AGROTECHNIQUES FOR ENHANCING ROOT PRODUCTION IN *Desmodium gangeticum* (L.) DC. UNDER PARTIAL SHADE

by

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THESIS

Submitted in partial fulfilment of the requirements for the degree of

MASTER OF SCIENCE IN AGRICULTURE

Faculty of Agriculture Kerala Agricultural University



DEPARTMENT OF AGRONOMY COLLEGE OF AGRICULTURE VELLAYANI, THIRUVANANTHAPURAM- 695 522 KERALA, INDIA 2019

DECLARATION

I, hereby declare that this thesis entitled "AGROTECHNIQUES FOR ENHANCING ROOT PRODUCTION IN *Desmodium gangeticum* (L.) DC. UNDER PARTIAL SHADE" 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 or other similar title, of any other University or Society.

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CERTIFICATE

Certified that this thesis entitled "AGROTECHNIQUES FOR ENHANCING ROOT PRODUCTION IN *Desmodium gangeticum* (L.) DC. UNDER PARTIAL SHADE" is a record of research work done independently by Mr. Abhijith S. S 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 him.

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ACKNOWLEDGEMENT

First and foremost, I bow my head before the Almighty God whose grace has endowed me the strength and confidence to complete the thesis work successfully on time.

From the inner core of my heart, I acknowledge my major advisor Dr. A. S. Anilkumar, ADR (SZ, Vellayani) and Professor, Department of Agronomy, College of Agriculture, Vellayani for his inspiring guidance, valuable suggestions and fruitful criticisms were always a source of inspiration to me. His painstaking efforts, scientific acumen and ever helping attitude is irreplaceable. Also, his close monitoring and stimulating influence made the work complete in time with perfection.

I would express my sincere gratitude to Dr. Sheela. K.R., Dr. Sansamma George and Dr. Elizabeth K Syriac, former Professor and Head, Department of Agronomy for their continuous and timely advices and guidance at crucial stages of research work.

My deep sense of obligation goes to Dr. O. Kumari Swadija, Professor and Head, Department of Agronomy, College of Agriculture, Vellayani for her guidance and encouragement during the course of work and for valuable suggestions during the preparation of the thesis.

I deem it a great pleasure to exhibit my inexplicable gratitude to the members of my advisory committee Sri. V. Jayakrishnakumar, Associate Professor, Department of Agronomy, Dr. K. N. Anith, Professor, Department of Agricultural Microbiology and Dr. Biju Joseph, Assistant Professor, Instructional Farm for their affectionate advice, critical comments, constructive criticism and treasured suggestions and kind help rendered in all phases of my work. I am sincerely grateful for the affectionate encouragement and guidance.

I would like to thank each and every teaching and non-teaching staff of Department of Agronomy and the labourers for their continuous support and whole-hearted cooperation throughout the research work.

I express my deep love to my classmates, my seniors and juniors for their constant support and suggestion.

I acknowledge with high sense of regards, to my parents and my brother who has been a great support during every stages of my life and filling the confidence in me to be optimistic during every hurdles and inspired me to follow my dreams.

At the end, I bow down my head before the almighty who has shown a beam of spiritual light in the darkness. I still seek his blessings to proceed further.

ABHIJITH .S. S

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LIST OF ABBREVIATIONS

AICRP	;	All India co-ordinated research programme
AMPRS	;	Aromatic and medicinal Plant Research Station
В	:	Boron
B : C ratio	:	Benefit-cost ratio
BAP	:	6-benzyl amino purine
Ca	;	Calcium
CD (0.05)	:	Critical difference at 5 % level
CGR	:	Crop growth rate
cm	:	Centimeter
CNS	:	Central nervous system
CPE	ą.	Cumulative pan evaporation
CU	3	Consumptive use
CWUE	•	Crop water use efficiency
DAS	:	Days after sowing
DMSO	:	Dimethyl sulphoxide
et al.	:	Co-workers/ Co-authors
Fe	:	Iron
Fig.	•	Figure
FWUE	,	Field water use efficiency
FYM	:	Farm yard manure
g	:	Gram
g m ⁻³	:	Gram per cubic meter
GA ₃	:	Gibberellic acid
h	:	Hour
H_2SO_4	:	Sulphuric acid
ha ⁻¹	:	Per hectare

HCl	:	Hydrochloric acid
HDP	:	High density planting
IAA	:	Indol-acetic acid
K	:	Potassium
KAU	:	Kerala Agricultural University
kg ha ⁻¹	•	Kilogram per hectare
КОН		Potassium hydroxide
m	3	Meter
M Pa	;	Mega pascal
Mg	;	Magnesium
mg L ⁻¹	:	Milligram per litre
mm	1	Millimeter
MS medium	:	Murashige and Skoog's medium
MSL	:	Mean sea level
Ν	;	Nitrogen
Na	:	Sodium
NAA	:	Naphthalene acetic acid
NAR	;	Net assimilation rate
NRP	:	Normal row planting
NS	:	Not significant
OD	:	Optical density
Р	;	Phosphorus
P. indica	;	Piriformospora indica
PDA	:	Potato dextrose agar
PDB	:	Potato dextrose broth
PiPT	:	Piriformospora indica phosphate transporter
RGR	î	Relative growth rate
RH	:	Relative humidity
RLWC	:	Relative leaf water content

rpm	:	Revolutions per minute
S	:	Sulphur
SE m	:	Standard error of mean
t ha ⁻¹	;	Tonnes per hectare
USWB	:	United States Weather Bureau
viz.,	:	Namely
WP	:	Water productivity
WUE	:	Water use efficiency
Zn	:	Zinc

LIST OF SYMBOLS

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%	*	Per cent
@	:	at the rate of
μΜ	:	Micromole
2n	:	Diploid chromosome number
°C	:	Degree Celsius

Introduction

1. INTRODUCTION

Desmodium gangeticum is one among the well-known group of medicinal plants called Dashamoola. It is a perennial under shrub, belonging to the Fabaceae family. The plant is known commonly as orila, salpani and shalparni in Malayalam, Hindi and Sanskrit respectively. Besides, it is also known as tick-tre-foil, beggar lice, hitch hikers or tick clover.

Many plants among the *Desmodium* species such as *Desmodium gangeticum* (L.) DC., *Desmodium triquetrum* Linn. and *Desmodium triflorum* Linn. have been used in the conventional as well as folklore medicines against various ailments in India, China and African counties.

Desmodium gangeticum is one of the major constituent of several Ayurvedic preparations like Dashamularishta, Chyavanaprasam and Agasthyarasayanam. It is also prescribed routinely in the treatment of fever, asthma, respiratory ailments and colic pain. Pharmacological investigations have unveiled the potency of the extracts of *Desmodium gangeticum* and its major principles (gangetin, gangetinin, desmodin and hordenine) as anti-diabetic, immunostimulatory, anti-ulcer, anti-inflammatory, and cardio as well as hepato-protective drugs.

Kirubha et al. (2011) claimed that the plant *Desmodium gangeticum* has hailed the title of the 'Master of medicinal plant in Ayurveda' considering the wide use in folklore medicine and demand in various Ayurvedic preparations for the treatment of different kind of diseases. It is a traditional medicinal herb that has crowned a unique role in many of the Ayurvedic preparations which in turn justifies its predominant disease curing potentials and immuno-modulatory functions (Ganjhu et al., 2014).

D. gangeticum is one of the major ingredients of famous Ayurvedic preparations like Dashmoolarishta and Dashmoolakwaath. Besides, the roots are also used in the preparation of Chitrak haritika, Dhanvantar tailum, Brahma rasayan, Dashmoola kadha and Dashmoola ark and many other Ayurvedic products (Niranjan and Tewari, 2008). Dashmoola is a combination of roots of ten different

medicinal plants in different proportions and which have been classified into lakhu panchamoola and bruhath panchmoola according to the relative quantities in which these roots are added to the preparation (Singh *et al.*, 2015).

Iwu *et al.* (1992) observed that the root system of the drug plant is mainly used in ethno-pharmacological preparations than the aerial parts due to higher concentration of curing principles like pterocarpenoids, desmodin, gangetin as well as gangetinin with expectorant, diuretic, alterative and antipyretic attributes. The root system of the crop is best for the treatment of neurological disorders.

Hemlal and Ravi (2012) revealed that the original species of the genus that forms the major ingredient of many of the Ayurvedic preparations are not geographically available in sufficient quantities to meet the need of pharmacological industries leading to adulterations and substitutions with related species having similar or different structural and phytochemical profiles. So, it is clear that there exist a huge gap between demand and need of the plant raw materials which in turn demands for improved cultivation practices for enhanced root production. Hence, there is an urgent need to develop agrotechniques for enhancing root production. The crop responds very well to management practices as well. Rhizosphere modulation with organic/inorganic fertilizers along with imparting moisture stress through adjusting the depth of irrigation and enhancing plant population besides maintaining the rhizosphere above an impervious layer may help to evolve cost effective techniques for enhancing root yield in *Desmodium gangeticum*.

In this situation, the present study entitled "Agrotechniques for enhancing root production in *Desmodium gangeticum* (L.) DC. under partial shade" was carried out with the objective to study the integrated effect of root endophyte fungus, planting density, source efficacy of nutrients, moisture stress and subsurface mulching on the growth, yield and quality constituents of *Desmodium gangeticum* (L.) DC. under partial shade.

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Review of Literature

2. REVIEW OF LITERATURE

Desmodium gangeticum (L.) DC. is a perennial under shrub distributed throughout the Indian sub-continent. India has rich species diversity with 38 species under the genus Desmodium but only Desmodium adscendens and Desmodium gangeticum are intensively exploited all over the globe. In Ayurvedic medicine, Desmodium gangeticum is the only exploited species of the genus Desmodium.

Substantial exploitation of *Desmodium gangeticum* by innumerable pharmaceutical industries along with an increasing interest in the field of herbal medicines have resulted in a greater demand for this peculiar species. Root availability of the plant is also not sufficient so as to meet the demand of ayurvedic medicine manufacturing industries. In order to bridge the gap between demand and plant root availability, current state of knowledge regarding the crop geographical distribution, soil and botanical description, seed biology and propagation, effect of fertilization, plant response to partial shade, prospects of intercropping, phytochemistry, effect of *Piriformospora indica* on crop production, cow dung slurry on plant and soil, irrigation and effect of high density planting on root growth, subsurface mulching, raised bed planting and coconut husk pitching and harvest and yield are reviewed in this chapter.

2.1 GEOGRAPHICAL DISTRIBUTION

Desmodium gangeticum, (Family -Fabaceae) a perennial medicinal plant is widely seen in tropical countries like India, China, Africa, and Australia (Kurian *et al.*, 2010). Tropical as well as subtropical climatic conditions are found favourable for crop cultivation (NMPB, 2008).

The drug plant grows throughout India up to an altitude of 1000 m from mean sea level (Meena *et al.*, 2010). It is distributed all over the Indian subcontinent and thus pronounced genetic variability exists within the species as a cause of the extensive adaptation and naturalization to diverse agro-climatic conditions (Nandanwar *et al.*, 2015).

2.2 SOIL

The plant, *Desmodium gangeticum* can be grown in a wide range of soils from coarse sandy to heavy clayey soils, however sandy loam to clay loam soils are most appropriate (NMPB, 2008).

According to Prakash *et al.* (2000), neutral to slightly alkaline soil reaction of pH 8 to 9 is preferred by the plant for optimal growth and good quality drug yield. The crop requires moist soils with partial shade to grow profusely which is mostly available in orchards (NMPB, 2008).

2.3 BOTANICAL DESCRIPTION

According to Kumar et al. (2014), Desmodium gangeticum (L.) DC. is an important medicinal plant originated in India, which have been extensively used in the ancient treatment systems of Ayurveda. Ganjhu et al. (2014) observed that Desmodium gangeticum belonging to the Leguminosae family was synonymous to Hedyserum gangeticum. Nandanwar and Manivel (2014) reported the diploid chromosome number of the species as 2n=22.

According to Gu *et al.* (2007) Rhizobia induced nodulation has been observed in the root system of the plant which has formed by a group of soil bacteria that facilitates crop growth under diverse environmental conditions. Numerous strains of rhizobial microsymbionts such as *Bradyrhizobium elkanii*, *Bradyrhizobium japonicum* and *Bradyrhizobium yuanmingense* including unique strains related to *Rhizobium*, *Sinorhizobium* or *Mesorhizobium* are found to colonize the root system of *Desmodium* sp.

Desmodium gangeticum is a perennial undershrub that grows either erect or prostrate to a height of 60 to 130 cm and produces angular branches (Rastogi *et al.*, 2011). Kawale *et al.* (2012) reported that the leaves were simple or unifoliate with oblong to lanceolate shaped lamina having a dimension of 3 to 3.5 cm x 2 to 2.5 cm. Apex of the leaves were acute or even acuminate mostly with wavy margins. The plant has got a unique leaf colour pattern with yellowish green patches on the lamina. Length of the petiole ranges from 1 to 2 cm which were often triangular shaped. Stipules of 6 to 8 mm length are also found at the base of the petiole. Verma *et al.* (2015) evaluated that abaxial surface of the leaf is light green coloured compared to adaxial surface.

Stem of the young plant appears angular but transformed to irregularly cylindrical in shape once they became mature and presence of trichomes has also been observed at every angular ends (Kawale *et al.*, 2012).

Inflorescence is a terminal axillary raceme and rarely form panicles. Length of inflorescence ranges from 10 to 40 cm having 2 to 6 flowers at every node (Nandanwar and Manivel, 2014). Colour of the blooms ranges from pink to purple and are small sized with typical papilionaceous attributes (Meena *et al.*, 2010). Pink colour of the flower is attributed by a single gene exhibiting Mendelian pattern of inheritance (Nandanwar and Manivel, 2014).

Plant came to flower from October to December. The plant has complete flowers with five hairy sepals of two mm size which are triangular shaped, petals are also five in number (4 mm), purple coloured (Kawale *et al.*, 2012). Flowers are zygomorphic with gamosepalous calyx (Aleman *et al.*, 2014).

Flowers after being fertilized form pods enclosing 5 to 8 seeds in each bearing curved beak like structures at the ends (Kawale *et al.*, 2012). Pods are thin and laterally compressed (Verma *et al.*, 2015) and are sub-falcate and curved having straight upper suture which is not indented and with deeply indented, hairy as well as hooked lower suture (Rastogi *et al.*, 2011). At normal conditions of temperature and pressure, harvested seeds retain viability for three years under storage (NMPB, 2008).

Tap root system of the plant is poorly developed with a number of primary roots arising from the base of the stem very close to original tap root (Vedpal *et al.*, 2016a). These roots are smooth textured, light yellow coloured and cylindrical shaped with a girth of 0.4 to 1.2 cm that penetrate to a depth of 20 to 50 cm (Kawale *et al.*, 2012). Kirubha *et al.* (2011) pointed out that even if the tap root was poorly developed, the lateral roots were strong enough with uniform cylindrical shape,

smooth in texture and pale yellow in colour. Root also has got a thick and tough strand of wood at the centre which is surrounded by a relatively thin bark. Besides this, a layer of hard and dead tissues which is tough and dry known as periderm were observed at the peripheral portion of roots (Vedpal *et al.*, 2016a).

The locations where the bark has broken, presence of lenticels has been observed that protects the interior root tissues like parenchymatous epidermis and lignified cork cambium. Interior region of the cork tissues are composed of variably compressed and thin walled cortex cells. Patches of secondary phloem tissues or elements that are separated by cambium from thick secondary xylem has been observed. Besides this, single layered or double layered radially elongated bands of medullary rays has been found which has noticed to differentiate the root secondary xylem and has extended from primary xylem to the secondary xylem (Kawale *et al.*, 2012).

Numerous and small but much branched fibrous rootlets harbouring various size of bacterial nodules adhered to their distal ends are the additional characteristic feature of the root system (Kirubha *et al.*, 2011). Ravindra (2011) evaluated that fresh and dry weight of the stem has a direct influence and root fresh weight has only an indirect influence over the root dry weight of the plant.

Serious pest and disease incidence have not been observed during any stage of the plant. However, in many of the dry areas, root system of those plantations having a duration of more than one year has been found to be extensively damaged by rats (NMPB, 2008).

The relative similarities of the plant with plants of other species often leads to confusion with identification of correct species and thus many times different materials are found to be mixed or adulterated at the time of preparation of ayurvedic medicines (Vedpal *et al.*, 2016b). Thus, a thorough knowledge of the plant biology is inevitable.

2.4 SEED BIOLOGY AND PROPAGATION

Seeds of *Desmodium gangeticum* are small and kidney shaped. Seeds are non-endospermic having bent axile type of embryo and recorded a test weight (1000 seed weight) of 1.5 g (Mukhopadhyay *et al.*, 2011).

Datta and Sen (1987) revealed that seed coat of *Desmodium gangeticum* has a thickness of 0.083 mm and it acts as a strong barrier to water entry. Datta and Sen (1987) observed that seeds of many leguminous crops, with dormancy due to some physical barriers, were not germinated even with adequate supply of water until the impermeable layer has removed. Mukhopadhyay *et al.* (2011) reported that seeds exhibiting physical dormancy which is associated with thickened malpighian cells of outer testa (palisade epidermal tissues) that restricts entry of water for seed germination.

When the un-sprouted seeds of *Desmodium gangeticum* were soaked in water for 1, 2, 4, 8 and 20 hours at room temperature, the percentage of water taken up by them has been recorded as 0, 4.2, 25.7, 34.4 and 35.1 per cent respectively (Datta and Sen, 1987). Mukhopadhyay *et al.* (2011) reported that different pregermination treatments such as dry heat treatment, hot water treatment and scarification with H₂SO₄ for various duration were given to overcome physical dormancy related germination barriers and shown higher germination percentage for each than the normal (without pre-treatment). Among these prior germination seed treatments given, scarification with H₂SO₄ for a period of 20 minutes was proved the best with a remarkable germination of 92 per cent.

Datta and Sen (1987) found out that when the desmodium seeds were brought back to room temperature after being treated at 70°C for four hours have shown 87 per cent germination. Prior treated seeds of *Desmodium gangeticum* germinated after six days of sowing under room temperature.

Seeds of the plant when dried to a moisture content of 5 to 7 per cent exhibited 80 to 90 per cent viability under conditions of normal temperature and pressure in seed storage and were noted ideal in seed banks for the purpose of exsitu conservation (Datta and Sen, 1987). Mukhopadhyay *et al.* (2011) opined that seed coat of *Desmodium gangeticum*, which is impermeable to water, helps the seeds to maintain a much lower moisture condition inside the seed even in highly humid external environments, and thus to retains viability for a significantly longer period of time. Seeds of the plant also showed best imbibition of water with good germination when stored at 50°C with low relative humidity.

Isaac and Lissy (2007) found out that when IAA and GA₃ were used at lower concentrations exhibited enhanced seed germination whereas NAA showed adverse effects on seed germination as well as seedling growth. In addition, in-vitro technique for rapid propagation of *Desmodium gangeticum (L.)* DC. on MS medium with the use of cotyledonary nodal explants have been developed (Vishwakarma *et al.*, 2009).

Preeti *et al.* (2013) evaluated that MS medium supplemented with 6-benzyl amino purine (4.44 μ M BAP) was the most effective in-vitro medium for shoot production and recorded highest response of 98 per cent shoot proliferation of explants with maximum number of shoots per explant. Patil *et al.* (2016) reported that multiple shoots were produced with increase in concentration of 6-benzyl amino purine (BAP) up to 0.5 mg L⁻¹.

2.5 EFFECT OF FERTILIZATION

Increased rates of fertilizers revealed an improvement in plant height, number of branches and leaves and fresh and dry weight of *Datura innoxia* with an additional advantage of enriched total alkaloid and drug concentrations (Al-Humaid, 2003). Aina *et al.* (2018) reported that soil application of organic manures and inorganic fertilizers showed a profound improvement in the physical and chemical properties of the soil.

Remarkable effects were not observed on total alkaloid and plant N content with varied level, form and time of application of nitrogenous fertilizers (Ruminska and Gamal, 1978). But, Sreevalli *et al.* (2003) pointed out improvement in the root and leaf alkaloid content with increased nitrogen (N) level were due to increased root and leaf production. The rate as well as type of fertilizers applied have a critical role in plant dry matter yield, phyto-chemistry and pharmacological potentials of medicinal herbs (Fung *et al.*, 2018).

2.6 RESPONSE OF PLANTS TO PARTIAL SHADE

Desmodium gangeticum can be cultivated as a sole crop or as an intercrop along with trees such as *Populus deltoidea* (poplar) that permit partial sunlight to pass through the canopy as the plant is tolerant to partial shading. It can also be raised as an intercrop in the orchards of mango, aonla and guava (NMPB, 2008).

Yang *et al.* (2018) revealed that accumulation of secondary metabolites of the plant has been found to be strongly associated with a wide range of environmental parameters like soil, water, light, temperature, fertility and soil salinity and changes to any of the individual factor may reflect in the concentration of secondary metabolites even though rest of the factors remains the same. Phytochemistry of the herbal medicinal plant has been found to be much complex and highly variable. Every changes in the environmental conditions were often found to alter the type and quantity in addition to the biological effects of the secondary metabolites. Plants respond variably according to the variations in solar radiation either by the release or accumulation of different secondary metabolites such as phenolic components, flavonoids and tri-terpenoids as most of them are highly valuable in their monitory as well as utilization value due to antioxidant potentials.

Prakash *et al.* (2000) also evaluated that ethanol and aqueous extracts of crops cultivated under shade yield greater amount of extracts compared to that grown under full illumination. Similar results were obtained from wild plants collected from shaded sites to that from open space. Thus intercropping of *Desmodium gangeticum* is most suitable for profitable crop production with the additional advantage of increased alkaloid accumulation which is the key principle. Devkota *et al.* (2010) revealed that quantitative and qualitative evaluation of

Centella asiatica showed higher concentration of bioactive principles like asiatic acid under conditions of 70 per cent shade.

2.7 PROSPECTS OF INTERCROPPING

Nandanwar *et al.* (2015) studied that during noon time, the plant shows a sharp decline in leaf water potential compared to that in the morning hours and evening as a consequence of active photosynthesis and transpiration. The mean leaf water potential during the early morning hours was recorded as -0.39 M Pa which declined to -1.90 M Pa during noon and again recorded less negative potential of -0.47 M Pa during evening hours. Decline in leaf water potential during noon act as a barrier to the rate of photosynthesis which could be managed by intercropping.

In the case of widely spaced crops like aonla, *D. gangeticum* can be planted in two adjacent rows at 30 cm \times 30 cm spacing (NMPB, 2008). When *D. gangeticum* was intercropped with black pepper, it produced 55 kg ha⁻¹ of root yield with a benefit-cost ratio (B: C ratio) of 2.6 (Thankamani *et al.*, 2012).

Akre *et al.* (2016) pointed out that intercropping of *D. gangeticum* help farmers to earn more profit from a given piece of land. In addition, the plant can come up well even under low soil fertility conditions and thus cost for fertilizers can be saved. In rubber plantations up to the age of four years it can be broadcast after initial ploughing and could be raised profitably as a cover crop.

2.8 PHYTOCHEMISTRY

At least 19 bioactive principles were recovered from the whole plant extracts of *D. gangeticum*. Most of the active ingredients were coming under the group of flavonoid, glycosides, lipids, glycolipids, and alkaloids (Mishra *et al.*, 2005). These bioactive constituents are primarily responsible for antiinflammatory, anti-nociceptive, analgesic, anti-amnesic, anti-diabetic, anti-oxidant, anti-ulcer, CNS depressant, antibacterial, antipyretic (Vaghela *et al.*, 2013) and wound healing (Jain *et al.*, 2006) activities. Nanda and Tiwari (2016) also reported that these bioactive principles functions as a carminative, rejuvenative and aphrodisiac besides, acting against the ill effects of fever, oedema, kidney disorders and post-delivery complications.

Bioassay mediated isolation and identification of active principles in combination with stereochemistry of the plant was suggested. Roots of the crop was found to be dominated by three major pterocarpinoids specifically gangetin, gangetinin and desmodin (Purushothaman *et al.*, 1971). According to Niranjan and Tewari (2008) not only roots but shoot portions also contained phenolic and phytochemical compounds, responsible for antioxidant activities and its high curing potency in different ayurvedic formulations. Kurian and Paddikkala (2009) reported that gangetin possess strong anti-inflammatory and analgesic activities. Bhattacharjee *et al.* (2013) reported that even though most of the pharmacological attributes of the plant have been studied, the real molecular mechanism for the final effects are still under siege.

Isolates from the anterior parts of *D. gangeticum* showed the presence of alkaloids like β -carbolines and indole-3-alkyl-amines which shows therapeutic stimulatory effect on smooth muscles, CNS and anticholinesterase activities (Ghosal and Bhattacharya, 1972) besides, strengthening the heart muscles and cholesterol declining properties (Verma *et al.*, 2015).

2.9 EFFECT OF PIRIFORMOSPORA INDICA ON CROP PRODUCTION

Root endophytic fungus *Piriformospora indica* (Hymenomycetes, Basidiomycota) belonging to Sebacinaceae family, colonizes the roots of plants and enhances crop growth in a manner similar to that of arbuscular mycorrhizal fungi (Berghofer *et al.*, 2004). It also exhibits wide host range in addition to imparting different positive aspects to the plants in which they colonizes (Franken, 2012).

Close interaction of *P. indica* with many medicinal herbs were identified and some of them are *Artemisia annua*, *Bacopa monniera*, *Abrus precatorius*, *Stevia rebaudiana*, *Linum album*, *Trigonella foenumgraecum*, *Coleus forskohlii*, *Withania somniferra*, *Chlorophytum borivilianu*, *Tridax procumbens*, *Curcuma longa*, *Podophyllum peltatum*, *Azadirachta indica*, *Foeniculum vulgare*, *Oscimum* *sanctum* (Das *et al.*, 2012a). Such beneficial interaction leads in reprogramming of transcriptome, proteome and metabolome, in response to the plant hormone levels and its signaling, mineral uptake and metabolism and finally contributes to resistance against biotic-abiotic stress (Johnson *et al*, 2011).

Roots of the plant colonized by *Piriformospora indica* exhibits accelerated growth during the initial stages of the crop (Rai and Varma, 2005), biomass yield (Varma *et al.*, 1999), increased tolerance to biotic and abiotic stress, besides enhanced growth and yield (Achatz *et al.*, 2010a) early flowering, higher seed yield, alteration in the secondary metabolites (Varma *et al.*, 2012). According to Sirrenberg *et al.* (2007) the beneficial effects of *P. indica* is primarily due to the enhanced soil exploration contributed by auxin induced root proliferation. The fungus once colonized found to persist and retain the interaction throughout the crop season (Serfling *et al.*, 2007).

Anith *et al.* (2011) reported that plantlets of pepper exhibited greater root proliferation when inoculated with the biological agent compared to un-inoculated plants. The fungus also imparts resistance against various root and shoot pathogens and improves biomass yield (Fakhro *et al.*, 2010). *P. indica* inoculated black pepper showed higher number of leaves and leaf area per plant and total chlorophyll content compared to the un-inoculated plants in addition to increased total oleoresin and piperine content in the berries (Anith *et al.*, 2018).

The fungus confers systemic resistance to the host plant associated with an increased antioxidant potential induced by active glutathione-ascorbate cycle (Waller *et al.*, 2005). Enhanced nitrate assimilation by gene induced nitrate reductase and starch-degrading enzyme glucan-water dikinase in the root and shoot proved to favour growth promotion in tobacco and Arabidopsis (Sherameti *et al.*, 2005). According to Deshmukh and Kogel (2007) upon colonization with *P. indica*, root system were protected from *Fusarium* injury as evidenced by decreased root rot symptoms. Sherameti *et al.* (2008) revealed that Arabidopsis plants co-cultivated with *P. indica* were found more resistant to drought induced stress under

greenhouse conditions. Kumar *et al.* (2009) observed that when *P. indica* was inoculated to maize plants, 10 days after the infection of pathogenic fungi *Fusarium verticillioides*, showed enhanced biomass, root length and root number compared to the plants grown with the pathogen alone.

Gene encoding for a phosphate transporter (PiPT) is actively engaged in phosphate transport and, in turn, *P. indica* helps to improve the nutritional status of the host plant (Yadav *et al.*, 2010). *P. indica* inoculated mustard on nutritional analysis revealed increased accumulation of N, P, K, Ca, Mg, S, Zn, Fe and B as well as significant reduction in erucic acid and glucosinolates which are harmful to human health (Su *et al.*, 2017).

In the absence of host, plant fungus can be cultured axenically on synthetic media (Johnson *et al.*, 2011) which produces characteristic pear-shaped chlamydospores and hence named *Piriformospora indica* (Verma *et al.*, 1998). Rodriguez *et al.* (2004) reported salt tolerance and higher yield in the mutualistic root endophyte colonized cultivated plants grown in saline soils.

Accelerated production of bio-active constituents are observed in medicinal plants colonized with the fungus and the effects were much pronounced in nutrient deficient conditions and aids in the hardening of micro-propagated plants (Johnson *et al*, 2014).

Deshmukh *et al.* (2006) reported that with the maturation of root tissues of the host plant, the fungal colonization grew much stronger. Colonization in older root tissues were associated with host cell death but without any adverse effects on the plant growth. The root apical meristem did not show any colonization whereas the zone of differentiation exhibited active fungal infestation producing intracellular hyphae and chlamydospores. Majority of the hyphae were found in dead rhizodermal and cortical cells that were completely filled by chlamydospores.

Immense potential of *P. indica* for commercial cultivation of *Spilanthes* calva and *Withania somnifera* was revealed due to the enhancement in different growth and yield attributes of the host plant with an additional quality enrichment

such as increase in shoot and root length, biomass, basal stem, leaf area, overall size, number of inflorescences and flowers and seed production compared to non-colonized plants (Rai *et al.*, 2001).

P. indica inoculated medicinal herb *Coleus forskohlii* exhibited increased biomass yield under field conditions and was found beneficial as the aerial parts are highly valuable for pharmacological industries (Das *et al.*, 2012b). Shahollari *et al.* (2007) reported that fungal colonized Arabidopsis produce 22 per cent more seeds in comparison to control plants.

Apart from increased biomass production, it contributes in nullifying the ill effects caused by phyto-pathogenic fungi like *F. culmorum*, *Pseudocercosporella herpotrichoides*, and *Blumeria graminis f.* sp. *tritici* on winter wheat grown in the pots as well as in the field (Serfling et al., 2007).

The colonised roots of barley exhibited improved photosynthetic rates even under low light intensities along with high root branching, more tillers per plant and early ear development (Achatz *et al.*, 2010b). Root system of barley seedlings exhibited enhanced root development in *P. indica* inoculated axenic system (Varma *et al.*, 2012).

The fungus was found to lower the decline in drought induced photosynthetic efficacy by up-regulating the activities of peroxidases, catalases and super-oxide-dis-mutase and through protecting chlorophylls and thylakoid proteins in the leaves from degradation (Sun *et al.*, 2010). Vadassery *et al.* (2009) opined that plants under drought stress were heavily colonized by the endophyte than irrigated plants.

2.10 EFFECT OF COW DUNG SLURRY ON PLANT AND SOIL

Taylor and Ratliff (1969) opined that low bulk density contributes to a decline in soil strength that promote the soil moisture content and rate of root elongation. Concheri *et al.* (1996) pointed out that organic manuring induces root elongation by enhancing mineral ions and humic substances that facilitates

proliferation of lateral roots and root hairs. It also contributed to faster root cell differentiation rates with effect on nutrient uptake, plant growth and yield.

Application of cow dung slurry not only improves the nutrient status but also enhances the size and diversity of microbial population and creates better physical, chemical and biological conditions. Soil health was found to be enhanced many folds by enhanced soil biological activity with the application of organic components (Albiach *et al.*, 2000).

Plants applied with farmyard manure (FYM) revealed higher ethanol-water extracts compared to control plants (Prakash *et al.*, 2000). Some physiologically active principles in cow dung slurry promotes tap root length rather than inducing number of laterals (Dobbss *et al.*, 2007).

Bacillus subtilis strains isolated from cow dung slurry was found to promote root length up to 70 to 74 per cent when compared to untreated plants (Swain and Ray, 2009). Trevisan *et al.* (2010) opined that humic substances contributed to accelerated root growth and elongation through the production of auxin or auxin like components. In addition to improve the plant availability of macronutrients, organic manures also help to retain soil moisture and reduce bulk density (Aimiuhi *et al.*, 2013).

Various adverse effects due to uninterrupted use of inorganic chemical fertilizers suggest the use for organic manures for maintenance of soil fertility and improvement in crop production (Sheikh *et al.*, 2015). Roots of khirni (*Manilkara hexandra* L.) showed increased seedling height, leaf number, primary root length and tertiary root number on 12 h soaking in cow urine along with 12 h keeping in cow dung slurry (Shinde and Malshe, 2015). But, application of organic manures at very high doses hinder crop growth (Sheikh *et al.*, 2015).

Sharma and Singh (2015) reported that application of cow dung slurry improved the nutrient status of the soil in addition to the enhanced crop resistance against pest and diseases. Bio-control effects of the bacteria, *Bacillus subtilis* isolated from cow dung was observed against phyto-pathogenic fungi such as *Fusarium oxysporum, Aspergillus niger* and *Aspergillus flavus* (Mangalanayaki and Thamizhmarai, 2016).

Mahabub *et al.* (2016) opined that cow dung application improved plant height, leaf number, number of branches, and number of seeds per pod and also resulted in precocity in flowering along with early maturity, improved test weight, seed as well as stover yield and N, phosphorus (P), potassium (K) and sulphur (S) content in mung bean compared to control treatment.

When the seeds of *Tectona grandis* were given pre-treatment with alternate wetting and drying of cow dung slurry, they exhibited significant increase in seedling root length and root dry weight after 120 days of treatment (Pamei *et al.*, 2017).

2.11 EFFECT OF IRRIGATION ON ROOT GROWTH

According to Green *et al.* (1971), root cell growth occurs as a result of the driving turgor pressure, experienced on yielding cell wall and the roots elongate as a result of turgor pressure in cells of elongation zone.

Cullen *et al.* (1972) reported that root dry weight of the plants increases with increase in depth of irrigation. Root system of mature field grown lettuces under irrigated condition exhibited 75 per cent more root length compared to un-irrigated control plants (Rowse, 1974).

Klepper (1991) reported that root length densities of well irrigated crops exhibited a pattern of exponential decline. Under irrigated conditions, the most important and inter dependent attributes that contribute to root growth are oxygen diffusion rate, soil water status and soil strength. Excessive irrigation restrict the root growth as a result of poor oxygen diffusion rates, especially in heavy soils. But in poorly irrigated situations, root length is reduced with increased soil strength and low water availability. Under rainfed situation, during the winter period irrigation once in a month is enough and it varies depending up on the intensity and frequency of rainfall during monsoon season (NMPB, 2008).

2.12 EFFECT OF HIGH DENSITY PLANTING ON PLANT GROWTH

High density system of planting (HDP) showed enhanced crop density and spatial uniformity, better weed suppression and in turn contributed to yield advantage by eliminating the weed competition factor (Olsen *et al.*, 2005).

Dalvi *et al.* (2010) revealed that in HDP system of mango, greater yield advantage was achieved without sacrificing the fruit quality attributes like size, shape and colour. HDP also ensures better resource utilization, assured cropping system uniformity and enhanced plant protection, cultural management, increasing sustainability and biodiversity in the field (Weiner *et al.*, 2010).

Kerutagi and Deshetti (2018) suggested that HDP is a modern crop management technique that ensures better utilization of land, solar radiation and secure high yield per unit area through increased photosynthetic efficiency. High density planting also ensures maximum land exploitation to achieve maximum yield per unit area with greater easiness in crop management practices (Kumar, 2019).

2.13 EFFECT OF SUBSURFACE MULCHING

Plastic mulching showed obvious improvements on different soil properties like soil temperature, soil moisture content, bulk density, aggregate stability as well as nutrient availability (Lalitha *et al.*, 2010). Black polythene mulch materials were found effective in enhancing root penetration, nutrient uptake, water use efficiency (WUE) and yield (Kumar and Dey, 2011).

Thick plastic films act as impervious pans and restrict the vertical and deep penetration of roots. Hard pans restrict plant root growth, root volume and crop yields under conventional small-scale agriculture in all fields (Esser, 2016). Mingming *et al.* (2018) reported that sub soil plastic film mulching exhibited enhanced water use efficiency and yield under rainfed situations. Zhang *et al.* (2018) evaluated that soil salinity levels can be effectively suppressed and crop WUE can be increased many folds by sub soil mulching when compared to no mulch situations.

Electrical conductivity of soil is significantly reduced under polythene mulched situations and also improved the plant nutrient uptake efficiency with additional improvement in soil health (Haque *et al.*, 2018).

2.14 RAISED BED PLANTING AND COCONUT HUSK PITCHING

Mapa (1996) revealed that coconut fibre matting showed substantial reduction in run off and soil erosion along with improvement in soil moisture availability and reduction in weed growth.

Plants grown on raised beds exhibited better resource use efficiency, reduced weed growth and higher yield advantage over plants raised on flat beds (Mollah *et al.*, 2009).

Khan *et al.* (2012) reported that the plants grown in raised beds of 45 cm height produced well organised root system with longer primary root having numerous laterals and greater total root yield. The overall improvement in the root system was found to be attributed by higher WUE, better resource use efficacy and low weed growth.

Dey *et al.* (2015) revealed that plants in the raised beds exhibited lower water stress when compared to conventional planting. Root length density was also found greater in the top 45 cm of raised beds due to porous soil conditions. Moreover, yield obtained from raised bed planting system was remarkably higher than that of flat-bed planting system with low water requirement.

According to Dey *et al.* (2015), higher yield obtained from raised bed planting system was due to the improved soil physical conditions and enhanced water availability that contributed to better root.

Permanently established raised bed planting systems impart improved WUE, nutrient uptake, reduced soil compaction in root zone and thus improve the soil structure and reduced Na content and electrical conductivity in addition to energy conservation and greater timelines of operations (Naresh *et al.*, 2017).

2.15 HARVEST AND YIELD

Prakash et al. (2000) pointed out that D. gangeticum harvested during hot summer and scorching winter exhibited higher alkaloid content with greatest therapeutic potential.

MATERIALS AND METHODS

3

3. MATERIALS AND METHODS

The study entitled "Agrotechniques for enhancing root production in *Desmodium gangeticum* (L.) DC. under partial shade" was conducted during May 2018 to May 2019 in the Instructional farm, College of Agriculture, Vellayani. The main objective was to study the integrated effect of root endophyte fungus, planting density, source efficacy of nutrients, moisture stress and subsurface mulching on the growth, yield and quality constituents of *Desmodium gangeticum* (L.) DC. under partial shade.

3.1 GENERAL DETAILS

3.1.1 Experiment Site

The experiment was conducted in the Instructional Farm, College of Agriculture, Vellayani, Kerala, India. The farm is located at 8° 5' North latitude and 76° 9' East longitude and at an altitude of 29 m above MSL.

3.1.2 Soil

A composite soil sample was taken for initial analysis from the experimental site prior to the conduct of experiment. The soil texture was sandy clay loam, acidic in reaction, low in organic carbon content, medium available N and P and high in available K status. The important physic-chemical properties of the soil are presented in Table 1.

3.1.3 Climate

The monthly weather parameters, *viz.*, maximum and minimum temperature, relative humidity (RH) and monthly rainfall (mm) were recorded during the cropping period. The data were collected from the Class B Agro-meteorological observatory, College of Agriculture, Vellayani.

The rainfall received during the crop season extending from 05/05/2018 to 28/05/2019 was 2253.70 mm. The average of maximum and minimum temperature recorded during the crop season were 35.80 and 18.80 °C respectively. The summary of weather data during the cropping period is presented in Appendix I and Fig. 1.

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Table 1. Physico-chemical properties of the soil before the experiment

Sl. No.	Fractions	Content in soil (%)	Method
1	Coarse sand	21.92	
2	Fine sand	30.89	Bouyoucous hydrometer
3	Silt	23.83	method (Bouyoucos,1962)
4	Clay	18.68	
5	Soil texture	Sandy clay loam	

A. Mechanical composition

B. Soil moisture characteristics

Sl. No. Fractions		Content	Method
1	Maximum water holding capacity	31.20	Core method (Gupta and Dakshinamoorthi,
2	Field capacity	29.50	1980)
3	Permanent wilting point	11.60	

C. Physico-chemical properties

Sl. No.	Parameters	Content	Method adopted
1	Bulk density (mg m ⁻³)	1.68	Pycnometer method (Black, 1965)
2	Soil reaction	5.6	pH meter (1:2.5 soil water ratio)
		(Acidic)	(Jackson, 1973)
3	Organic carbon (%)	0.680	Walkley and Black rapid titration
		(low)	method (Walkley and Black, 1934)
4	Available N (kg ha ⁻¹)	301.10	Alkaline permanganate method
		(Medium)	(Subbiah and Asijia, 1956)
5	Available P (kg ha ⁻¹)	23.77	Bray colorimetric method
	A 512 A	(Medium)	(Jackson, 1973)
6	Available K (kg ha ⁻¹)	328.53	Ammonium acetate method
		(low)	(Jackson, 1973)

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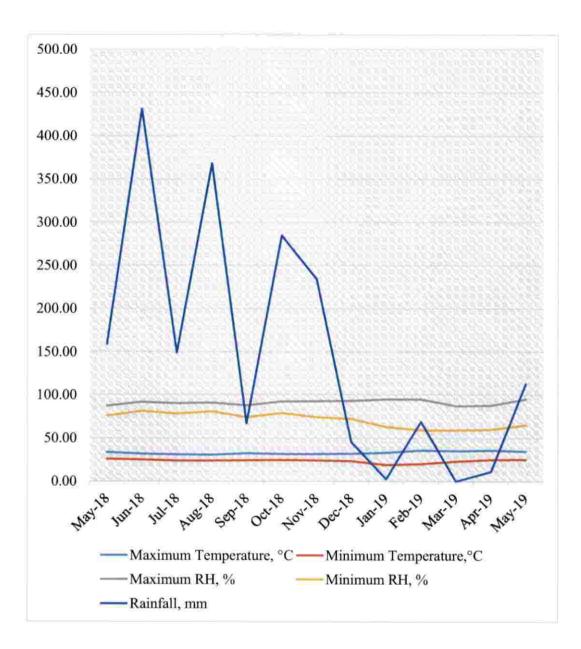


Fig.1 Weather data during the crop season (May 2018 to May 2019)

3.2. MATERIALS

3.2.1 Source of Seed

The seeds for the experiment were procured from Aromatic and Medicinal Plant Research Station (AMPRS), Odakkali, Ernakulum and AICRP on Medicinal and Aromatic plants, College of Horticulture, Vellanikkara, Thrissur.

3.2.2 Manures and Fertilizers

Well rotten cow dung (0.45 % N, 0.17 % P_2O_5 and 0.5 % K_2O) was used as the source of organic manure. Source of NPK for the experiment were urea (46 % N), rajphos (20 % P_2O_5) and muriate of potash (60 % K_2O).

3.3 METHODS

3.3.1 Design and Lay Out

Design	: Randomized block design (RBD)
Treatments	: 12
Replication	: 3
Spacing	
a) Normal row planting	: 40 cm x 40 cm
b) High Density Plantin	g: 40 cm x 20 cm
Gross Plot Size	: 2.4 m x 2.4 m
Net Plot size	: 1.6 m x 1.6 m
Total number of plots	: 36

3.3.2 Treatment Details

- T₁ : Inoculation with *Piriformospora indica* alone
- T₂ : T₁ + Soil application of cow dung slurry
- T_3 : T_1 + Soil application of NPK
- T_4 : T_2 + Irrigation at 15 mm depth
- T_5 : T_2 + Irrigation at 30 mm depth
- T_6 : T_3 + Irrigation at 15 mm depth
- T_7 : T_3 + Irrigation at 30 mm depth
- T_8 : T_5 at high density planting
- T₉ : T₇ at high density planting
- T₁₀ : T₈ under subsurface mulching with polythene

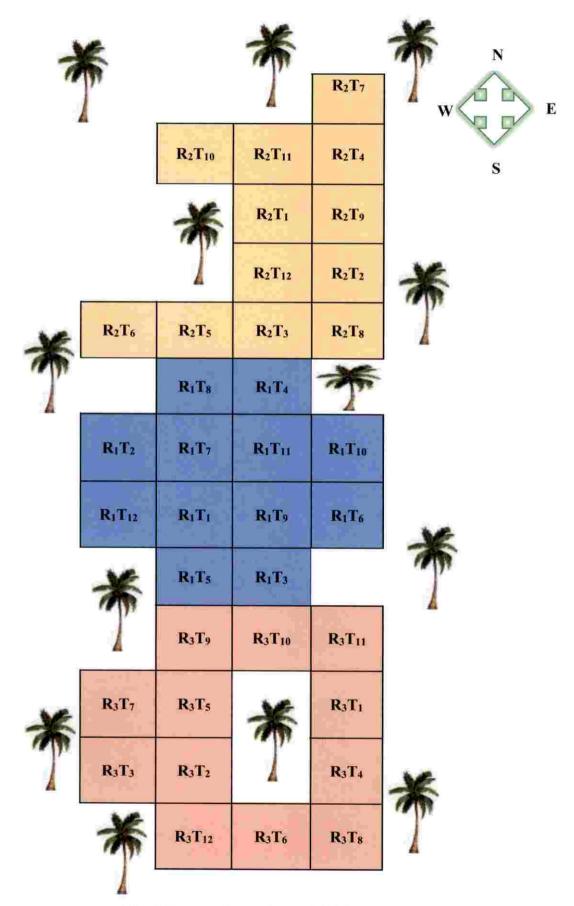


Fig. 2 Layout of experimental field

- T₁₁ : T₉ under subsurface mulching with polythene
- T₁₂ : Control at normal row planting

3.3.3 Field Preparation and Lay Out

The experimental area was cleared by removing weeds and stubbles and ploughed (excluding coconut basins) with power tiller. The clods were crushed and brought the soil to a fine tilth.

Raised beds to a height of 45 cm having a size of 2.4 m x 2.4 m were taken for all the treatments as per the lay out plan. In plots having subsurface mulching with black polythene, raised beds to a height of 45 cm was maintained over polythene mulch. Coconut husk pitching was given along the four sides to prevent soil loss by erosion.

3.3.4 Preparation of Piriformospora indica Culture

P. indica was cultivated in potato dextrose agar (PDA; pH 6.5) at 28°C (Kumar *et al.*, 2011). The fungus was mass multiplied in 100 ml potato Dextrose broth (PDB; pH 6.5) in 250 ml Erlenmeyer flasks after inoculating with a mycelial disc from a freshly grown PDA plate, and incubating at 28°C for 15 days with constant shaking in a rotary shaker (Scigenics, India) at 90 rpm. The mycelium was harvested by filtration through a strainer.

3.3.5 Preparation of Potting Medium

One part of coco-peat and two parts of vermicompost was thoroughly mixed together to prepare potting medium and strained out *P. indica* mycelium was added @ 10 g fungal mycelium for each kilogram (kg) of potting mixture.

3.3.6 Seeds and Sowing

The seeds exhibited dormancy due to hard seed coat and hence subjected to seed scarification. The scarified seeds were soaked in luke warm water for three minutes followed by soaking in normal water for three days.

The treated seeds were dibbled in the seedling portrays filled with potting mixture inoculated with fungal culture of *P. indica*. Two to three seeds were dibbled





Plate 1. General view of the experimental field

in each cell. The seedlings were transferred to paper cups filled with 1:1 mixture of soil and cow dung 15 day after sowing in portrays.

3.3.7 Transplanting

The seedlings were ready for transplanting in the main field 45 days after sowing. Seedlings were transplanted at a spacing of 40 cm x 40 cm and 40 cm x 20 cm as per the treatments.

3.3.8 Application of Manures and Fertilizers

Dried cow dung was applied to all the plots @ 10 t ha⁻¹ before transplanting the seedlings. Entire dose of N, P and K was applied @ 40:40:40 kg ha⁻¹ year⁻¹ as basal for specified treatments. Fresh cow dung slurry (5 %) was applied to specified treatments at monthly interval.

3.3.9 Irrigation

Irrigation was scheduled based on cumulative pan evaporation (CPE) values as per the technical programme. Life-saving irrigation was given to other treatments as and when required. Details of irrigation given are presented in Table 2. The following formula was used for calculating the volume of water required for irrigating each plot.

Volume of water required = depth of irrigation water x area

3.3.10 Weed Management

Weed management was done through hand weeding. Three hand weeding at 30, 60 and 90 DAT were done.

3.3.11 Plant Protection

Serious incidence of pest and disease was not observed. But a minor infestation of cowpea pod bug (*Riptortus pedestris*) was observed during the seed setting stage which was managed very early by spraying Imidacloprid @ 1 ml per 10 L of water

3.3.12 Harvest

The crop was harvested on 28/05/2019. Whole plant was uprooted by digging with spade. The roots are separated from shoot, weighed, dried in shade and stored in low humidity environment. The root weight was recorded separately and expressed in kg ha⁻¹ on dry weight basis.

Treatments	No. of	Irrigation	Effective	Total water
	irrigations	requirement	rainfall during	requirement
		(L plant ⁻¹)	the crop	(L plant ⁻¹)
			season (cm)	
T ₁	0	0	82.35	132.4
T ₂	0	0	82.35	132.4
T3	0	0	82.35	132.4
T 4	67	2.4	82.35	292.56
T5	44	4.8	82.35	237.36
T ₆	67	2.4	82.35	292.56
T7	44	4.8	82.35	237.36
T ₈	44	4.8	82.35	118.68
T9	44	4.8	82.35	118.68
T ₁₀	44	4.8	82.35	118.68
T11	44	4.8	82.35	118.68
T ₁₂	0	0	82.35	132.4

Table 2. Details of irrigation during the experiment

3.4 OBSERVATIONS

Observations were taken at monthly intervals starting from 2 MAT to 7 MAT and followed by the final observation at harvest. Methods followed for recording field observations are detailed below.

3.4.1 Morphological Characters

Observations on morphological characters were taken at monthly interval from 2 month after transplanting (MAT) to 7 MAT and at final harvest. From the net plot area three plants were randomly selected and tagged for recording the observations on growth parameters viz., plant height, leaf number, leaf area and number of branches per plant.

3.4.1.1 Plant Height

Plant height during the vegetative stage was measured from the base to the tip of the top most leaf of tagged plants. During reproductive stage, the height was recorded from the base to the tip of the inflorescence. Average plant height was recorded in cm.

3.4.1.2 Leaf Number per Plant

Total number of functional leaves from the observation plants were recorded and computed the mean.

3.4.1.3 Leaf Area per Plant

The leaf area of index leaf was estimated by graphical method.

3.4.1.4 Number of Branches per Plant

Number of branches of the observation plants were counted and worked out the average.

3.4.2 Root Characters

Observations on root characters were noted at monthly interval from 2 MAT to 7 MAT as well as at harvest.

3.4.2.1 Root Number per Plant

Representative plants from the plot were identified and uprooted. Total number of roots were counted and worked out the mean.



Seedlings at transplanting stage (45 DAS)



Vegetative stage

Plate 2. Crop growth at seedling and vegetative stage

3.4.2.2 Root Spread per Plant

The maximum root spread was measured using a meter scale and recorded in cm.

3.4.2.3 Root Volume per Plant

Water displacement method was adopted to determine the root volume (Misra and Ahmed, 1989) and was expressed in cm³ per plant.

3.4.2.4 Length of Tap Root per Plant

The tap root length in cm was measured from the collar region of the plant to the tip of the tap root using a meter scale.

3.4.2.5 Girth of Primary Root per Plant

The maximum girth of the primary root was measured using a meter scale and noted in cm.

3.4.2.6 Length of Lateral Root per Plant

Length of lateral root was measured using a meter scale and recorded in cm.

3.4.2.7 Root Fresh Weight per Plant

The roots were washed, cleaned and weighed. Weight was recorded in g per plant.

3.4.2.8 Root Dry Weight per Plant

The roots were washed, cleaned, weighed and dried in an oven at 65 ± 5 °C to a constant weight. It was expressed in g per plant.

3.4.2.9 Root Yield at Harvest

The roots uprooted from the net plot area were washed, cleaned, weighed and dried in an oven at 65 ± 5 °C to a constant weight. The root dry weight thus recorded in kg m⁻² was converted to t ha⁻¹.

3.4.3 Physiological Parameters

Physiological parameters were determined at monthly interval from 2 MAT to 7 MAT and also at harvest.

3.4.3.1 Chlorophyll Content

Total chlorophyll content of the index leaf was determined using DMSO (dimethyl sulphoxide) method suggested by Yoshida *et al.* (1976).

V

Total Chlorophyll = [20.2 (OD at 645) - 8.02 (OD at 663)] x -----

W x 1000

3.4.3.2 Relative Leaf Water Content (RLWC)

The procedure given by Slatyer and Barrs (1965) was adopted to determine the RLWC of leaf and was expressed in percentage.

RLWC = Turgid weight – Dry weight X 100

3.4.3.3 Root-Shoot Ratio

Root and shoot dry weight of the uprooted plants were recorded separately and the root and shoot ratio worked out.

3.4.3.4 Leaf Area Index

Leaf area of the plant was determined by graphical method. By multiplying the leaf area with total number of leaves, total leaf area per plant was obtained. LAI was thus calculated as follows:

LAI = $\frac{\text{Total leaf area of plant in m}^2}{\text{Area occupied by plant in m}^2}$

3.4.3.5 Crop Growth Rate (CGR)

Sample plants were uprooted and the CGR was computed in the unit, g m⁻² day⁻¹ using the formula put forward by Watson (1958).

$$CGR = \frac{(W_2 - W_1)}{(t_2 - t_1)} \times \frac{1}{A}$$

where, W_2 is weight of crop at stage n_2 (g), W_1 is the weight of crop at stage n_1 (g), t_2 is days after transplanting at stage n_2 , t_1 is days after transplanting at stage n_1 and A is ground area.

3.4.3.6 Relative Growth Rate (RGR)

From net plot area representative plants were uprooted, washed and dried to a constant weight in hot air oven at 65 \pm 5°C. The amount of dry matter unit⁻¹ dry weight of plant unit⁻¹ time was stated as mg g⁻¹ day⁻¹ by the formula given by Evans (1972).

$$RGR = \frac{(\log_e W_2 - \log_e W_1)}{(t_2 - t_1)}$$

where $\log_e W_2$ and $\log_e W_1$ are the logarithmic values of dry weight of plant at two stages n_2 and n_1 respectively and t_2 and t_1 are duration in days between the crop growth stages.

3.4.3.7 Net Assimilation Rate (NAR)

From net plot area representative plants were uprooted washed and dried to a constant weight in hot air oven at 65 ± 5 °C. Corresponding leaf area of the sample plants was also recorded. NAR was worked out and expressed in mg cm⁻² day using the formula shown below.

NAR =
$$\frac{(W_2 - W_1) \times (\log L_2 - \log L_1)}{(t_2 - t_1) \times (L_2 - L_1)}$$

where, W_1 and W_2 are the initial and final plant dry weights respectively, L_1 and L_2 are the respective plant leaf area and logeL₂ and logeL₁ are the logarithmic value of plant leaf area at two stages n_2 and n_1 respectively and t_2 and t_1 are duration in days between the crop growth stages.

3.4.4 Biochemical Parameters at Harvest

3.4.4.1 Total Alkaloid

For the extraction of total alkaloid from root system of the crop soxhlet extraction process was adopted. The soxhlet assembly is a continuous extractor generally suitable for the extraction of alkaloids from powdered plant materials with the help of organic solvents. Ethanol (95 %) was used as the solvent. Dried and powdered root of *D. gangeticum* was loosely packed in a thimble of soxhlet apparatus and on boiling ethanol penetrate deeply into the powdered root thereby allowing the greatest possible extraction of alkaloids from the exposed surfaces of the cells and tissues of the crude drug. The distillation process was stopped when the ethanol leaving the thimble appears clear indicating the complete extraction of alkaloid fraction from crude drug. Extraction is followed by filtration and evaporation of solvent in a rotary thin-film evaporator.

3.4.5 Microbiological Studies

3.4.5.1 Per cent Root Colonization by P. indica

Roots collected from *P. indica* inoculated plants were examined for the presence of fungal colonization. The root system was washed in running water to remove the adhered soil particles. Then it was cut in to small bits of roughly one cm length. These bits were cleared by boiling in 10 per cent KOH for 10 minutes followed by washing in distilled water.

Root bits were acidified with 2 per cent HCl for 5 to 10 minutes and directly transferred to staining agent, lactophenol-tryphan blue for 10 minutes. After 10 minutes, excess stain was removed through de-staining with lactophenol and was examined for fungal colonization under microscope.

The per cent root colonization was calculated using the formula,

No. of root bits with chlamydospores x 100

Per cent root colonization =

Total root bits examined

3.4.6 Seed Production Parameters

3.4.6.1 Time Taken for First Flowering

Number of days taken for the first flower opening for each treatment was noted separately.

3.4.6.2 Time Taken for 50 Per cent Flowering

Number of days from sowing to 50 per cent flowering was recorded from observation plants and worked out the mean.

3.4.6.3 Number of Inflorescence per Plant

Total number of inflorescence from observation plants was counted and the mean recorded.

3.4.6.4 Length of Inflorescence per Plant

Length of inflorescence was measured from the base of the inflorescence stalk to the tip.

3.4.6.5 Number of Seeds per Inflorescence per Plant

Mature seeds from the inflorescence were separated, counted and noted the mean.

3.4.6.6 Thousand Seed Weight

Mature seeds were separated from the harvested pods, dried, counted and weighed. The test weight was expressed in g.

3.4.7 Soil Moisture Studies

3.4.7.1 Moisture Content

Standard moisture meter was used to measure the soil moisture status before and after irrigation. The moisture content was recorded in percentage.

3.4.7.2 Consumptive Use of Water

Consumptive use of water by *Desmodium gangeticum* under different treatments were computed using the formula developed by Dasthane (1972).

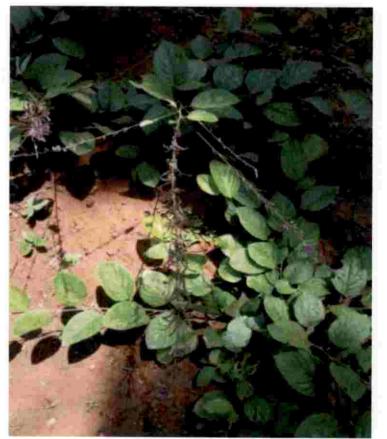
ⁿ

$$CU = \sum (Ep \ge 0.6) + \sum \frac{(Mai - Mbi)}{1} x Asi \ge Di + ER$$
1
1
1
1
1
N
(Mai - Mbi)
1
N
(Mai - Mbi)
N
(Mai - M

where, CU = Consumptive use in mm.



Flowering stage



Seed setting stage

Plate 3. Crop during reproductive phase

Ep = Pan-evaporation value from USWB class A open pan-evaporimeter from the date of irrigation to the date of soil sampling after irrigation.

0.6 = A constant used for obtaining ET value from pan evaporation value for the given period of time.

Mai = Percentage soil moisture (w/w) of the ith layer of soil at the time of sampling after irrigation.

Mbi = Percentage soil moisture (w/w) of the ith layer of soil at the time of sampling before irrigation.

Asi = Apparent specific gravity of ith layer of soil, g cc-1

Di = Depth (mm) of the ith layer of soil

ER = Effective rainfall if any within the season (mm)

N = Number of soil layers

n = Number of days between irrigation and post irrigation soil sampling.

3.4.7.3 Irrigation Requirement

Irrigation requirement was computed directly by adding the quantities of water delivered during irrigation for each treatment.

3.4.7.4 Crop Coefficient (Kc)

Crop coefficient was worked out by dividing the consumptive use during a given period by pan evaporation value during that period.

3.4.7.5 Crop Water Use Efficiency (CWUE)

Crop water use efficiency was computed by the formula given below and was expressed in g m⁻³.

Yield

CWUE = _____

Consumptive use

3.4.7.6 Field water use efficiency (FWUE)

Field water use efficiency was measured using the following formula and expressed in g m⁻³.

Yield

FWUE = _____

Total water requirement

3.4.7.7 Water Productivity (WP)

Water productivity was estimated using the formula put forwarded by Kijne *et al.* (2003) and expressed as g m⁻³.

Total biomass

WP = _____

Total water depleted

3.5 CHEMICAL ANALYSIS

3.5.1 Plant Analysis at Harvest

The root and shoot portions of the harvested plant were analyzed separately for the total N, P and K content. The samples were dried in hot air oven at 65 ± 5 °C to constant weight, ground in a mixer and analyzed. The required quantities of samples were weighed out accurately, subjected to acid extraction and N, P and K content were determined.

3.5.1.1 Total N Content

Total nitrogen content was estimated by modified micro-kjheldal method (Jackson, 1973).

3.5.1.2 Total P Content

Total phosphorus content was estimated by vanado-molybdate phosphoric yellow colour method (Jackson, 1973).

3.5.1.3 Total K Content

Total potassium content was determined using flame photometer (Jackson, 1973).

3.5.2 Soil Analysis

For initial soil sample analysis, representative samples were drawn to a depth of 15 cm from four different spots of the experimental area, shade dried and composite samples were obtained by quartering. After crop harvest also, composite soil samples were taken from each treatment plot for the analysis of organic carbon and available N, P and K status adopting the methods given in Table 1. 3.6 ECONOMIC ANALYSIS

Based on cost of cultivation, yield and the prevailing market price of the produce, gross income, net income and B: C ratio were worked out.

3.6.1 Net Income

Net income was computed using the formula

Net income (₹ ha⁻¹) = Gross income - Cost of cultivation

3.6.2 Benefit Cost Ratio

Benefit cost ratio was computed using the formula

Gross income

B: C ratio =

Cost of cultivation

3.7 STATISTICAL ANALYSIS

The data was statistically analyzed as per the procedure outlined by Panse and Sukhatme (1985) for Randomised Block Design.

Results

6

4. RESULTS

A field experiment was conducted during May 2018 to May 2019 in the Instructional farm, College of Agriculture, Vellayani, Thiruvananthapuram, Kerala, India with the objective to study the integrated effect of root endophyte fungus, planting density, source efficacy of nutrients, moisture stress and subsurface mulching on the growth, yield and quality constituents of *Desmodium gangeticum* (L.) DC. under partial shade. The results of the experiment are described in this chapter.

4.1 MORPHOLOGICAL CHARACTERS

4.1.1 Plant Height

Data relating to the effect of agrotechniques on mean plant height at 2, 3, 4, 5, 6 and 7 MAT and at harvest are presented in Table 3.

Agrotechniques significantly influenced plant height at all stages of plant growth except at 2 MAT. The treatment T_7 recorded the tallest plants at 3 and 4 MAT and was on par with the treatments T_5 and T_9 at 3 MAT and T_5 , T_8 and T_9 at 4 MAT. Appreciable difference in plant height was observed due to different agrotechniques as growth progressed. The treatment T_9 registered the tallest plants at 5 MAT and was on par with T_5 and T_6 . The same treatment expressed the tallest plants at 6 MAT and was on par with T_8 . At 7 MAT and at harvest, plant height recorded by T_9 was significantly higher and were 113.97 and 150.33 respectively and was 30.26 per cent higher compared to the control.

4.1.2 Number of Branches per Plant

Data recorded on the effect of agrotechniques on mean number of branches at 2, 3, 4, 5, 6 and 7 MAT and at harvest stage are depicted in Table 4.

Number of branches per plant was significantly affected by agrotechniques at all growth stages of the crop except at 2 MAT. Results indicated that number of branches in T₇ was higher at all growth stages except at 2 MAT where it was found non-significant. At 3 MAT, the treatments T₂, T₅ and T₁₂ were statistically on par with T₇ At 4 MAT, T₂, T₃, T₄, and T₁₂ were statistically comparable with T₇. At 5 MAT, T₂, T₉ and T₁₂ were on par with T₇. Treatments T₂, T₃, and T₉ were on par at 6 MAT while at 7 MAT, T₅ and T₈ were statistically comparable with T₇. At harvest, only T₈ was on par with T₇.

4.1.3 Leaf Number per Plant

The effect of treatments on mean leaf number recorded at 2, 3, 4, 5, 6 and 7 MAT and at harvest are given in Table 5.

From 3^{rd} month onwards leaf production was significantly influenced by the treatment effects. At 3 MAT, T₃, T₄, T₅ and T₇ were statistically comparable with T₆ for higher leaf production. At 4 MAT, T₇ produced the highest number of leaves which was statistically on par with T₃, T₄, T₅ and T₆. T₆ recorded the highest number of leaves of leaves at 5 MAT and was found to be on par with T₅, T₇ and T₈. During 6 and 7 MAT as well as at harvest, T₇ registered the highest leaf number.

4.1.4 Leaf Area per Plant

Data showing the effect of agrotechniques on mean leaf area per plant recorded at 2, 3, 4, 5, 6 and 7 MAT and at harvest are presented in Table 6.

Leaf area was significantly influenced by agrotechniques at all plant growth stages except at 2 and 7 MAT. At 3 MAT, T₆ recorded the highest leaf area and was on par with T₁, T₂, T₃, T₅, T₇, and T₁₂. At 4 MAT, T₆ recorded the highest leaf area and was statistically comparable with T₁, T₂, T₃, T₄, T₅, T₇, and T₁₂. At 5 and 6 MAT, T₇ showed the highest leaf area which was on par with T₆ and T₂ respectively. At harvest, T₅ expressed greater leaf area and was statistically on par with T₁, T₂, T₃, T₆, T₇, T₈, and T₉.

4.2 ROOT CHARACTERS

4.2.1 Root Number per Plant

Observations recorded on mean number of roots at 2, 3, 4, 5, 6 and 7 MAT and at harvest are presented in Table 7.

Observations on the number of roots were significantly influenced by the agrotechniques at all growth stages except at 2 and 4 MAT. The treatment T_5 registered the highest root number 3 MAT and was on par with all the treatments with the exception of T_2 , T_3 , T_{10} and T_{12} . At 5, 6, 7 MAT and at harvest T_{10} recorded

Treatments	2 MAT	3 MAT	4 MAT	5 MAT	6 MAT	7 MAT	Harvest
T 1	14.80	48.23	59.10	71.70	78.90	81.53	113.83
T2	17.67	53.03	61.07	71.73	73.20	84.80	114.10
T3	17.10	57.37	66.83	72.00	74.53	87.83	121.80
T4	16.90	57.23	65.67	71.00	78.80	82.97	110.67
T5	17.37	69.20	76.33	82.97	84.23	81.03	115.20
T ₆	16.20	64.33	64.77	81.30	87.07	87.63	123.20
T7	18.20	69.90	77.03	80.80	82.27	87.33	122.63
T ₈	19.03	49.73	73.73	79.03	96.07	101.20	141.73
T9	17.77	66.20	75.83	85.50	99.57	113.97	150.33
T10	17.67	36.83	47.23	61.77	79.37	101.80	116.27
T11	19.63	40.40	48.83	68.23	80.63	108.07	124.93
T ₁₂	14.60	55.60	58.20	68.50	70.17	82.53	104.83
SEm (±)	1.26	1.48	1.93	1.51	2.71	1.95	1.99
CD (0.05)	NS	4.377	5.698	4.447	8.006	5.763	5.880

Table 3. Effect of agrotechniques on plant height, cm

Table 4. Effect of agrotechniques on number of branches per plant

Treatments	2 MAT	3 MAT	4 MAT	5 MAT	6 MAT	7 MAT	Harvest
T ₁	3.33	7.33	7.33	7.67	6.00	16.00	12.67
T ₂	3.33	9.33	8.33	9.00	7.67	13.33	16.67
T_3	3.67	8.00	8.00	6.67	8.33	17.00	12.33
T ₄	4.67	8.33	8.33	8.33	6.00	16.33	15.00
T ₅	3.00	8.67	6.67	8.33	6.67	18.67	11.33
T ₆	4.33	8.33	7.33	8.33	5.33	13.67	17.00
T7	4.33	11.00	9.33	11.00	10.00	20.67	21.00
T8	4.33	4.67	6.00	7.00	6.67	17.33	20.67
T9	3.00	5.00	7.33	9.67	7.67	13.00	16.33
T ₁₀	3.00	4.67	6.00	6.33	6.67	13.00	13.00
T ₁₁	3.00	4.33	4.67	6.67	6.67	13.67	11.33
T ₁₂	3.33	10.00	7.67	8.67	5.67	16.33	15.00
SEm (±)	0.50	0.80	0.64	0.84	0.86	1.17	1.24
CD (0.05)	NS	2.349	1.882	2.489	2.525	3.450	3.651

Treatments	2 MAT	3 MAT	4 MAT	5 MAT	6 MAT	7 MAT	Harvest
T_1	13.00	59.00	66.67	65.33	97.33	113.33	212.33
T ₂	14.00	59.67	67.33	67.67	107.00	135.67	244.00
T_3	14.00	60.67	73.00	65.67	100.33	134.67	234.33
T4	13.67	64.33	71.00	66.33	98.00	119.67	217.67
T ₅	14.33	63.33	66.33	75.33	100.00	127.67	243.67
T ₆	13.33	68.67	73.67	83.33	105.33	139.00	250.00
T7	16.33	64.33	75.67	81.00	108.00	139.33	255.67
Τ8	16.00	55.33	66.67	76.33	100.00	102.33	210.33
T9	16.00	50.33	59.00	62.67	83.33	108.67	212.00
T ₁₀	18.33	43.33	57.67	51.00	89.67	117.33	199.67
T ₁₁	19.67	39.67	52.00	51.00	88.00	109.67	205.00
T ₁₂	14.33	55.33	61.33	61.33	94.00	111.67	209.00
SEm (±)	1.81	2.85	3.95	3.52	2.23	1.80	2.78
CD (0.05)	NS	8.402	11.659	10.397	6.567	5.317	8.208

Table 5. Effect of agrotechniques on number of leaves per plant

Table 6. Effect of agrotechniques on leaf area per plant, cm²

Treatments	2 MAT	3 MAT	4 MAT	5 MAT	6 MAT	7 MAT	Harvest
T_1	170.76	1628.65	1589.50	1725.56	2479.77	2028.05	3499.41
T ₂	172.33	1495.04	1639.99	1692.22	2650.42	2354.31	3901.66
T ₃	191.13	1530.41	1722.37	1573.04	2463.45	2239.75	3960.51
T ₄	197.49	1290.68	1777.83	1470.41	2323.58	2032.03	3143.61
T ₅	284.34	1569.06	1563.65	1729.71	2527.19	2601.05	4085.86
T ₆	239.09	1777.00	1798.74	1896.31	2457.55	2563.71	3900.94
T7	244.49	1464.71	1713.18	2334.32	3021.85	2497.80	3821.86
Τ8	230.68	1403.41	1090.35	1367.25	1720.45	1915.99	3654.65
Т9	226.66	1122.40	967.95	1162.49	1461.49	2078.66	3632.27
T10	212.26	1033.97	919.73	963.52	1502.45	2045.28	2458.92
T ₁₁	280.36	924.20	920.12	843.72	1458.61	2053.05	2834.33
T ₁₂	213.79	1535.03	1553.66	1541.21	2074.88	2047.40	3264.74
SEm (±)	33.44	124.52	106.18	152.34	139.68	177.05	264.70
CD (0.05)	NS	367.557	313.429	449.670	412.314	NS	781.337

the highest root number. At 5 and 6 MAT, T_{10} was significantly superior in root number which was also statistically comparable with T_8 at 7 MAT and both T_8 and T_{11} at harvest. Control treatment showed the least root proliferation at 5, 6 and 7 MAT.

4.2.2 Root Spread per Plant

Data on mean root spread at 2, 3, 4, 5, 6 and 7 MAT and at harvest stage are presented in Table 8.

The root spread was significantly affected by treatment effects at all the growth stages except at 4 and 5 MAT. At 2 MAT, T₉ registered the highest root spread and was on par with T₅, T₆ and T₁₀ while control showed the poorest spread. At 3, 6, and 7 MAT as well as at harvest, T₇ exhibited greater root spread. It was also statistically comparable with T₅ at 3 MAT, T₄ and T₅ at 6 MAT, T₄, T₅ and T₆ at 7 MAT and T₁, T₄, T₅ and T₆ at harvest.

4.2.3 Root Volume per Plant

Data relating to the effect of agrotechniques on root volume recorded at 2, 3, 4, 5, 6 and 7 MAT and at harvest are given in Table 9.

Treatment effects significantly influenced root volume at all the growth stages except at 2 and 4 MAT. At 3 MAT, T₅ recorded the highest root volume and was on par with T₆. During 5 and 6 MAT, T₇ recorded the greatest root volume which was statistically comparable with all the treatments with the exception of T₈, T₉, T₁₀ and T₁₁. At 7 MAT, T₆ showed the highest root volume which was on par with all the treatments except T₈, T₉, T₁₀ and T₁₂. At harvest, T₇ exhibited the greatest root volume and was on par with all the treatments except T₈, T₉, T₁₀, T₁₁ and T₁₂.

4.2.4 Length of Tap Root per Plant

Data on mean tap root length as influenced by the effect of agrotechniques at 2, 3, 4, 5, 6 and 7 MAT and at harvest are furnished in Table 10.

Agrotechniques significantly influenced the length of tap root at all growth stages except 2 and 5 MAT. At 3 MAT, the treatments T₄, T₆, T₇ and T₈ were statistically comparable with T₉ for longer tap root. At 4 and 6 MAT and at harvest also T₉ exhibited longer tap root. Only T₄ was on par with T₉ at 4 MAT whereas T₄,

T₇, T₈ and T₁₁ at 6 MAT and T₆, T₇, T₈ and T₁₀ were statistically comparable with T₉ at harvest. At 7 MAT, T₈ excelled in primary root length which was on par with T₆, T₇, T₉ and T₁₀ while, the shortest root was noted in T₁.

4.2.5 Girth of Primary Root per Plant

The effect of agrotechniques on mean girth of primary root recorded at 2, 3, 4, 5, 6 and 7 MAT and at harvest are shown in Table 11.

The primary root girth was significantly influenced by treatment effects from 5 MAT onwards. At 3 MAT, T₅ registered the highest tap root girth which was on par with T₆, T₇ and T₈. During 5, 6 and 7 MAT, T₁, T₂, T₃, T₄, T₅ and T₆ were statistically comparable with T₇ for greater primary root girth. At harvest, T₆ registered the highest primary root girth which was statistically on par with T₁, T₂, T₃, T₄, T₅, T₇ and T₁₁.

4.2.6 Length of Lateral Root per Plant

Data relating to the effect of agrotechniques on mean length of lateral roots at 2, 3, 4, 5, 6 and 7 MAT and at harvest are given in Table 12.

From 5 MAT onwards, lateral root length was found to be significantly influenced by treatment effects. T₇ registered the longest lateral root at 5, 6 and 7 MAT and at harvest. T₇ was observed to be on par with T₁, T₂, T₄, T₅ and T₆ at 5 MAT and T₁, T₂, T₃, T₄, T₅, T₆ and T₁₂ at 6 MAT and T₂, T₃, T₄, T₅ and T₆ at 7 MAT. But at harvest, the treatments T₁, T₂, T₃, T₄, T₅ and T₆ were on par with T₇.

4.2.7 Root Fresh Weight per Plant

Data on root fresh weight per plant recorded at 2, 3, 4, 5, 6 and 7 MAT and at harvest are furnished in Table 13.

Root fresh weight per plant was significantly influenced by agrotechniques at all growth stages except at 2 and 4 MAT. The highest root fresh weight was recorded by T₉ at 3 MAT. At 5 and 7 MAT, T₆ registered the highest root fresh weight which was statistically comparable with T₁, T₂, T₃, T₄, T₅ and T₇. At 6 MAT and harvesting, T₇ registered the highest root fresh weight and was on par with T₁, T₂, T₃, T₄, T₅ and T₆.

Treatments	2 MAT	3 MAT	4 MAT	5 MAT	6 MAT	7 MAT	Harvest
T 1	3.00	8.33	8.33	9.33	9.67	14.33	19.67
T ₂	3.33	6.67	8.67	9.33	9.00	15.00	18.00
T3	4.33	6.67	9.00	9.33	10.33	14.33	19.00
T4	4.33	8.67	8.67	8.67	8.33	14.67	20.33
T ₅	3.00	9.67	9.33	9.67	10.00	17.00	22.67
T ₆	2.67	8.33	8.33	8.00	10.00	16.33	22.00
T7	2.67	8.67	9.00	7.33	9.33	16.67	23.00
T ₈	3.33	8.00	8.33	11.00	12.00	18.00	24.33
T9	3.00	8.67	8.67	7.67	10.67	15.67	22.67
T ₁₀	3.67	6.00	8.67	13.67	14.67	21.33	27.33
T11	3.33	9.67	9.33	7.67	10.00	15.33	23.33
T ₁₂	2.67	6.00	8.00	7.00	8.00	13.67	20.33
SEm (±)	0.41	0.78	1.00	0.79	0.76	1.22	1.37
CD (0.05)	NS	2.315	NS	2.344	2.240	3.614	4.042

Table 7. Effect of agrotechniques on root number per plant

Table 8. Effect of agrotechniques on root spread per plant, cm

Treatments	2 MAT	3 MAT	4 MAT	5 MAT	6 MAT	7 MAT	Harvest
T_1	4.80	13.60	14.73	16.23	23.87	28.07	39.20
T ₂	5.07	13.23	13.87	16.23	22.00	27.23	35.57
T_3	5.23	13.10	14.33	14.77	23.63	26.93	35.10
T ₄	5.00	14.90	15.30	16.83	26.90	31.83	38.50
T5	5.93	15.47	13.73	16.77	27.70	31.73	40.23
T ₆	6.20	14.43	14.80	15.07	25.90	31.30	40.00
T ₇	5.10	16.80	17.50	19.90	30.00	34.17	43.17
Τ8	4.60	13.37	15.27	17.00	24.07	25.43	37.57
Т9	6.63	15.37	15.23	15.37	24.93	26.13	33.50
T ₁₀	6.17	13.53	15.27	14.53	23.73	25.33	31.83
T11	4.70	12.63	13.90	14.53	23.10	24.53	32.07
T ₁₂	4.17	11.97	15.07	15.23	23.67	26.53	37.17
SEm (±)	0.38	0.46	1.17	1.29	1.36	1.68	1.72
CD (0.05)	1.125	1.362	NS	NS	4.014	4.954	5.080

Treatments	2 MAT	3 MAT	4 MAT	5 MAT	6 MAT	7 MAT	Harvest
T 1	2.00	3.60	3.93	14.53	17.87	26.33	28.33
T ₂	1.33	3.73	3.97	14.40	17.73	25.77	27.77
T ₃	1.50	3.73	4.27	14.57	17.90	25.90	27.90
T ₄	2.00	3.83	4.27	14.53	18.20	26.47	28.47
T ₅	2.00	4.50	4.67	14.60	18.60	28.37	30.37
T ₆	1.67	4.10	4.43	14.77	18.53	28.90	30.90
T7	1.83	4.03	4.60	14.83	18.77	28.83	31.17
Τ8	1.67	3.60	4.17	12.93	15.73	20.67	22.67
T9	1.83	3.60	3.87	13.07	16.07	20.90	22.90
T ₁₀	1.67	3.23	4.00	12.37	14.90	19.37	21.37
T ₁₁	1.83	3.53	3.77	13.10	15.43	20.50	22.53
T ₁₂	1.60	3.57	4.07	14.10	17.43	20.73	25.73
SEm (±)	0.19	0.15	0.30	0.38	0.73	2.26	1.45
CD (0.05)	NS	0.428	NS	1.128	2.160	6.658	4.271

Table 9. Effect of agrotechniques on root volume per plant, cm³

Table 10. Effect of agrotechniques on tap root length per plant, cm

Treatments	2 MAT	3 MAT	4 MAT	5 MAT	6 MAT	7 MAT	Harvest
T_1	13.67	15.53	18.60	30.43	32.62	40.59	48.57
T ₂	14.73	15.67	19.87	31.30	32.90	42.30	48.15
T ₃	13.03	16.53	20.10	31.17	34.72	44.28	50.84
T_4	11.80	18.27	19.70	30.60	38.36	45.45	52.18
T5	13.17	15.90	18.80	29.83	33.39	42.93	51.25
T ₆	15.53	17.17	18.20	30.87	34.44	47.70	54.77
T7	14.37	17.67	20.87	29.83	37.10	46.35	53.22
Τ8	14.13	18.87	22.40	31.10	39.62	52.92	58.49
T9	13.77	19.60	24.30	31.10	41.16	50.94	60.76
T ₁₀	13.90	16.83	18.27	29.23	35.35	49.32	56.63
T11	14.10	16.40	19.37	29.73	36.05	44.64	49.29
T ₁₂	13.30	15.03	17.23	29.13	31.57	41.94	46.60
SEm (±)	1.06	0.90	0.76	1.52	1.89	2.43	2.79
CD (0.05)	NS	2.654	2.231	NS	5.574	7.166	8.229

0

Treatments	2 MAT	3 MAT	4 MAT	5 MAT	6 MAT	7 MAT	Harvest	
T ₁	0.27	0.43	1.20	1.23	1.40	1.53	2.07	
T ₂	0.27	0.43	1.17	1.23	1.33	1.47	1.90	
T ₃	0.30	0.43	1.07	1.23	1.47	1.57	2.13	
T ₄	0.37	0.63	1.13	1.27	1.50	1.60	2.17	
T ₅	0.37	0.87	1.10	1.23	1.40	1.57	2.20	
T ₆	0.33	0.67	1.10	1.23	1.40	1.63	2.30	
T7	0.30	0.67	1.10	1.33	1.57	1.67	2.23	
T_8	0.33	0.87	1.10	1.10	1.20	1.33	1.83	
T9	0.23	0.53	0.97	1.03	1.13	1.23	1.73	
T ₁₀	0.30	0.50	0.90	1.10	1.20	1.27	1.67	
T11	0.30	0.57	0.90	1.07	1.17	1.30	1.90	
T ₁₂	0.33	0.43	1.03	1.13	1.23	1.37	1.77	
SEm (±)	0.04	0.08	0.10	0.05	0.08	0.08	0.14	
CD (0.05)	NS	0.226	NS	0.151	0.246	0.221	0.408	

Table 11. Effect of agrotechniques on girth of primary root per plant, cm

Table 12. Effect of agrotechniques on length of laterals per plant, cm

Treatments	2 MAT	3 MAT	4 MAT	5 MAT	6 MAT	7 MAT	Harvest
T1	5.67	13.50	15.50	19.43	23.80	28.20	38.80
T ₂	5.23	12.67	19.03	18.43	24.10	29.23	40.10
T3	4.77	15.43	15.30	17.20	25.20	28.97	40.50
T4	5.27	12.70	16.40	19.67	25.37	31.27	40.47
T ₅	4.60	15.47	15.93	18.90	24.87	31.30	40.93
T6	5.67	15.93	17.00	18.73	23.47	28.83	41.77
T7	5.03	16.70	15.90	21.10	25.57	33.87	42.97
T8	5.27	17.27	16.57	16.57	19.83	23.83	34.67
T9	5.10	15.47	15.73	14.53	20.33	26.47	35.00
T ₁₀	5.07	12.43	16.33	14.87	18.83	25.90	33.03
T11	5.13	12.40	17.43	15.40	20.00	24.27	33.73
T ₁₂	5.13	15.40	17.30	17.60	24.83	27.73	33.50
SEm (±)	0.32	1.55	1.77	0.96	1.67	1.85	1.81
CD (0.05)	NS	NS	NS	2.830	4.929	5.467	5.355

4.2.8 Root Dry Weight per Plant

Data recorded on the effect of treatments on root dry weight per plant at 2, 3, 4, 5, 6 and 7 MAT and at harvest are presented in Table 14.

Root dry weight was significantly affected by agrotechniques at all growth stages from 5 MAT to harvest. Treatments T_1 , T_2 , T_3 , T_4 , T_5 , T_7 and T_8 were statistically on par with T_6 for higher root dry weight at 5 MAT. At 6 and 7 MAT and at harvest, T_7 showed the highest root dry weight per plant and was on par with T_1 , T_2 , T_3 , T_4 , T_5 and T_6 .

4.2.9 Root Yield at Harvest

Data on root yield at harvest expressed in t ha⁻¹ are presented in Table 15.

At harvest, root yield in *D. gangeticum* was found to be significantly influenced by agrotechniques. The treatment, T_8 registered the highest root yield which was statistically on par with T_9 , T_{10} and T_{11} . The control treatment showed the lowest yield.

4.3 PHYSIOLOGICAL PARAMETERS

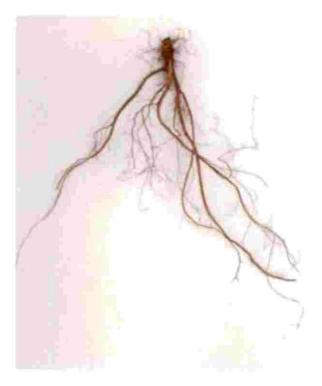
4.3.1 Chlorophyll Content

Observations recorded on total chlorophyll content at 2, 3, 4, 5, 6 and 7 MAT and at harvest are given in Table 16.

Total chlorophyll content was found significantly influenced by treatment effects at all growth stages except at 3 MAT. At 2 MAT, T₅ showed the highest total chlorophyll content and was on par with all other treatments except T₁ and T₁₂. At 4 MAT, T₉ was superior and on par with T₃, T₄ and T₆. T₆ registered maximum chlorophyll content at 5 MAT which was on par with all other treatments except of T₂, T₁₀ and T₁₂. T₁₁ showed the highest chlorophyll content at 6 MAT and T₅, T₆, T₇, T₈, T₉ and T₁₀ were on par. At 7 MAT and at harvest, T₉ recorded the highest total chlorophyll content.



Treatment T₈ (P. indica + cow dung slurry + 30 mm irrigation @ HDP)



Control treatment (T₁₂) Plate 4. Effect of agrotechniques on root growth

4.3.2 Relative Leaf Water Content

Data on RLWC at 2, 3, 4, 5, 6, 7 MAT and at harvest are given in Table 17.

Relative leaf water content was significantly influenced by treatment effects at all growth stages except at 2 MAT. T₂ showed the highest RLWC at 3 MAT and was on par with T₁, T₃, T₄, T₅, T₆, T₇ and T₁₁. At 4 MAT, T₂ showed the highest RLWC which was on par with T₁, T₄, T₆ and T₇. During 5 and 6 MAT and at harvest T₇ registered the highest RLWC. At 7 MAT, T₆ showed the highest RLWC which was on par with T₁ and T₇.

4.3.3 Root-Shoot Ratio

The data pertaining to root-shoot ratio at 2, 3, 4, 5, 6 and 7 MAT and at harvest are furnished in Table 18.

Root-shoot ratio was significantly influenced by the treatment effects at 5 and 7 MAT and at harvest. At 5 MAT, T_7 recorded the highest root-shoot ratio which was statistically on par with T_1 , T_2 and T_4 . At 7 MAT, T_1 , T_4 , T_6 and T_7 were statistically comparable with T_5 . At harvest, T_6 registered the highest root-shoot ratio which was on par with T_3 , T_4 , T_5 and T_7 .

4.3.4 Leaf Area Index

Data pertaining to the effect of agrotechniques on LAI at 2, 3, 4, 5, 6 and 7 MAT and at harvest stage are given in Table 19.

Treatment effects significantly influenced LAI at all growth stages except at 4 MAT. At 2 MAT, T_{11} exhibited the highest LAI and was on par with T₈ and T₉. At 3, 5, 6 MAT and at harvest, T₈ recorded the highest LAI whereas T₉ was superior at 7 MAT. At 5 MAT, T₇ and T₉ were statistically similar to T₈. T₇, T₉, T₁₀ and T₁₁ were on par with T₈ at 6 MAT. T₈, T₁₀ and T₁₁ were on par with T₇ which showed the highest LAI at 7 MAT. At harvest, T₈ was on par with T₉.

4.3.5 Crop Growth Rate

The data on CGR recorded at various growth stages are furnished in Table 20.

Treatments	2 MAT	3 MAT	4 MAT	5 MAT	6 MAT	7 MAT	Harvest	
T ₁	0.41	1.85	3.75	14.59	17.89	26.67	30.17	
T ₂	0.40	1.78	4.27	14.45	17.77	26.27	29.28	
T ₃	0.35	1.71	3.99	14.61	17.96	26.23	29.62	
T4	0.38	1.73	4.08	14.59	18.22	26.97	29.52	
T ₅	0.42	1.84	4.40	14.64	18.80	28.70	31.67	
T ₆	0.35	2.22	4.41	14.88	18.63	29.40	31.90	
T7	0.37	2.28	4.01	14.81	19.11	29.17	32.55	
T8	0.42	2.44	4.11	12.99	15.74	21.17	24.67	
Т9	0.41	2.98	3.44	13.11	16.09	21.23	25.27	
T ₁₀	0.39	1.37	3.10	12.51	15.02	19.87	24.37	
T ₁₁	0.42	1.56	3.14	13.27	15.62	20.83	25.13	
T ₁₂	0.36	1.07	3.44	13.71	16.18	21.23	27.40	
SEm (±)	0.03	0.18	0.35	0.38	0.78	2.21	1.50	
CD (0.05)	NS	0.539	NS	1.123	2.300	6.521	4.432	

Table 13. Effect of agrotechniques on root fresh weight per plant, g

Table 14. Effect of agrotechniques on root dry weight per plant, g

Treatments	2 MAT	3 MAT	4 MAT	5 MAT	6 MAT	7 MAT	Harvest
T_1	0.15	0.72	1.70	10.48	13.88	19.16	23.07
T ₂	0.14	0.60	1.87	10.61	14.77	18.66	23.53
T ₃	0.12	0.68	1.47	10.59	13.87	18.64	23.21
T ₄	0.13	0.66	1.57	10.61	14.81	19.40	23.10
T ₅	0.16	0.75	1.35	10.54	14.25	21.09	24.02
T ₆	0.12	0.73	1.30	10.88	13.89	21.71	23.24
Τ7	0.14	0.73	1.14	10.81	15.19	21.75	24.04
Τ8	0.16	0.69	1.55	9.71	11.76	13.57	17.61
T9	0.16	0.74	1.39	8.27	12.07	13.65	16.71
T ₁₀	0.15	0.64	1.42	8.51	10.87	12.34	16.23
T ₁₁	0.17	0.68	1.29	8.99	11.53	13.46	16.58
T ₁₂	0.11	0.56	1.42	9.11	11.97	13.33	18.68
SEm (±)	0.02	0.08	0.28	0.53	0.79	2.05	1.78
CD (0.05)	NS	NS	NS	1.550	2.323	6.041	5.257

Treatments	Root dry weight (t ha ⁻¹)				
T ₁ : Inoculation with <i>Piriformospora indica</i> alone	1.14				
$T_2: T_1 + Soil application of cow dung slurry$	1.16				
$T_3: T_1 + Soil application of NPK$	1.14				
T ₄ : T ₂ + Irrigation at 15 mm depth	1.14				
T ₅ : T ₂ + Irrigation at 30 mm depth					
T ₆ : T ₃ + Irrigation at 15 mm depth	1.15				
T ₇ : T ₃ + Irrigation at 30 mm depth	1.21				
T ₈ : T ₅ at high density planting	1.73				
T ₉ : T ₇ at high density planting	1.67				
T ₁₀ : T ₈ under sub-surface mulching with polythene	1.59				
T ₁₁ : T ₉ under sub-surface mulching with polythene	1.63				
T12: Control at normal row planting	0.91				
SEm (±)	0.11				
CD (0.05)	0.320				

Table 15. Effect of agrotechniques on root yield at harvest, t ha-1

Table 16. Effect of agrotechniques on total chlorophyll content, mg g-1

Treatments	2 MAT	3 MAT	4 MAT	5 MAT	6 MAT	7 MAT	Harvest
Tı	2.69	2.92	3.23	3.61	2.92	3.44	3.46
T ₂	3.58	2.86	3.59	3.24	3.42	3.93	3.76
T ₃	3.63	4.00	3.71	3.72	3.31	3.83	4.19
T4	3.63	3.58	3.67	3.00	3.34	3.85	3.72
T ₅	3.70	4.20	3.32	3.50	3.72	4.23	3.83
T ₆	3.55	3.50	4.00	4.01	3.47	3.99	4.40
T7	3.66	3.51	3.07	3.82	3.66	4.17	4.35
T8	3.49	3.53	3.43	3.84	3.93	4.44	4.38
T9	3.28	3.32	4.34	3.79	3.62	4.70	4.69
T10	3.10	3.10	3.22	3.35	3.48	4.00	4.43
T11	3.38	3.84	2.62	3.65	4.18	4.14	4.46
T ₁₂	2.47	3.22	3.04	3.08	2.83	3.34	3.08
SEm (±)	0.21	0.40	0.26	0.21	0.25	0.25	0.26
CD (0.05)	0.629	NS	0.758	0.618	0.729	0.729	0.752

VY9

Treatments	2 MAT	3 MAT	4 MAT	5 MAT	6 MAT	7 MAT	Harvest
T_1	84.40	90.12	90.93	89.22	90.57	81.63	79.94
T ₂	73.53	92.18	93.20	89.34	90.44	71.43	76.53
T3	83.55	88.94	90.30	90.19	89.50	75.39	76.50
T ₄	79.11	91.23	90.83	91.46	90.18	70.44	74.54
T5	86.73	89.96	90.30	92.31	91.31	66.66	76.52
T ₆	79.72	90.83	93.03	92.32	87.80	82.02	79.64
T7	86.55	91.27	93.10	92.43	91.67	81.87	80.55
T8	76.89	86.64	85.44	87.47	86.41	71.45	72.58
T9	82.88	86.54	85.04	88.16	90.25	72.44	72.44
T ₁₀	79.35	73.82	68.76	79.05	85.09	71.60	71.21
T11	85.82	87.45	87.73	82.95	85.61	69.23	72.56
T ₁₂	85.78	84.53	89.06	89.44	90.35	72.07	74.48
SEm (±)	4.82	1.80	0.97	1.04	0.99	1.56	1.42
CD (0.05)	NS	5.328	2.871	3.082	2.932	4.604	4.201

Table 17. Effect of agrotechniques on relative leaf water content, per cent

Table 18. Effect of agrotechniques on root-shoot ratio

Treatments	2 MAT	3 MAT	4 MAT	5 MAT	6 MAT	7 MAT	Harvest
\overline{T}_1	0.22	0.19	0.20	0.35	0.38	0.45	0.52
T ₂	0.22	0.17	0.21	0.34	0.42	0.42	0.50
T3	0.17	0.17	0.21	0.31	0.39	0.42	0.55
T ₄	0.26	0.17	0.20	0.34	0.39	0.44	0.54
T ₅	0.23	0.18	0.21	0.31	0.42	0.46	0.54
T ₆	0.20	0.19	0.22	0.32	0.41	0.44	0.56
T7	0.21	0.17	0.21	0.35	0.41	0.44	0.54
Τ8	0.23	0.17	0.21	0.31	0.38	0.40	0.50
Т9	0.21	0.20	0.19	0.31	0.38	0.40	0.51
T10	0.34	0.15	0.18	0.30	0.37	0.40	0.50
T ₁₁	0.18	0.12	0.18	0.30	0.37	0.40	0.49
T ₁₂	0.14	0.13	0.20	0.32	0.42	0.39	0.50
SEm (±)	0.07	0.02	0.01	0.01	0.03	0.01	0.01
CD (0.05)	NS	NS	NS	0.027	NS	0.035	0.033

Agrotechniques significantly influenced CGR at all growth stages except during 2 to 3 MAT and 3 to 4 MAT. During 4 to 5 MAT, T₈ recorded the highest CGR which was on par with T_{10} and T_{11} . T₉ was significantly superior and was statistically on par with T_{11} during 5 to 6 MAT. During 6 to 7 MAT, T₆ was superior and on par with T₅. During 7 MAT to harvest T₉ was found significantly superior to other treatments.

4.3.6 Relative Growth Rate

The effect of agrotechniques on RGR recorded at various growth stages are given in Table 21.

Relative growth rate was significantly influenced by treatment effects at all growth stages except during 5 to 6 MAT. During 2 to 3 MAT, the treatment T_4 showed the highest RGR which was on par with T_6 and T_{10} . During 3 to 4, 4 to 5 and 6 to 7 MAT, T_6 registered the highest RGR. T_6 was on par with T_1 , T_2 and T_3 during 3 to 4 MAT. During 4 to 5 MAT, T_6 was significantly superior. However, during 6 to 7 MAT, T_6 was found statistically on par with T_5 . During harvest T_9 exhibited the highest RGR and was on par with T_{10} and T_{12} .

4.3.7 Net Assimilation Rate

The data on NAR recorded at various growth stages are furnished in Table 22.

NAR was found to be significantly influenced by agrotechniques at crop growth stages during 2 to 3 MAT and 4 to 5 MAT alone. During 2 to 3 MAT, T_{11} registered the highest NAR and was statistically on par with T_{10} . During 4 to 5 MAT, T_{10} recorded the highest NAR which was on par with T_8 , T_9 and T_{11} .

4.4 BIOCHEMICAL PARAMETERS AT HARVEST

4.4.1 Total Alkaloid

Data corresponding to the effect of agrotechniques on total root alkaloid content at harvest in percentage are given in Table 23.

Total root alkaloid content in *D. gangeticum* was found significantly influenced by agrotechniques at harvest. At harvest, T_{12} registered the highest alkaloid yield which was statistically similar to T_1 , T_2 and T_3 .

Treatments	2 MAT	3 MAT	4 MAT	5 MAT	6 MAT	7 MAT	Harvest
T_1	0.10	0.98	0.95	1.03	1.49	1.22	2.10
T_2	0.10	0.90	0.98	1.02	1.59	1.41	2.34
T3	0.12	0.92	1.03	0.94	1.48	1.34	2.38
T ₄	0.12	0.77	1.07	0.88	1.39	1.22	1.88
T5	0.17	0.94	0.94	1.04	1.52	1.56	2.45
T ₆	0.14	1.06	1.08	1.14	1.48	1.54	2.34
T7	0.15	0.88	1.03	1.40	1.81	1.50	2.76
Τ8	0.28	1.68	1.31	1.64	2.06	2.30	4.38
T 9	0.27	1.35	1.16	1.38	1.75	2.49	4.36
T ₁₀	0.26	1.24	1.10	1.16	1.80	2.45	2.95
T11	0.34	1.11	1.11	1.01	1.75	2.46	3.40
T ₁₂	0.13	0.92	1.29	0.92	1.24	1.23	1.96
SEm (±)	0.02	0.11	0.13	0.13	0.11	0.14	0.20
CD (0.05)	0.071	0.322	NS	0.381	0.326	0.416	0.594

Table 19. Effect of agrotechniques on leaf area index

Table 20. Effect of agrotechniques on crop growth rate, g m⁻² day⁻¹

Treatments	2 to 3 MAT	3 to 4 MAT	4 to 5 MAT	5 to 6 MAT	6 to 7 MAT	7 MAT to Harvest
T 1	0.74	1.17	6.01	2.12	2.23	1.52
T ₂	0.67	1.29	6.28	1.65	2.56	1.88
T ₃	0.77	0.80	7.27	1.49	2.58	1.51
T4	0.77	0.96	6.59	2.18	2.17	1.06
T ₅	0.89	0.73	7.32	1.43	3.63	1.24
T ₆	0.76	1.04	7.57	1.88	4.39	0.17
T ₇	0.86	0.84	7.02	2.37	3.54	1.02
T8	1.46	1.75	12.72	1.98	1.52	2.64
T9	1.37	1.69	10.51	3.24	1.41	5.16
T10	1.66	1.64	11.12	1.96	2.38	3.26
T11	2.23	1.44	12.27	2.96	1.91	2.47
T ₁₂	1.21	1.10	5.86	0.61	1.52	1.84
SEm (±)	0.33	0.40	0.57	0.22	0.29	0.25
CD (0.05)	NS	NS	1.689	0.654	0.849	0.747

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able 21. Effe	ect of agrot	echniques o	n relative gr	owth rate, n	ng g ⁻¹ day ⁻¹	0	2.0.1	
Treatments	2 to 3 MAT	3 to 4 MAT	4 to 5 MAT	5 to 6 MAT	6 to 7 MAT	7 MAT to Harvest		
T_1	24.09	11.82	19.76	3.29	2.71	1.95		
T ₂	22.95	12.64	20.53	2.54	3.13	1.85		
T ₃	25.24	12.15	24.56	2.12	3.03	2.00		
T_4	27.69	10.09	22.01	3.34	2.48	1.42		
T ₅	25.27	8.61	26.46	2.19	4.74	1.29		
T ₆	26.70	13.07	29.86	2.85	5.47	0.31		
T ₇	26.02	6.45	27.41	3.33	4.30	1.02		
T ₈	22.03	9.04	21.94	2.00	1.33	2.19		
T9	21.54	10.37	20.13	3.34	1.44	2.96		
T ₁₀	26.95	8.80	20.35	1.83	2.21	2.77		
T11	25.07	5.55	22.54	2.55	1.66	1.83		
T ₁₂	24.55	8.31	21.34	46.00	2.16	2.65		
SEm (±)	0.53	0.52	0.45	12.97	0.28	0.18		
CD (0.05)	1.561	1.536	1.313	NS	0.828	0.524		

Table 22. Effect of agrotechniques on net assimilation rate, mg cm⁻² day⁻¹

Treatments	2 to 3 MAT	3 to 4 MAT	4 to 5 MAT	5 to 6 MAT	6 to 7 MAT	7 MAT to Harvest
T ₁	0.08	0.05	0.27	0.07	0.04	0.04
T ₂	0.08	0.06	0.28	0.06	0.04	0.04
T ₃	0.09	0.04	0.32	0.06	0.04	0.04
T ₄	0.10	0.05	0.29	0.09	0.04	0.05
T ₅	0.09	0.03	0.32	0.05	0.05	0.01
T ₆	0.08	0.04	0.31	0.07	0.05	0.00
T7	0.10	0.04	0.26	0.06	0.04	0.01
T8	0.16	0.10	0.77	0.09	0.05	0.07
T9	0.18	0.13	0.73	0.18	0.05	0.27
T ₁₀	0.23	0.12	0.87	0.12	0.07	0.13
T ₁₁	0.29	0.12	0.68	0.19	0.06	0.08
T12	0.13	0.05	0.29	0.02	0.04	0.07
SEm (±)	0.04	0.03	0.10	0.05	0.01	0.07
CD (0.05)	0.104	NS	0.295	NS	NS	NS

4.5 MICROBIOLOGICAL STUDIES

4.5.1 Per Cent Root Colonization by P. indica

When the root bits of *D. gangeticum* was examined under microscope for root colonization by the root endophyte at harvest, presence of characteristic fungal chlamydospores were not evident. So, the inoculation procedure was repeated further in the nursery conditions and confirmed the colonization of *Piriformospora indica* in un-lignified tender roots through microscopic investigation.

4.6 SEED PRODUCTION PARAMETERS

4.6.1 Time taken for first flowering

Data relating to the effect of agrotechniques on time taken for first flowering are given in Table 24.

The time taken for first flowering from the date of sowing was not significantly influenced by the treatments.

4.6.2 Time Taken for 50 Per cent Flowering

Data on the effect of agrotechniques on time taken for 50 per cent flowering are given in Table 25.

Influence of agrotechniques on the time taken for 50 per cent flowering from the date of sowing was not significant.

4.6.3 Number of Inflorescence per Plant

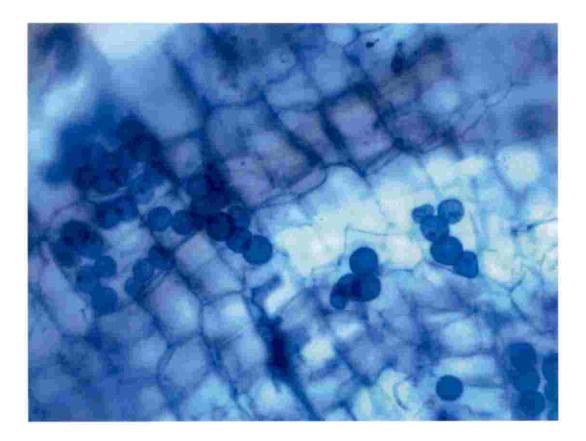
Observations on the number of inflorescence per plant as influenced by treatment effects are furnished in Table 26.

Number of inflorescence per plant was not significantly influenced by the agrotechniques.

4.6.4 Length of Inflorescence

Data on length of inflorescence as influenced by the treatment effects are furnished in Table 27.

Length of inflorescence was not significantly influenced by agrotechniques.



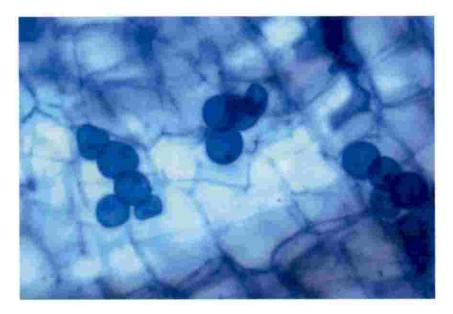


Plate 5. Characteristic chlamydospores of *Piriformospora indica* under microscope (40 X magnification)

Treatments	Harvest
T ₁ : Inoculation with Piriformospora indica alone	6.20
T ₂ : T ₁ + Soil application of cow dung slurry	6.33
$T_3: T_1 + Soil application of NPK$	6.60
$T_4: T_2 + Irrigation at 15 mm depth$	4.33
$T_5: T_2 + Irrigation at 30 mm depth$	4.33
$T_6: T_3 + $ Irrigation at 15 mm depth	4.67
T ₇ : T ₃ + Irrigation at 30 mm depth	4.67
T ₈ : T ₅ at high density planting	4.53
T ₉ : T ₇ at high density planting	4.33
T10: T8 under sub-surface mulching with polythene	4.47
T ₁₁ : T ₉ under sub-surface mulching with polythene	4.20
T ₁₂ : Control at normal row planting	6.60
SEm (±)	0.16
CD (0.05)	0.470

Table 23. Effect of agrotechniques on total alkaloid content at harvest, per cent

Table 24.	Effect of as	grotechniques	on time	taken	for	first	flowering
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Treatments	DAS
T ₁ : Inoculation with Piriformospora indica alone	120.67
T ₂ : T ₁ + Soil application of cow dung slurry	120.67
T ₃ : T ₁ + Soil application of NPK	117.67
T ₄ : T ₂ + Irrigation at 15 mm depth	115.33
$T_5: T_2 + Irrigation at 30 mm depth$	120.67
$T_6: T_3 + Irrigation at 15 mm depth$	118.33
T ₇ : T ₃ + Irrigation at 30 mm depth	122.00
T ₈ : T ₅ at high density planting	117.67
T ₉ : T ₇ at high density planting	121.67
T ₁₀ : T ₈ under sub-surface mulching with polythene	122.33
T ₁₁ : T ₉ under sub-surface mulching with polythene	117.33
T ₁₂ : Control at normal row planting	119.33
SEm (±)	5.84
CD (0.05)	NS

Treatments	DAS
T ₁ : Inoculation with <i>Piriformospora indica</i> alone	133.00
$T_2: T_1 + Soil application of cow dung slurry$	133.67
T ₃ : T ₁ + Soil application of NPK	132.67
T ₄ : T ₂ + Irrigation at 15 mm depth	132.33
$T_5: T_2 + Irrigation at 30 mm depth$	131.33
T ₆ : T ₃ + Irrigation at 15 mm depth	133.33
$T_7: T_3 + Irrigation at 30 mm depth$	135.00
T ₈ : T ₅ at high density planting	132.67
T9: T7 at high density planting	133.33
$T_{10}$ : $T_8$ under sub-surface mulching with polythene	129.00
T ₁₁ : T ₉ under sub-surface mulching with polythene	132.67
T ₁₂ : Control at normal row planting	134.33
SEm (±)	4.97
CD (0.05)	NS

Table 25. Effect of agrotechniques on time taken for 50 per cent flowering

Table 26. Effect of agrotechniques on number of inflorescence per plant

Treatments	5 MAT	6 MAT	7 MAT
$T_1$	5.67	24.67	17.33
T ₂	0.33	17.67	21.33
<b>T</b> ₃	5.67	20.33	24.33
T ₄	6.67	21.33	20.00
T5	4.33	14.33	16.00
<b>T</b> 6	2.00	14.00	15.67
T ₇	4.00	23.67	25.00
Τ8	1.67	15.00	26.33
Т9	4.67	15.33	28.67
T ₁₀	2.33	13.00	16.00
T ₁₁	5.00	18.67	22.67
T ₁₂	2.67	14.33	21.33
SEm (±)	2.906	3.818	5.38
CD (0.05)	NS	NS	NS

# 4.6.5 Number of Seeds per Inflorescence

Observation on number of seeds per inflorescence as influenced by treatment effects are furnished in Table 28.

Influence of agrotechniques on number of seeds per inflorescence was significant at 6 MAT. At 6 MAT, T₁ recorded the highest number of seeds per inflorescence and was statistically on par with T₂, T₃, T₆ and T₇.

#### 4.6.6 Thousand Seed Weight

Data relating to the effect of agrotechniques on thousand seed weight are presented in Table 29.

Influence of agrotechniques on thousand seed weight in *Desmodium* gangeticum was observed to be not significant under partial shade.

# **4.7 SOIL MOISTURE STUDIES**

Mean data relating to soil moisture content before and after irrigation, CU, daily consumptive use, Kc, CWUE, FWUE and WP are presented in Table 30 and 31.

Mean soil moisture content before and after irrigation, crop consumptive use, daily consumptive use, crop co-efficient, crop water use efficiency, field water use efficiency and water productivity were found to be significantly influenced by the treatment effects.

The highest mean soil moisture content prior to irrigation was registered by  $T_8$  which was statistically similar to  $T_7$  and  $T_9$ . While examining the average soil moisture content after irrigation,  $T_9$  showed the highest content which was on par with  $T_{10}$  and  $T_{11}$ .

The treatment  $T_4$  on par with  $T_6$  registered the highest CU, daily consumptive use and Kc. The treatments,  $T_3$ ,  $T_1$  and  $T_2$  recorded the lowest CU, daily consumptive use and Kc respectively.

The highest CWUE was registered by  $T_8$  and was statistically on par with  $T_9$ ,  $T_{10}$  and  $T_{11}$  which was 39.73 per cent higher compared to the control.

Treatments	5 MAT	6 MAT	7 MAT
$T_1$	29.50	27.70	23.33
T2	30.33	25.37	23.20
T3	27.27	26.47	30.33
T ₄	27.27	21.70	29.20
T5	29.50	27.80	23.03
T ₆	24.33	22.73	30.53
$T_7$	24.27	17.53	29.23
T ₈	30.03	35.83	29.83
T9	31.17	24.33	29.40
T ₁₀	26.07	25.33	31.67
T11	20.53	28.40	24.43
T ₁₂	26.90	27.57	29.67
SEm (±)	4.293	3.390	2.87
CD (0.05)	NS	NS	NS

Table 27. Effect of agrotechniques on length of inflorescence, cm

Table 28. Effect of agrotechniques on number of seeds per inflorescence

Treatments	6 MAT	7 MAT
T1	303.00	240.00
T ₂	248.67	167.33
T ₃	277.33	174.33
T ₄	147.33	196.33
T5	180.33	206.33
T ₆	257.00	250.00
T ₇	221.33	266.67
$T_8$	207.00	165.67
T۹	190.67	143.67
T ₁₀	161.33	146.67
T ₁₁	131.00	126.00
T ₁₂	195.33	201.00
SEm (±)	28.940	34.10
CD (0.05)	85.427	NS

The treatment  $T_2$  on par with  $T_1$ ,  $T_3$  and  $T_8$  exhibited the highest FWUE and WP. The treatment  $T_2$  exhibited the highest WP and it was on par with  $T_1$  and  $T_3$ .

# 4.8 CHEMICAL ANALYSIS

# 4.8.1 Plant Nutrient Uptake at Harvest

Data pertaining to the effect of agrotechniques on plant nutrient uptake at harvest in kg ha⁻¹ are presented in Table 32.

Uptake of N, P and K was found to be significantly influenced by the treatment effects at harvest. The highest N uptake was recorded by T₉ which was on par with  $T_{11}$ . P uptake was found significantly superior in T₉. T₉ also recorded the highest K uptake which was on par with T₃, T₆, T₇, T₈, T₁₀ and T₁₁. The lowest uptake of N, P and K was observed in the control plot.

# 4.8.2 Soil Analysis

Data on organic carbon content and N, P and K status of soil after the experiment are given in Table 33.

Organic carbon status and available K content of post-harvest soil were significantly affected by agrotechniques though available N and P were not significant.  $T_{10}$  on par with  $T_2$ ,  $T_4$ ,  $T_5$  and  $T_8$  registered the highest soil organic carbon content in the soil. The highest post-harvest soil available K content was found in  $T_3$  and was on par with all the treatments except  $T_4$ ,  $T_7$  and  $T_8$ .

# 4.9 ECONOMIC ANALYSIS

Data pertaining to the effect of agrotechniques on net income and B: C ratio are presented in Table 34.

Net income and B: C ratio were significantly influenced by treatment effects. The highest net income of  $\gtrless$  47,902 ha⁻¹ and B: C ratio of 1.74 were registered by the treatment T₈. It was on par with T₉ and T₁₀ with respect to net income. B: C ratio recorded by T₈ was significantly superior.

Treatments	5 MAT	6 MAT	7 MAT
T ₁	1.40	1.37	1.33
T2	1.43	1.40	1.37
$T_3$	1.33	1.33	1.47
T ₄	1.50	1.47	1.30
T5	1.40	1.27	1.47
T ₆	1.30	1.30	1.37
T ₇	1.27	1.27	1.37
$T_8$	1.43	1.43	1.30
T9	1.43	1.43	1.37
T ₁₀	1.37	1.23	1.47
T ₁₁	1.27	1.37	1.23
T ₁₂	1.47	1.47	1.47
SEm (±)	0.094	0.079	0.09
CD (0.05)	NS	NS	NS

Table 29. Effect of agrotechniques on thousand seed weight, g

Table 30. Effect of agrotechniques on mean soil moisture content before and after irrigation, per cent

	Soil moisture content (%)			
Treatments	Before irrigation	After irrigation		
Tı	10.78	15.40		
T ₂	11.00	14.89		
<b>T</b> ₃	10.31	15.62		
T4	11.02	22.73		
T ₅	9.82	23.73		
T ₆	9.94	24.00		
T7	11.58	23.47		
$T_8$	12.44	23.87		
T9	11.42	26.36		
T ₁₀	10.29	25.18		
T11	10.40	26.29		
T ₁₂	10.54	16.67		
SEm (±)	0.460	0.46		
CD (0.05)	1.359	1.352		

Treatments	CU (cm)	Daily CU (cm day ⁻¹ )	Kc	CWUE (g m ⁻³ )	FWUE (g m³)	WP (g m ⁻³ )
$T_1$	82.35	0.21	0.58	184.38	154.15	538.12
T ₂	82.35	0.21	0.58	188.56	158.31	567.42
T ₃	82.35	0.21	0.58	185.55	155.31	525.29
T ₄	100.36	0.26	0.70	151.51	53.81	240.45
T5	94.62	0.24	0.66	167.28	77.36	249.08
T ₆	100.36	0.26	0.70	152.47	54.34	234.29
T7	94.54	0.24	0.66	170.32	79.20	253.63
$T_8$	94.53	0.24	0.66	243.88	126.08	380.87
Τ9	94.71	0.24	0.66	235.00	120.69	361.46
T ₁₀	94.67	0.24	0.66	223.36	113.20	349.10
T ₁₁	94.74	0.24	0.66	228.86	116.81	361.46
T ₁₂	82.35	0.21	0.58	146.99	116.94	445.19
SEm (±)	0.06	0.09	0.12	15.61	11.03	27.62
CD (0.05)	0.168	0.290	0.260	46.078	32.551	81.519

Table 31. Effect of agrotechniques on consumptive use (cm), crop co-efficient crop water use efficiency (g m⁻³), field water use efficiency (g m⁻³) and water productivity (g m⁻³)

Table 32. Effect of agrotechniques on crop nutrient uptake, kg ha-1

Treatments	N uptake	P uptake	K uptake
$T_1$	68.29	10.80	12.17
$T_2$	79.59	14.33	12.64
$T_3$	81.36	18.46	18.45
$T_4$	71.18	13.09	12.89
T5	82.88	13.27	12.51
T ₆	103.34	19.52	19.19
T7	117.03	22.10	18.56
$T_8$	109.89	22.65	18.04
Τ9	167.83	32.30	19.95
T10	114.59	18.94	18.12
T11	154.74	19.96	18.63
T ₁₂	43.80	7.42	10.16
SEm (±)	8.89	3.04	1.24
CD (0.05)	26.246	8.970	3.673

Treatments	Organic carbon (%)	Available N (kg ha ⁻¹ )	Available P (kg ha ⁻¹ )	Available K (kg ha ⁻¹ )
T1	0.40	342.87	25.24	246.40
T ₂	0.50	351.23	28.61	238.93
T ₃	0.27	342.87	37.93	313.60
<b>T</b> 4	0.56	351.23	29.45	212.80
T5	0.55	334.51	22.06	261.33
T ₆	0.38	393.05	36.12	298.67
T7	0.36	384.68	19.43	153.07
T8	0.56	259.24	23.77	216.53
T9	0.27	275.97	24.73	246.40
T ₁₀	0.57	250.88	30.30	231.47
T ₁₁	0.46	284.33	30.35	302.40
T ₁₂	0.28	300.81	25.24	309.87
SEm (±)	0.03	34.01	3.72	29.71
CD (0.05)	0.082	NS	NS	87.684

Table 33. Effect of agrotechniques on soil organic carbon and available NPK status after the experiment

Table 34. Effect of agrotechniques on net income and benefit-cost ratio at harvest

Treatments	Net income (₹ ha ⁻¹ )	B: C ratio
T ₁	24,021	1.48
T ₂	20,698	1.38
T3	14,490	1.24
T4	14,121	1.24
T5	19,668	1.34
T ₆	9,589	1.15
T7	15,997	1.26
$T_8$	47,902	1.74
<b>T</b> 9	39,005	1.56
T ₁₀	28,584	1.38
T11	26,194	1.33
T ₁₂	14,012	1.31
SEm (±)	7,067	0.02
CD (0.05)	20,860	0.049

# DISCUSSION



#### DISCUSSION

The results of the experiment presented in the previous chapter are discussed in the following paragraphs.

### 5.1 GROWTH CHARACTERS

Desmodium gangeticum responded differentially to different agrotechniques. The crop response in relation to growth characters namely plant height and number of leaves, branches (Table 4) and leaf area per plant (Table 6) varied widely during different stages of crop growth and could not establish any definite trend in growth pattern. In general, robust plants in terms of the above growth characters were observed when transplanted plants of *D. gangeticum* inoculated seedlings were given basal dressing of NPK followed by scheduling irrigation at 30 mm depth. In addition, when the above agrotechniques were integrated with high density planting, *D. gangeticum* grew taller (Table 3).

In general, the impact of agrotechniques on growth characters is worth mentioning compared to control at normal row planting. Height measures both the photosynthetic capacity of the crop and their transpirational area which is closely related to field establishment and better growth. It is generally accepted as a better measure of growth and survival. The growth of a plant is influenced by the metabolic activities which required adequate amounts of nutrients and water. When basal dressing of NPK was carried out, the crop received sufficient quantities of nutrients for better growth and development. Crop response to depth of irrigation varied with stages of growth and it favourably influenced all the growth characters compared to control. This has resulted because D. gangeticum under irrigation at 30 mm depth never faced water stress unlike treatments under rainfed condition where life saving irrigation was given. Water deficit is likely to affect two vital processes namely cell division and cell enlargement resulting in poor growth under rainfed conditions (Begg and Turner, 1976). The favourable influence of higher moisture regime is likely due to stimulation of metabolic activities resulting in better growth.

Effect of soil moisture on the rate of leaf production was remarkable. Maintenance of readily available soil moisture by bringing the rhizosphere to field capacity and maintaining moisture in the readily available range for a longer period resulted in favourable rhizosphere situation for better development. Reduction in leaf number under  $T_{10}$  (integrated crop management involving HDP of *Piriformospora indica* inoculated *Desmodium* seedlings raised over subsurface mulched raised beds under partial shade followed by basal application of NPK and scheduling irrigation at 30 mm depth once in six days) might be due to water stress induced inhibition of cell division and cell expansion for effectively conserving water by reducing transpiration because of limited water supply in the soil over a period of time. One of the mechanism of water stress is to reduce the transpirational surface area which helps the plant to reduce the heat load on the leaf (Nath, 1993). Increase in leaf number as evident in Table 5 has resulted in maximization of LAI.

When stress induces senescence and early abscission of leaves, which when combined with reduced primodial initiation, result in reduced number of leaves per plant. Decrease in leaf number may be a mechanism of the species to reduce water loss in response to restricted water availability. The leaf area showed significant reduction due to water deficit during different growth stages. The reduction in LAI could be attributed to the reduction in the number of leaves per plant and leaf area (Shubhra *et al.*, 2004).

# **5.2 ROOT PARAMETERS**

The treatment which were beneficial for improving growth characters were also useful in enhancing root spread (Table 8) and volume (Table 9). However, number of roots was found to be higher when *P. indica* inoculated seedlings which were planted in high density rows on raised beds under subsurface mulching with black polythene and given soil application of cow dung slurry and irrigation at 30 mm depth.

Variations in root parameters namely root number (Table 7), spread, volume, length of tap root (Table 10), girth of primary root (Table 11) and length of lateral roots were conspicuous due to treatment effects. Management practices were effective for promoting root proliferation. Impact of agrotechniques on root

growth was not consistent. Maintenance of readily available water in the rooting zone increased root number, length and spread. On the other hand, moisture stress in the root zone promoted expansion of root surface and improvement in root volume.

Profound influence of treatments was observed on the length of tap roots and girth of primary root. Integrated crop management involving HDP of *P. indica* inoculated seedlings on raised beds under subsurface mulching and soil application of either cow dung slurry or NPK along with irrigation at 30 mm depth was found useful in enhancing length of tap root and girth of primary root.

The purpose of providing a nutrient-moisture rich rooting medium is to establish vigorous and healthy growth of the crop throughout its growth stages. The rooting medium physically supports the growing crop and stores and releases nutrients, water and air to the root system in an optimal manner if appropriate nutrient, moisture and oxygen regime is maintained. Better the medium, better will be the root development and crop establishment and productivity.

If the natural habitat of the root is disturbed by manipulation of the planting site, the root growth pattern can be permanently altered with positive or negative effects. Root weight, root volume, root length and root surface area are the common measurements. Various soil factors particularly moisture, penetrability and porosity play an important part in the development of the root system. The treatments which improved root characters like spread and volume of roots were also beneficial for improving the length of laterals (Table 12).

# 5.3 ROOT PRODUCTIVITY

The influence of treatments on fresh and dry weight per plant was different at different growth stages. In general, T₇ improved these parameters. At harvest, the fresh and dry weight of the plant ranged from 24.37 g to 32.55 g and 16.23 g to 24.04 g respectively which were 15.82 and 22.30 per cent higher compared to control (Table 13 and 14).

Better crop growth and development especially improvement in root production when soil application of cow dung slurry or soil application of NPK was resorted to. The root production was reduced in  $T_{12}$  (control treatment at normal row planting) due to the effect of nutrient and water stress. Water deficits generally have negative effects on dry matter production in plants as it influenced many of the physiological characters which determine growth. Root yield is the final manifestation of several intricate morphological and physiological traits which initiate at germination and terminate at harvest. Better yields are obtained by encouraging vegetative growth which is influenced by various management practices including efficient use of fertilizers. In the present study, soil application of cow dung slurry and soil application of NPK proved effective in maximizing LAI and root yield. Shukla and Shukla (2012) reported that crop is more efficient to use solar radiation and soil nutrients when it is grown at closer spacing. Pakkiyanathan *et al.* (2004) found that weight of dry root yield per unit area is significantly affected by plant geometry unlike yield per plant.

Even though, root weight per plant was more for the above treatment (T₇), the highest root yield per hectare (Fig. 3) was obtained when *P. indica* inoculated seedlings transplanted under HDP along with basal dressing of cow dung slurry and irrigation at 30 mm depth (T₈). The highest root yield of 1.73 t ha⁻¹ recorded in T₈ which was on par with T₉, T₁₀ and T₁₁ was 47.4 per cent higher compared to control.

# 5.4 PHYSIOLOGICAL PARAMETERS

Physiological parameters namely total chlorophyll (Table 16), RLWC (Table 17), root-shoot ratio (Table 18) and LAI (Table 19) were found to be influenced by treatment effects. Crop performance with respect to the above parameters varied with growth stages and no definite pattern could be observed.

Integrated crop management involving inoculation of *P. indica*, soil application of NPK and irrigation at 15 mm depth enhanced root-shoot ratio. Increasing the depth of irrigation to 30 mm depth increased the RLWC. LAI and chlorophyll content were improved when high density planting was resorted along with the adoption of the above techniques including soil application of NPK.

Q.

Chlorophyll content ranged from 2.47 to 4.70 mg g⁻¹ and T₉ (integrated crop management involving HDP of *P. indica* inoculated seedlings under partial shade followed by basal application of NPK and scheduling irrigation at 30 mm depth once in six days). RLWC ranged from 66.66 to 93.20 per cent and T₇ (integrated crop management involving normal row planting of *P. indica* inoculated seedlings under partial shade followed by basal application of NPK and scheduling irrigation at 30 mm depth once in six days) registered the highest value. T₆ (integrated crop management involving normal row planting of *P. indica* inoculated seedlings under partial shade followed by basal application of NPK and scheduling irrigation at 30 mm depth once in six days) registered the highest value. T₆ (integrated crop management involving normal row planting of *P. indica* inoculated seedlings under partial shade followed by basal application of NPK and scheduling irrigation at 15 mm depth once in four days) recorded the highest root-shoot ratio which ranged from 0.30 and 0.56. LAI ranged from 0.34 to 4.38 and T₈ (integrated crop management involving high density planting of *P. indica* inoculated seedlings under partial shade followed by basal application of NPK and scheduling irrigation at 30 mm depth once in six days) recorded the highest value.

Relative leaf water content is an important physiological indicator for plant water stress conditions; a species with higher RLWC indicates that it is highly drought resistant. Studies have shown that maximum RLWC is useful to differentiate between drought resistant and drought susceptible cultivars. Modification of above and below ground microclimatic parameters by altering the planting geometry and imposing restrictions on root growth by subsurface mulching with black polythene might have contributed to wide variation in physiological parameters studied. The above results are in line with the findings of Leopold *et al.* (1981). Root production is influenced by metabolic activities which require adequate amount of nutrients and moisture. Growth characters, root traits and physiological parameters indicate that *D. gangeticum* respond very well to cultural inputs and agroclimatic conditions.

Leaf area index is an important parameter determining crop productivity and efforts should be directed towards improving LAI. In general, maintenance of readily available water in the rooting zone by scheduling irrigation at 30 mm depth favoured higher LAI compared to moisture stress situation consequent to lifesaving irrigation. Variations in LAI is due to changes in leaf number or leaf size. Leaf number depends up on plant height (Table 3) and the rate of leaf production (Table 5) (Gupta, 1975).

Root-shoot ratio is an indication of ability of the plant to survive under moisture stress situations by strengthening the root system without proportionate development of shoot. It is evident that nutrient and moisture present in the root zone decide the ratio.

The effect of treatments was evident on CGR (Fig. 4) after 3 to 4 months of transplanting. RGR varied significantly at all growth stages except 5 to 6 MAT. Net assimilation rate was significant only during 2 to 3 and 4 to 5 MAT. A critical analysis of Table 20, 21 and 22 on CGR, RGR and NAR respectively revealed the significant effect of management practices in influencing growth expressions during certain stages and no specific trend could be inferred.

An important determinant of plant yield is the light environment in which the plants grow. Both light quantity (incident radiation) and light quality (solar spectrum) affect plant growth and development and ultimately determine plant yield (Chory, 2010). Biomass accumulation is dependent on radiation use efficiency and light interception and the latter is defined by the architecture of canopy as well as planting density (Byrt, 2011). Planting at increased densities proved to be an effective mechanism to increase the crop yields (Duvick, 1997).

# 5.5 CHLOROPHYLL CONTENT

Chlorophyll is said to be an index of productivity; hence, any alteration in chlorophyll concentration may change the morphological, physiological and biochemical characters of the plant. The photosynthetic capacity of the plant increased with chlorophyll concentration.

The root and shoot production relies on inter regulation of multiple physiological processes. To regulate these processes efficiently, crops need an adequate supply of resources. Sufficient supply of nutrients stimulate metabolic activities and development so as to adapt efficiently to the nutritional status. The

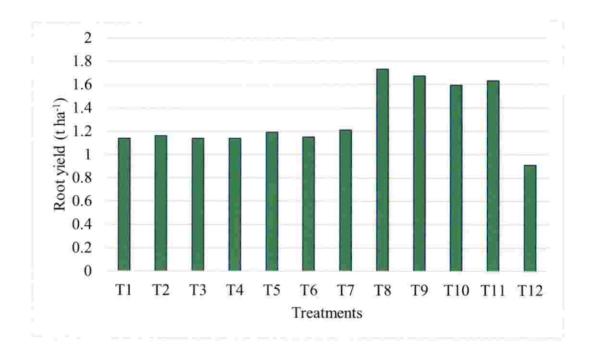


Fig. 3 Effect of agrotechniques on root yield, t ha-1

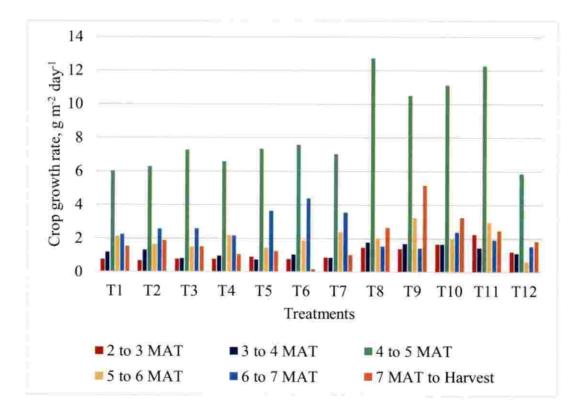


Fig. 4 Effect of agrotechniques on crop growth rate, g m⁻² day⁻¹

results revealed that applied nutrients result in positive responses for root and shoot yield. Nitrogen is a constituent of many important biomolecules namely nucleic acid, protein, hormones and chlorophyll. Since large amount of chlorophyll per unit area is needed to capture solar energy efficiently, N is intimately related to photosynthesis (Lawlor, 2002). Lower rates of photosynthesis under conditions of N limitations are often attributed to lower chlorophyll content (Toth *et al.*, 2002). Also, a positive correlation has been reported between N and chlorophyll content by several workers (Evans and Terashima, 1988). Similarly P is an integral component of plant cells including the sugar phosphate intermediaries in respiration and photosynthesis and the phospholipid that constitute the plant membranes. It is also an important component of ATP, DNA and RNA (Taiz and Zeiger, 2006). A significant increase in chlorophyll content due to N and P application has been observed by several workers in many plants suggesting that biosynthesis of the pigment molecule was depended on the uptake of N and P within certain limits (Prsa *et al.*, 2007).

The crop yield per unit area is a function of plant density and per plant yield. With increase in number of leaves per unit area, the crowding coefficient of plant community also increases which leads to the decrease in freeness to the individual plant and increase in competition for growth factors from effective root zone. In the present investigation, high root yield per unit area recorded in closer spacing was primarily due to an increase in plant population despite compromise in per plant yield.

# 5.6 SEED PRODUCTION POTENTIAL

*D. gangeticum* is propagated through seeds. Flowering and pod formation began after five month after transplanting. Precocity in flowering was observed in  $T_4$  (integrated crop management involving normal row planting of *P. indica* inoculated seedlings under partial shade followed by monthly application of cow dung slurry and scheduling irrigation at 15 mm depth once in four days). The treatment  $T_1$  (integrated crop management involving normal row planting of *P. indica* inoculation alone) registered the highest pod number. The seed yield (Table

28) ranged from 131 to 303. Seed production potential of *D. gangeticum* was influenced by treatment effects and the treatment  $T_1$  (integrated crop management involving normal row planting of *P. indica* inoculation alone) was found to be significant in increasing seed production. Favourable rhizosphere conditions for growth and development might have contributed for precocity in flowering. Higher number of pods per plant and seeds per pod have contributed for prolific seed production. Similar results have reported in several crop plants (Chathropadhya and Pathra, 1992), (Reddy and Khan, 1998), (Mahendy *et al.*, 2002). The crop produces both axillary and terminal inflorescences and the emergence coincides with hot summer which necessitates supplemental irrigations to mitigate moisture stress. Seedlings of the plant raised under nursery inoculation of *P. indica* alone and maintained as a rainfed crop recorded maximum seed production.

# 5.7 ROOT QUALITY

Total alkaloid content ranged from 4.2 to 6.6 per cent (Table 23). Control treatment (T12) at normal row planting recorded the highest alkaloid content of 6.6 per cent and was on par with T1 (inoculation of P. indica alone) with 6.2 per cent, T₂ (integrated crop management involving normal row planting of P. indica inoculated seedlings under partial shade followed by monthly application of cow dung slurry) with 6.33 per cent and T₃ (integrated crop management involving normal row planting of P. indica inoculated seedlings under partial shade followed by basal application of NPK) with 6.6 per cent. The crop responded favourably to P. indica inoculation, soil application of cow dung slurry and soil application of NPK. All the treatments which received irrigation either at 15 mm or 30 mm depth recorded significantly lower values though P. indica inoculation and soil application of cow dung slurry or soil application of NPK or HDP were practiced. This clearly indicates that the soil edaphic condition under rainfed situation is highly congenial for the accumulation of total alkaloids in D. gangeticum. A critical analysis of the root characters furnished in Table 10 and 12 on length of tap root and length of lateral roots revealed that length of lateral roots was less in control

treatment under rainfed condition compared to irrigated. It is inferred that, the total alkaloids might be accumulating in the tap roots rather than laterals.

#### **5.8 SOIL MOISTURE STUDIES**

# 5.8.1 Soil Moisture Content

Significant variation was observed with respect to soil moisture before and after irrigation (Table 30). Before irrigation soil moisture content ranged from 9.82 to 12.44 per cent. The highest soil moisture content before irrigation was 12.44 per cent which was 15.28 per cent higher compared to control. The higher moisture content recorded in  $T_8$  (*P. indica* inoculated seedlings were transplanted under high density planting along with basal dressing of cow dung slurry and irrigation at 30 mm depth) might be due to improvement in physical properties of the soil particularly higher water holding capacity of the soil consequent to repeated monthly application of cow dung slurry. After irrigation also, the soil moisture ranged from 14.89 to 26.36 per cent. After irrigation, the highest soil moisture content of 26.36 per cent was recorded in T₉ and it was 36.76 per cent higher compared to control.

# 5.8.2 Crop Consumptive Use

Consumptive use (Fig. 5) ranged from 82.35 to 100.36 cm. The highest CU and Daily CU of 100.36 cm and 0.26 cm per day were recorded by the same treatment  $T_6$  which was 17.95 and 19.23 per cent higher than control.

#### 5.8.3 Crop Co-efficient

The treatments  $T_4$  and  $T_6$  recorded the highest Kc because of high CU of this treatment compared to other treatments. Crop co-efficient ranged from 146.99 to 243.88 g m⁻³. The above two treatments increased the Kc value as more soil moisture was available for meeting the evapotranspiration requirement of the crop consequent to reduced rate of soil evaporation coupled with high retention of moisture.

#### 5.8.4 Water Use Efficiency

The results revealed the superior performance of the treatment T₈ on par with T₉, T₁₀ and T₁₁ with respect to CWUE (Fig. 6). In T₈, CWUE was 39.73 per cent higher compared to T₁₂ (the control treatment). T₈ recorded maximum efficiency due to the increase in root yield and decrease in seasonal CU when compared to other treatments. The higher FWUE (Fig. 6), 158.31 g m⁻³ was registered in T₂ which was 26.13 per cent higher compared to control. It ranged from 53.81 to 158.31 g m⁻³. The trend was almost similar to WP (Fig. 7) also. The higher WP (567.42 g m⁻³) was registered in T₂ which was 21.54 per cent higher compared to control.

# 5.9 UPTAKE OF NUTRIENTS

Significant variations were observed with respect to N, P and K uptake by *D. gangeticum*. The highest NPK uptake of 167.83, 32.30 and 19.95 kg ha⁻¹ were recorded by T₉. Quantitative expression of nutrient uptake (Table 32) is the product of nutrient content of plant tissue and total dry matter. The highest nutrient status and uptake in plants were observed in T₉ which helped in better availability and absorption of nutrients by the plants. The greater uptake of nutrients can also be related to higher dry matter production due to greater accumulation of metabolites. These observations are in confirmation with the findings of Mahendran and Kumar (1998) in potato and Suja *et al.* (2005) and Kalyanasundaram *et al.* (2008) in sweet potato.

The positive effects of basal application of nutrients especially soil application of NPK might be due to the adequate supply of these nutrients ensuring their continuous absorption by roots, followed by smooth translocation to the shoot. This would result finally in the satisfactory distribution throughout the foliage. An increase in the level of N, P and K content as a result of application of fertilizers has also be noted by Singh and Ram, 1992.

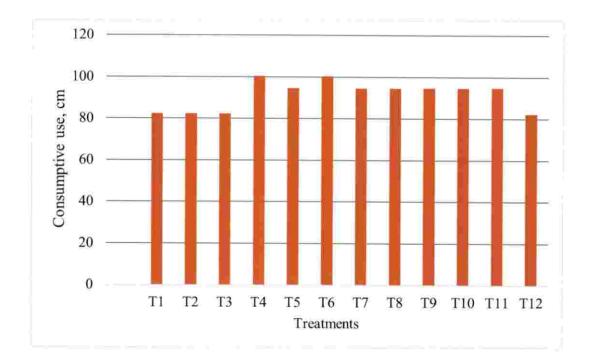


Fig. 5 Effect of agrotechniques on consumptive use, cm

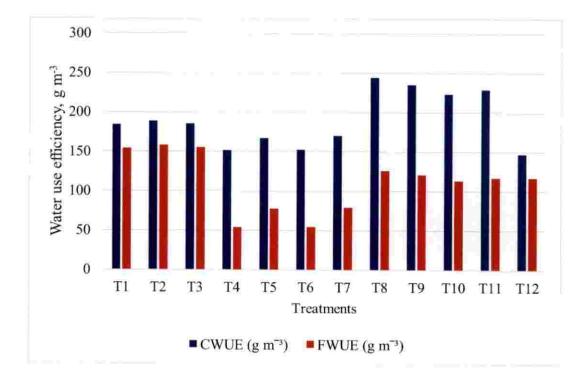


Fig. 6 Effect of agrotechniques on crop and field water use efficiency, g m⁻³

# 5.10 EFFECT OF PIRIFORMOSPORA INDICA

Higher root yield (Table 15) in *D. gangeticum* was recorded in all the treatments in which *Piriformospora indica* was inoculated compared to the control. This is in conformity with the findings of several researchers.

Being immobile organisms, plants have to cope with unfavourable conditions such as nutrient deficiencies, salinity, drought and pathogen attack. To avoid such adverse situations plants tend to establish their associations with beneficial organisms (Lum and Hirsh, 2003). In particular, symbiosis with beneficial fungi are vital for nutrient acquisition by the root systems of most plants (Sirrenberg et al., 2007). Thus, application of beneficial organisms as bio-fertilizers plays a key role in today's agricultural scenario through enhancement of soil fertility and crop production. Endophytic fungi P. indica which is phylogenetically close to mycorrhizal endosymbiosis has also been recognized as a growth parameter of numerous plant species (Varma et al., 1999). P. indica is a mycorrhiza like endophytic fungus which exhibits its versatility for colonizing the plant species with direct manipulation of plant hormone signalling and induces both local and systemic resistance to several fungal and viral plant diseases through signal transduction. P. indica is multifunctional in providing it services such as nutrient uptake, disease resistance, stress tolerance and growth promotion (Unnikumar et al., 2013). P. indica infestation in a number of medicinal plants has been reported to stimulate the synthesis of valuable secondary metabolites (Prasad et al., 2013)

# 5. 11 ECONOMIC ANALYSIS

Economic Analysis proved the significance of  $T_8$  (*P. indica* inoculated seedlings were transplanted under HDP along with basal dressing of cow dung slurry and irrigation at 30 mm depth) in achieving the highest net income (₹ 47,902 ha⁻¹) and B: C ratio (1.74). The treatment T₈ recorded the highest root yield which is the economic part of the plant. The treatment T₈ recorded 70.75 per cent higher net income (Fig. 8) compared to the control.

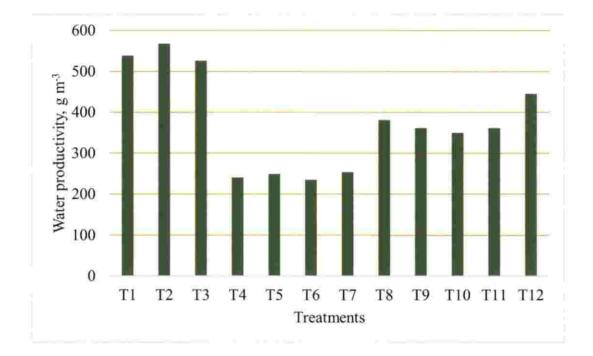


Fig. 7 Effect of agrotechniques on water productivity, g m-3

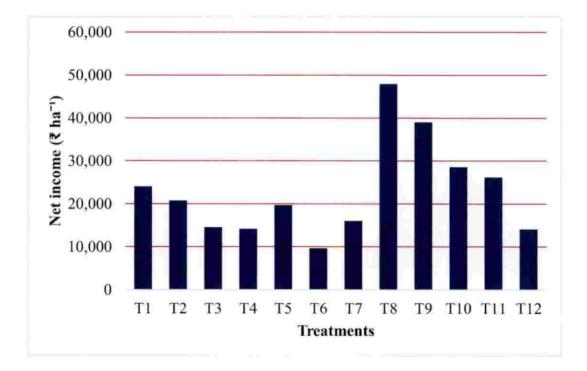


Fig. 8 Effect of agrotechniques on net income, ₹ ha⁻¹



Plate 6. Pod bug infestation during seed development stage

# SUMMARY

#### 6. SUMMARY

A field experiment was conducted during May 2018 to May 2019 in the Instructional farm, College of Agriculture, Vellayani, Thiruvananthapuram, Kerala, with the objective to study the integrated effect of root endophyte fungus, planting density, source efficacy of nutrients, moisture stress and subsurface mulching on the growth, yield and quality constituents of *Desmodium gangeticum* (L.) DC. under partial shade.

The experiment was laid out in RBD with 12 treatments and three replications. The treatments were,  $T_1$  – Inoculation with *Piriformospora indica* (root endophyte) alone,  $T_2 - T_1$  + Soil application of cow dung slurry (5% at monthly intervals),  $T_3 - T_1$  + Soil application of NPK (basal- @ 40:40:40 kg ha⁻¹ year⁻¹),  $T_4 - T_2$  + Irrigation at 15 mm depth,  $T_5 - T_2$  + Irrigation at 30 mm depth,  $T_6 - T_3$  + Irrigation at 15 mm depth,  $T_7 - T_3$  + Irrigation at 30 mm depth,  $T_8 - T_5$  at high density planting (40 cm x 20 cm),  $T_9 - T_7$  at high density planting,  $T_{10} - T_8$  under subsurface mulching with black polythene and  $T_{12}$  – Control at normal row planting (40 cm x 40 cm). The salient findings of the study are presented here.

Agrotechniques significantly influenced plant height at all stages of plant growth except at 2 MAT. The treatment  $T_7$  recorded the tallest plants at 3 and 4 MAT and it was on par with the treatments  $T_5$  and  $T_9$  at 3 MAT and  $T_5$ ,  $T_8$  and  $T_9$ at 4 MAT. Appreciable difference in plant height was observed due to different agrotechniques as growth progressed.  $T_9$  registered the tallest plants at 5 MAT and was on par with  $T_5$  and  $T_6$ . The same treatment recorded the tallest plants 6 MAT and was on par with  $T_8$ . At 7 MAT and at harvest, plant height recorded by  $T_9$  was significantly higher and were 113.97 cm and 150.33 cm.

Number of branches were significantly affected by the agrotechniques at all growth stages of the crop except at 2 MAT. Number of branches in  $T_7$  was higher at all growth stages except at 2 MAT. At 3 MAT, the treatments  $T_2$ ,  $T_5$  and  $T_{12}$  were statistically on par with  $T_7$ . At 4 MAT,  $T_2$ ,  $T_3$ ,  $T_4$ , and  $T_{12}$  were statistically

comparable with T₇. Treatments T₂, T₃, and T₉ were on par at 6 MAT while at 7 MAT, T₅ and T₈ were statistically comparable with T₇. At harvest, only T₈ was on par with T₇.

From  $3^{rd}$  month on wards leaf production was significantly influenced by treatment effects. At 3 MAT, T₃, T₄, T₅ and T₇ were statistically comparable with T₆ for the highest leaf production. T₆ recorded the highest number of leaves at 5 MAT and was found to be on par with T₅, T₇ and T₈. During 6 and 7 MAT as well as at harvest stage, T₇ registered highest leaf number while T₉, T₈ and T₁₀ showed lesser number respectively. Leaf area was significantly influenced by agrotechniques at all growth stages except at 2 and 7 MAT. At 3 MAT, T₆ recorded the highest leaf area and was on par with T₁, T₂, T₃, T₅, T₇, and T₁₂. At 5 and 6 MAT, T₇ showed the highest leaf area which was on par with T₆ and T₂ respectively. At harvest, T₅ expressed higher leaf area and was statistically on par with T₁, T₂, T₃, T₆, T₇, T₈, and T₉.

Number of roots were significantly influenced by the agrotechniques at all growth stages except at 2 and 4 MAT. T₅ registered the highest root number at 3 MAT and was on par with all the treatments with the exception of T₂, T₃, T₁₀ and T₁₂. At 5, 6, 7 MAT and at harvest T₁₀ recorded the highest root number. At 5 and 6 MAT, root number was significantly higher in T₁₀ which was also statistically comparable with T₈ at 7 MAT and both T₈ and T₁₁ at harvest. Control treatment showed the least root proliferation at 5, 6 and 7 MAT.

The root spread was significantly affected by treatment effects at all the growth stages except at 4 and 5 MAT. At 2 MAT, T₉ registered the highest root spread and was on par with T₅, T₆ and T₁₀ while control showed the poorest spread. At 3, 6, and 7 MAT as well as at harvest, T₇ exhibited greater root spread. It was also statistically comparable with T₅ at 3 MAT, T₄ and T₅ at 6 MAT, T₄, T₅ and T₆ at 7 MAT and T₁, T₄, T₅ and T₆ at harvest.

Treatment effects significantly influenced root volume at all the growth stages except at 2 and 4 MAT. At 3 MAT,  $T_5$  recorded the highest root volume and was on par with  $T_6$ . At 5 and 6 MAT,  $T_7$  recorded the highest root volume which was statistically comparable with all the treatments with the exception of  $T_8$ ,  $T_9$ ,  $T_{10}$ 

and  $T_{11}$ . At 7 MAT,  $T_6$  showed the highest root volume which was on par with all the treatments except  $T_8$ ,  $T_9$ ,  $T_{10}$ ,  $T_{11}$  and  $T_{12}$ . At harvest,  $T_7$  exhibited the greatest root volume and was on par with all the treatments except  $T_8$ ,  $T_9$ ,  $T_{10}$ ,  $T_{11}$  and  $T_{12}$ .

Agrotechniques significantly influenced the length of tap root at all the growth stages except at 2 and 5 MAT. At 3 MAT, the treatments  $T_4$ ,  $T_6$ ,  $T_7$  and  $T_8$  were statistically comparable with T₉ for longer tap root whereas  $T_{12}$  showed the least length. At 4 and 6 MAT and at harvest also T₉ exhibited longer tap root. Only T₄ was on par with T₉ at 4 MAT whereas T₄, T₇, T₈ and T₁₁ at 6 MAT and T₆, T₇, T₈ and T₁₀ were statistically comparable with T₉ at harvest. At 7 MAT, T₈ excelled in primary root length which was on par with T₆, T₇, T₉ and T₁₀ while, the shortest root was noted in T₁.

The primary root girth was significantly influenced by treatment effects from 5 MAT onwards. At 3 MAT, T₅ registered the highest tap root girth which was on par with T₆, T₇ and T₈. During 5, 6 and 7 MAT, T₁, T₂, T₃, T₄, T₅ and T₆ were statistically comparable with T₇ for greater primary root girth. At harvest, T₆ registered the highest primary root girth which was statistically on par with T₁, T₂, T₃, T₄, T₅, T₇ and T₁₁.

From 5 MAT onwards, lateral root length was found to be significantly influenced by treatment effects. T₇ registered the longest lateral root at 5, 6 and 7 MAT and at harvest. T₇ was observed to be on par with T₁, T₂, T₄, T₅ and T₆ at 5 MAT and T₁, T₂, T₃, T₄, T₅, T₆ and T₁₂ at 6 MAT and T₂, T₃, T₄, T₅ and T₆ at 7 MAT. But at harvest, the treatments T₁, T₂, T₃, T₄, T₅ and T₆.

Root fresh weight per plant was significantly influenced by agrotechniques at all growth stages except at 2 and 4 MAT. Significantly higher root fresh weight was recorded by T₉ at 3 MAT. At 5 and 7 MAT, T₆ registered the highest root fresh weight which was statistically comparable with T₁, T₂, T₃, T₄, T₅ and T₇. At 6 MAT and harvesting, T₇ registered the highest root fresh weight and was on par with T₁, T₂, T₃, T₄, T₅ and T₆. Treatment T₁₀ recorded the lowest root fresh weight at 5, 6 and 7 MAT and at harvest.

Root dry weight was significantly affected by agrotechniques at all growth stages from 5 MAT to harvest. Treatments T₁, T₂, T₃, T₄, T₅, T₇ and T₈ were

statistically on par with  $T_6$  for greater root dry weight at 5 MAT. At 6 and 7 MAT and at harvest,  $T_7$  showed the highest root dry weight per plant and was on par with  $T_1$ ,  $T_2$ ,  $T_3$ ,  $T_4$ ,  $T_5$  and  $T_6$ .

At harvest, root yield in *D. gangeticum* was found to be significantly influenced by agrotechniques. The treatment,  $T_8$  registered the highest root yield which was statistically on par with  $T_9$ ,  $T_{10}$  and  $T_{11}$ . The control treatment showed the lowest yield.

Total chlorophyll content was found to be significantly influenced by treatment effects at all growth stages except at 3 MAT. At 2 MAT, T₅ showed the highest chlorophyll content and was on par with all other treatments except T₁ and T₁₂. T₃, T₄. At 4 MAT, T₉ was found superior and on par with T₆. T₆ registered maximum chlorophyll content at 5 MAT which was on par with all other treatments except of T₂, T₁₀ and T₁₂. T₁₁ showed the highest chlorophyll content at 6 MAT and T₅, T₆, T₇, T₈, T₉ and T₁₀ were on par. At 7 MAT and at harvest, T₉ recorded the highest chlorophyll content.

Relative leaf water content was significantly influenced by treatment effects at all growth stages except at 2 MAT. T₂ showed the highest RLWC at 3 MAT and was on par with T₁, T₃, T₄, T₅, T₆, T₇ and T₁₁. At 4 MAT, T₂ showed the highest RLWC which was on par with T₁, T₄, T₆ and T₇. During 5 and 6 MAT and at harvest T₇ registered the highest RLWC. At 7 MAT, T₆ showed highest RLWC which was on par with T₁ and T₇.

Root-shoot ratio was significantly influenced by the treatment effects at 5 and 7 MAT and at harvest. At 5 MAT,  $T_7$  recorded the highest root-shoot ratio which was statistically on par with  $T_1$ ,  $T_2$  and  $T_4$ . At 7 MAT,  $T_1$ ,  $T_4$ ,  $T_6$  and  $T_7$  were statistically comparable with  $T_5$ . At harvest,  $T_6$  registered the highest root-shoot ratio which was on par with  $T_3$ ,  $T_4$ ,  $T_5$  and  $T_7$ .

Treatment effects significantly influenced LAI at all growth stages except at 4 MAT. At 2 MAT, T₁₁ exhibited highest LAI and was on par with T₈ and T₉. At 3, 5, 6 MAT and at harvest, T₈ recorded the highest LAI whereas T₉ was the superior at 7 MAT. At 5 MAT, T₇ and T₉ were statistically similar to T₈. T₇, T₉, T₁₀ and T₁₁ were on par with T₈ at 6 MAT. T₈, T₁₀ and T₁₁ were on par with T₇ which showed the highest LAI at 7 MAT. At harvest, T₈ was on par with T₉.

Agrotechniques significantly influenced CGR at all growth stages except during 2 to 3 MAT and 3 to 4 MAT. During 4 to 5 MAT, T₈ recorded the highest CGR which was on par with  $T_{10}$  and  $T_{11}$ . CGR was significantly higher in T₉ and was statistically on par with  $T_{11}$  during 5 to 6 MAT. During 6 to 7 MAT, T₆ was on par with T₅ and recorded significantly high CGR. During 7 MAT to harvest T₉ was found significantly superior to other treatments.

Relative growth rate was significantly influenced by treatment effects at all growth stages except during 5 to 6 MAT. During 2 to 3 MAT, the treatment  $T_4$  showed the highest RGR which was on par with  $T_6$  and  $T_{10}$ . During 3 to 4, 4 to 5 and 6 to 7 MAT,  $T_6$  registered the greatest RGR.  $T_6$  was on par with  $T_1$ ,  $T_2$  and  $T_3$  during 3 to 4 MAT. During 4 to 5 MAT,  $T_6$  was significantly superior. However, during 6 to 7 MAT,  $T_6$  was found statistically on par with  $T_5$ . During harvest,  $T_9$  exhibited the highest RGR and was on par with  $T_{10}$  and  $T_{12}$ .

Net assimilation rate was found to be significantly influenced by agrotechniques at crop growth stages during 2 to 3 MAT and 4 to 5 MAT alone. During 2 to 3 MAT,  $T_{11}$  registered the highest NAR and was statistically on par with  $T_{10}$ . During 4 to 5 MAT,  $T_{10}$  recorded the highest NAR which was on par with  $T_8$ ,  $T_9$  and  $T_{11}$ .

Total root alkaloid content in *D. gangeticum* was found significantly influenced by agrotechniques at harvest. At harvest,  $T_{12}$  registered the highest alkaloid yield which was statistically on par with to  $T_1$ ,  $T_2$  and  $T_3$ .

When the root bits of *D. gangeticum* was examined under microscope for root colonization by the root endophyte at harvest, presence of characteristic fungal chlamydospores were not evident. So, the inoculation procedure was repeated further in the nursery conditions and confirmed the colonization of *Piriformospora indica* in unlignified tender roots through microscopic investigations.

The treatment effects on the time taken for first and fifty per cent flowering, number of inflorescence per plant, length of inflorescence and thousand seed weight in *D. gangeticum* were found to be not significant. Influence of agrotechniques on

number of seeds per inflorescence was significant at 6 MAT. At 6 MAT,  $T_1$  recorded the highest number of seeds per inflorescence and was statistically on par with  $T_2$ ,  $T_3$ ,  $T_6$  and  $T_7$ .

Mean soil moisture content before and after irrigation, crop CU, daily CU, Kc, CWUE, FCWUE and WP were significantly influenced by the treatment effects. The highest mean soil moisture content prior to irrigation was registered by T₈ which was statistically similar to T₇ and T₉. While examining the average soil moisture content after irrigation, T₉ showed the highest content which was on par with T₁₀ and T₁₁. The treatment T₄ on par with T₆ registered the highest CU, daily CU and Kc. The highest CWUE was registered by T₈ and was statistically on par with T₉, T₁₀ and T₁₁ which was 60.27 per cent higher compared to the control. The treatment T₂ on par with T₁, T₃ and T₈ exhibited the highest FCWUE and WP. The treatment T₂ exhibited the highest WP and it was on par with T₁ and T₃.

Uptake of N, P and K was significantly influenced by the treatment effects at harvest. Among the various agrotechniques, the highest N, P and K uptake was recorded by T₉ which was on par with  $T_{11}$ . P uptake was found significantly superior in T₉. However, T₉ recorded the highest K uptake which was on par with T₃, T₆, T₇, T₈, T₁₀ and T₁₁.

Organic carbon status and available K content of post-harvest soil were significantly affected by agrotechniques though available N and P not significantly influenced.  $T_{10}$  on par with  $T_2$ ,  $T_4$ ,  $T_5$  and  $T_8$  registered the highest organic carbon content in the soil. The highest post-harvest soil available K content was found in  $T_3$  and was on par with all the treatments except  $T_4$ ,  $T_7$  and  $T_8$ .

Net income and B: C ratio were significantly influenced by treatment effects. The highest net returns of  $\gtrless$  47,902 ha⁻¹ was registered by the treatment T₈. It was on par with T₉ and T₁₀. B: C ratio recorded by T₈ was significantly superior. The lowest net returns of  $\gtrless$  9,589 ha⁻¹ and B: C ratio of 1.15 were shown by the treatment, T₆ which was on par with T₁₂ (control).

#### FUTURE LINE OF WORK

- Micro-meteorological parameters and agrotechniques influencing accumulation of biochemical enrichments in *D. gangeticum* may be investigated.
- Habit-habitat analysis of *D. gangeticum* may be carried out to identify appropriate agro-ecological zone for commercial mediculture.
- Cultural practices may be standardized for introduction of *D. gangeticum* in the predominant cropping system of Kerala.
- Optimum stage of root harvest of the crop with respect to alkaloid accumulation may be identified.
- Use of eco-friendly biodegradable plastic utilizing locally available materials may be developed for in-situ rain water harvest, conservation and utilization.

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# Appendix

## Appendix I

Year	Month	Temperature (°C)		RH (%)		
						Rainfall
		Maximum	Minimum	Maximum	Minimum	(mm)
2018	May	33.56	25.73	87.13	75.57	158.80
	June	31.56	24.89	91.53	81.22	431.10
	July	30.74	23.70	90.23	78.30	148.90
	August	30.38	23.66	90.88	80.96	368.30
	September	32.40	24.20	87.70	74.10	67.00
	October	31.48	24.40	92.40	79.00	284.80
	November	31.58	23.98	92.75	74.25	234.00
	December	32.10	23.49	93.05	72.18	45.50
2019	January	32.90	18.80	95.00	63.00	2.40
	February	35.80	20.00	95.00	59.00	68.70
	March	35.10	23.00	87.00	59.00	0.00
	April	35.60	25.00	88.00	60.00	11.20
	May	34.70	25.30	95.00	65.00	113.00

## Weather data during the crop season (May 2018 to May 2019)

# Abstract

## AGROTECHNIQUES FOR ENHANCING ROOT PRODUCTION IN *Desmodium gangeticum* (L.) DC. UNDER PARTIAL SHADE

by

#### ABHIJITH S. S (2017-11-016)

### ABSTRACT

Submitted in partial fulfilment of the requirements for the degree of

### MASTER OF SCIENCE IN AGRICULTURE

Faculty of Agriculture Kerala Agricultural University



DEPARTMENT OF AGRONOMY COLLEGE OF AGRICULTURE VELLAYANI, THIRUVANANTHAPURAM- 695 522 KERALA, INDIA 2019

#### ABSTRACT

The study entitled "Agrotechniques for enhancing root production in *Desmodium gangeticum* (L.) DC. under partial shade" was undertaken during 2017-2019, in the Instructional farm, College of Agriculture, Vellayani, Thiruvananthapuram, Kerala, with an objective to study the integrated effect of root endophyte fungus, planting density, source efficacy of nutrients, moisture stress and subsurface mulching on the growth, yield and quality constituents of *Desmodium gangeticum* (L.) DC. under partial shade.

The field experiment was laid out in randomized block design with 12 treatments and three replications. The treatments were,  $T_1$  – Inoculation with *Piriformospora indica* (root endophyte) alone,  $T_2 - T_1 +$ Soil application of cow dung slurry (5% at monthly interval),  $T_3 - T_1 +$ Soil application of NPK (basal- @ 40:40:40 kg ha⁻¹ year⁻¹),  $T_4 - T_2 +$ Irrigation at 15 mm depth,  $T_5 - T_2 +$ Irrigation at 30 mm depth,  $T_6 - T_3 +$ Irrigation at 15 mm depth,  $T_7 - T_3 +$ Irrigation at 30 mm depth,  $T_8 - T_5$  at high density planting (40 cm x 20 cm),  $T_9 - T_7$  at high density planting,  $T_{10} - T_8$  under subsurface mulching with black polythene,  $T_{11} - T_9$  under subsurface mulching with black polythene and  $T_{12}$  – control at normal row planting (40 cm x 40 cm). *Piriformospora indica* was inoculated with the potting medium @ 10g fungal culture kg⁻¹ of potting medium.

Results of the experiment revealed that integrated management practices have significant effects on growth and yield attributes of *D. gangeticum*.

The treatment T₇ recorded the tallest plants at 3 and 4 months after transplanting (MAT) whereas, T₉ was superior at 5, 6 and 7 MAT and at harvest. The treatment T₇ registered the highest number of branches at all stages of growth. At 3 and 5 MAT, T₆ recorded the highest leaf number whereas T₇ was superior at 4, 6 and 7 MAT and at harvest. T₅ registered the highest root number at 3 MAT but T₁₀ was found superior at 5, 6 and 7 MAT and at harvest. At 2 MAT, T₉ and at all other growth stages, T₇ recorded the highest root spread. T₅ at 3 MAT, T₇ at 5 and 6 MAT and at harvest and T₆ at 7 MAT registered the highest root volume. With

respect to length of tap root, the treatments  $T_9$  at 3, 4, and 6 MAT and at harvest and  $T_8$  at 7 MAT were found superior.

At 3 MAT, T₅ revealed the highest girth of primary root but at 5, 6 and 7 MAT, T₇ and at harvest T₆ were found superior. The treatment, T₇ registered the longest laterals at 5, 6 and 7 MAT and at harvest. The root fresh and dry weight showed a similar trend. The treatments T₉ at 3 MAT, T₆ at 5 and 7 MAT and T₇ at 6 MAT and at harvest showed the highest fresh and dry root weight. The highest root yield at harvest was recorded by T₈ which was on par with T₉, T₁₀ and T₁₁.

Chlorophyll content varied with different growth stages.  $T_5$  at 2 MAT,  $T_6$  at 5 MAT,  $T_{11}$  at 6 MAT and  $T_9$  at 4 and 7 MAT and at harvest registered the highest total chlorophyll content. Like chlorophyll content, RLWC also showed variations with respect to different growth stages.  $T_2$  at 3 and 4 MAT,  $T_6$  at 7 MAT and  $T_7$  at 5 and 6 MAT and at harvest recorded the highest values. At 5 and 7 MAT and at harvest  $T_7$ ,  $T_5$  and  $T_6$  respectively recorded the highest root-shoot ratio. The treatments  $T_{11}$  and  $T_9$  at 2 and 7 MAT and  $T_8$  at 3, 5 and 6 MAT and at harvest registered the highest leaf area index. Observations on crop growth rate showed the significance of  $T_8$  at 4 to 5 MAT,  $T_6$  at 6 to 7 MAT and  $T_9$  at 2 to 3 MAT and  $T_6$  at 3 to 4, 4 to 5 and 6 to 7 MAT followed by  $T_9$  at 7 MAT to harvest were found superior. At 2 to 3 and 4 to 5 MAT, the treatments  $T_{11}$  and  $T_{10}$  respectively recorded the highest values for net assimilation rate.

Ethanol extract of plant roots grown under control at normal row planting (40 cm x 40 cm) ( $T_{12}$ ) recorded the highest total alkaloids at harvest. Among seed parameters, only number of seeds per inflorescence was significantly influenced by the treatments at 6 MAT and it was the highest in  $T_1$ .

Soil moisture studies revealed the significance of  $T_8$  and  $T_9$  in enhancing soil moisture retention before and after irrigation.  $T_4$  on par with  $T_6$  registered the highest consumptive use, daily consumptive use and Kc. Crop water use efficiency was the highest for the treatment  $T_8$ .  $T_2$  registered the highest field water use efficiency and water productivity.  $T_9$  recorded the highest up take of primary plant nutrients. After the experiment, organic carbon and available K status of soil were found superior in  $T_3$  and  $T_{10}$ . Even though nursery seedlings exhibited *P. indica* root colonization at harvest, it was not clearly evident through microscopic investigation.

Economic analysis of the system revealed the significance of  $T_8$  (₹ 47,902 ha⁻¹) which was on par with T₉ and T₁₀ with respect to net income. The highest benefit-cost ratio was also registered by T₈ which was significantly superior to all other treatments.

It is concluded that high density planting of *P. indica* inoculated seedlings under partial shade followed by monthly application of cow dung slurry (5 %) and scheduling irrigation at 30 mm depth once in six days (T₈) was found beneficial for enhancing leaf area index, root production, crop water use efficiency, net income (₹ 47,902 ha⁻¹) and benefit-cost ratio (1.74).

## സംഗ്രഹം

#### സംഗ്രഹം

"ചെറുചോലയിൽ ഓരിലയുടെ വേരുത്പാദനം ഉയർത്തുവാനുതകുന്ന കാർഷിക സങ്കേതിക വിദ്യകൾ" എന്ന ഗവേഷണ പദ്ധതി 2017-19 കാലയളവിൽ വെള്ളായണി കോളേജിനോടനുബന്ധിച്ചുള്ള കാർഷിക ഫാമിൽ നടത്തുകയുണ്ടായി. വേരുകളിൽ ഇൻസ്ട്രക്ഷണൽ പടരുന്ന അന്തർവ്യാപന ശേഷിയുള്ള മിത്രകുമിളുകൾ, വിളയുടെ നടീൽ സാന്ദ്രത, സ്രോതസുകളുടെ സസ്യമുലക ഉപയോഗക്ഷമത, ഈർപ്പരാഹിത്യം, വേരുപടലത്തിനു കീഴിലുള്ള പുതയിടൽ എന്നീ കാർഷിക മുറകളുടെ സംയോജനം ദശമൂലത്തിലെ ഒരു പ്രധാന ചേരുവയായ ഓരിലയുടെ കായികവളർച്ചയേയും വിളവിനേയും വേരിന്റെ ഗുണഗണങ്ങളേയും ഏതെല്ലാം രീതിയിൽ സ്വാധീനിക്കുന്നു എന്നതായിരുന്നു പഠനത്തിന്റെ പ്രധാന ലക്ഷ്യം.

റാൻഡമൈസ്ല് ബ്ലോക്ക് ഡിസൈനിൽ പന്ത്രണ്ടു കാർഷിക മുറകളുടെ സംയോജന രീതികൾ മൂന്നു റെപ്ലിക്കേഷനിൽ വിന്യസിക്കുകയുണ്ടായി. സംയോജന മുറകൾ താഴെ പറയുന്നവയാണ്. ടി-1= പിരിഫോർമോസ്പോറ ഇൻഡിക്ക മാത്രം, ടി-2= ടി-1 + ചാണകക്കുഴമ്പ് മാസംതോറും അഞ്ചു ശതമാനം (5%), ടി-3= ടി-1 + എൻ:പി:കെ വർഷത്തിൽ 40:40:40 കിലോഗ്രാം /ഹെക്ടർ എന്ന നിരക്കിൽ അടിവളമായി, ടി-4= ടി-2 + 15 മില്ലിമീറ്റർ താഴ്ച്ചയിൽ വേനൽക്കാല നന, ടി-5= ടി-2 + 30 മില്ലിമീറ്റർ താഴ്ച്ചയിൽ വേനൽക്കാല നന, ടി-6=ടി-3 + 15 മില്ലിമീറ്റർ താഴ്ച്ചയിൽ വേനൽക്കാല നന, ടി-7= ടി-3 + 30 മില്ലിമീറ്റർ താഴ്ച്ചയിൽ വേനൽക്കാല നന, ടി-7= ടി-3 + 30 മില്ലിമീറ്റർ താഴ്ച്ചയിൽ വേനൽക്കാല നന, ടി-8= ടി-5 + അതിസാന്ദ്രതാ നടീൽ സമ്പ്രദായം, ടി-9= ടി-7 + അതിസാന്ദ്രതാ നടീൽ സമ്പ്രദായം, ടി-10= ടി-8 + കറുത്ത പോളിത്തീൻ ഉപയോഗിച്ച് വേരുപടലത്തിനു കീഴിലുള്ള പുതയിടൽ, ടി-11= ടി-9 + കറുത്ത പോളിത്തീൻ ഉപയോഗിച്ച് വേരുപടലത്തിനു കീഴിലുള്ള പുതയിടൽ, ടി-12= കൻട്രോൾ.

പത്തു ഗ്രാം പിരിഫോർമോസ്പോറ ഇൻഡിക്കയുടെ കൾച്ചർ ഒരു കിലോഗ്രാം പോട്ടിങ് മിശ്രിതത്തിൽ കലർത്തിയാണ് ഓരിലയുടെ തൈകൾ പ്രോട്രേയിൽ വളർത്തിയെടുത്തത്. അനുവർത്തിച്ച സംയോജിത കാർഷിക മുറകളെല്ലാം തന്നെ ഓരിലയുടെ കായിക വളർച്ചയേയും ഉൽപ്പാദന ഘടകങ്ങളേയും ഉൽപ്പാദനത്തേയും കാര്യമായി സ്വാധീനിക്കുന്നതു കണ്ടു. പൊതുവേ പറയുകയാണെങ്കിൽ ടി-7 എന്ന ട്രീറ്റ്മെന്റിനു വിളയുടെ കായിക വളർച്ച വർധിപ്പിക്കുവാൻ കഴിഞ്ഞു. വേരിന്റെ എണ്ണം, വ്യാപനം, വ്യാപ്പം, നാരായ വേരിന്റെ തൂക്കം, വേരുൽപ്പാദനം തുടങ്ങിയ മാനദണ്ഡങ്ങളെ സ്വാധീനിക്കുന്ന സാങ്കേതിക മുറകളിൽ എടുത്തു പറയത്തക്കത് ടി-7, ടി-8 ട്രീറ്റ്മെന്റുകളായിരുന്നു.

ഫിസിയോളജി സംബന്ധമായ ഘടകങ്ങളായ ഹരിതകം, ഇലയുടെ ആപേക്ഷിക ജലസംഗ്രഹശേഷി, വേര്-തണ്ട് അനുപാദം, ഇലപ്പരപ്പു സൂചിക കൂടാതെ വളർച്ചാസൂചകങ്ങളായ ക്രോപ് ഗ്രോത്ത് റേറ്റ്, റിലേറ്റിവ് ഗ്രോത്ത് അസിമിലേഷൻ റേറ്റ് തുടങ്ങിയവയെ പ്രയോഗിക്കപ്പെട്ട റേറ്റ്, നെറ്റ് സംയോജിത കാർഷികമുറകൾ കാര്യമായി സ്വാധീനിക്കുകയുണ്ടായി. ഒരു പ്രത്യേക കാർഷികമുറയ്ക്ക് എന്നിരുന്നാലും ഏതെങ്കിലും ഈ സ്വാധീനിക്കുന്ന ഘടകങ്ങളെയെല്ലാം ഒരേ പോലെ പ്രവണത പ്രകടിപ്പിക്കുവാൻ കഴിഞ്ഞില്ല.

ഒരു ഹെക്ടർ വിളവുൽപ്പാദനത്തിൽ മുൻ പന്തിയിൽ നിന്നത് ടി-ഃ എന്ന സംയോജിതമുറ ആയിരുന്നു. വേനൽക്കാല നന മണ്ണിലെ ജലാംശത്തെയും അതു വഴി തദ്വാര വേരിന്റെ ആൽക്കലോയിഡ് ഉൽപ്പാദനത്തേയും ഹാനികരമാംവിധം സ്വാധീനിക്കുന്നതു കാണുകയുണ്ടായി. ജലസേചനം നടത്തിയും നടത്താതെയുമുള്ള കൃഷിരീതികളെ താരതമ്യം ചെയ്യപ്പോൾ മഴയെ മാത്രം ആശ്രയിച്ചു മൂപ്പെത്തിയ വിളയുടെ വേരിലാണ് ഔഷധഗുണമുള്ള ആൽക്കലോയിഡ് കൂടുതൽ അടങ്ങിയിരിക്കുന്നത് എന്ന് തെളിയുകയുണ്ടായി.

മണ്ണിന്റെ ജലസംഗ്രഹ ശേഷി വർധിപ്പിക്കുന്നതിനായി ടി-8 ഉം ടി-9 ഉം വളരെ ഫലപ്രദമാണെന്നു തെളിയുകയുണ്ടായി. കൂടുതൽ കൺസംട്വിവ് യൂസും നിത്യേന കൺസംട്വിവ് യൂസും ക്രോപ് കോ-എഫിഷ്യന്റും കണ്ടത് ടി-4 കാർഷിക മുറയിലായിരുന്നു. വിളയുടെ ജല ഉപയോഗ കാര്യക്ഷമത കൂടുതൽ കണ്ടത് ടി-8 കാർഷിക മുറയിലായിരുന്നു. എന്നാൽ ഫീൽഡിലെ ജല ഉപയോഗ ക്ഷമതയും ജല ഉൽപ്പാദന ക്ഷമതയും കൂടുതൽ കണ്ടത് ടി-2 കാർഷിക മുറയിലായിരുന്നു. ടി-9 ട്രീറ്റ്മെന്റ് ഏറ്റവും കൂടുതൽ സസ്യമൂലകങ്ങൾ വലിച്ചെടുക്കുകയുണ്ടായി. ടി-8 ട്രീറ്റ്മെന്റിൽ നിന്ന് ഏറ്റവും കൂടുതൽ അറ്റാദായം (₹ 47,902 /ഹെക്ടർ) ലഭിക്കുകയുണ്ടായി. വരവു-ചിലവനുപാതവും ഈ ട്രീറ്റ്മെന്റിൽ അധികരിച്ചിരുന്നു (1.74).

പിരിഫോർമോസ്പോറ ഇൻഡിക്ക എന്ന അന്തർവ്യാപന ശേഷിയുള്ള മിത്രകുമിളുകൾ സന്നിവേശിപ്പിച്ച ഓരിലതൈകൾ ചെറുചോലയിൽ അതിസാന്ദ്രതാസമ്പ്രദായത്തിൽ വച്ചുപിടിപ്പിച്ച് മാസം തോറും അഞ്ചു വീര്യത്തിൽ ഒഴിച്ച് ശതമാനം ചാണക കുഴമ്പ് കലക്കി ആറു ദിവസത്തിലൊരിക്കൽ 30 മില്ലിമീറ്റർ ആഴത്തിൽ വേനൽക്കാല നന അനുവർത്തിക്കുമ്പോൾ ദശമൂലത്തിലെ പ്രധാന ചേരുവയായ ഓരിലയുടെ ഇലപ്പരപ്പു സൂചികയും വേരിന്റെ വിളവും ജലത്തിന്റെ കാര്യക്ഷമമായ ഉപയോഗവും അറ്റാദായവും വരവു-ചിലവനുപാതവും അഭിവൃദ്ധിപ്പെടുന്നതായി തെളിഞ്ഞു.