



Allelopathic Effects of *Spermacoce hispida* L. on *Arachis hypogaea* L. under *In Vitro* and Pot Conditions

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Abstract

The study was conducted during January to April, 2023 in the Department of Botany at Government Arts College (Autonomous), Salem-7, Tamil Nadu, India, aimed to investigate the interaction between the crop (groundnut) and the weed nattaichuri (*Spermacoce hispida* L.) in two different culture conditions: *in vitro* and pot culture. Groundnut (*Arachis hypogaea* L.), an important pulse crop with high protein content, used for edible purposes and to extract cooking oil. Nattaichuri (*Spermacoce hispida* L.), a common weed found in groundnut fields in Salem District. 10 g of leaf and stem powder separately extracted with 100 ml distilled water and considered as 100% extract. The extracts (both leaf and stem) were diluted and then tested at various concentrations (25%, 50%, 75% and 100%) for their effects on germination and growth of groundnut using distilled water as control. In the control, 100% seed germination was observed on 2nd and 5th day of seed sowing, but in the stem and leaf extract treatments, seed germination decreased with higher extract concentrations. In pot culture, 80% seed germination was observed with 100% leaf extract, whereas in control and other treatments, with stem and leaf extracts, 100% seed germination was recorded. Seedlings treated with stem and leaf extracts showed an increase in the growth of shoot and root, as well as the fresh weight and dry weight, than the control in the pots. The study reported two different interactions between the crop and the weed under different conditions. In *in vitro* culture, the allelopathic interaction was inhibitory, while in pot culture, the interaction was stimulatory.

Keywords: Biomass, dry weight, fresh weight, seed germination, seedling growth

1. Introduction

Allelopathy is a natural phenomenon where the survival of one organism in the environment is determined by inhibiting other organisms through the production of secondary metabolites. Such secondary metabolites are termed as allelopathic chemicals or allelochemicals in reference to the interaction of two organisms. Broadly, allelopathy is defined as the interaction of two organisms (positive or negative) exerted by secondary metabolites. The weeds release various allelochemicals, which inhibit or stimulate the germination and growth of other plants (Mandal and Bharti, 2026). Allelopathy is also exerted by microflora or fauna through the release of allelochemicals that interfere with the growth of plants (Rithiga et al., 2024). The harmful effects of allelopathic chemicals include the inhibition of seed germination or delays in seed germination, a decline in root, radicle, and stem development, and a decrease in total dry matter (Abou El-Enin and Abdel-Ghffa, 2017). In recent years, more focus has been given to studying the interaction of weeds with crop

plants to evaluate the extent of inhibition by assessing seed germination and seedling growth (Dai et al., 2022), which are crucial for the establishment of a plant in its environment (Vivek and Duraisamy, 2017). Weeds exhibit fast growth and reproduction, simultaneously inhibiting the overall growth and development of the crop plant through the production of allelochemicals (Shahena et al., 2021). Phytotoxicity of allelochemicals in the soil environment is determined by the physical, chemical, and biological conversions of them in the soil (Shahena et al., 2021). Beneficial soil microorganisms are reported to promote plant growth, increase nutrient availability, reduce the impact of growth inhibitors, and also secrete growth-promoting substances (Abraheem et al., 2024). India was the second-largest exporter of groundnut (*Arachis hypogaea* L.) during 2017–18 with 5.22 lakh t in the world (Meena et al., 2020). The seeds of groundnut contain 10% carbohydrate, 3% minerals, and 17 mg niacin (100 g)⁻¹ nut (Vaghasiya and Nair, 2021). The genus *Spermacoce* of Rubiaceae family of 275 species, and nine species are



reported in India, including *Spermacoce hispida* L. (Patel et al., 2022). Nattaichuri (*Spermacoce hispida* L.) is an herb, commonly called “shaggy button weed”, reported useful in the treatments of fever, respiratory disorders, digestive disorders, diabetes, nephrotoxicity, anxiety, and also reported to possess hypolipidemic, antioxidant, and antimicrobial properties (Meti and Dabburu, 2017, Durgadevi et al., 2022, Sitafale et al., 2022, Nithin Krishna et al., 2024). Nattaichuri is present throughout India as a weed, and this plant is reported to contain phenolic Compounds, tannins, flavonoids, saponins, steroids, triterpenoids, alkaloids, proteins, carbohydrates and glycosides (Sundaram and Vasanthi, 2022). Nattaichuri is a common weed in the groundnut field in Salem District, and there was no previously reported literature available on the allelopathic interaction of Nattaichuri with groundnut. The interaction of the weed with the crop is determined by other factors present in the soil, including the microorganisms. Hence the present investigation aimed to study the interaction of Nattaichuri with groundnut using aqueous extract of stem and leaf under two different conditions; *in vitro* culture (petri dish filled with filter paper) and in pot culture (pot filled with soil mixture to assess the seed germination and seedling growth).

2. Materials and Methods

The experiment was conducted during January to April, 2023 in the Department of Botany at Government Arts College (Autonomous), Salem-7, Tamil Nadu, India.

2.1. Plant material collection

A seed germination experiment was conducted using an aqueous extract of the leaf and stem of *Spermacoce hispida* L. (Nattaichuri). Nattaichuri was collected from a groundnut field in Veeriyanthanda, Salem district, Tamil Nadu, India. The harvested groundnut seeds were obtained from a farmer in the same agricultural land where Nattaichuri was collected (the farmer had preserved groundnut seeds for the next cropping season).

2.2. Preparation of plant extracts

The collected *Nattaichuri* was washed thoroughly in water, shade dried, and the leaf and stem portions were separated and powdered. The powder (10 g) of the stem and leaf were soaked separately in 100 ml of distilled water for 24 h, filtered, made up to 100 ml, and used as a stock solution (considered as a 100% solution). The stock of stem and leaf aqueous extracts was diluted with distilled water to obtain 25%, 50% and 75% extracts.

2.3. Seed germination-in vitro and pot conditions

For each treatment, ten uniform and vigorous seeds (seeds that immediately sink in water) were soaked in distilled water (control) and 25%, 50%, 75%, and 100% leaf and stem extracts for 24 h before sowing. The pre-soaked seeds were grown in Petri dishes (in vitro culture) filled with absorbent filter paper and in pots (pot culture) filled with a soil mixture

(composed of sand, garden soil, and farmyard manure in a 1:2:1 ratio). The Petri dishes were 100 mm (10 cm) in width (diameter) and 15 mm (1.5 cm) in height. The pots were uniform in size, consisting of a 20 cm diameter on the upper side, a 14 cm diameter on the lower side, and 17cm in depth. The light intensity ranges from 220 Lux to 700 Lux, the average temperature was 22–38°C, and the environmental relative humidity was 50–70%. Stock solutions were preserved in the refrigerator and used for irrigation of the seedlings with the appropriate dilutions for the initial five days, and then irrigation was done with distilled water whenever needed. Seed germination results were observed on the 2nd day and the 5th day after seed sowing. In in vitro culture, the emergence of the radicle was considered germination on the 2nd day, and thereafter, both radicle and plumule growth were considered as germination. In pot culture, seedlings that emerged from the soil were counted and tabulated. The effect of the extracts on seedling growth was evaluated in the seedlings, which were grown in pots. On the 15th day of seed sowing, the length of stem and root (in cm scale) of the seedlings was measured. Biomass analysis of the seedlings was done by weighing the fresh and dry weights in grams. Uprooted seedlings were thoroughly washed, air dried and weighed in a digital electronic balance to determine the fresh weight. Later, seedlings were dried in a hot air oven for 24 h at 45°C to measure the dry weight.

2.4. Statistical analysis

The experiments were conducted in a randomized block design. Each replica consisted of 10 seeds in a petri dish and in a pot. Three sets, 10 seeds in a set were maintained for all the treatments, including the control. Statistical analysis of the results was conducted by subjecting the data to one-way ANOVA, and then a comparison of means was done with DMRT (Duncan’s Multiple Range Test) at the significance level 0.05 using SPSS software version 10.0.

3. Results and Discussion

3.1. Effect of nattaichuri on the germination of groundnut

Inhibition of seed germination was observed in in vitro culture with both stem and leaf extracts. The inhibition was more severe with the leaf extract than the stem extract; inhibition also increased with an increase in extract concentration (Table 1).

In the control group, 100% seed germination was observed on 2nd and 5th days of seed sowing. In stem extract treatment, on 2nd and 5th days of seed sowing, the seed germination response was 90% with 25% extract. In other concentrations, a difference in seed germination response was observed between the 2nd and 5th day. For example, with a 50% extract treatment, germination percentage was 90% on the 2nd day and 70% on the 5th day. With a 75% stem extract, seed germination was 80% on the 2nd day and 50% on the 5th day. Seed germination was 30% on the 2nd day and 20% on the



Table 1: Effect of Nattaichuri on seed germination of groundnut sown in Petri dishes

Treatment	Extract Concentration	Percentage of seed germination	
		After two days	After five days
Control	0	100 ^a	100 ^a
Stem	25%	90 ^{ab}	90 ^{ab}
	50%	90 ^{ab}	70 ^c
	75%	80 ^b	50 ^d
	100%	30 ^{de}	20 ^{ef}
Leaf	25%	90 ^{ab}	70 ^c
	50%	60 ^c	30 ^e
	75%	40 ^d	20 ^{ef}
	100%	10 ^e	0 ^g

Data represent the mean value of 10 replica. Results followed by different alphabets in each column indicate statistical difference at $p \leq 0.05$ as per DMRT

5th day with 100% stem extract. In leaf extract-treated seeds, on 2nd day of seed sowing, seed germination was 90%, 60%, 40%, and 10% respectively, in 25%, 50%, 75% and 100% treatments. Delay in seed germination was also evident with the observation; germination was 70% with a 25% extract, 30% with 50% extract, 20% with 75% extract, and no seed germination was observed with 100% leaf extract on 5th day of seed sowing. In vitro culture, inhibition and delay of seed germination were observed in groundnut when treated with Nattaichuri extract. Similarly, *Euphorbia dracunculoides* Lam. was reported to delay seed germination in chickpea and wheat (Tanveer et al., 2012). In negative allelopathic interactions, a decrease in seed germination, shoot length, root length, and total dry weight was reported and concluded that inhibition and delay of seed germination were important indicators of negative allelopathic interactions (Abou El-Enin and Abdel-Ghffa, 2017). Allelochemicals present in weeds helped them to inhibit or delay the germination of seeds of other plants in that specific zone, where all the plants compete for food, water, and sunlight (Cherigo et al., 2017). Low seed germination in the aqueous extract might be due to disruption of the host plant's osmotic balance, inhibition of water uptake, increased respiration, and a decrease in cell division and embryo enlargement (Shahena et al., 2021).

In pot culture, 80% seed germination was observed with 100% leaf extract treatment, whereas in other treatments and in the control, 100% seed germination was observed (Table 2).

3.2. Effect of nattaichuri on the growth of groundnut

After 15 days of seed sowing, the length of the stem and root was measured in seedlings (in cm) grown in a pot, and the average was calculated and presented in Table 3. Compared

Table 2: Effect of Nattaichuri on seed germination of groundnut sown in pots

Treatment	Extract concentration	Percentage of seed germination
Control	0	100 ^a
Stem	25%	100 ^a
	50%	100 ^a
	75%	100 ^a
	100%	100 ^a
Leaf	25%	100 ^a
	50%	100 ^a
	75%	100 ^a
	100%	80 ^b

Data represent the mean value of 10 replica. Results followed by different alphabets in each column indicate statistical difference at $p \leq 0.05$ as per DMRT

Table 3: Effect of Nattaichuri on seedling growth in groundnut grown in pots

Treatment	Extract concentration	Shoot length in cm	Root length in cm
Control	0	10 ^h	9.19 ^g
Stem	25%	12.58 ^a	11.00 ^c
	50%	11.29 ^e	10.88 ^d
	75%	10.9 ^{fg}	11.40 ^b
	100%	11.7 ^d	10.01 ^f
Leaf	25%	11.81 ^{cd}	10.94 ^{cd}
	50%	10.94 ^f	10.46 ^e
	75%	12.5 ^{ab}	11.81 ^a
	100%	11.9 ^c	10.31 ^{ef}

Data represent the mean value of 8 replica. Results followed by different alphabets in each column indicate statistical difference at $p \leq 0.05$ as per DMRT

to the control, the length of the root was higher in both types of extract (leaf and stem) treated seedlings. In leaf extract treated seedlings, growth of the root was highest with 75% extract than in other concentrations (11.81 cm). Similarly, in stem extract, the length of the root was higher with a 75% extract treatment compared to other concentrations. In control, the root length was 9.19 cm and in 75% stem extract treatment it was 11.4 cm. Root growth is a crucial factor for the establishment of a plant in its environment. The deeper the root (length) and the more branching, the higher the chance of survival.

The length of the shoot was higher in all the treatments compared to the control (Table 3).

The shoot length was measured at 10 cm in the control,



whereas in the stem extract treated seedlings, it was 12.58 cm with a 25% extract concentration; this was the highest result observed among stem extract treatments. In 50%, 75%, and 100% stem extract treatments, shoot length was 11.29 cm, 10.9 cm, and 11 cm, respectively. Similarly, in all leaf extract-treated seedlings, shoot length was higher than in the control. Maximum increase in shoot length was observed with 75% leaf extract as 12.5 cm.

On 15th day of seed sowing, the fresh weight and dry weight of the seedlings of pot culture were assessed and presented in Table 4. Compared to the control, stem and leaf extract treatments resulted in higher fresh and dry weights (biomass) in the seedlings.

Table 4: Effect of nattaichuri on biomass of the seedlings of groundnut grown in pots

Treatment	Extract concentration	Seedling fresh weigh in g	Seedling dry weight in g
Control	0	1.708 ^f	0.21 ^g
Stem	25%	2.24 ^{cd}	0.27 ^b
	50%	2.02 ^e	0.246 ^{ef}
	75%	2.33 ^{ab}	0.26 ^c
	100%	2.13 ^{de}	0.25 ^d
Leaf	25%	2.2 ^d	0.257 ^{de}
	50%	2.245 ^c	0.26 ^c
	75%	2.35 ^a	0.29 ^a
	100%	2.2 ^d	0.25 ^e

Data represent the mean value of 8 replica. Results followed by different alphabets in each column indicate statistical difference at $p \leq 0.05$ as per DMRT

The maximum increase in biomass was observed with the 75% leaf extract treatment, with 2.35 g fresh weight and 0.29 g dry weight. In the control seedlings, fresh weight was 1.708 g and dry weight was 0.21 g. Though stem extract increased the biomass of seedlings compared to the control, its effect was less than the leaf extract treatments. The maximum increase in fresh weight in the seedlings was observed with a 75% stem extract, where the fresh weight was 2.33 g.

All the results of the present investigation revealed that the plant extract was beneficial in improving the growth parameters such as root length, shoot length, and biomass of groundnut in pot culture experiment. Positive interactions result in high seed germination, shoot and root growth, and high dry matter, which were associated with high longevity of the seedlings due to the production of phytohormones and the increase in the bioavailability of minerals by seed-priming microorganisms (Somasundaram and Bhaskaran, 2017). In sorghum, drought-tolerant varieties were reported to have maximum values for shoot and root length and more fresh and dry weight of the roots (Rajarajan et al., 2018). In the present study, an increase in seed germination was observed

when seedlings were grown in pots compared to seeds grown in petri dishes (*in vitro* culture). This might be due to the interaction of soil microorganisms with plant extracts and the degradation of allelochemicals by the soil microorganisms. Similarly, the microbial group present in the fermented organic matter was reported to enhance the plant growth by acting as a decomposer in addition to being a biofertilizer and an organic pesticide (Irwandhi et al., 2025).

4. Conclusion

Though *in vitro* culture showed inhibitory allelopathic interaction of Nattaichuri (*Spermacoce hispida* L.) with groundnut (*Arachis hypogaea* L.), the pot culture showed enhanced growth of groundnut when treated with Nattaichuri leaf and stem extracts. Further studies might be conducted to identify the microbial flora which were responsible for the growth promotion effect.

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