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# EFFECT OF DROUGHT STRESS ON CONTENT AND COMPOSITION OF TOTAL ROOT ALKALOIDS IN SHALPARNI (*Desmodium gangeticum* L. DC.)

**Abhijith S.S.[1], Gautham Suresh S.P.[2]* and Anilkumar, A.S.[1]**

[1]Department of Agronomy, College of Agriculture, Vellayani, Kerala Agricultural University, Thiruvananthapuram-695522, Kerala, India

[2]Department of Vegetable Science, University of Horticultural Sciences, Bagalkot, Karnataka – 587104, India

*Corresponding author E-mail: gauthamhort@gmail.com

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**ABSTRACT**

The leguminous medicinal species *Desmodium gangeticum* (L.) DC., commonly known as Shalparni (Orila) was subjected to an experiment to evaluate the effect of drought stress on the growth, yield and composition of total root alkaloids. The field experiment was conducted at the Instructional Farm, College of Agriculture, Vellayani, Kerala. The bioactive principles accumulated by the medicinal plants are essentially responsible for their healing effects. However, the quality and quantity of these chemical metabolites are influenced by a multitude of factors, chief among them being abiotic factors. The trial adopted a randomized block design, with 12 treatment groups and 3 replicates. Variables incorporated into the trial included NPK fertilization, cow dung slurry application, 15/30 mm irrigation, high density panting and root endophyte fungal colonization (*Piriformospora indica*). Total alkaloids were extracted from pulverized root samples using 95% ethanol *via.*, the Soxhlet extraction method, and the extraction yields were quantified. The experiment demonstrated a significant trade-off between biomass production and alkaloid concentration in the tested medicinal plants. The treatment high-density planting ($T_8$) group with 30 mm irrigation had the highest root dry weight, reaching up to 1.73 t ha$^{-1}$, while the $T_3$ extreme water stress group had the lowest root dry weight of only 0.91 t ha$^{-1}$. The total alkaloid content was observed to change in the opposite direction. The $T_{12}$ blank control group recorded the highest value at 6.60%, while irrigation groups had values ranging from 4.20% to 4.67%. It was also found that colonization by Inoculation with *Piriformospora indica* (root endophyte) can accelerate alkaloid synthesis.

***Keywords* :** Desmodium, drought stress, root alkaloid, yield, medicinal plant.

## Introduction

*Desmodium gangeticum* is a well-known medicinal plant belonging to the Dashamoola group. It is a perennial under shrub, belonging to the Fabaceae family. The plant is commonly known as Orila, tick clover, and Shalparni in Malayalam, English, and Sanskrit, respectively. It is a major constituent of several Ayurvedic preparations, such as Dashamularishta, Chyavanaprasam, and Agasthyarasayanam. It is also routinely prescribed for the treatment of fever, asthma, respiratory ailments, and colic pain (Linga-rao and Savithramma, 2012). 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.

The substantial exploitation of *Desmodium gangeticum* by numerous pharmaceutical industries, along with increasing interest in herbal medicines, has resulted in a greater demand for this peculiar species. The whole plant extracts of *Desmodium gangeticum* revealed the presence of more than 19 bioactive principles such as flavonoid, glycosides, lipids, glycolipids, and alkaloids (Mishra *et al*., 2005). These different bio-active plant components were responsible for imparting anti-inflammatory, anti-nociceptive, analgesic, anti-amnesic, anti-diabetic, anti-oxidant, anti-ulcer, CNS depressant, antibacterial and antipyretic activities (Vaghela *et al*., 2013) wound healing (Jain *et al*., 2006) and their function as a carminative, rejuvenative, aphrodisiac, used in fevers, oedema, kidney disorders and postdelivery complications (Nanda and Tiwari, 2016).

Yasmeen *et al*. (2011) reported that ethanol extracts of whole plants showed the presence of iso-flavonoids besides flavonoids and alkaloids and which might be responsible for the antioxidant and anti-diabetic properties. Govindarajan *et al*. (2007) reported that flavonoid fractions were found highly potent antioxidants compared to the alkaloid fraction of *D. gangeticum*. Antioxidant attributes in aqueous extract of *Desmodium gangeticum* were found responsible for the cardio-protective and hypo-cholesterolemic activities (Kurian *et al*., 2005). Roots of the crop was found to be dominated by three major pterocarpinoids specifically gangetin, gangetinin, and desmodin (Purushothaman *et al*., 1971). Currently, the large-scale exploitation of the medicinal plant *D. gangeticum* by the pharmaceutical industry has created resource pressures. Alkaloids in its roots are the core source of its pharmacological value. This study intends to understand the concentration of therapeutic secondary metabolites by regulating water stress, so as to secure the stable supply of high-quality raw materials.

## Materials and Methods

The experiment was conducted at 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. 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 (pH-5.6), low in organic carbon content (0.68), medium N (301.10 kg ha$^{-1}$) and P (23.77 kg ha$^{-1}$) with high K (328.53 kg ha$^{-1}$) status.

The field experiment was laid out in randomized block design with 12 treatments and 3 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 sub-surface mulching with black polythene, $T_{11}$ – $T_9$ under sub-surface mulching with black polythene and $T_{12}$ – absolute control at normal row planting (40 cm x 40 cm). *Piriformospora indica* was inoculated with the potting medium @ 10g fungal culture per kg of potting medium.

The seeds for the experiment were procured from Aromatic and medicinal Plant Research Station (AMPRS), Odakkali, Ernakulum and College of Horticulture, Vellanikkara, Thrissur. The total alkaloid content during harvest was determined. For the extraction of total alkaloid from root system of the crop soxhlet extraction process was adopted. The soxhlet assembly was a continuous extractor generally suitable for the extraction of alkaloids from powdered plant materials with the help of organic solvents. 95 % ethanol was used as the solvent. The powdered drug was loosely packed in a thimble of Soxhlet apparatus and on boiling ethanol penetrate deeply in to the powdered drug 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. The data was statistically analyzed as per the procedure outlined by Panse and Sukhatme (1985) for completely randomized block design.

## Results

### Root Yield at Harvest

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. Data on root yield at harvest expressed in t ha$^{-1}$ are presented in Table 1.

**Table 1 :** Effect of drought stress on root yield in desmodium at harvest, t ha$^{-1}$

| Treatments | Root dry weight (t ha$^{-1}$) |
|---|---|
| $T_1$ : Inoculation with *Piriformospora indica* 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 | 1.19 |
| $T_6$ : $T_3$ + 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 |
| $T_{12}$ : Control at normal row planting | 0.91 |
| SEm (±) | 0.11 |
| CD (0.05) | 0.320 |

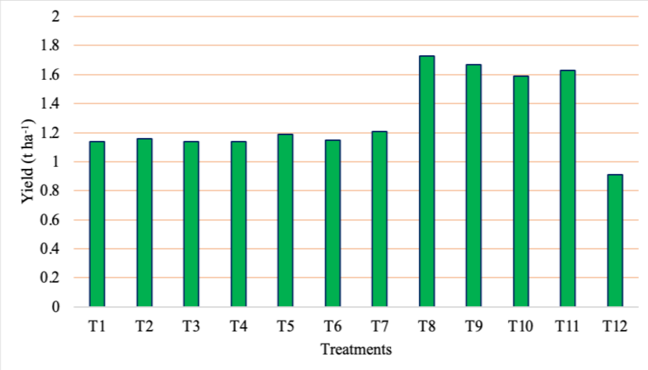


**Fig. 1 :** Effect of drought stress on dry matter production of *Desmodium* at harvest, t ha$^{-1}$

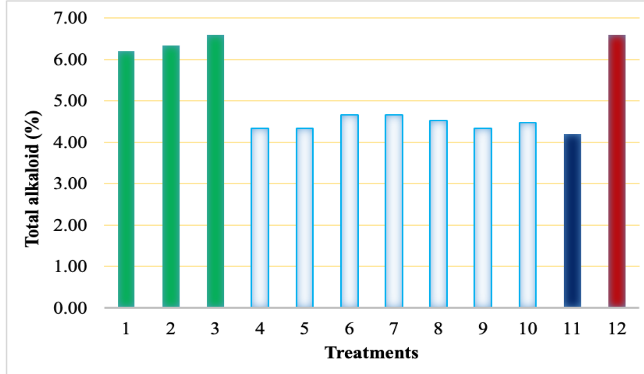


**Fig. 2 :** Variation in the total root alkaloid content with drought stress

## Alkaloid Yield at Harvest

Total root alkaloid content in *Desmodium gangeticum* was found significantly influenced by the moisture stress during the growth stages. Effect of moisture stress on total root alkaloid yield during harvest in per cent is shown in Table 2. During harvest, absolute control treatment ($T_{12}$) under extreme moisture stress registered the highest alkaloid yield which was statistically comparable to *Piriformospora indica*, a root endophyte fungi inoculated treatment ($T_1$), *P. indica* inoculation followed by monthly application of 5% cow dung slurry ($T_2$) and *P. indica* inoculation coupled with soil application of NPK @ 40:40:40 kg ha$^{-1}$ year$^{-1}$ ($T_3$) whereas, of *P. indica* inoculated seedlings under partial shade grown over sub-surface black polythene mulched beds followed by soil application of NPK and scheduling irrigation at 30 mm depth once in six days ($T_{11}$) yield the minimum alkaloid contents.

**Table 2 :** Effect of moisture stress on total alkaloid yield at harvest, %

| 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 |
| $T_{10}$ : $T_8$ 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 |

## Discussion

The study demonstrates that moisture status is a key determinant of total root alkaloids in the medicinal plant Salapani (*Desmodium gangeticum*), and it supports the established consensus in the field that the secondary metabolism of medicinal plants exhibits plasticity under abiotic stress. It is well known that moisture deficit is an important factor that limits the plant growth and establishment. Water deficient suppress the cell division as well as cell elongation and thus results in lower dry mater production. The reduction in plant dry weight was associated with a decline in the cell enlargement and more leaf senescence (Bhatt and Rao, 2005). Stress inhibits the efficacy of translocation and assimilation of photosynthates (Xiong and Zhu, 2002) and that might have resulted in the decline in root dry yield.

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. Prakash *et al.* (2000) pointed out that *Desmodium gangeticum* harvested during hot summer and scorching winter exhibited higher alkaloid content with greatest therapeutic potential. One year old plants yield more root with high quality alkaloid and withheld irrigation shall be given three weeks prior to uprooting (NMPB, 2008). Also, inoculation of *Centella asiatica* with *P. indica* improved the production of secondary metabolite asiaticoside (Satheesan *et al.,* 2011).

The total alkaloid accumulation in shoot of *Catharanthus roseus* was found increased significantly under drought stress (Amirjani, 2013). The content of alkaloids in *C. roseus* has been found influenced by factors, such as stage of plant growth, drought and other stress (Misra and Gupta, 2006). But, in shalparni the useful part is the root system and it is an important ingredient of more than 50 Ayurvedic formulations like Chyavanaprasam, Dhanuantharam kuzhambu, Dasamularishtam etc (Kumar *et al*., 2014). The root alkaloid content in *Desmodium gangeticum* is principally responsible for the curing effects and wide pharmacological acceptance.

The results showed that alkaloid content got concentrated with drought that could be associated with decline in total dry matter production. Increase in alkaloids might be a defense response of Shalparni. On the basis of present study, it could be concluded that the biochemical and growth parameters were significantly reduced under the induced drought stress on *Desmodium gangeticum*. Under drought stress the plant, fresh and dry weight has been reduced. But, increased the total alkaloid yield at harvest. Similar results were obtained by Babitha *et al*. (2024). The percent alkaloid content of *Desmodium gangeticum* increases with increasing moisture stress, even the root yield decreases. Also, the alkaloid yield was found influenced by the crop duration as one year crop showed higher yield compared to 7 month duration. Besides, crop uprooted at hot summer revealed superior to that harvested during monsoon. Colonization with *Piriformospora indica*, a root endophytic fungus also enhances accelerated alkaloid synthesis.

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**Conflicts of Interest:** There are no conflicts among authors

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