp*; (E):control;(F): *Bam*; (G): *Bp*; (H): *Bv*

Plate 12: Effect of bacterization with *Bacillus* spp. on root length of *W.somnifera;* (A): *Bam+ Bp +Bv*; (B): *Bam +Bv;* (C): *Bp+Bv*; (D): *Bv*; (E): *Bp*;(F): *Bam+Bp*; (G): control; (H): *Bam*

4.2.2.16 Root Dry Yield Plant^{-1}

The data of dry root yield per plant of *W. somnifera*is presented in Table 14. A significant difference with respect to the dry root yield among the treatments was noticed. T7 with 1.44 g plant^{-1}dry root yield recorded the significantly higher value and was on par with T5 (1.36 g plant^{-1}). The lowest yield (0.45 g plant^{-1}) was observed in T4 which was on par with T1, T2 and T8 (0.66, 0.73, and 0.71 g plant^{-1}, respectively).

4.2.2.17 Total Dry Matter Production

The data on total dry matter production in response to the treatments applied is depicted in Table 15. T5 recorded a maximum of 18.89 g plant^{-1} total dry matter and it was on par with T6 and T7 (15.85 and 18.22 g plant^{-1} , respectively). The lowest total dry matter (11.50 g plant^{-1}) was recorded in T3 which was observed to be on par with T1, T2, T4 and T8 (12.01, 13.35, 13.68 and 11.93 g plant^{-1}, respectively).

4.2.2.18 Harvest Index

A significant variation in harvest index was observed among the treatments with respect to the root yield. But harvest index in relation to leaf yield did not vary significantly (Table 15). The highest harvest index (0.10) with respect to root was recorded in T7. It was on par with T6 (0.08). T4 recorded the least harvest index (0.04) which was on par with T1, T2 and T8 (0.05).

4.2.3. Biochemical Estimation

The data on biochemical parameters of *W. somnifera* recorded at harvest was analyzed statistically and is detailed below.

4.2.3.1 Chlorophyll Content

4.2.3.1.1 Chlorophyll a

The chlorophyll a content differed significantly among the treatments (Table 16). T3 recorded the highest value of 0.33 mg g^{-1} fresh weight and the least was observed in T8 (0.17 mg g^{-1} fresh weight)

4.2.3.1.2 Chlorophyll b

Chlorophyll b content significantly varied among the treatments (Table 16). T3 recorded a significantly higher value of 0.07 mg g^{-1} fresh weight of chlorophyll b and was on par with T1, T2, T4, T6 and T7 (0.06, 0.05, 0.06, 0.06 and 0.06 mg g^{-1} fresh weight, respectively). T8 with 0.03 mg g^{-1} fresh weight was found to be the lowest among the treatments.

4.2.3.1.3 Total Chlorophyll

The data on total chlorophyll content in response to the treatments is tabulated in Table 16. The total chlorophyll content (0.40 mg g^{-1} fresh weight) observed in T3 was significantly higher among the different treatments applied. The least total chlorophyll was observed in T8 (0.21 mg g^{-1} fresh weight).

4.2.3.2.Total Carbohydrate Content

The total carbohydrate content significantly differed among the treatments tried (Table 16). T5 recorded significantly higher carbohydrate content of 23.30 mg $100g^{-1}$. This was found to be on par with T7 (21.26 mg $100g^{-1}$). The least value was observed in T8 with a carbohydrate content of 16.47 mg $100g^{-1}$ and it was on par with T1 (16.67 mg $100g^{-1}$).

4.2.3.3 Total Protein Content

The data on total protein content of the roots is presented in Table 16. The total protein content varied significantly among the treatments. The significantly higher total

protein content (2.96 mg $100g^{-1}$) was recorded in T5. T5 was on par with T7 (2.92 mg $100g^{-1}$). The lowest value was recorded in T8 (2.69 mg $100g^{-1}$) and was on with T1, T3 and T4 (2.73, 2.72 and 2.72 mg $100g^{-1}$, respectively).

4.2.3.4.Total Alkaloid Content

A significant difference in total alkaloid content and yield $plant^{-1}$ in the leaves was noticed in response to the various treatments tried (Table 17). T6 recorded the highest total alkaloid content (7.86 µg 100 mg^{-1}) this was found to be on par with T7 and T5 (7.76 and 7.84 µg 100 mg^{-1} respectively). T4 yielded 3.76 µg mg^{-1} of total alkaloid which was the lowest among all the treatments. The control treatment recorded 4.56 µg 100 mg^{-1}.

A significant variation in the yield of total leaf alkaloid was observed among the treatments tried. The total leaf alkaloid yield per plant (397.44 µg $plant^{-1}$) was found to be higher in T7 which was on par with T5 (385.52 µg $plant^{-1}$). These were followed by T6, which recorded 251.90 µg $plant^{-1}$. T8 recorded 79.66 µg $plant^{-1}$ of total leaf alkaloid yield, which was the lowest and was observed to be on par with T1 and T4 (175.14 and 109.15 µg $plant^{-1}$, respectively).

4.2.3.5.Total Withanolide Content

The data on total withanolide content and yield $plant^{-1}$ from the roots of *W. somnifera* is given in Table 17. The total withanolide content in the roots differed significantly among the treatments. T7 recorded a maximum of 7.46 µg mg^{-1} total withanolide content. T7 was on par with T5 and T6 (7.09 and 7.30 µg mg^{-1} respectively). T8 recorded the lowest of 3.35 µg mg^{-1}. T8 was on par with T2 and T3 (3.68 and 3.46 µg mg^{-1}, respectively).

A significant variation in the yield of total withanolides per plant from the roots was observed among the treatments applied (Table 17). T7 recorded significantly higher value (10.77 mg $plant^{-1}$) of total withanolide content per plant which was observed to be

on par with T5 (9.65 mg plant^{-1}). T4 yielded 2.19 mg plant^{-1} of total withanolides from the root which was the lowest among the treatments and was found to be on par with T2, T3 and T8 (2.68, 2.84 and 2.37 mg plant^{-1}).

Table 5. Effect of bacterization with *Bacillus* spp. on days to first flowering and fruit set in *W. somnifera.*

T. No.	ST	Days to first flowering (DAS)	Days to fruit set (DAF)
T1	*Bam*	94.56±9.00^{b}	11.00±1.20
T2	*Bp*	99.22±5.50^{ab}	11.00±0.88
T3	*Bv*	97.44±3.08^{ab}	11.11±1.26
T4	*Bam+Bp*	102.33±3.22^{ab}	11.00±0.00
T5	*Bam+Bv*	83.22±2.91^{c}	10.67±0.58
T6	*Bp+Bv*	101.56±1.64^{ab}	11.00±1.00
T7	*Bam+Bp+Bv*	100.44±6.00^{ab}	10.33±0.58
T8	Control	106.33±9.26^{a}	10.67±0.67
SE m (±)		3.24	0.50
C.D. (0.05)		9.71	NS

T.No: Treatment number; ST: Seedling treatment; DAS: Days after sowing; DAF: Days after flowering. Each figure represents mean (± SD) of three replications. Figures followed by same letter in a column do not differ significantly (P>0.05).

Table 6. Effect of bacterization with *Bacillus* spp. on shoot length of *W.somnifera.*

T. No.	ST	AT	30 DAT	60 DAT	90 DAT	120 DAT	H
T1	*Bam*	4.29±0.55^{bc}	6.56±1.29^{cde}	29.04±5.40^{bc}	49.52±6.26^{b}	55.90±5.50^{bc}	61.51±5.15^{bc}
T2	*Bp*	4.84±0.28^{b}	6.01±1.38^{de}	27.47±1.00^{cd}	47.77±3.33^{b}	55.34±4.38^{bc}	60.51±4.98^{bc}
T3	*Bv*	4.40±0.20^{bc}	5.93±0.78^{e}	26.92±5.03^{cd}	47.74±3.40^{b}	54.10±1.88^{bc}	60.12±2.25^{bc}
T4	*Bam+Bp*	4.92±0.43^{b}	8.27±0.63^{bcd}	31.09±3.00^{bc}	53.06±2.90^{b}	60.33±2.76^{b}	66.31±2.78^{b}
T5	*Bam+Bv*	4.98±0.13^{b}	9.87±1.27^{b}	36.36±5.87^{b}	66.97±6.57^{a}	72.94±6.95^{a}	78.20±7.51^{a}
T6	*Bp+Bv*	4.39±0.39^{bc}	8.61±0.6^{bc}	36.26±7.28^{b}	62.98±3.05^{a}	70.68±3.00^{a}	76.48±3.59^{a}
T7	*Bam+Bp+Bv*	5.77±0.56^{a}	13.07±2.29^{a}	47.51±3.22^{a}	67.26±3.71^{a}	73.50±3.41^{a}	78.99±4.24^{a}
T8	Control	4.13±0.50^{c}	5.63±1.39^{e}	20.00±5.94^{d}	46.33±4.77^{b}	53.12±2.26^{c}	57.41±2.62^{c}
SE m (±)		0.24	0.76	2.87	2.58	2.37	2.57
C.D. (0.05)		0.71	2.27	8.60	7.72	7.10	7.70

T.No: Treatment number; ST: Seedling treatment; AT: At transplanting; DAT: Days after transplanting; H: Harvest. Each figure represents mean (± SD) of three replications. Figures followed by same letter in a column do not differ significantly (P>0.05)

Table 7. Effect of bacterization with *Bacillus* spp. on number of branches of *W. somnifera.*

T. No.	ST	30 DAT	60 DAT	90 DAT	120 DAT	H
T1	*Bam*	1.00±0.88^{bc}	3.00±1.528^{ab}	4.33±1.20^{ab}	4.56±1.20^{b}	5.78±0.77^{bc}
T2	*Bp*	1.56±0.19^{ab}	2.78±0.509^{ab}	3.44±0.51^{b}	4.44±0.84^{b}	5.11±0.77^{cd}
T3	*Bv*	1.11±0.39^{bc}	2.00±0.67^{bc}	3.33±1.00^{bc}	4.22±1.02^{bc}	5.56±1.68^{cd}
T4	*Bam+Bp*	0.56±0.39^{c}	2.33±0.00^{abc}	3.56±0.19^{b}	4.56±0.51^{b}	5.00±0.56^{cd}
T5	*Bam+Bv*	2.11±0.84^{a}	3.67±1.00^{a}	4.89±0.77^{a}	5.44±1.02^{ab}	7.33±1.73^{ab}
T6	*Bp+Bv*	0.33±0.33^{c}	2.56±0.84^{abc}	3.56±0.51^{b}	4.56±0.19^{b}	5.33±0.00^{cd}
T7	*Bam+Bp+Bv*	2.44±0.51^{a}	3.67±0.67^{a}	4.56±0.51^{ab}	6.22±0.19^{a}	8.78±0.19^{a}
T8	Control	0.33±0.58^{c}	1.11±0.84^{c}	2.11±0.51^{c}	3.00±0.67^{c}	3.89±1.02^{d}
SE m (±)		0.32	0.50	0.41	0.44	0.59
C.D. (0.05)		0.97	1.49	1.24	1.31	1.77

T.No: Treatment number; ST: Seedling treatment; DAT: Days after transplanting; H: Harvest. Each figure represents mean (± SD) of three replications. Figures followed by same letter in a column do not differ significantly (P>0.05)

Table 8. Effect of bacterization with *Bacillus* spp. on number of leaves of *W. somnifera.*

T. No.	ST	AT	30 DAT	60 DAT	90 DAT	120 DAT	H
T1	*Bam*	5.07±0.31^{cd}	13.00±1.86^{c}	45.78±0.84^{d}	64.56±0.39^{e}	61.56±0.69^{d}	55.00±2.60^{c}
T2	*Bp*	5.87±0.12^{a}	14.56±1.07^{bc}	53.56±0.84^{c}	74.67±1.33^{d}	64.22±2.69^{cd}	62.78±1.35^{b}
T3	*Bv*	5.33±0.31^{bc}	13.11±1.64^{c}	44.89±2.55^{d}	63.56±0.77^{e}	56.22±2.04^{e}	51.44±2.50^{c}
T4	*Bam+Bp*	5.67±0.23^{ab}	15.56±0.51^{b}	38.11±1.26^{e}	54.67±1.73^{f}	51.11±0.77^{f}	38.00±5.86^{e}
T5	*Bam+Bv*	5.80±0.40^{a}	21.90±0.39^{a}	77.33±0.88^{a}	86.67±0.67^{b}	72.78±1.17^{b}	65.33±0.88^{ab}
T6	*Bp+Bv*	4.80±0.20^{d}	11.00±0.58^{d}	65.33±2.08^{b}	84.44±0.69^{c}	66.67±2.19^{c}	65.00±5.70^{b}
T7	*Bam+Bp+Bv*	6.07±0.12^{a}	23.22±1.39^{a}	76.44±1.54^{a}	95.00±2.40^{a}	85.89±2.91^{a}	71.00±3.06^{a}
T8	Control	4.93±0.23^{cd}	9.00±0.58^{e}	33.67±5.78^{f}	44.56±1.89^{g}	48.78±2.91^{f}	44.22±5.16^{d}
SE m (±)		0.15	0.66	1.449	0.812	1.218	1.984
C.D. (0.05)		0.44	1.96	4.345	2.434	3.651	5.947

T.No: Treatment number; ST: Seedling treatment; AT: At transplanting; DAT: Days after transplanting; H: Harvest. Each figure represents mean (± SD) of three replications. Figures followed by same letter in a column do not differ significantly (P>0.05)

Table .9 Effect of bacterization with *Bacillus* spp. on collar girth of *W. somnifera*.

T. No.	ST	AT (cm)	30 DAT (cm)	60 DAT (cm)	90 DAT (cm)	120 DAT (cm)	H (cm)
T1	*Bam*	0.64±0.07	1.13±0.12^{c}	1.93±0.25	2.41±0.20	2.63±0.22	2.74±0.14^{bc}
T2	*Bp*	0.64±0.07	1.12±0.08^{c}	2.12±0.17	2.51±0.33	2.68±0.36	2.77±0.39^{bc}
T3	*Bv*	0.68±0.14	1.10±0.23^{c}	1.94±0.71	2.53±0.95	2.90±0.95	3.02±0.95^{bc}
T4	*Bam+Bp*	0.63±0.00	1.24±0.12^{bc}	1.87±0.09	2.22±0.05	2.38±0.10	2.46±0.05^{c}
T5	*Bam+Bv*	0.66±0.10	1.50±0.09^{a}	2.46±0.08	2.86±0.07	3.19±0.18	3.30±0.21^{ab}
T6	*Bp+Bv*	0.77±0.07	1.20±0.17^{bc}	2.26±0.54	2.11±0.69	2.94±0.81	3.04±0.83^{bc}
T7	*Bam+Bp+Bv*	0.81±0.13	1.43±0.03^{ab}	2.49±0.30	3.06±0.17	3.48±0.21	3.91±0.24^{a}
T8	Control	0.53±0.12	1.14±0.23^{c}	1.67±0.27	1.92±0.25	2.20±0.15	2.30±0.07^{c}
SE m (±)		0.06	0.086	0.211	0.261	0.276	0.28
C.D. (0.05)		NS	0.257	NS	NS	NS	0.84

T.No: Treatment number; ST: Seedling treatment; AT: At transplanting; DAT: Days after transplanting; H: Harvest. Each figure represents mean (± SD) of three replications. Figures followed by same letter in a column do not differ significantly (P>0.05)

Table .10 Effect of bacterization with *Bacillus* spp. on leaf area of *W. somnifera*.

T. No.	ST	AT (cm^2)	30 DAT (cm^2)	60 DAT (cm^2)	90 DAT (cm^2)	120 DAT (cm^2)	H (cm^2)
T1	*Bam*	4.64±0.53^{c}	86.49±7.04^{c}	1798.14±68.79^{e}	3432.93±102.58^{e}	1807.80±95.60^{d}	2712.46±79.50^{d}
T2	*Bp*	8.09±0.46^{b}	124.30±3.96^{b}	2926.67±241.37^{c}	4565.84±15.36^{c}	2872.96±95.60^{b}	3650.32±74.51^{b}
T3	*Bv*	5.35±1.18^{c}	90.56±1.51^{c}	1913.59±144.94^{e}	3146.58±129.14^{f}	1908.64±10.82^{d}	2430.80±48.93^{d}
T4	*Bam+Bp*	5.30±0.61^{c}	76.80±1.92^{cd}	1292.11±74.01^{f}	2374.68±154.75^{g}	1670.85±124.06^{e}	1584.76±270.77^{e}
T5	*Bam+Bv*	12.04±0.09^{a}	463.55±27.80^{a}	4147.40±89.65^{b}	5328.63±105.42^{b}	3412.87±136.36^{b}	3857.85±24.06^{b}
T6	*Bp+Bv*	6.84±0.49^{b}	134.08±1.61^{b}	2594.73±117.96^{d}	4138.48±100.45^{d}	2502.29±39.00^{c}	3073.76±282.04^{c}
T7	*Bam+Bp+Bv*	13.38±1.66^{a}	462.62±5.15^{a}	4758.31±189.52^{a}	7324.60±200.55^{a}	4538.95±163.13^{a}	5146.81±199.10^{a}
T8	Control	3.94±0.28^{c}	66.96±2.53^{d}	1706.22±68.20^{e}	2359.44±42.35^{g}	1710.26±69.51^{e}	1478.31±141.62^{e}
SE m (±)		0.472	6.05	79.58	69.10	61.30	97.34
C.D. (0.05)		1.415	18.15	238.60	207.17	183.77	291.83

T.No: Treatment number; ST: Seedling treatment; AT: At transplanting; DAT: Days after transplanting; H: Harvest. Each figure represents mean (± SD) of three replications. Figures followed by same letter in a column do not differ significantly (P>0.05)

Table 11. Effect of bacterization with *Bacillus* spp. on number of flowering branches of *W.somnifera.*

T. No.	ST	60 DAT	90 DAT	120 DAT	H
T1	*Bam*	0.44±0.51^{abc}	2.44±1.02^{ab}	4.00±0.33^{ab}	4.89±0.39^{bc}
T2	*Bp*	0.22±0.19^{bc}	2.00±0.67^{bc}	3.56±0.84^{ab}	4.67±0.67^{bc}
T3	*Bv*	0.11±0.19^{bc}	1.44±0.51^{bc}	2.78±1.07^{bc}	4.56±1.39^{bc}
T4	*Bam+Bp*	0.00±0.00^{c}	2.00±0.00^{bc}	3.56±0.39^{ab}	4.78±0.69^{bc}
T5	*Bam+Bv*	0.56±0.39^{ab}	3.33±1.20^{a}	4.67±0.58^{a}	6.78±1.07^{a}
T6	*Bp+Bv*	0.11±0.19^{bc}	1.78±0.69^{bc}	3.22±0.19^{abc}	5.00±0.33^{b}
T7	*Bam+Bp+Bv*	0.89±0.19^{a}	2.44±0.51^{ab}	4.67±1.76^{a}	7.89±0.39^{a}
T8	Control	0.11±0.19^{bc}	0.78±0.51^{c}	1.78±0.84^{c}	3.56±1.02^{c}
SE m (±)		0.16	0.42	0.51	0.48
C.D. (0.05)		0.47	1.25	1.53	1.43

T.No: Treatment number; ST: Seedling treatment; DAT: Days after transplanting; H: Harvest. Each figure represents mean (± SD) of three replications. Figures followed by same letter in a column do not differ significantly (P>0.05)

Table 12. Effect of bacterization with *Bacillus* spp. on shoot yield parameters of *W. somnifera.*

T. No.	ST	LFW (g plant^{-1})	LDW (g plant^{-1})	SFW (g plant^{-1})	SDW (g plant^{-1})	BFW (g plant^{-1})	BDW (g plant^{-1})	ShFW (g plant^{-1})	ShDW (g plant^{-1})
T1	*Bam*	25.63±1.56^{c}	3.10±0.81^{bc}	32.32±2.44^{c}	7.27±0.65^{c}	3.56±0.31^{c}	1.77±0.17^{c}	65.30±3.45^{d}	11.35±0.41^{c}
T2	*Bp*	35.24±1.80^{b}	3.21±1.12^{bc}	25.92±10.80^{cd}	7.08±0.42^{c}	3.75±0.18^{c}	1.83±0.61^{c}	67.27±5.11^{cd}	12.62±3.03^{bc}
T3	*Bv*	24.48±1.63^{c}	3.57±1.84^{ab}	25.75±6.67^{cd}	6.96±0.26^{c}	3.34±0.43^{c}	1.83±0.39^{c}	64.20±6.74^{d}	10.68±1.14^{c}
T4	*Bam+Bp*	18.68±2.08^{d}	2.67±0.50^{bc}	44.03±1.64^{b}	7.79±0.08^{bc}	4.28±0.64^{c}	2.31±0.42^{c}	73.26±7.37^{cd}	13.23±0.23^{bc}
T5	*Bam+Bv*	38.18±3.18^{b}	4.97±0.55^{a}	49.07±2.46^{b}	9.28±1.14^{a}	8.85±2.31^{a}	5.33±1.51^{a}	97.48±7.54^{a}	17.52±2.48^{a}
T6	*Bp+Bv*	36.42±2.02^{b}	3.26±0.84^{b}	51.20±4.22^{b}	8.70±0.78^{ab}	6.24±0.78^{b}	2.70±0.23^{bc}	81.32±12.46^{bc}	14.86±0.46^{ab}
T7	*Bam+Bp+Bv*	45.89±1.80^{a}	5.07±0.31^{a}	61.85±2.76^{a}	9.78±0.45^{a}	7.85±1.10^{ab}	3.96±0.35^{ab}	90.39±6.80^{ab}	16.84±1.21^{a}
T8	Control	16.66±1.03^{d}	1.64±0.34^{c}	22.58±3.77^{d}	6.94±0.71^{c}	3.60±1.16^{c}	2.59±1.54^{bc}	60.58±16.43^{d}	11.22±2.63^{c}
SE m (±)		1.139	0.531	2.997	0.37	0.619	0.481	5.269	1.03
C.D. (0.05)		3.415	1.591	8.984	1.108	1.855	1.441	15.796	3.087

T.No: Treatment number; ST: Seedling treatment; LFW: Leaf fresh weight; LDW: Leaf dry weight; SFW: Stem fresh weight; SDW: Stem dryweight; BFW: Berry fresh weight; BDW: Berry dry weight ShFW: Shoot fresh weight; ShDW: Shoot dry weight. Each figure represents mean (± SD) of three replications. Figures followed by same letter in a column do not differ significantly (P>0.05)

Table 13. Effect of bacterization with *Bacillus* spp. on number of berries, seed yield and 100 seed weight of *W. somnifera*

T. No.	ST	NB	SY(g plant^{-1})	100 seed weight (g)
T1	*Bam*	31.56±2.17^{c}	1.88±0.15^{c}	0.20±0.02^{bcd}
T2	*Bp*	29.11±1.93^{c}	1.70±0.07^{c}	0.19±0.01^{cd}
T3	*Bv*	26.00±4.81^{c}	1.59±0.21^{c}	0.21±0.01^{bcd}
T4	*Bam+Bp*	38.33±8.84^{c}	2.71±0.70^{c}	0.22±0.02^{b}
T5	*Bam+Bv*	90.56±24.20^{a}	7.35±1.99^{a}	0.25±0.00^{a}
T6	*Bp+Bv*	68.00±11.79^{b}	5.03±0.83^{b}	0.22±0.03^{bc}
T7	*Bam+Bp+Bv*	87.56±8.47^{ab}	7.34±0.50^{a}	0.26±0.00^{a}
T8	Control	38.67±12.12^{c}	2.04±0.64^{c}	0.18±0.00^{d}
SE m (±)		6.62	0.50	0.01
C.D. (0.05)		19.85	1.49	0.026

T.No: Treatment number; ST: Seedling treatment; NB: Number of berries; SY: Seed yield. Each figure represents mean (± SD) of three replications. Figures followed by same letter in a column do not differ significantly (P>0.05)

Table 14. Effect of bacterization with *Bacillus* spp. on root yield parameters of *W. somnifera*

T. No.	**ST**	**RL (cm)**	**RD (cm)**	**RV (cm^{3})**	**RFW (g plant^{-1})**	**RDW (g plant^{-1})**
T1	*Bam*	13.43±0.97^{c}	0.93±0.09^{cd}	2.50±0.60^{bcd}	2.64±0.68^{bc}	0.66±0.18^{cd}
T2	*Bp*	14.90±0.30^{c}	0.92±0.02^{cd}	2.89±0.10^{bc}	2.91±0.06^{b}	0.73±0.02^{bcd}
T3	*Bv*	15.62±1.17^{bc}	0.90±0.15^{cd}	3.22±0.77^{bc}	3.11±0.57^{b}	0.82±0.15^{bc}
T4	*Bam+Bp*	13.96±1.60^{c}	0.81±0.08^{d}	1.61±0.26^{d}	1.82±0.08^{c}	0.45±0.01^{d}
T5	*Bam+Bv*	20.31±2.42^{a}	1.29±0.04^{ab}	5.06±0.26^{a}	5.37±0.26^{a}	1.36±0.03^{a}
T6	*Bp+Bv*	17.77±1.04^{b}	1.11±0.12^{bc}	3.56±0.86^{b}	3.58±0.71^{b}	1.00±0.27^{b}
T7	*Bam+Bp+Bv*	21.27±1.47^{a}	1.33±0.03^{a}	5.39±0.35^{a}	5.47±0.24^{a}	1.44±0.05^{a}
T8	Control	13.81±1.70^{c}	0.82±0.28^{d}	2.39±1.21^{cd}	2.65±1.18^{bc}	0.71±0.30^{cd}
SE m (±)		0.84	0.07	0.38	0.34	0.10
C.D. (0.05)		2.52	0.22	1.13	1.03	0.29

T.No: Treatment number; ST: Seedling treatment; RL: Root length; RD: Root diameter; RV: Root volume; RFW: Root fresh weight; RDW: Root dry weight. Each figure represents mean (± SD) of three replications. Figures followed by same letter in a column do not differ significantly (P>0.05)

Table 15. Effect of bacterization with *Bacillus* spp. on total dry matter production and harvest index of *W. somnifera*.

T. No.	ST	TDM (g plant^{-1})	HI	
			Root	Leaf
T1	*Bam*	12.01±0.34^{c}	0.05±0.01^{cde}	0.28±0.03
T2	*Bp*	13.35±3.03^{bc}	0.05±0.01^{de}	0.23±0.04
T3	*Bv*	11.50±1.29^{c}	0.06±0.01^{bcd}	0.24±0.01
T4	*Bam+Bp*	13.68±0.22^{bc}	0.04±0.01^{e}	0.25±0.06
T5	*Bam+Bv*	18.89±2.51^{a}	0.08±0.01^{bc}	0.25±0.06
T6	*Bp+Bv*	15.85±0.28^{ab}	0.08±0.02^{ab}	0.25±0.01
T7	*Bam+Bp+Bv*	18.22±1.13^{a}	0.10±0.02^{a}	0.25±0.07
T8	Control	11.93±2.90^{c}	0.05±0.01^{de}	0.26±0.05
SE m (±)		1.062	0.009	0.026
C.D. (0.05)		3.183	0.026	NS

T.No: Treatment number; ST: Seedling treatment; TDM: Total dry matter; HI: Harvest index. Each figure represents mean (± SD) of three replications. Figures followed by same letter in a column do not differ significantly (P>0.05)

Table 16. Effect of bacterization with *Bacillus* spp. on biochemical parameters of *W. somnifera*.

T. No.	ST	Chlorophyll content (mg/g fresh wt.)			TC(mg 100 mg^{-1})	TP (mg 100 mg^{-1})
		Chl a	Chl b	Total Chl		
T1	*Bam*	0.29±0.00^{bc}	0.06±0.01^{ab}	0.35±0.01^{bc}	16.67±0.27^{f}	2.73±0.01^{c}
T2	*Bp*	0.29±0.00^{bc}	0.05±0.01^{ab}	0.34±0.01^{bc}	18.04±0.13^{d}	2.79±0.01^{b}
T3	*Bv*	0.33±0.00^{a}	0.07±0.01^{a}	0.40±0.02^{a}	19.36±0.14^{c}	2.72±0.01^{c}
T4	*Bam+Bp*	0.31±0.02^{b}	0.06±0.00^{ab}	0.36±0.02^{b}	17.46±0.59^{e}	2.72±0.05^{c}
T5	*Bam+Bv*	0.25±0.02^{d}	0.05±0.00^{b}	0.30±0.02^{d}	23.30±0.21^{a}	2.96±0.02^{a}
T6	*Bp+Bv*	0.27±0.01^{c}	0.06±0.01^{ab}	0.33±0.03^{c}	21.37±0.38^{b}	2.80±0.05^{b}
T7	*Bam+Bp+Bv*	0.27±0.01^{c}	0.06±0.01^{ab}	0.33±0.01^{c}	23.26±0.25^{a}	2.92±0.02^{a}
T8	Control	0.17±0.01^{e}	0.03±0.01^{c}	0.21±0.02^{e}	16.47±0.21^{f}	2.69±0.02^{c}
SE m (±)		0.007	0.005	0.01	0.177	0.016
C.D. (0.05)		0.021	0.014	0.029	0.53	0.048

T.No: Treatment number; ST: Seedling treatment; TC: Total carbohydrate; TP: Total protein content; Each figure represents mean (± SD) of three replications. Figures followed by same letter in a column do not differ significantly (P>0.05)

Table 17. Effect of bacterization with *Bacillus* spp. on yield of biochemical compounds of *W. somnifera.*

T. No.	ST	TA(µg 100 mg^{-1})	TA(µg plant^{-1})	TW (µg mg^{-1})	TW (mg plant^{-1})
T1	*Bam*	5.58±0.13^{c}	175.14±41.31^{bcd}	6.36±0.31^{b}	4.21±1.16^{c}
T2	*Bp*	6.64±0.006^{b}	217.19±57.08^{bc}	3.68±0.01^{d}	2.68±0.06^{cd}
T3	*Bv*	6.76±0.018^{b}	222.61±13.77^{b}	3.46±0.04^{d}	2.84±0.53^{cd}
T4	*Bam+Bp*	3.76±0.28^{e}	109.15±9.90^{cd}	4.83±0.01^{c}	2.19±0.05^{d}
T5	*Bam+Bv*	7.76±0.131^{a}	385.52±42.10^{a}	7.09±0.20^{a}	9.65±0.22^{a}
T6	*Bp+Bv*	7.86±0.34^{a}	251.90±87.91^{b}	7.30±0.57^{a}	7.26±1.95^{b}
T7	*Bam+Bp+Bv*	7.84±0.141^{a}	397.44±24.10^{a}	7.46±0.50^{a}	10.77±0.34^{a}
T8	Control	4.56±0.026^{d}	79.66±7.59^{d}	3.35±0.09^{d}	2.37±1.00^{d}
SE m (±)		0.049	37.76	0.174	0.524
T C.D. (0.05)		0.147	113.204	0.521	1.571

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No: Treatment number; ST: Seedling treatment; TA: Total alkaloid; TW: Total withanolide. Each figure represents mean (± SD) of three replications. Figures followed by same letter in a column do not differ significantly (P>0.05)

DISCUSSION

5. DISCUSSION

The study entitled “Growth, yield and secondary metabolite production responses to microbial elicitation in *Withania somnifera* (L.) Dunal.” was conducted at the Department of Plantation Crops and Spices, College of Agriculture, Vellayani, Thiruvananthapuram during 2019-2021. The results obtained from this study are discussed in this chapter.

In the first phase of the study, the seeds of *W. somnifera* were pretreated with different bacterial inoculants individually or in combination. In the second phase, roots of the randomly selected seedlings from each treatment in the first phase were soaked in the respective bacterial suspensions to evaluate their effect on morphological, biochemical and yield related attributes.

5.1 PHASE 1- EFFECT OF BIOPRIMING ON SEED GERMINATION AND SEEDLING GROWTH

5.1.1 Effect of Biopriming of Seed on Germination Parameters of *W. somnifera*

The results from the first phase of the study indicated that, the seed of *W. somnifera* subjected to different microbial treatments with PGPRs (*Bacillus* spp.) either individually or in combination exhibited better results in all the germination related parameters over untreated control and water soaking treatments. Among the bacterial inoculants tried, mixed application of *B. amyloliquefaciens* (*Bam), B. pumilus (Bp)* and *B. velezensis* (*Bv)* recorded the earliest germination (5.33 days). The untreated control followed by hydropriming treatment took the longest time (9.00 and 8.00 days, respectively) to germinate. Sengupta *et al*. (2015) opined that the enhancement of seed germination and seedling growth parameters on application of rhizobacteria may be due to the enhanced production of hormones like gibberellins that triggers the activity of specific enzymes responsible for early germination. In consensus with our finding, Wydnyana (2019) reported that, seed soaking in the mixture of bacterial suspension of

PGPRs could accelerate seed germination by two or more days than that by hydropriming.

Both the trio combination, *Bam+ Bp+ Bv* and the dual combination, *Bam+ Bv* exhibited similar trend in germination and survival per cent also. The highest germination and survival per cent (96.67 and 92.67 per cent, respectively) was observed in *Bam+ Bp+ Bv* which was on par with *Bam+ Bv* (92.67 and 82.00 per cent, respectively). The lowest mean germination time and the highest germination index were observed in the dual combination of *Bp+ Bv*. The seeds primed with *Bacillus* spp. recorded superior germination and seedling parameters over the untreated control and hydropriming. Kumar *et al*. (2015) reported that, tomato seedlings treated with microbial consortium led to more significant results than that by their single application on seedling vigour index, root and shoot length, root and shoot weight and leaf area. The performance of untreated control was inferior to all the microbial treatments. Among the individual treatments tried, *B. amyloliquefaciens* recorded higher seedling vigour index. Gowtham *et al*. (2018) reported that *B. amyloliquefaciens* improved both seed and plant growth parameters in chilli.

The morphological parameters of the seedlings reflected the same trend as the seed germination parameters. The trio combination, *Bam+Bp+Bv* recorded the higher leaf area (13.38 cm^2), basal shoot girth (0.81 cm), number of leaves (6.07), shoot length (5.77), root length (4.16 cm), seedling length (9.92 cm) and root volume (0.54 cm^3). The dual combination treatment, *Bp+Bv* was on par with *Bam+Bp+Bv* for all these parameters except for number of leaves and seedling length. A combination of *Bam+Bv* was found to be on par with the trio combination treatment on leaf area, basal shoot girth and number of leaves. The combination treatments followed by individual treatments recorded higher values of allometric index. The highest seedling vigour index was recorded in *Bam+Bp+Bv*. In line with this finding, the positive effect of the mixture of bacterial inoculants over their individual application on plant growth parameters was reported by Chakraborty *et al*. (2011) in mandarin plants and by Kaushal *et al*. (2019) in

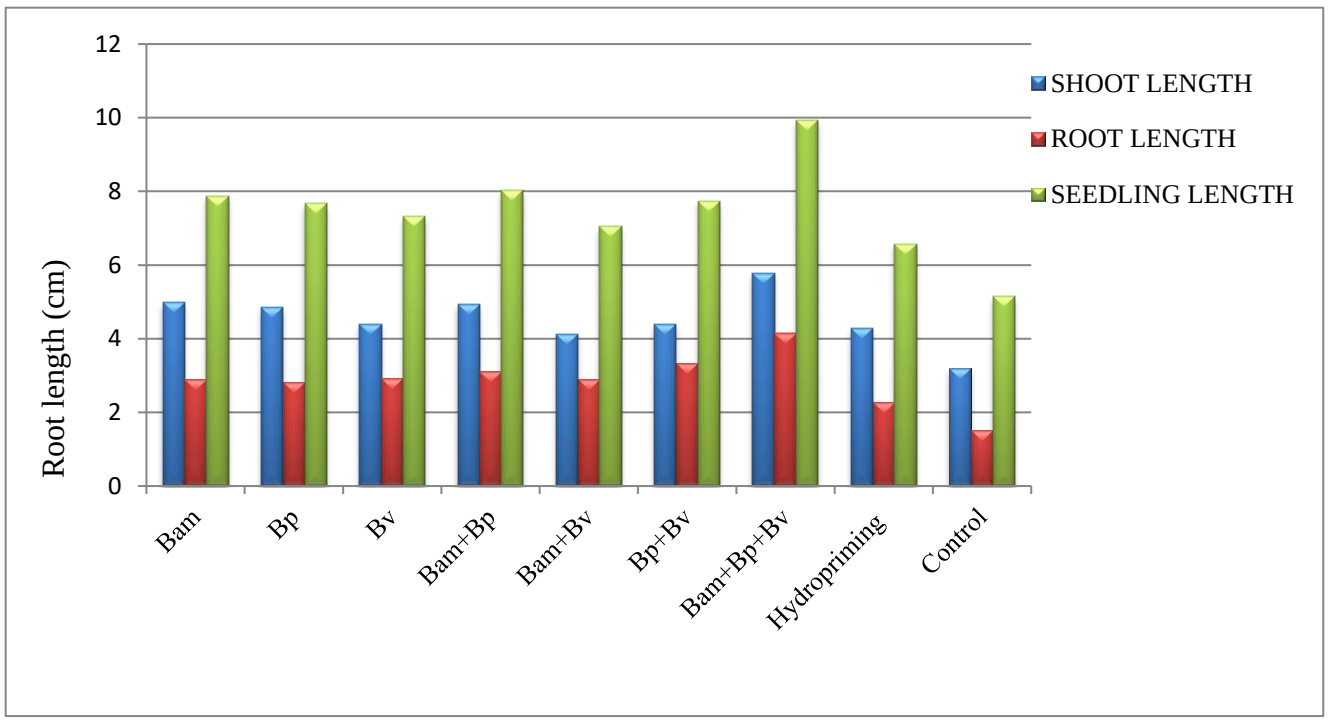

Fig 1. Effect of biopriming on shoot length, root length and seedling length of *W. somnifera* at 45 DAS

bell pepper. Similarly, diverse strains of *Bacillus* sp. are reported to enhance plant growth and secondary metabolite production in several medicinal plants (Koberl *et al*., 2013). The untreated control and hydropriming treatments recorded the lowest values with respect to seedling growth parameters compared to priming with *Bacillus* spp. The effect of biopriming treatments on shoot, root and seedling length of *W. somnifera* at 45 days after sowing is presented in Figure 1.

5.2 PHASE II- EFFECT OF *BACILLUS* SPP. ON GROWTH, YIELD AND SECONDARY METABOLITE PRODUCTION.

5.2.1 Effect of *Bacillus* spp. on Plant Growth Parameters of *W. somnifera*

The earliest flowering (83.22 DAS) was observed in combination of *Bam+ Bv*. The control and the trio combination, *Bam+Bp+Bv* took the longest time to flower, among the treatments applied. According to Kaymak (2011), plant growth promoting rhizobacteria can induce early flowering in crops. It is observed that, inspite of early germination and higher seedling vigour index exhibited by *Bam+Bp+Bv*, flowering initiated late in the treatment. Hence, it can be inferred that a better seed germination or seedling vigour index need not always impart earliness in flowering and fruit set in the crop.

The pronounced effect of *Bam+Bp+Bv* in seed germination and seedling growth was found to be continued in the second phase of the study with respect to morphological parameters. The morphological parameters such as shoot length (78.99 cm), number of branches (8.78), number of leaves (71.00), collar girth (3.91 cm), leaf area (5146.81 cm^2) and number of flowering branches (7.89) were the highest in the trio combination treatment and was on par with combination of *Bam+Bv* in all the parameters except leaf area. In agreement with this finding, Ju *et al*. (2019) opined that a combination of different strains of the same species can boost the beneficial traits in crops over their single application. He *et al*. (2019) reported that a bacterial consortium recorded higher

values of total biomass, root fresh and dry weight and root:shoot ratio over uninoculated control.

In the first phase of the study, though the dual combination, *Bp+Bv* was found to be comparable with *Bam+Bp+Bv* for seedling growth parameters, it did not show similar response in morphological parameters in the second phase. The control treatment continued to be the least significant treatment among all. It was observed to be on par with *Bam+Bp* in terms of morphological parameters, *viz.*, number of branches, collar girth, leaf area and number of flowering branches. The inhibitory effect of *Bam+Bp* may be due to the competing nature of *B. pumilus* over *B. amyloliquefaciens*, though they are biocompatible (He *et al*., 2019).

The individual bacterial treatments- *Bam, Bp* and *Bv* exhibited on par values with respect to morphological parameters observed. Among the dual combinations tried *Bam+Bv* followed by *Bp+Bv* recorded better results.

5.2.2 Effect of *Bacillus* spp. on Yield Parameters of *W. somnifera*

Bam+Bp+Bv recorded the highest leaf weight- fresh and dry (45.89 g plant $^{-1}$ and 5.07 g plant $^{-1}$, respectively) and were on par with the dual combination treatments, *Bam+Bv* and *Bp+Bv*. The effect of bacterization *Bacillus* spp. on leaf yield at harvest is depicted in Figure 2. Stem weight also showed a similar trend with *Bam+Bp+Bv* recording higher fresh and dry weight (61.85 g plant $^{-1}$ and 9.78 g plant $^{-1}$, respectively) and this also was found to be on par with *Bam +Bv* and *Bp+ Bv*. The highest values of berry weight – fresh and dry (8.85 g plant $^{-1}$ and 5.33 g plant $^{-1}$, respectively) shoot weight – fresh and dry (97.48 g plant $^{-1}$ and 17.52 g plant $^{-1}$, respectively) were observed in *Bam+ Bv*, which was on par with *Bam+ Bp+ Bv*. The higher values of stem fresh and dry weight, shoot fresh weight, berry fresh weight and berry dry weight were recorded in combination treatments followed by individual treatments. The said parameters were observed to be lower in control except for fresh and dry weight of berries. The leaf fresh and dry weight was the lowest in control treatments. Other than *Bam+Bp*, all the

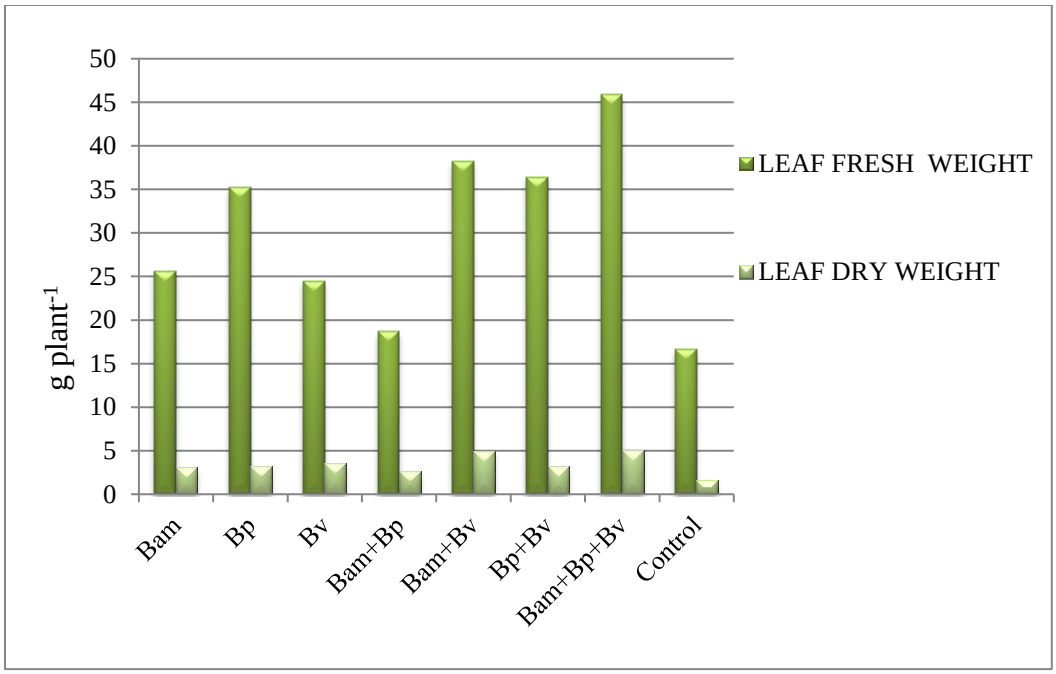

Fig 2. Effect of bacterization with *Bacillus* spp. on leaf yield of *W. somnifera* at harvest

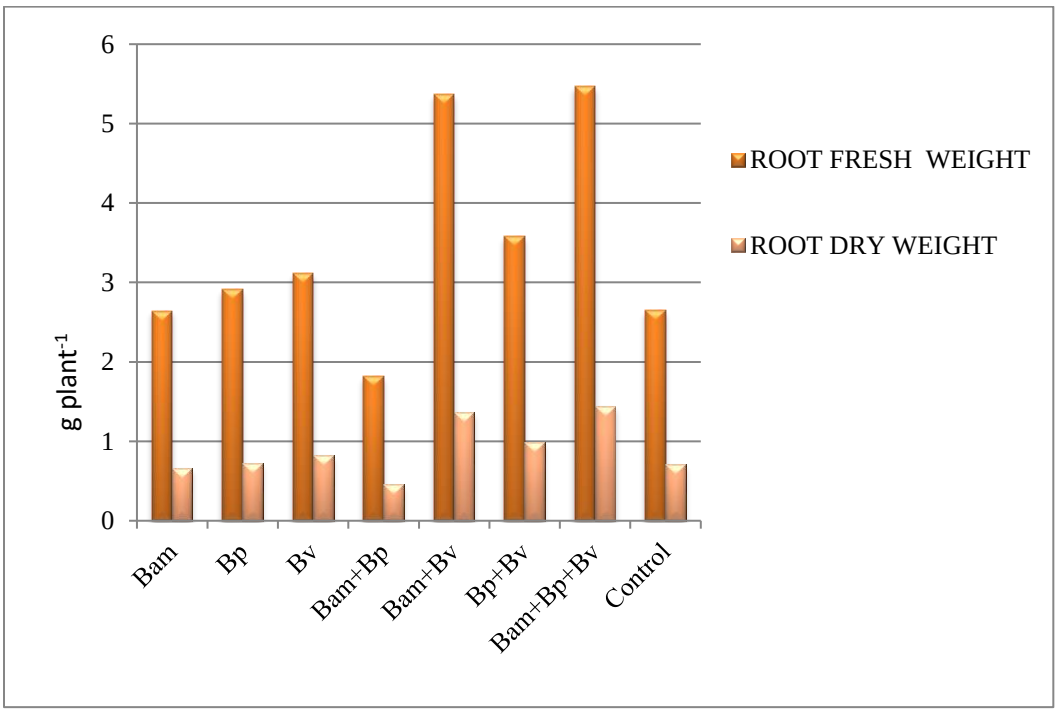

Fig 3. Effect bacterization with *Bacillus* spp. on root yield of *W. somnifera* at harvest

combination treatments recorded higher fresh and dry weight of leaves over individual treatments. The control treatment recorded the lowest stem fresh and dry weight, leaf fresh and dry weight and shoot fresh weight. The lowest stem dry weight was recorded in *Bv* and it was on par with other individual treatments, Bam *+Bv* and control. The total dry matter production was the highest (18.89 g plant^{-1}) in *Bam+ Bv* which was on par with *Bp+Bv* and *Bam+Bp+Bv*. The individual treatment, *Bv* recorded the lowest total dry matter. It was on par with other individual treatments and control. The pronounced effect of bacterial inoculation either individually or in combination over water soaking was also reported by Yadav (2017). He stated that co inoculation followed by individual application of bacterial inoculants yielded better leaf fresh weight in *Ocimum basilicum* over the uninoculated control. Similarly He *et al*. (2019) reported 34.17 per cent higher total biomass from bacterial treatments in combination over uninoculated control. The combination treatment of *Bam +Bp* exhibited good response with respect to yield parameters compared to other two dual combinations *(Bam+ Bv* and *Bp+Bv*).

The number of berries and seed yield per plant was highest in *Bam+Bv* which was on par with *Bam+Bp+Bv* combination. The lower number of berries and seed yield was observed in all the individual inoculation (*Bam, Bp* and *Bv*) and in the dual combination *Bam+Bp*. These were on par with the control treatment. 100 seed weight was highest (0.26 g) in *Bam+Bp+Bv* and it was on par with *Bam+ Bv*. The control treatment recorded the lowest (0.19 g) and was on par with the individual treatments, *Bam, Bp* and *Bv*. In contrast to this, better fruit or seed yield over the untreated control by the application of *B. amyloliquefaciens* in chilli (Hernandez *et al.*, 2018), *B. pumilus* in strawberry (Kaushal *et al*., 2017) and *B. velezensis* in tomato (Siripornvisal *et al*., 2021) were reported.

Root yield related attributes such as root length, root diameter, root volume, root fresh and dry yield were the highest (21.27cm, 1.33cm, 5.39 cm^3, 5.47 g plant^{-1} and 1.44 g plant^{-1} respectively) in *Bam+Bp+Bv* and was on par with *Bam +Bv*. These were followed by *Bp+ Bv* with respect to root parameters. The dual combination, *Bam+Bp*

recorded the lower root length (13.96 cm) root diameter (0.81 cm) root volume (1.61 cm^3) root fresh yield (1.82 g $plant^{-1}$) and root dry yield (0.45 g $plant^{-1}$) and was observed to be on par with the control treatment. All the individual bacterial treatment also showed on par value with the control. The effect of bacterization with *Bacillus* spp. on root yield of *W. somnifera* is presented in Figure 3. In dissension to this finding, *B. pumilus* inoculated red pepper seedlings showed the greatest increase in root fresh weight by 20% compared to the control (Joo *et al*., 2004). Similarly, Shao *et al.* (2014) reported that individual application of *B. amyloliquefaciens* in cucumber seedlings enhanced root growth parameters over the untreated control. Chilli seedlings when treated with bacterial suspension of *B. velezensis* exhibited higher root length and root fresh weight compared to untreated plants (Shin *et al*., 2021). However, Sibponkrung *et al*. (2020) observed higher root yield in soybean when treated with bacterial consortium rather than their individual inoculation.

Plant growth promoting rhizobacteria such as *Bacillus* spp. enhances root growth by the production of phytohormones like auxins that take part in primary and secondary root elongation, cytokinins that control root meristem differentiation and induce root hair proliferation, and gibberellins that are responsible for abundance of root hairs (Riefler *et al*., 2006; Spaepen *et al*., 2007; Sansinenea , 2019).

The harvest index with respect to the leaf yield did not vary significantly. But a significant difference in the harvest index with respect to the root yield was observed. The highest harvest index (0.10) was recorded in *Bam+Bp+Bv* which was on par with dual combination of *Bam+Bv*. A combination of *Bam+Bp* recorded the lowest harvest index with respect to the root yield. Among the individual microbial treatments tried, *Bv* showed higher values

5.2.3 Effect of *Bacillus* spp. on Biochemical Parameters of *W. somnifera*

At the time of harvest, the chlorophyll a, b and total chlorophyll were reported to be higher in *Bv*. The individual application of bacterial inoculants (*Bam*, *Bp* and *Bv*)

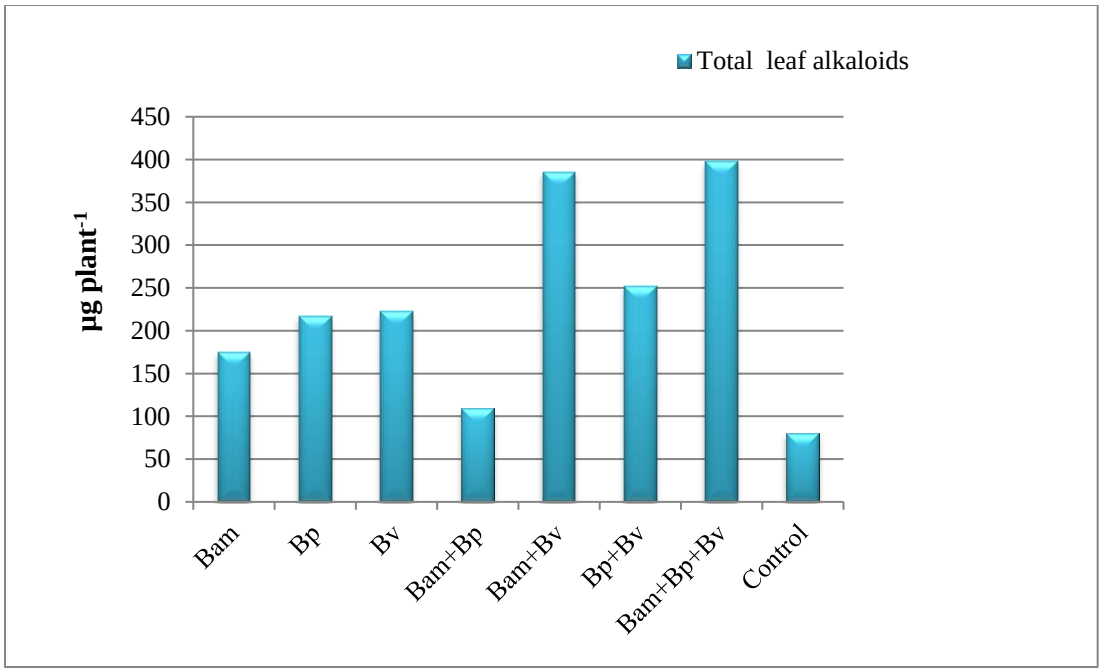

Fig 4. Effect of bacterization with *Bacillus* spp. on total leaf alkaloids yield in *W. somnifera* at harvest

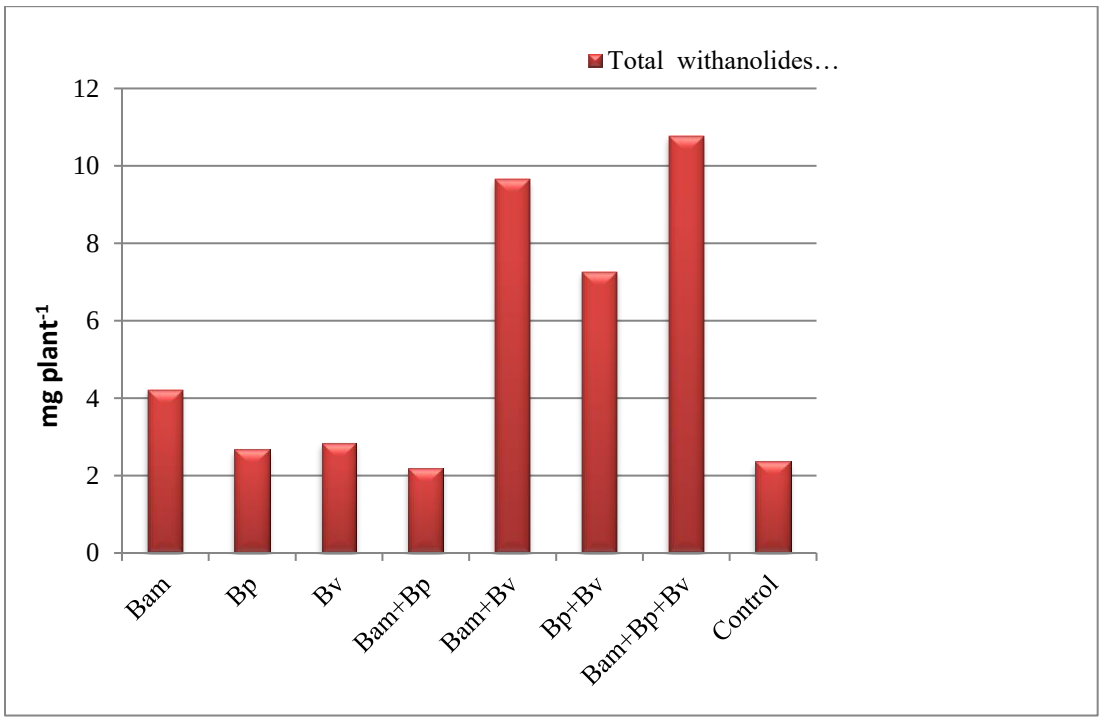

Fig 5. Effect of bacterization with *Bacillus* spp. on total withanolides yield in roots of *W. somnifera* at harvest

recorded better results in total chlorophyll content than their combination and mixture. The hydropriming treatment recorded the lowest among the treatments. *Bam* + *Bv* recorded the least values on chlorophyll a, b and total chlorophyll content among the microbial treatments tried. In agreement with this finding, Marious *et al*. (2013) reported the highest chlorophyll content in runner bean leaves by the single application of bacterial inoculants over their combination which exhibited more pronounced effect during vegetative and harvest stages. And also, Bayisa *et al*. (2020) noticed enhanced chlorophyll content in sesame plants treated with *B. velezensis*.

Rajasekar and Elango (2011) opined that microbial consortium could elicit the total alkaloid content along with plant growth in *W. somnifera.* In the present study, among the treatments tried, all the combination treatments except *Bam*+ *Bp* recorded higher total alkaloid content in the leaves. The highest total alkaloid content in leaves (7.86 µg 100 mg^{-1}) was recorded in *Bp*+ *Bv* which was on par with *Bam*+ *Bv* and *Bam*+ *Bp*+ *Bv*. The least recorded treatment was *Bam*+ *Bp* (3.76 µg 100 mg^{-1}) and was on par with *Bam, Bp* and the control. According to He *et al*. (2019) this inhibitory effect may be due to the competing nature of *B. pumilus* over *B. amyloliquefaciens* even if they are biocompatible. The yield of total alkaloids on per plant basis was higher (397.44 µg $plant^{-1}$) in the trio combination, *Bam*+ *Bp*+ *Bv* which was on par with *Bam*+ *Bv*. The lowest (79.66 µg $plant^{-1}$) was recorded in the control and was found to be on par with the individual treatment, *Bam*. This is due to the variation in the dry leaf yield among the treatments. Among the individual bacterial inoculants tried, *Bv* recorded the highest and *Bam* recorded the least values in terms total leaf alkaloid content and total alkaloid yield per plant. The effect of bacterization with *Bacillus* spp. on total leaf alkaloid yield per plant is presented in Figure 4.

The total protein content in roots of *W. somnifera* varied between 3 and 8 per cent (Kujur *et al*., 2021). The highest protein content (2.96 mg 100 mg^{-1}) in the roots was recorded in *Bam*+*Bv* which was on par with *Bam*+ *Bp*+ *Bv* (2.92 mg 100 mg^{-1}). The lowest protein content (2.64 mg 100 mg^{-1}) was observed in the control. It was on par

with *Bam, Bv* and *Bam+ Bp*. Enhanced crude protein content in amaranthus treated with a bacterial combination over untreated control was reported by Panday *et al*. (2018). Also, Guo *et al*. (2020) found that a combination of bacterial inoculants enhanced soluble protein level of chilli fruits.

In a study by Kujur *et al*. (2021), the total carbohydrate content in the roots of *W. somnifera* was reported to be varying between 13 and 39 per cent. In consensus with this, the carbohydrate content in the roots of *W. somnifera* varied between 16 and 23 per cent in the present study. The highest (23.30 mg 100 mg^{-1}) was recorded in the dual combination of *Bam+Bv*. *Bam+Bv* was on par with *Bam+Bp+Bv*. The lowest value was observed in hydroprming treatment which recorded 16.47 mg 100 mg^{-1} of carbohydrate content in roots. The positive impact of microbial combination was justified by Pandey *et al*. (2018) who reported 49.08 per cent enhancement in carbohydrate content of amaranthus treated with two *Bacillus* strains, *B. pumilus* and *B. subtilis.*

The total withanolide content in the roots of *W. somnifera* ranged between 0.33 and 0.75 per cent. The highest value (7.46 µg mg^{-1}) was recorded in *Bam+Bp+Bv* which was on par with *Bam+Bv* and *Bp+Bv*. The hydropriming treatment recorded the lowest total withanolide content. All the combination treatments were superior to individual treatments and control except *Bam+Bp* which recorded total withanolide lower than *Bam*. The additive effect of bacterial treatments as a consortium on plant growth and quality stated by Kumar *et al*. (2013) was in conformation with this finding. The per plant yield of total withanolide was the highest in *Bam+Bp+Bv* and was on par with *Bam +Bv*. The lowest total withanolide yield was observed in *Bam+Bp* which was on par with *Bp, Bv* and the control treatment. This is because the *Bam+Bp* recorded lowest dry root yield among all the treatments tried. The effect of bacterization with *Bacillus* spp. on total withanolide yield per plant is depicted in Figure 5.

Among the biochemical parameters analyzed, the overall performance of the trio combination, *Bam+Bp+Bv* and the dual combination, *Bam +Bv* was the best followed by the dual combination, *Bp+Bv*. The trio combination of *B. amyloliquefaciens, B. pumilus*

and *B. velezensis* and the dual combination, *B. amyloliquefaciens* and *B. velezensis* was observed to give better performance with respect to the biochemical parameters, total alkaloid content in the leaves, withanolide content, carbohydrate content and protein in the roots. Among individual treatments tried *B. velezensis* was found to be better. The control recorded the least in all biochemical parameters except in case of total alkaloids.

In the present investigation, seed priming with bacterial inoculants, *Bacillus* spp. either individually or in combination recorded better results over the control in germination, and seedling growth, parameters. In the second phase of the study, wherein the seedlings were given a dip in the respective bacterial suspension, the combination treatments gave better performance with respect to plant growth, yield and biochemical parameters except for one combination *Bam+ Bp*. Inspite of *Bam+ Bp* showing higher germination percent and seedling vigour index, it did not show better performance with respect to plant growth, yield and biochemical parameters in the second phase of the study. However, *Bam+ Bv* which recorded on par values with *Bam+ Bp*, in terms of germination per cent and seedling vigour index in the first phase, *Bam+ Bv* also recorded the best performance with respect to plant growth, yield and biochemical parameters, on par with the trio combination.

In the first phase of the study, the trio combination of *Bam + Bp+ Bv* gave the best performance in terms of seed germination, seedling growth and seedling vigor index, In the second phase, *Bam + Bp+ Bv* and *Bam+ Bv* gave superior performance, in terms of plant growth, yield and biochemical parameters.

Future Line of Work

- The effect of seed priming and seedling treatments with bacterial inoculants in *W. somnifera* has to be investigated in field conditions.

- The effect of *Bacillus* spp. on *W. somnifera* at varying frequencies, concentration and mode of application has to be explored.

- The trio combination of PGPRs of the present study has to be formulated and its effects on other medicinally important crops have to be investigated.

SUMMARY

6. SUMMARY

The study entitled “Growth, yield and secondary metabolite production responses to microbial elicitation in *Withania somnifera* (L.) Dunal.” was conducted at the Department of Plantation Crops and Spices, College of Agriculture, Vellayani, Thiruvananthapuram during 2019-2021 with an objective to evaluate the effect of bacterial inoculants on seed germination, seedling vigour, growth, yield and secondary metabolite production in *W. somnifera.*

Seeds of *W. somnifera* for the study were procured from Anand Agricultural University, Gujarat and the microbial cultures were obtained from the Department of Agricultural Microbiology, College of Agriculture, Vellayani. The study was conducted in two phases. Phase 1- Effect of bioprimimg of seeds on germination and seedling growth; Phase 2-Effect of bacterial inoculants on plant growth, yield and secondary metabolite production. The seeds of *W. somnifera* were subjected to biopriming with *B. amyloliquefaciens, B. pumilus* and *B. velezensis* at 1x 10^8cfu mL^{-1} individually and in combination followed by root dipping in respective bacterial suspension. Water soaked seeds and seeds without biopriming treatments were taken as control in the first phase. In the second phase, untreated seedlings were taken as the control.

In the first phase of the study, the seeds primed with a combination of *B.amyloliquefaciens, B.pumilus* and *B.velezensis* (T7) recorded the earliest germination (5.33 days), the highest germination per cent (96.67) and survival per cent (92.67). It was on par with T5 (*Bam*+ *Bp*). A combination of *Bp*+ *Bv* (T6) recorded the lowest mean germination time (8.67) and the highest germination index (6.15) which was on par with combination treatments, T5 (*Bam*+ *Bp*) and T7(*Bam*+ *Bp*+ *Bv*).

The morphological parameters of the seedlings *viz.,* basal shoot girth, number of leaves, leaf area, shoot length, root length and root volume were the highest (0.81 cm, 6.07, 13.38 cm^2, 5.77cm, 4.16 cm and 0.54 cm^3 , respectively) in T7, trio combination of *Bam*+ *Bp*+ *Bv*. The highest allometric index (0.76) was observed in (*Bp*+ *Bv*) which was

on par with dual the combination treatments, T5 and T7. The highest seedling vigour index was observed in T7. All the biopriming treatments with *Bacillus* spp. recorded superior germination and seedling parameters over the untreated control (T9) and hydropriming (T8).

In the second phase of the study the 45 days old bacterized seedlings were transplanted in polybags after root dip in the respective bacterial suspension for 30 min. The plants were evaluated for growth, yield and biochemical parameters.

The morphological parameters such as shoot length (78.99 cm), root length (21.27 cm), root diameter (1.33 cm), root volume (5.39 cm^3), number of branches (8.78), number of leaves (71.00), leaf area (5146.81 cm^2) and number of flowering branches (7.89) were observed to be higher in trio combination, *Bam+Bp+Bv* and were on par with T5 in the said parameters. The highest collar girth and earliest flowering was observed in T7 (*Bam+Bp+Bv*) and T5 (*Bam+ Bv*) respectively. The control treatment were observed to be the lowest with respect to morphological parameters, *viz.*, number of leaves, collar girth, leaf area and number of flowering branches and was found to be on par with T4 (*Bam+Bp*). There were no any significant difference days to fruit set among the treatments tried.

Yield determining parameters such as shoot fresh and dry weight, berry fresh and dry weight, number of berries and seed yield per plant and total dry matter production were the highest (97.48, 17.51, 8.85 and 5.32 g plant $^{-1}$, 90.56, 7.35, 18.89 g $plant^{-1}$ respectively) in T5 (*Bam+ Bv*), which was on par with T7 (*Bam+Bp+Bv*). Stem fresh and dry weight, leaf fresh and dry weight, root fresh and dry weight, and 100 seed weight (61.85, 9.78, 45.89, 5.07, 5.47 and 1.44 g $plant^{-1}$ and 0.26g respectively) were observed to be the highest in T7 (*Bam+Bp+Bv*) and was on par with T5 (*Bam+ Bp*). The control treatment recorded the lowest stem fresh and dry weight, leaf fresh and dry weight and shoot fresh weight. The lowest stem dry weight and total dry matter production were recorded in T3 (*Bv*) and it was on par with other individual treatments, T4 and control. The individual treatments (T1, T2 and T3) recorded lower values in berry and root yield

related characters which were on par with the control treatment. The harvest index with respect to the leaf yield did not vary significantly. However, the harvest index in terms of root yield varied significantly among the treatments. The highest harvest index (0.10) was observed in T7, which was also observed to be on par with T5.

The chlorophyll content was reported to be higher in T3 (*Bv*). The highest total alkaloid content in leaves (7.86 µg 100 mg^{-1}) was recorded in T6 (*Bp+Bv*) which were on par with T5 (*Bam +Bv*) and T7 (*Bam+Bp+Bv*). T5 recorded the highest protein and carbohydrate content (2.96 and 23.30 µg $mg^{-1,}$ respectively) in the roots which was on par with T7. The withanolide content was superior (7.46 µg mg^{-1}) in T7 (*Bam+Bp+Bv*) which was on par with T5 and T6. The yield of biochemical parameters on per plant basis *viz.*, total leaf alkaloids, total root withanolides were the highest (397.44 µg $plant^{-1}$, 10.77 mg $plant^{-1}$ respectively) in trio combination of T7 (*Bam+Bp+Bv*) which was on par with dual combination, T5 (*Bam +Bv*).

In the present investigation, seed priming with bacterial inoculants, *Bacillus* spp. either individually or in combination recorded better results over the control in germination, and seedling growth parameters. In the second phase of the study, wherein the seedlings were given a dip in the respective bacterial suspension, it was observed that the combination treatments gave better performance with respect to plant growth, yield and biochemical parameters except for one combination T4 *(Bam+ Bp).* Inspite of T4 showing higher germination per cent and seedling vigour index, it did not show better performance with respect to plant growth, yield and biochemical parameters in the second phase of the study. However, T5 which recorded on par values in terms of germination per cent and seedling vigour index with T4 in the first phase recorded the best performance with respect to plant growth, yield and biochemical parameters, on par with the trio combination.

In the first phase of the study, the trio combination of *Bam+ Bp+ Bv* (T7) gave the best performance in terms of seed germination, seedling growth and seedling vigor index, In the second pahse, *Bam + Bp+ Bv* (T7) and *Bam+ Bv* (T5) gave superior performance, in terms of plant growth, yield and biochemical parameters.

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ABSTRACT

GROWTH, YIELD AND SECONDARY METABOLITE PRODUCTION RESPONSES TO MICROBIAL ELICITATION IN *Withania somnifera* (L.) DUNAL

by

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8. ABSTRACT

The study entitled “Growth, yield and secondary metabolite production responses to microbial elicitation in *Withania somnifera* (L.) Dunal.” was conducted at the Department of Plantation Crops and Spices, College of Agriculture, Vellayani, Thiruvananthapuram during 2019-2021 with a view to evaluate the effect of bacterial inoculants on seed germination, seedling vigour, growth, yield and secondary metabolite production in *W. somnifera.*

Seeds of *W. somnifera* were primed with *B. amyloliquefaciens* (*Bam*), *B. pumilus* (*Bp*) and *B. velezensis* (*Bv*) at 1x 10^8cfu mL^{-1} individually and in combination for 24 h. Among these treatments, T7, the trio combination of *Bam+Bp+Bv* recorded the earliest germination (5.33 days) highest germination per cent (96.67), survival per cent (92.67) seedling vigour index (958.93), basal shoot girth (0.81 cm), number of leaves (6.07), leaf area (13.38 cm^2), shoot length (5.77cm), root length (4.16 cm) and root volume (0.54 cm^3). All the biopriming treatments with *Bacillus* spp. recorded superior germination and seedling parameters over the untreated control (T9) and hydropriming (T8).

The seedlings from the first phase were subjected to root dip with the respective bacterial suspension for 30 min on transplanting. The morphological and yield determining parameters such as shoot length(78.99 cm), root length (21.27cm), number of branches (8.78), number of leaves (71.00), collar girth (3.91 cm), leaf area (5146.81 cm^2) number of flowering branches (7.89), stem fresh weight (61 .85 g $plant^{-1}$), stem dry weight(9.78 g $plant^{-1}$), leaf fresh weight (45.89 g $plant^{-1}$), leaf dry weight (5.07g $plant^{-1}$), root fresh weight (5.47g $plant^{-1}$), root dry weight (1.44 g $plant^{-1}$) 100 seed weight (0.26g) root diameter (1.33cm), root volume (5.39 cm^3) and harvest index (0.10) were observed to be significantly higher in T7, the trio combination of (*Bam+ Bp+ Bv*), which was observed to be on par with T5, dual combination of (*Bam+ Bv*). T5 was found to be superior in shoot fresh and dry weight, berry fresh and dry weight, number of berries and

seed yield per plant and total dry matter production (97.48, 17.51, 8.85 and 5.32 g plant^{-1}, 90.56, 7.35, 18.89 g plant^{-1} respectively, which was observed to be on par with T7. All the said parameters were significantly lower in untreated control.

Seedlings treated with bacterial suspension of *B. velezensis* (*Bv*) recorded highest chlorophyll content in the leaves of *W. somnifera* at the time of harvest. The highest total alkaloid content in leaves (7.86 µg 100 mg^{-1}) was recorded in dual combination of *Bp+Bv* which was on par with the other combinantions, *Bam+Bv* (T5) and *Bam+Bp+Bv* (T7). T5 recorded the highest protein and carbohydrate content (2.96 and 23.30 mg 100 mg^{-1} respectively) in the roots which was on par with T7. The withanolide content was superior (7.46 µg mg^{-1}) in T7, *Bam+Bp+Bv* which was on par with T5, *Bam+Bv* and T6, *Bp+Bv*. The yield of biochemical parameters on per plant basis *viz.*, total leaf alkaloids, total root withanolides were the highest (397.44 µg plant$^{-1,}$ and 10.77 mg plant^{-1} respectively) in trio combination of T7 which was on par with dual combination T5. The control treatment recorded significantly lower values in all the biochemical parameters observed.

In the first phase of the study, the trio combination of *Bam + Bp+ Bv* (T7) gave the best performance in terms of seed germination, seedling growth and seedling vigor index, In the second phase, *Bam + Bp+ Bv* (T7) and *Bam+ Bv* (T5) gave superior performance, in terms of plant growth, yield and biochemical parameters.

അശ്വഗന്ധയുടെ (വിതാനിയ സോമിനിഫെറ) വളർച്ചയിലും വിളവിലും ദ്വിതീയ മെറ്റാബോലൈറ്റ് ഉൽപ്പാദനത്തിലും സൂക്ഷ്മാണു പ്രയോഗം ഉളവാക്കുന്ന പ്രതികരണം

കാർഷിക കോളേജ് വെള്ളായണിയിലെ തോട്ട സുഗന്ധവിള വിഭാഗത്തിൽ അശ്വഗന്ധ ചെടികളുടെ വിത്ത് മുളയ്ക്കൽ, തൈകളുടെ വീര്യം, വളർച്ച, വിളവ്, ദ്വിതീയ മെറ്റാബോലൈറ്റ് ഉൽപ്പാദനം എന്നിവയിൽ ജൈവസൂക്ഷ്മാണുക്കളുടെ പ്രഭാവം വിലയിരുത്തുക എന്ന ലക്ഷ്യത്തോടെ "അശ്വഗന്ധയുടെ (വിതാനിയ സോമിനിഫെറ) വളർച്ചയിലും വിളവിലും ദ്വിതീയ മെറ്റാബോലൈറ്റ് ഉൽപ്പാദനത്തിലും സൂക്ഷ്മാണു പ്രയോഗം ഉളവാക്കുന്ന പ്രതികരണം " എന്ന വിഷയത്തിൽ 2019-2021 കാലയളവിൽ പഠനം നടത്തുകയുണ്ടായി.

ബാസില്ലസ് അമൈലോലിക്വിഫേഷ്യൻസ്, ബാസില്ലസ് പ്യൂമിലസ്, ബാസില്ലസ് വലസൻസിസ് എന്നീ ബാക്റ്റീരിയകളെ $1x10^8$ cfu mL^{-1} എന്ന തോതിൽ വ്യക്തിഗതമായും സംയോജിപ്പിച്ചും അശ്വഗന്ധയുടെ വിത്തുകളുമായി 24 മണിക്കൂർ പരിചരിച്ചു. മേല്പറഞ്ഞ പരീക്ഷണങ്ങളിൽ മൂന്ന് സൂക്ഷ്മാണുക്കളുടെയും മിശ്രിതം (T7) ഉപോയോഗിച്ച് പരിചരിച്ച വിത്തുകൾ ആണ് ഏറ്റവും നേരത്തെ മുളച്ചത് (5.33 ദിവസം). കൂടാതെ, ഏറ്റവും ഉയർന്ന മുളയ്ക്കൽ ശതമാനം (96.67), അതിജീവന ശതമാനം (92.67) തൈകളുടെ വീര്യ സൂചിക (958.93), ബേസൽ ഷൂട്ട് ചുറ്റളവ് (0.81 സെ.മീ.), ഇലകളുടെ എണ്ണം (6.07), ഇലകളുടെ വിസ്തീർണ്ണം (13.38 സെ.മീ^{2}), ഷൂട്ടിന്റെ നീളം (5.77 സെ.മീ.), വേരുകളുടെ നീളം (4.16 സെ.മീ.), വേരുകളുടെ വ്യാപ്തി (0.54 സെ.മീ^{3}) എന്നിവയും രേഖപ്പെടുത്തിയത് ഈ മിശ്രിതത്തിലാണ്. ബാസില്ലസ് ഉപയോഗിച്ചുള്ള എല്ലാ പരീക്ഷണങ്ങളും ബാസില്ലസ് രഹിത നിയന്ത്രണത്തെക്കാളും (T9) വെള്ളത്തിൽ മുക്കി വച്ച് നട്ടതിനെക്കാളും (T8) മികച്ചതായിരുന്നു.

ബാക്റ്റീരിയകളുമായി മുൻകൂർ പരിചരിച്ച് മുളപ്പിച്ച തൈകൾ പരീക്ഷണത്തിന്റെ രണ്ടാം ഘട്ടത്തിൽ അതേ സൂക്ഷ്മജീവികളുടെ ലായനികളിൽ 30 മിനിറ്റ് നേരത്തേക്ക് മുക്കിവക്കുകയും ഗ്രോബാഗുകളിൽ മാറ്റി നടുകയും ചെയ്തു. ചെടികളുടെ രൂപഘടനയും വിളവും നിർണയിക്കുന്ന

ഘടകങ്ങളായ ഷൂട്ടിന്റെ നീളം (78.99 സെ.മീ), വേരിന്റെ നീളം (21.27 സെ.മീ), ശാഖകളുടെ എണ്ണം (8.78), ഇലകളുടെ എണ്ണം (71.00), കോളർ ചുറ്റളവ് (3.91 സെ.മീ), ഇലകളുടെ വിസ്തീർണ്ണം (5146.81 സെ.മീ ^{2}) പൂവിടുന്ന ശാഖകളുടെ എണ്ണം (7.89), തണ്ടിന്റെ ഭാരം (പച്ച- 61 .85 ഗ്രാം ചെടി$^{-1}$, ഉണക്ക -9.78 ഗ്രാം ചെടി$^{-1}$), ഇലയുടെ ഭാരം (പച്ച- 45.89 ഗ്രാം ചെടി$^{-1}$,, ഉണക്ക- 5.07 ഗ്രാം ചെടി$^{-1}$) വേരിന്റെ ഭാരം (പച്ച- 5.47 ഗ്രാം ചെടി$^{-1}$, ഉണക്ക- 1.44 ഗ്രാം ചെടി$^{-1}$) 100 വിത്തുകളുടെ ഭാരം (0.26 ഗ്രാം) വേരുകളുടെ വ്യാസം (1.33 സെ.മീ), വ്യാപ്തി (5.39 സെ.മീ^{3}), വിളവെടുപ്പ് സൂചിക (0.10) എന്നിവ മൂന്നു ബാക്റ്റീരിയകളുടെയും സംയോജിത പരീക്ഷണത്തിൽ ഗണ്യമായി ഉയർന്നതായി നിരീക്ഷിച്ചു. ബാസില്ലസ് അമൈലോലികഫേഷ്യൻസ്, ബാസില്ലസ് വലസൻസിസ് എന്നീ ബാക്റ്റീരിയകളുടെ ഇരട്ട സംയോജനത്തിലും മൂന്നു സൂക്ഷ്മാണുക്കളുടെയും മിശ്രിതത്തിനു തത്തുല്യമായ ഫലങ്ങളാണ് രേഖപ്പെടുത്തിയത്. ചെടികളുടെ ഭാരം (പച്ച-97.48 ഗ്രാം ചെടി$^{-1}$, ഉണക്ക-17.51 ഗ്രാം ചെടി$^{-1}$), കായ്കളുടെ ഭാരം (പച്ച- 8.85 ഗ്രാം ചെടി$^{-1}$, ഉണക്ക- 5.32 ഗ്രാം ചെടി$^{-1}$), വിത്തുകളുടെ എണ്ണം (90.56), വിത്തുകളുടെ ഭാരം (7.35 ഗ്രാം ചെടി$^{-1}$), മൊത്തം ഉണങ്ങിയ സസ്യ വസ്തുക്കളുടെ ഉത്പാദനം (18.89 ഗ്രാം ചെടി$^{-1}$) എന്നിവയിൽ ബാസില്ലസ് അമൈലോലികഫേഷ്യൻസ്, ബാസില്ലസ് വലസൻസിസ് എന്നീ ബാക്റ്റീരിയകളുടെ ഇരട്ട സംയോജനം മികച്ചതായി കണ്ടെത്തി. ഇത് മൂന്നു ബാക്റ്റീരിയകളുടെയും സംയോജിത പരീക്ഷണത്തിന് തുല്യമാണെന്ന് നിരീക്ഷിച്ചു. മേൽപറഞ്ഞ ഘടകങ്ങൾ ബാസില്ലസ് രഹിത പരീക്ഷണത്തിൽ വളരെ കുറഞ്ഞ അളവിലാണ് രേഖപ്പെടുത്തിയത്.

ബാസില്ലസ് വലസൻസിസ് (T3) ഉപയോഗിച്ച് പരിചരിച്ച തൈകളിൽ നിന്നുള്ള ചെടികളിൽ ഉത്പാദിപ്പിച്ച ഹരിതകം വിളവെടുപ്പ് സമയത്ത് ഏറ്റവും ഉയർന്നതായി കാണപ്പെട്ടു. ഇലകളിലെ ഏറ്റവും ഉയർന്ന ആൽക്കലോയിഡ് അംശം (7.86 µg 100 mg-1) ബാസില്ലസ് പ്യൂമിലസ്, ബാസില്ലസ് വലസൻസിസ് (T6) എന്നിവയുടെ ഇരട്ട സംയോജനത്തിലാണ് രേഖപ്പെടുത്തിയത്. ഇത് മറ്റ് സമ്മിശ്രങ്ങളായ ബാസില്ലസ് അമൈലോലികഫേഷ്യൻസ് + ബാസില്ലസ് വലസൻസിസ് (T5), ബാസില്ലസ് അമൈലോലികഫേഷ്യൻസ് + ബാസില്ലസ് പ്യൂമിലസ്+ബാസില്ലസ് വലസൻസിസ് (T7) എന്നിവയ്ക്ക് തുല്യമായിരുന്നു. വേരുകളിൽ ഏറ്റവും ഉയർന്ന മാംസ്യവും അന്നജവും (യഥാക്രമം 2.96, 23.30 mg

100 mg^{-1}) രേഖപ്പെടുത്തിയത് ബാസില്ലസ് അമൈലോലികഫേഷ്യൻസ് + ബാസില്ലസ് വലസൻസിസ് സമയോചിത പരീക്ഷണത്തിലായിരുന്നു. ഇത് ബാസില്ലസ് അമൈലോലികഫേഷ്യൻസ് + ബാസില്ലസ് പ്യൂമിലസ് + ബാസില്ലസ് വലസൻസിസ് മിശ്രിതത്തിനു തത്തുല്യമാണെന്നു കണ്ടെത്തി. വേരിലടങ്ങിയിട്ടുള്ള വിത്തനോലൈഡ് ഏറ്റവുമധികം രേഖപ്പെടുത്തിയത് T7-ൽ ആണ് (7.46 µg mg^{-1}), ഇത് T5- ബാസില്ലസ് അമൈലോലികഫേഷ്യൻസ് + ബാസില്ലസ് വലസൻസിസ്, T6- ബാസില്ലസ് പ്യൂമിലസ് + ബാസില്ലസ് വലസൻസിസ് എന്നിവയ്ക്ക് തുല്യമായിരുന്നു. ഓരോ ചെടിയുടെയും ആകെ ഇലകളിലുള്ള ആൽക്കലോയിഡുകളും ആകെ വേരുകളിലുള്ള വിത്തനോലൈഡുകളും (യഥാക്രമം 397.44 µg ചെടി$^{-1}$, 10.77 മില്ലിഗ്രാം ചെടി$^{-1}$എന്നിവ) ഏറ്റവും ഉയർന്ന നിരക്കിൽ രേഖപ്പെടുത്തിയത് T7- ൽ ആയിരുന്നു. ബാസില്ലസ് അമൈലോലികഫേഷ്യൻസ് + ബാസില്ലസ് വലസൻസിസ് സംയോജിത പരീക്ഷണം, T7 നു തുല്യമായ നിരക്കുകൾ രേഖപ്പെടുത്തി. നിരീക്ഷിച്ച എല്ലാ ബയോകെമിക്കൽ പാരാമീറ്ററുകളിലും ബാസില്ലസ് രഹിത പരീക്ഷണത്തിൽ ഗണ്യമായ കുറവ് കണ്ടെത്തി .

പഠനത്തിന്റെ ആദ്യ ഘട്ടത്തിൽ, ബാസില്ലസ് അമൈലോലികഫേഷ്യൻസ്, ബാസില്ലസ് പ്യൂമിലസ്, ബാസില്ലസ് വലസൻസിസ് (T7) എന്ന സംയോജിത സൂക്ഷ്മാണു പ്രയോഗം വിത്ത് മുളയ്ക്കൽ, തൈകളുടെ വളർച്ച, തൈകളുടെ വീര്യ സൂചിക എന്നിവയിൽ മികച്ചതായി കണ്ടു. രണ്ടാം ഘട്ടത്തിൽ, ബാസില്ലസ് അമൈലോലികഫേഷ്യൻസ് + ബാസില്ലസ് പ്യൂമിലസ് + ബാസില്ലസ് വലസൻസിസ് (T7), ബാസില്ലസ് അമൈലോലികഫേഷ്യൻസ് + ബാസില്ലസ് വലസൻസിസ് (T5) എന്നീ സംയോജിത സൂക്ഷ്മാണു പ്രയോഗ ങ്ങൾ ചെടികളുടെ വളർച്ച, വിളവ്, ബയോകെമിക്കൽ പാരാമീറ്ററുകൾ എന്നിവയിൽ മികച്ച തായി കണ്ടു