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Verdant Legacy

Research Article

Evaluation of the Allelopathic Potential of *Caralluma tuberculata* N.E. Brown on Selected Vegetable Crops through Bioassays and Phytochemical Analysis

Marriam Khan[1], Noreen Kareem[2], Madiha Rashid[2*]

1. New World International School, Al Khobar, Saudi Arabia. marriamfahad22@gmail.com
2. Department of Botany, Division of Science and Technology, University of Education, Lahore, Pakistan. noreenkareem06@gmail.com; botanistpk@yahoo.com

Corresponding Email: botanistpk@yahoo.com

Abstract

Caralluma tuberculata N.E. Brown is a medicinal species valued for its pharmacological and nutritional properties. This study evaluated the allelopathic potential of *C. tuberculata* on selected vegetable crops using sandwich bioassays, pot experiments, and spectrophotometric analyses. In sandwich bioassays, dried plant material (0.05 and 0.10 g) was tested against *Lactuca sativa*, *Raphanus sativus*, *Spinacia oleracea*, *Cucumis sativus*, and *Abelmoschus esculentus*. Pot experiments employed *n*-hexane, acetone, and ethanol extracts at 1% and 3% (w/v) to assess seedling growth and morphology. Total phenolic and flavonoid contents were quantified and expressed as gallic acid equivalents (GAE) and quercetin equivalents (QE), respectively. The results revealed concentration-dependent inhibition of seed germination and seedling growth in most species, with significant reductions in radicle and hypocotyl lengths. Morphological abnormalities, including chlorosis, necrosis, wilting, reduced leaf production, and plant mortality, were observed. *Raphanus sativus* and *Spinacia oleracea* were the most sensitive species, whereas *Abelmoschus esculentus* exhibited partial tolerance and slight stimulation of hypocotyl growth under some treatments, suggesting a possible hormetic response. Phytochemical analyses confirmed the presence of phenolic and flavonoid compounds that may contribute to the observed phytotoxicity. However, inhibitory effects observed in solvent controls, particularly with *n*-hexane and acetone, indicate that solvent-induced phytotoxicity may have influenced the results. Overall, the findings demonstrate the allelopathic potential of *C. tuberculata* while highlighting the need for further isolation and characterization of its bioactive allelochemicals to clarify the mechanisms underlying its phytotoxic effects.

Keywords: Allelopathy, *Caralluma tuberculata*, germination, phytotoxicity, phenolic compounds, flavonoids, sandwich method, vegetable crops.

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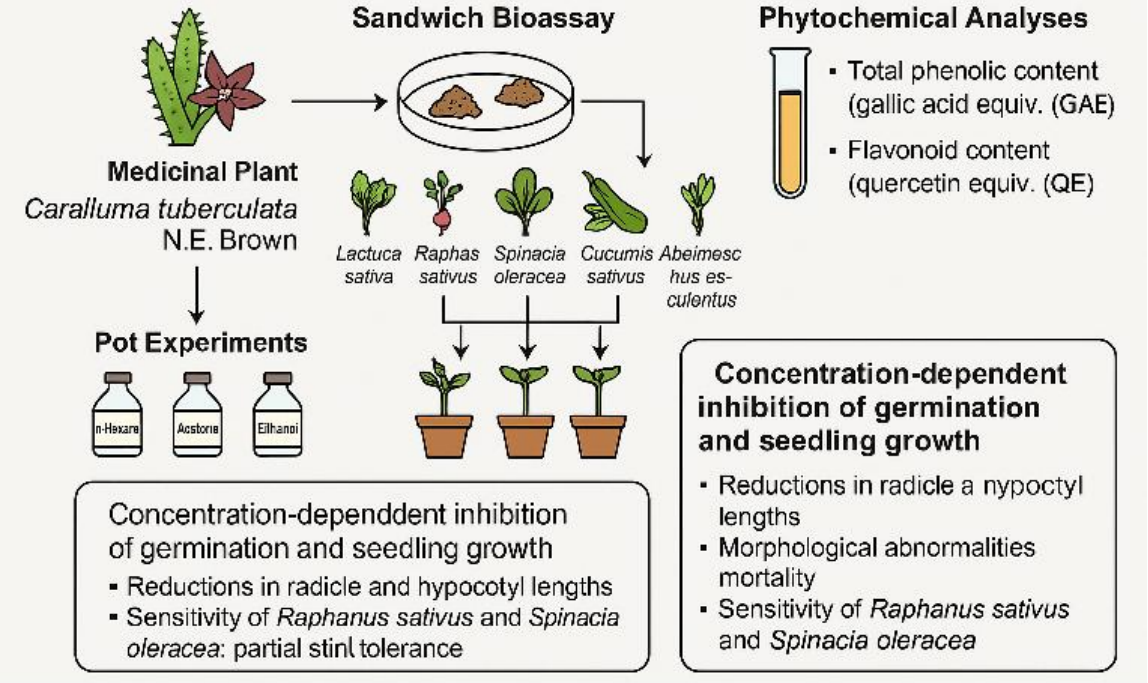
Graphical Abstract:



1: Introduction

Caralluma tuberculata N.E.Brown is an important medicinal plant belonging to the family Asclepiadaceae. It is commonly known as Chunga, Pamankay or Bittercress. The Genus Caralluma is succulent, perennial and branched herbs with clear latex (Ullah et al., 2022). Caralluma species are primarily distributed in arid and semi-arid regions of Africa, the Middle East, and South Asia, including Pakistan, India, and Saudi Arabia (Rehman et al., 2014). It is found in various countries such as Spain, Saudi Arabia, Africa, Middle East, India, and Pakistan. *Caralluma tuberculata* found abundantly on the mountains in the FATA and its surrounding districts in Pakistan. In a local Pashto language *C. tuberculata* is called Pamankay, which comes from combination of Paman and kay Paman means pimple and kay means thing (Ahmad et al., 2014).

Caralluma tuberculata is traditionally used in the treatment of various human diseases in Pakistan (Iftikhar *et al.*, 2023). *Caralluma* are used for the treatment of diabetes, cancer, snake and scorpion bites, joints pain, inflammation, skin rashes, and pain fever; and stimulate central nervous system (Alodaini et al., 2025). *Caralluma tuberculata* has juicy stem which is sour tonic, carminative, febrifuge, and stomachic valuable in rheumatism and can be consumed as vegetable (Ishaq et al., 2023). *Caralluma* species have been used for centuries as emergency foods in semi-arid areas of Pakistan (Waheed *et al.*, 2023).

Allelochemicals in plants are mostly secondary metabolites, or the by-products of primary metabolic processes that exert an allelopathic effect on the growth and development of the neighboring plants (Dar et al., 2025). The plants usually store these chemicals in cells in the bounded forms for example as water-soluble glycosides, alkaloids, flavonoids and phenol. Polyphenolics is a highly broad term that covers many different subgroups of simple phenols, phenolic acids and flavonoids and it is abundant in land plants (Anh et al., 2025).

According to the World Health Organization (WHO), more than 80% of the world's population relies on traditional herbal medicine for their primary health care needs (Hlatshwayo et

al., 2025). These traditional systems are culturally and psychologically more tolerable in most of the societies as compared to western allopathic medicines (Javed, et al., 2025). In addition, being the natural plant products, they are considered to be the safest way of treating diseases with least side effects on human health as compared to allopathic or homeopathic medicines (Sentayehu et al., 2026).

Existing medicinal plants face numerous threats. For threatened medicinal plant species, cultivation offers a viable conservation strategy because the continuous harvesting from wild populations often exceeds the annual sustainable yield (Takubessi et al., 2025). If the demand for these species can be met through cultivated sources, the pressure on wild populations can be alleviated. In such cases, strict conservation of remaining populations, secure ex-situ germplasm storage, and investment in selection and improvement programs become extremely urgent, as exemplified by Jaborandi (*Pilocarpus jaborandi*) in Brazil (Aftab et al., 2025, Misganaw et al., 2025). Since the utility of *Caralluma tuberculata* relies on its wild habitat, its natural populations are under considerable stress. Therefore, cultivating this plant is necessary to relieve pressure on wild populations. However, before initiating cultivation, understanding its interactions with other plants is essential.

Plants can release chemicals into the environment that influence the growth of surrounding plants, a process known as allelopathy. Allelopathic functions also play other ecological roles, such as plant defense, nutrient chelation, and regulation of soil biota, which can affect decomposition and soil fertility (Sun et al., 2026). The active biomolecules released by allelopathic plants, commonly called allelochemicals, can be found in any part of the plant, including leaves, flowers, roots, fruits, or stems (Wang et al., 2025). Allelochemicals are involved in chemical communication between plants, between plants and insects or herbivores, and in interactions with microorganisms (Fadiji et al., 2025). The aim of this research was to evaluate the allelopathic potential of the functional food species *Caralluma tuberculata*, specifically its effects on the growth of the test plants: Lettuce (*Lactuca sativa* L.), Radish (*Raphanus sativus* L.), Spinach (*Spinacia oleracea* L.), Cucumber (*Cucumis sativus* L.), and Ladyfinger (*Abelmoschus esculentus* L.).

Despite the increasing medicinal and nutritional importance of *Caralluma tuberculata,* limited information is available regarding its allelopathic interactions with agricultural crops. Understanding these interactions is essential before introducing this species into cultivation systems, particularly where intercropping or mixed cropping practices are employed. Therefore, the present study aimed to evaluate the allelopathic potential of *C. tuberculata* using sandwich bioassays and pot experiments and to determine whether its phytotoxic effects are associated with the presence of phenolic and flavonoid compounds. Specifically, the study investigated the effects of *C. tuberculata* on seed germination, radicle growth, hypocotyl growth, and seedling development of selected vegetable crops, including *Lactuca sativa* L., *Raphanus sativus* L., *Spinacia oleracea* L., *Cucumis sativus* L., and *Abelmoschus esculentus* L.

2: Materials and Methods:

2.1: Experimental Framework

The study was conducted in three sequential phases to evaluate the allelopathic potential of *Caralluma tuberculata* N.E. Brown. First, a sandwich bioassay was performed to screen the inhibitory effects of dried plant material on seed germination and seedling growth of selected vegetable crops. Second, pot experiments were conducted using solvent extracts of *C. tuberculata* to assess their effects on seedling growth and morphology under soil conditions. Finally, spectrophotometric analyses were carried out to estimate total phenolic and flavonoid contents, which may contribute to the observed allelopathic activity.

2.2: Plant Material Collection and Preparation

Whole plants of *C. tuberculata* were collected from their natural habitat and authenticated by a qualified taxonomist. Plant material was washed thoroughly with distilled water, air-dried under shade at room temperature, and ground into a fine powder using an electric

grinder. The powdered material was stored in airtight containers until further use.

2.3: Sandwich Bioassay

The sandwich method described by Fujii et al. (2003) was used to evaluate allelopathic activity. Two quantities of dried plant powder, 0.05 g and 0.10 g, were placed separately in sterile Petri dishes. A layer of agar medium was poured over the plant material, followed by a second agar layer after solidification. Seeds of *Lactuca sativa* L., *Raphanus sativus* L., *Spinacia oleracea* L., *Cucumis sativus* L., *and Abelmoschus esculentus* L. were placed on the agar surface.

Petri dishes containing agar without plant material served as controls. Each treatment was replicated three times and arranged according to a Completely Randomized Design (CRD).Pots were randomly positioned and re-randomized periodically throughout the experimental period to minimize positional effects. After incubation under controlled laboratory conditions, germination percentage, radicle length, and hypocotyl length were recorded after seven days of growth.

2.4: Preparation of Plant Extracts

For pot experiments, dried plant powder was extracted separately using n-hexane, acetone, and ethanol. The extracts were concentrated using a rotary evaporator and stored at 4 °C until use. Stock solutions were prepared and diluted to obtain final concentrations of 1% (w/v) and 3% (w/v).

2.5: Pot experiment

The pot experiment was conducted to evaluate the effects of solvent extracts on seedling growth and morphology. Plastic pots were filled with sterilized soil and sown with seeds of the selected vegetable crops. Treatments consisted of:

1. Water control
2. Solvent control (without plant extract)
3. 1% (w/v) extract
4. 3% (w/v) extract

The experiment was arranged according to a Completely Randomized Design (CRD) with three replicates per treatment. Pots were randomly positioned and re-randomized periodically throughout the experimental period to minimize positional effects caused by light, temperature, or airflow variation. Five test plants *Cucumis sativus* L., *Lactuca sativa* L., *Abelmoschus esculentus* L., *Raphanus sativus* L. and *Spinacia oleracea* L. were used to evaluate the effect of treatment. Five seeds of each test plant were sown in plastic pots. Different plants have different germination period. Under favorable conditions, seeds started to germinate after 4-10 days of sowing. After 7 days of growth changes in vegetative characters, number of leaves and changes in growth patterns parameters were observed by using different formulas for determination of germination percentage, growth percentage and growth inhibition percentage (Anon et al., 2013).

2.6: Spectrophotometric Analysis

A double beam spectrophotometer (BD Holo) was used to determine total phenolic and flavonoid contents in *C. tuberculata*. For extraction, plant material was dried at 55 °C in a hot air oven and ground into a fine powder. Approximately 0.089 g of the powdered sample was extracted with 10 mL of 80% methanol and kept for maceration for 48 hours. The extract was then sonicated for 25 minutes under chilled conditions and centrifuged at 4 °C for 20 minutes at 6500 rpm. The supernatant was collected, stored in amber-colored tubes, wrapped with aluminum foil to prevent light exposure, and preserved at −20 °C until analysis.

2.8: Total flavonoid content

For total flavonoid content (TFC), the aluminum chloride colorimetric method was used (Abate et. al 2018). Standard solutions of Quercetin ranging from 0 to 1.0 mg/mL were prepared in methanol. In the assay, 0.5 mL of standard or 1.5 mL of plant extract was mixed with distilled water, followed by the addition of 0.3 mL of 10% aluminum chloride and 0.15 mL of sodium nitrite.

$$\% \text{ germination } = \frac{Total\ no.of\ seeds\ germinated}{Total\ no\ of\ seed} \times 100$$

$$\% \ growth = \frac{Length\ of\ radical}{Average\ radical\ lenght\ of\ control} \times 100$$

$$\% \ growth = \frac{Length\ of\ Hypocotyle}{Average\ Hypocotyle\ Length} \times 100$$

$$\% \ growth\ inhibition = \frac{Pc - Pt}{Pc} \times 100$$

After 6 minutes, 1 mL of sodium hydroxide was added, and the mixture was incubated at room temperature. The absorbance was recorded at 510 nm, and flavonoid content was expressed as mg quercetin equivalents (QE) per gram of dry weight (Agbo et al., 2015). Total flavonoid content (TFC) was determined using the aluminium chloride colorimetric method. Absorbance was measured spectrophotometrically, and results were expressed as milligrams of quercetin equivalents (QE) per gram of dry weight.

2.9: Statistical analysis

The data was analyzed by SPSS 16.0 (statistical analysis of social procedures) and Microsoft Excel using one-way ANOVA. Data were analyzed using one-way analysis of variance under a Completely Randomized Design. Treatment means were compared using an appropriate post hoc test at a significance level of $P < 0.05$. Results are presented as mean ± standard error. Statistical analyses were performed using standard statistical software.

3: Results and Discussion

The present study was conducted to evaluate the biological effects of *C. tuberculata* on selected test species using pot experiment.

3.1: Allelopathic effect of 0.05g and 0.1g of *Caralluma tuberculata* on *Cucumis sativus* L. (as recipient plant) by sandwich method

The allelopathic effect of 0.05g of *Caralluma tuberculata* on *Cucumis sativus* was observed by measuring the germination percentage, growth percentage and growth inhibition of seedlings. Germination and seedling growth were observed in all plates. Under the influence of treatment, the germination percentage was 93.3% (Table 1 and Figure 1A) and the growth of hypocotyl was also reduced. Enhancement in growth of radicle was observed. The minimum growth inhibition percentage for radicle was -7.14% and maximum growth inhibition percentage for hypocotyl was 40.3% (Figure 1B). The maximum growth percentage for radicle was 107.4% and minimum growth percentage for hypocotyl was 59.86% (Figure 1C).

The allelopathic effect of 0.1g of *C. tuberculata* on *Cucumis sativus* was observed by measuring the germination percentage, growth percentage and growth inhibition of seedlings. Germination and seedling growth were observed in all plates. Under the influence of treatment, the germination percentage was 93.3% (Table 1 and Figure 1A) and the growth of radicle and hypocotyl was also reduced. The maximum growth inhibition percentage for radicle was 37.5% and for hypocotyl was 49.83% (Figure 1D). The minimum growth percentage for radicle was 62.15% and for hypocotyl was 50.16% (Figure 1E).

3.2: Allelopathic effect of 0.05g and 0.1g of *Caralluma tuberculata* on *Spinacia oleracea* L. (as recipient plant) by sandwich method

The allelopathic effect of 0.05g of *Caralluma tuberculata* on *Spinacia oleracea* was observed by measuring the germination percentage, growth percentage and growth inhibition of seedlings. Germination and seedling growth were observed in all plates. Under the influence of treatment, the germination percentage was 60% (Table 1 and Figure 2 A) and the growth of hypocotyl and radicle was also reduced. The maximum growth inhibition percentage for radicle was 61.01% and

for hypocotyl was 31.19% (Figure 2 B). The minimum growth percentage for radicle was 38.98% and for hypocotyl was 68.8% (Figure 2 C)

Table 1: Germination percentage of *Cucumis sativus* L., *Spinacia oleracea* L., *Raphanus sativus* L., and *Abelmoschus esculentus* L. in response to 0.05 g and 0.1 g *Caralluma tuberculata* using the sandwich method.

Replicate	Treatment	*Cucumis sativus* (%)	*Spinacia oleracea* (%)	*Raphanus sativus* (%)	*Abelmoschus esculentus* (%)
1	Control	100	100	100	100
1	0.05 g	100	60	60	80
1	0.1 g	100	60	60	60
2	Control	100	100	100	100
2	0.05 g	80	60	60	80
2	0.1 g	100	40	60	80
3	Control	100	100	100	100
3	0.05 g	100	60	60	60
3	0.1 g	80	60	40	80

The allelopathic effect of 0.1g of *Caralluma tuberculata* on *Spinacia oleracea* was observed by measuring the germination percentage, growth percentage and growth inhibition of seedlings. Germination and seedling growth were observed in all plates. Under the influence of treatment, the germination percentage was 53.33% (Table 1 and Figure 2 A) and the growth of hypocotyl and radicle was also reduced. The maximum growth inhibition percentage for radicle was 49.15% and for hypocotyl was 47.24% (Figure 2 D). The minimum growth percentage for radicle was 50.84% and for hypocotyl was 52.75% (Figure 2 E).

3.3: Allelopathic effect of 0.05g and 0.1g of *Caralluma tuberculata* on *Spinacia oleracea* L. (as recipient plant) by sandwich metho

The allelopathic effect of 0.05g of *Caralluma tuberculata* on *Spinacia oleracea* was observed by measuring the germination percentage, growth percentage and growth inhibition of seedlings. Germination and seedling growth were observed in all plates. Under the influence of treatment, the germination percentage was 60% (Table 1 and Figure 2 A) and the growth of hypocotyl and radicle was also reduced. The maximum growth inhibition percentage for radicle was 61.01% and for hypocotyl was 31.19% (Figure 2 B). The minimum growth percentage for radicle was 38.98% and for hypocotyl was 68.8% (Figure 2 C)

The allelopathic effect of 0.1g of *Caralluma tuberculata* on *Spinacia oleracea* was observed by measuring the germination percentage, growth percentage and growth inhibition of seedlings. Germination and seedling growth were observed in all plates. Under the influence of treatment, the germination percentage was 53.33% (Table 1 and Figure 2 A) and the growth of hypocotyl and radicle was also reduced. The maximum growth inhibition percentage for radicle was 49.15% and for hypocotyl was 47.24% (Figure 2 D). The minimum growth percentage for radicle was 50.84% and for hypocotyl was 52.75% (Figure 2 E).

3.4: Allelopathic effect of *Caralluma tuberculata* on *Abelmoschus esculentus* L. by sandwich method

The allelopathic effect of 0.05g of *Caralluma tuberculata* on *Abelmoschus esculentus* was observed by measuring the germination percentage, growth percentage and growth inhibition of seedlings. Germination and seedling growth were observed in all plates.

Table 2: Radicle and hypocotyl length (cm) of *Cucumis sativus* L., *Spinacia oleracea* L., *Raphanus sativus* L., and *Abelmoschus esculentus* L. in response to 005 g and 0.1 g *Caralluma tuberculata* N.E.Brown using the sandwich method.

Replicate	Treatment	1	2	3	4	5	6	7	8
1	Control	0.97 a	12 a	0.70 a	4.66 a	1.00 a	2.73 a	0.63 a	3.20 a
1	0.05 g	1.17 a	7.16 b	0.33 b	3 b	0.63 b	2.23 b	0.30 b	3.43 a
1	0.10 g	0.87 b	6 b	0.30 b	2.3 c	0.30 c	1.70 c	0.33 b	3.30 a
2	Control	1.10 ab	11.6 a	0.43 a	4.4 a	1.26 a	2.60 a	0.60 a	3.16 b
2	0.05 g	1.16 a	7.6 b	0.46 a	3.56 b	0.56 b	2.36 a	0.36 a	3.90 a
2	0.10 g	0.70 b	7 b	0.50 a	2.7 c	0.26 b	1.73 b	0.56 a	3.93 a
3	Control	1.30 a	12.3 a	0.66 a	4.03 a	1.00 a	2.66 a	0.60 a	3.66 b
3	0.05 g	1.20 a	9.33 b	0.23 ab	3.06 b	0.56 b	2.36 a	0.30 b	4.46 a
3	0.10 g	0.70 b	6 c	0.50 b	2.7 c	0.40 b	1.40 b	0.50 a	4.00 a

Key: 1. *Cucumis sativus* R (cm) 2: *Cucumis sativus* H (cm) 3: *Spinacia oleracea* R (cm) 4: *Spinacia oleracea* H (cm) 5: *Raphanus sativus* R (cm) 6: *Raphanus sativus* H (cm) 7: *Abelmoschus esculentus* R (cm) 8: *Abelmoschus esculentus* H (cm)

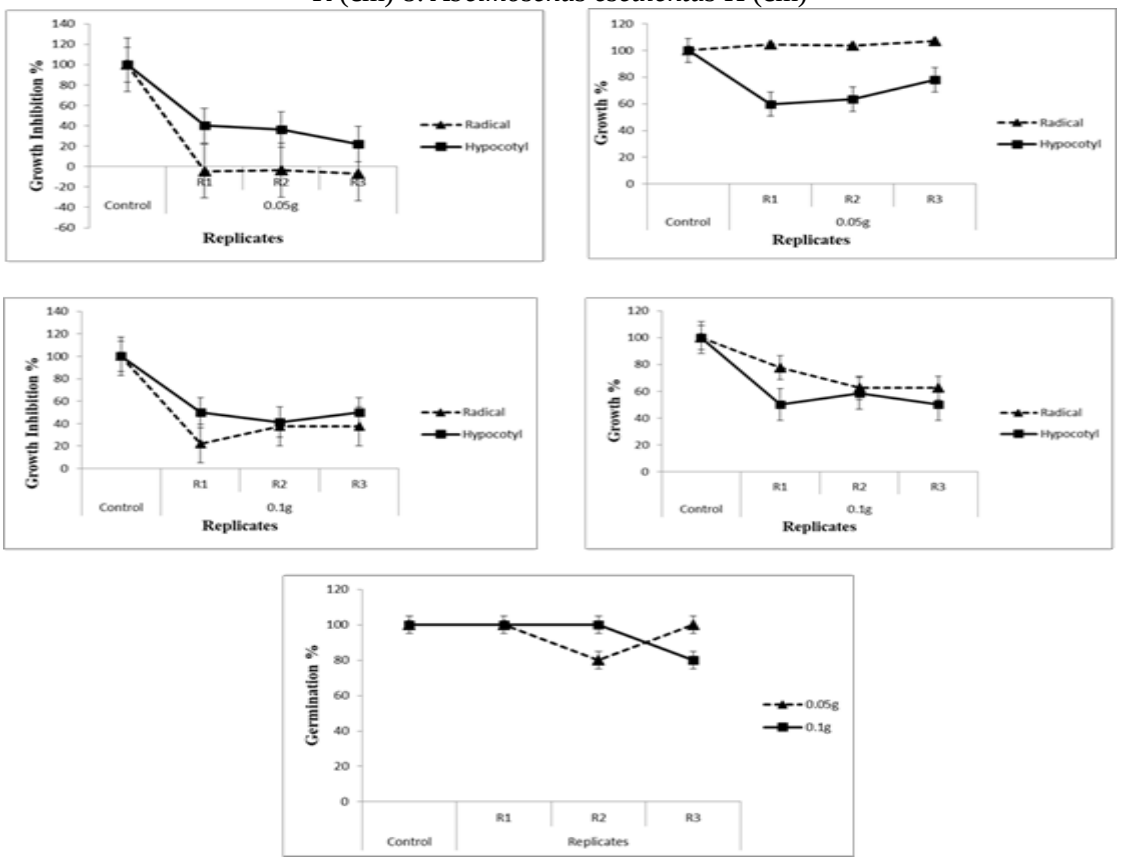


Figure 1: Right to left Hypocotyl and radicle length of *Cucumis sativus* L. on 0.05g **(**Hypocotyl and radicle length of *Cucumis sativus* L. on 0.05g **(**Hypocotyl and radicle length of *Cucumis sativus* L. on 0.1g **(**Hypocotyl and radicle length of *Cucumis sativus* L. on 0.1g **(**Germination percentage of *Cucumis sativus* L. in response to 0.05 g and 0.1g of *Caralluma tuberculata* N.E.Brown.

Under the influence of treatment, the germination percentage was 73.33% (Table 1 and Figure 4 A) and the growth of radicle was also reduced. Enhancement in growth of hypocotyl was observed. The maximum growth inhibition percentage for radicle was 50.81% and minimum growth inhibition percentage for hypocotyl was -33.53% (Figure 4 B). The minimum growth percentage for radicle was 49.18% and maximum growth percentage for hypocotyl was 133.53% (Figure 4 C).

The allelopathic effect of 0.1g of *Caralluma tuberculata* on *Abelmoschus esculentus* was observed by measuring the germination percentage, growth percentage and growth inhibition of seedlings. Germination and seedling growth were observed in all plates. Under the influence of treatment, the germination percentage was 73.33% (Table 1 and Figure 4 A) and the growth of radicle was also reduced. Enhancement in growth of hypocotyl was observed. The maximum growth inhibition percentage for radicle was 45.9% and minimum growth inhibition percentage for hypocotyl was -19.76% (Figure 4 D). The minimum growth percentage for radicle was 54.09% and maximum growth percentage for hypocotyl was 119.76% (Figure 4 E)

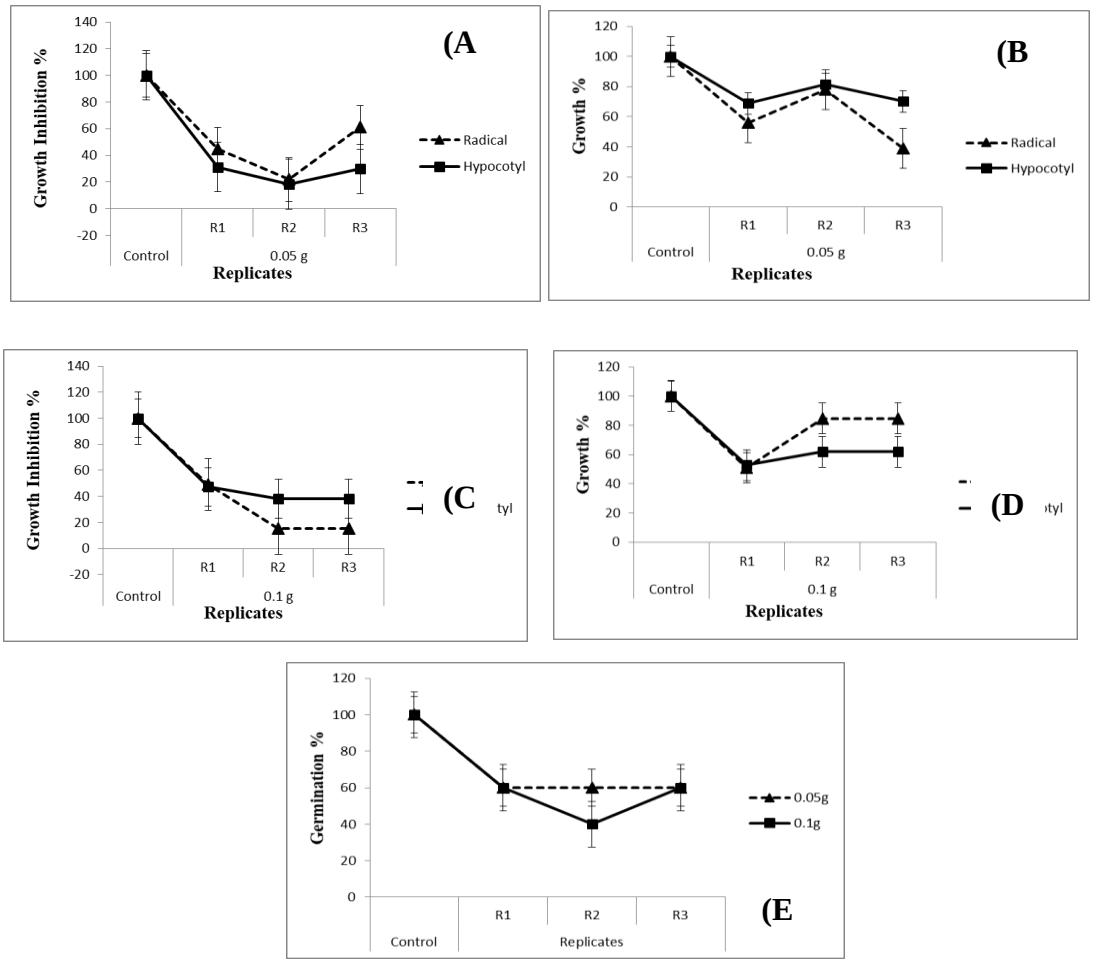


Figure 2: (A Hypocotyl and radicle length of *Spinacia oleracea* L. on 0.05g **(B** Hypocotyl and radicle length of *Spinacia oleracea* L. on 0.05g **(C** Hypocotyl and radicle length of *Spinacia oleracea* L. on 0.1g **(D** Hypocotyl and radicle length of *Spinacia oleracea* L. on 0.1g **(E** Germination percentage *Spinacia oleracea* L. in response to 0.05 g and 0.1g of *Caralluma tuberculata* N.E.Brown.

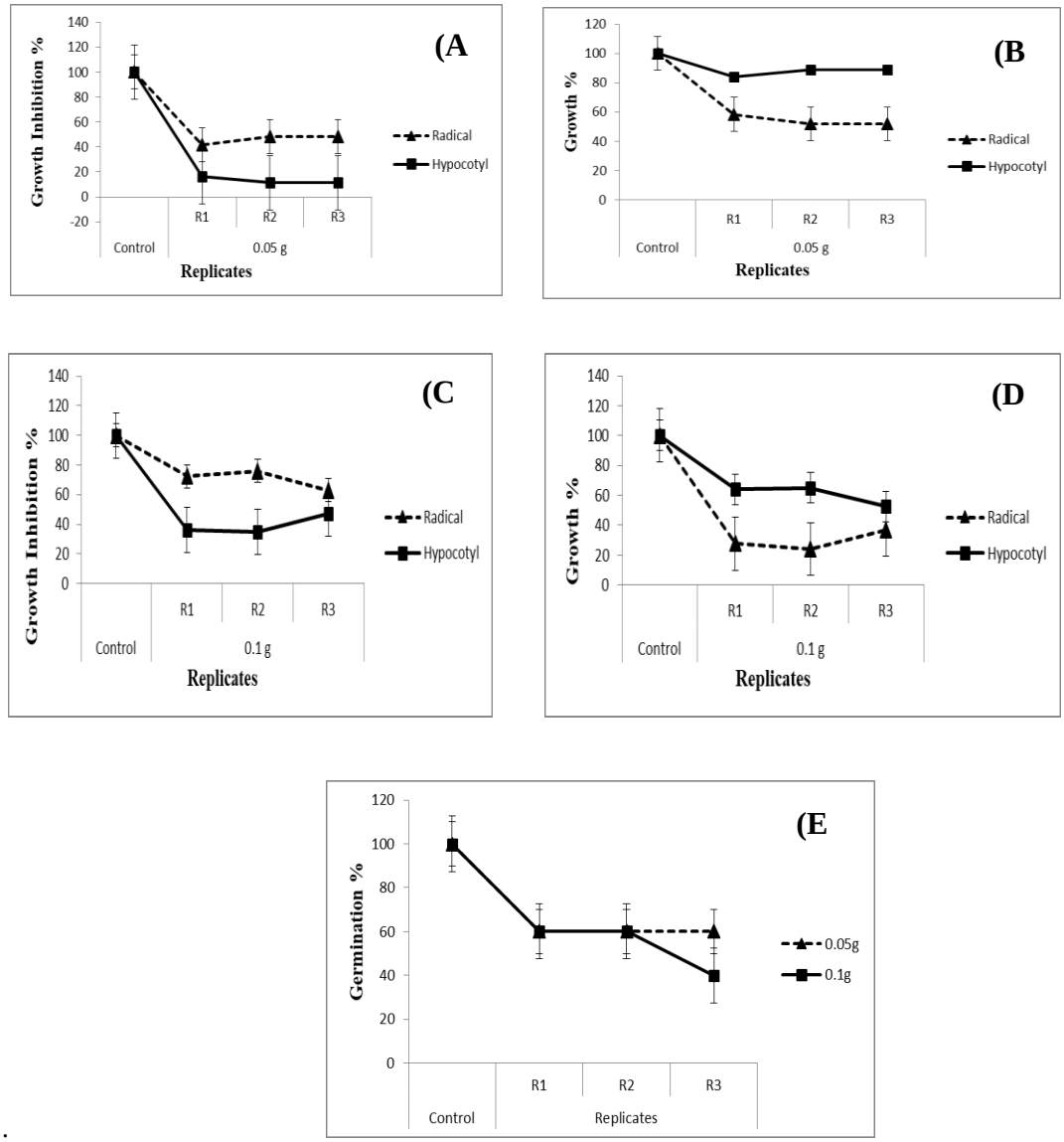


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Figure 3: (A Hypocotyl and radicle length of *Raphanus sativus* on 0.05g **(B** Hypocotyl and radicle length of *Raphanus sativus* L. on 0.05g **(C** Hypocotyl and radicle length of *Raphanus sativus* L. on 0.1g **(D** Hypocotyl and radicle length of *Raphanus sativus*. on 0.1g **(E** Germination percentage *Raphanus sativus* in response to 0.05 g and 0.1g of *Caralluma tuberculata* N.E.Brown.

Polarity of allellochemicals is different therefore different solvents were used for their extraction. For screening purpose different solvents which were non-polar to polar (n-hexane, acetone and ethanol) were utilized in the present research work.

3.5: Allelopathic potential of *Caralluma tuberculata* extract on *Cucumis sativus* L. by using pot method

The n-hexane extract of *Caralluma tuberculata* and n-hexane without plant extract showed changes in the morphology of *Cucumis sativus*. Under the influence of all three treatments (1%, 3% plant extract and without extract), the leaf color changed from green to brown, number of leaves were reduced from 9 to 2 and length of stem was also reduced from 10cm to 9cm as compared to control plant (Table 3). Plant health was also adversely affected. The extreme affect was observed when plant was treated

with 1% plant extract (n-hexane) as the growth was completely retarded by this treatment.

Changes in the morphology of *Cucumis sativus* were observed when it was treated with acetone extract of *Caralluma tuberculata* and acetone without plant extract. Under the influence of all three treatments (1%, 3% plant extract and without extract), the leaf color changed from green to yellowish green, number of leaves were reduced from 15 to 8 and length of stem was also reduced from 15cm to 9cm as compared to control plant (Table 3). Plant health was also affected. The extreme affect was observed when plants were treated with 3% plant extract (acetone) and acetone (without extract) as the leaves showed chlorosis. It indicated that photosynthetic activities were disturbed by these treatments. The ethanol extract of *Caralluma tuberculata* and ethanol without plant extract showed changes in the morphology of *Cucumis sativus*. Under the influence of all three treatments (1%, 3% plant extract and without extract), the leaf color changed from green to brown, number of leaves were reduced from 4 to 3 and length of stem was also reduced from 12cm to 6cm as compared to control plant (Table 3). Health was also affected in all plants. The extreme affect was observed when plant was treated with 3% plant extract (ethanol) as this treatment caused the death of plant.

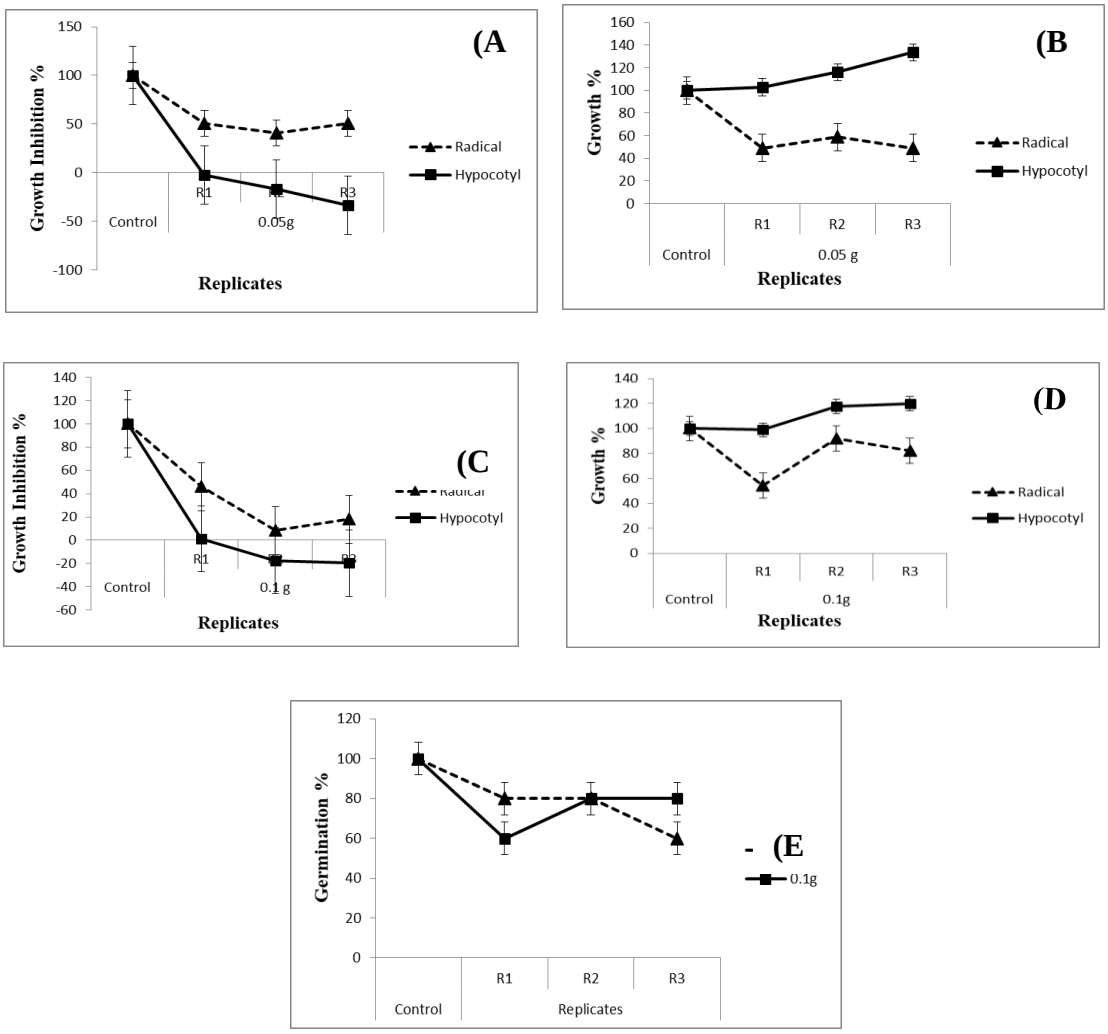

Figure 4: (A Hypocotyl and radicle length of *Abelmoschus esculentus* on 0.05g **(B** Hypocotyl and radicle length of *Abelmoschus esculentus*. on 0.05g **(C** Hypocotyl and radicle length of *Abelmoschus esculentus* on 0.1g **(D** Hypocotyl and radicle length of *Abelmoschus esculentus* on 0.1g **(E** Germination percentage *Abelmoschus esculentus* in response to 0.05 g and 0.1g of *Caralluma tuberculata* N.E.Brown.

Table 3: Growth response of tested plant species to *Caralluma tuberculata* extracts prepared using different solvents (n-hexane, acetone, and ethanol) under pot conditions. Treatments comprised control (water), solvent control, and plant extracts at 1% and 3% concentrations.

Experiment	Solvent	Treatment	Colour	Leaves	Stem(cm)	Plant Health
Cucumis sativus	n-Hexane	Control (Water)	Green	9	10	Fresh
	n-Hexane	Control (Solvent)	Green yellow	2	9	Chlorosis,
	n-Hexane	Extract 1%	Brown	3	9	Retarded
	n-Hexane	Extract 3%	Green brown	3	9	Chlorosis,
	Acetone	Control (Water)	Green	15	15	Fresh
	Acetone	Control (Solvent)	Yellowish green	9	12	Chlorosis
	Acetone	Extract 1%	Green	12	9	Fresh
	Acetone	Extract 3%	Yellowish green	8	13	Chlorosis
	Ethanol	Control (Water)	Green	4	12	Fresh
	Ethanol	Control (Solvent)	Green brown	3	11	Wilting
	Ethanol	Extract 1%	Yellowish green	3	9	Necrosis
	Ethanol	Extract 3%	Brown	4	6	Dead
Spinacia oleracea	n-Hexane	Control (Water)	Green	14	5	Fresh
	n-Hexane	Control (Solvent)	Yellowish green	6	3	Chlorosis,
	n-Hexane	Extract 1%	Green brown	11	3	Wilting
	n-Hexane	Extract 3%	Brown	2	2	Dead
	Acetone	Control (Water)	Green	15	5	Fresh
	Acetone	Control (Solvent)	Brown	4	0	Dead
	Acetone	Extract 1%	Brown	3	3	Wilting
	Acetone	Extract 3%	Brown	3	0	Dead
	Ethanol	Control (Water)	Green	20	6	Fresh
	Ethanol	Control (Solvent)	Yellowish green	16	6	Chlorosis
	Ethanol	Extract 1%	Yellowish green	14	5	Chlorosis
	Ethanol	Extract 3%	Green brown	17	4	Necrosis
Raphanus sativus	n-Hexane	Control (Water)	Green	8	6	Fresh
	n-Hexane	Control (Solvent)	Brown	3	0	Dead
	n-Hexane	Extract 1%	Brown	2	2	Dead
	n-Hexane	Extract 3%	Brown	5	3	Dead
	Acetone	Control (Water)	Green	18	10	Fresh
	Acetone	Control (Solvent)	Yellowish green	8	5	Chlorosis
	Acetone	Extract 1%	Brown	9	5	Dead
	Acetone	Extract 3%	Green brown	10	8	Necrosis
	Ethanol	Control (Water)	Green	17	9	Fresh
	Ethanol	Control (Solvent)	Brown	3	4	Dead
	Ethanol	Extract 1%	Brown	4	5	Dead
	Ethanol	Extract 3%	Brown green	7	4	Dead

Lactuca sativa	n-Hexane	Control (Water)	Green	2	2	Fresh
	n-Hexane	Control (Solvent)	Brown	1	0	Dead
	n-Hexane	Extract 1%	Brown	2	0	Dead
	n-Hexane	Extract 3%	Brown	2	0	Dead
	Acetone	Control (Water)	Green	3	3	Fresh
	Acetone	Control (Solvent)	Yellowish green	3	2	Fresh
	Acetone	Extract 1%	Yellowish green	2	2	Chlorosis
	Acetone	Extract 3%	Yellowish green	3	2	Chlorosis
	Ethanol	Control (Water)	Green	9	7	Fresh
	Ethanol	Control (Solvent)	Yellowish green	3	2	Chlorosis
	Ethanol	Extract 1%	Brown	2	0	Dead
	Ethanol	Extract 3%	Brown	2	0	Dead
Abelmoschus esculentus	n-Hexane	Control (Water)	Green	8	10	Fresh leaves
	n-Hexane	Control (Solvent)	Brown	4	7	Necrosis
	n-Hexane	Extract 1%	Green brown	5	9	Wilting
	n-Hexane	Extract 3%	Brown	6	9	Necrosis
	Acetone	Control (Water)	Green	8	24	Fresh
	Acetone	Control (Solvent)	Green brown	8	22	Necrosis
	Acetone	Extract 1%	Yellowish green	10	15	Chlorosis
	Acetone	Extract 3%	Green	3	13	Fresh
	Ethanol	Control (Water)	Green	8	30	Fresh
	Ethanol	Control (Solvent)	Green	3	20	Fresh
	Ethanol	Extract 1%	Yellowish green	14	20	Chlorosis
	Ethanol	Extract 3%	Yellowish green	10	28	Chlorosis

3.6: Allelopathic potential of *Caralluma tuberculata* extract in different solvents and control (only solvent) on *Spinacia oleracea* L. (as recipient plant) by using pot method

The n-hexane extract of *Caralluma tuberculata* and n-hexane without plant extract showed dramatic changes in the morphology of *Spinacia oleracea*. Under the influence of all three treatments (1%, 3% plant extract and without extract), the leaf color changed from green to brown, number of leaves were reduced from 14 to 2 and length of stem was also reduced from 5cm to 2cm as compared to control plant (Figure 3). Plant health was also adversely affected. The extreme affect was observed when plant was treated with 3% plant extract (n-hexane) as this treatment caused the death of plant.

Changes in the morphology of *Spinacia oleracea* were observed when it was treated with acetone extract of *Caralluma tuberculata* and acetone without plant extract. Under the influence of all three treatments (1%, 3% plant extract and without extract), the leaf color changed from green to brown, number of leaves were reduced from 15 to 3 and length of stem was also reduced from 5cm to 0cm as compared to control plant (Table 3). Plant health was also adversely affected. The extreme affect was observed when plants were treated with 3% plant extract (acetone) and acetone (without extract) as this treatment caused the death of plant. It indicated that acetone itself had a very adverse effect on *Spinacia oleracea.*

The ethanol extract of *Caralluma tuberculata* and ethanol without plant extract showed changes in the morphology of *Spinacia*

oleracea. Under the influence of all three treatments (1%, 3% plant extract and without extract), the leaf color changed from green to green brown, number of leaves were reduced from 20 to 14 and length of stem was also reduced from 6cm to 4cm as compared to control plant (Table 3). Health was also affected in all plants. The extreme affect was observed when plant was treated with 3% plant extract (ethanol) as the plant was wilted and exhibited necrosis by this treatment.

3.7: Allelopathic potential of *Caralluma tuberculata* extract in different solvents and control (only solvent) on *Lactuca sativa* L. (as recipient plant) by using pot method

The n-hexane extract of *Caralluma tuberculata* and n-hexane without plant extract showed morphological changes in *Lactuca sativa*. Under the influence of all three treatments (1%, 3% plant extract and without extract), the leaf color changed from green to brown, number of leaves were reduced from 2 to 1 and length of stem was also reduced from 2cm to 0cm as compared to control plant (Table 3). Plant health was also badly affected in all treatments. The death of all plants occurred due to adverse effect of n-hexane.

The acetone extract of *Caralluma tuberculata* and acetone without plant extract exhibited changes in the morphology of *Lactuca sativa*. Under the influence of all three treatments (1%, 3% plant extract and without extract), the leaf color changed from green to yellowish green, number of leaves were reduced from 3 to 2 and length of stem was also reduced from 3cm to 2cm as compared to control plant (Table 3). Plant health was also affected in all treatments. Plants treated with 1% plant extract (acetone) and 3% plant extract (acetone) showed extreme affects as the leaves showed chlorosis. It indicated that photosynthetic activities were disturbed by these treatments.

Changes in the morphology of *Lactuca sativa* were observed when it was treated with ethanol extract of *Caralluma tuberculata* and ethanol without plant extract. Under the influence of all three treatments (1%, 3% plant extract and without extract), the leaf color changed from green to brown, number of leaves were reduced from 9 to 2 and length of stem was also reduced from 7cm to 0cm as compared to control plant (Table 3). Plant health was also affected in all treatments. Plants treated with 1% plant extract (ethanol) and 3% plant extract (ethanol) showed extreme affects as this treatment caused the death of plant.

3.8: Allelopathic potential of *Caralluma tuberculata* extract in different solvents and control (only solvent) on *Abelmoschus esculentus* L. (as recipient plant) by using pot method

The n-hexane extract of *Caralluma tuberculata* and n-hexane without plant extract showed morphological changes in *Abelmoschus esculentus*. Under the influence of all three treatments (1%, 3% plant extract and without extract), the leaf color changed from green to brown, number of leaves were reduced from 8 to 4 and length of stem was also reduced from 10cm to 7cm as compared to control plant (Table 3). Plant health was also affected in all treatments. The extreme affect was observed when plant was treated with 1% plant extract (n-hexane) as the plant was wilted.

Changes in the morphology of *Abelmoschus esculentus* were observed when it was treated with acetone extract of *Caralluma tuberculata* and acetone without plant extract. Under the influence of all three treatments (1%, 3% plant extract and without extract), the leaf color changed from green to green brown, number of leaves were reduced from 8 to 3 and length of stem was also reduced from 24cm to 13cm as compared to control plant (Table 3). Plant health was also affected in all treatments. The extreme affect was observed when plant was treated with acetone (without extract) as the plant showed necrosis. It indicated that acetone had itself adverse effect on the plant.

The ethanol extract of *Caralluma tuberculata* and ethanol without plant extract showed changes in the morphology of *Abelmoschus esculentus.* Under the influence of all three treatments (1%, 3% plant extract and without extract), the leaf color changed from green to yellowish green and length of stem was also reduced from 30cm to 20cm as compared to control plant (Table 3). Plant health was also affected in all treatments. The extreme affect was observed when plants were treated with 1%

plant extract (ethanol) and 3% plant extract (ethanol) as the leaves showed chlorosis.

3.9: Spectrophotometric Analysis of *Caralluama tuberculata* N.E. Brown

The analysis of *Caralluma tuberculata* using a BD Holo double beam spectrophotometer confirmed the presence of polyphenolic compounds, including phenolics and flavonoids. Total phenolic content, measured using the Folin-Ciocalteu reagent and expressed as gallic acid equivalents, was found to be 2.695 with gallic acid identified as the specific phenolic compound. Flavonoid content, determined by the aluminum chloride colorimetric method and expressed as quercetin equivalents, was 5.464 mg/g with quercetin identified as the specific flavonoid present.

Caralluma tuberculata is a medicinal plant known to contain a diverse range of secondary metabolites, including phenolic compounds, flavonoids, glycosides, and other bioactive constituents (Javed et al., 2025; Sentayehu et al., 2026). Such compounds are often associated with allelopathic activity because they can influence seed germination, seedling growth, nutrient uptake, and physiological processes in neighboring plants Alodaini et al., 2025; Ali et al., 2024; Haider et al., 2022).The present study investigated the allelopathic potential of C. tuberculata using sandwich bioassays, pot experiments, and spectrophotometric analyses.

The results of the sandwich bioassay demonstrated that dried plant material of *C. tuberculata* inhibited germination and seedling growth of the tested vegetable crops in a concentration-dependent manner. Greater reductions in radicle and hypocotyl growth were generally observed at 0.10 g compared with 0.05 g of plant material (Table 2). These findings are consistent with previous reports showing that allelochemicals released from plant tissues can suppress seed germination and early seedling development by interfering with cell division, membrane permeability, nutrient absorption, and enzymatic activity(Dar et al., 2025; Fadiji et al., 2025;Sun et al., 2026).

Among the tested species, Raphanus sativus and Spinacia oleracea exhibited greater sensitivity to the treatments, whereas Abelmoschus esculentus showed comparatively higher tolerance. In some treatments, a slight stimulatory response was observed in the hypocotyl growth of A. esculentus. This response may represent a hormetic effect, a phenomenon in which low concentrations of biologically active compounds stimulate growth while higher concentrations become inhibitory. Similar hormetic responses have been reported in studies(Ullah et al., 2022; Ishaq et al., 2023) involving plant-derived allelochemicals and environmental stress factors.

The pot experiments further supported the phytotoxic potential of C. tuberculata extracts. Seedlings exposed to plant extracts exhibited chlorosis, necrosis, wilting, reduced leaf production, stunted growth, and, in some cases, plant mortality. These symptoms are commonly associated with physiological stress, disruption of photosynthetic processes, oxidative damage, and altered cellular metabolism. The severity of these symptoms generally increased with extract concentration, suggesting a dose-dependent response.

However, an important observation of the present study was that solvent controls also produced inhibitory effects in several crop species. In particular, n-hexane and acetone controls caused chlorosis, growth suppression, necrosis, and even plant death in some treatments. This finding indicates that part of the observed inhibition may have resulted from solvent-induced phytotoxicity rather than solely from plant-derived allelochemicals. Therefore, although the extracts generally produced stronger inhibitory effects than their corresponding solvent controls, the contribution of solvent toxicity cannot be completely excluded.

The spectrophotometric analyses revealed the presence of phenolic and flavonoid compounds in C. tuberculata extracts. Total phenolic content was expressed as gallic acid equivalents (GAE), whereas total flavonoid content was expressed as quercetin equivalents (QE). These results support the possibility that phenolic and flavonoid constituents contributed to the observed phytotoxic effects. Phenolic compounds have been reported to inhibit seed germination and seedling growth through interference with cellular respiration, nutrient

uptake, membrane integrity, and photosynthetic activity. Similarly, flavonoids may affect hormone regulation, enzymatic activity, and oxidative balance in plants.

Although the results indicate considerable phytotoxic and potential allelopathic activity of C. tuberculata, caution is required when interpreting these findings. The present study relied on crude extracts and spectrophotometric estimation of total phenolic and flavonoid contents, and therefore the specific allelochemicals responsible for the observed effects were not identified. Furthermore, the inhibitory effects of solvent controls highlight the need for improved extraction and application procedures in future investigations.

The ecological implications of these findings are noteworthy. As interest in the cultivation and commercial utilization of C. tuberculata continues to increase, understanding its interactions with neighboring plant species becomes increasingly important. The observed inhibitory effects suggest that the species may influence the establishment and growth of nearby crops under certain conditions. Consequently, careful evaluation should be undertaken before incorporating C. tuberculata into intercropping systems or mixed agricultural production systems.

Future research should focus on the isolation, identification, and characterization of individual allelochemicals responsible for the observed phytotoxic effects. Advanced analytical techniques such as HPLC, LC–MS, and GC–MS may provide valuable information regarding the chemical constituents involved. Additional field-based investigations are also required to determine whether the inhibitory effects observed under laboratory and greenhouse conditions occur under natural agricultural environments.

Overall, the findings suggest that *Caralluma tuberculata* possesses phytotoxic properties that may be associated with its phenolic and flavonoid constituents. However, because solvent controls also demonstrated inhibitory effects, the results should be interpreted as evidence of potential allelopathic activity rather than definitive proof of allelopathy. Further chemical and field investigations are necessary to confirm the mechanisms underlying these interactio (Anh and Xuan, 2025; Wang et al., 2025). Ali et al., 2024; Sun et al., 2026; Dar et al., 202; Fadiji et al., 2025; Agathokleous et al., 2020).

Caralluma tuberculata is a medicinal plant known to contain a diverse range of secondary metabolites, including phenolic compounds, flavonoids, glycosides, and other bioactive constituents, which are responsible for many of its pharmacological properties (Alodaini et al., 2025; Ali et al., 2024; Haider et al., 2022). Such compounds are often associated with allelopathic activity because they can influence seed germination, seedling growth, nutrient uptake, and physiological processes in neighboring plants (Dar et al., 2025; Sun et al., 2026). The present study investigated the allelopathic potential of C. tuberculata using sandwich bioassays, pot experiments, and spectrophotometric analyses.

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Among the tested species, Raphanus sativus and Spinacia oleracea exhibited greater sensitivity to the treatments, whereas Abelmoschus esculentus showed comparatively higher tolerance. In some treatments, a slight stimulatory response was observed in the hypocotyl growth of A. esculentus. This response may represent a hormetic effect, a phenomenon in which low concentrations of biologically active compounds stimulate growth while higher concentrations become inhibitory. Similar hormetic responses have been widely reported in plant stress biology and allelopathy studies (Agathokleous et al., 2020).

The pot experiments further supported the phytotoxic potential of C. tuberculata extracts. Seedlings exposed to plant extracts exhibited chlorosis, necrosis, wilting, reduced leaf production, stunted growth, and, in some cases, plant mortality. These symptoms are commonly associated with physiological stress, disruption of photosynthetic processes, oxidative damage, and altered cellular metabolism. The severity of these symptoms generally increased with extract concentration, suggesting a dose-dependent response (Sun et al., 2026; Dar et al., 2025).

However, an important observation of the present study was that solvent controls also produced inhibitory effects in several crop species. In particular, n-hexane and acetone controls caused chlorosis, growth suppression, necrosis, and even plant death in some treatments. This finding indicates that part of the observed inhibition may have resulted from solvent-induced phytotoxicity rather than solely from plant-derived allelochemicals. Therefore, although the extracts generally produced stronger inhibitory effects than their corresponding solvent controls, the contribution of solvent toxicity cannot be completely excluded.

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Although the results indicate considerable phytotoxic and potential allelopathic activity of C. tuberculata, caution is required when interpreting these findings. The present study relied on crude extracts and spectrophotometric estimation of total phenolic and flavonoid contents, and therefore the specific allelochemicals responsible for the observed effects were not identified. Furthermore, the inhibitory effects of solvent controls highlight the need for improved extraction and application procedures in future investigations.

The ecological implications of these findings are noteworthy. As interest in the cultivation and commercial utilization of C. tuberculata continues to increase, understanding its interactions with neighboring plant species becomes increasingly important. The observed inhibitory effects suggest that the species may influence the establishment and growth of nearby crops under certain conditions. Consequently, careful evaluation should be undertaken before incorporating C. tuberculata into intercropping systems or mixed agricultural production systems (Riaz et al., 2026).

Furthermore, the increasing demand for medicinal plants has led to overexploitation of wild populations, threatening their long-term survival (Aftab et al., 2025; Takubessi et al., 2025). Cultivation of C. tuberculata can help reduce harvesting pressure on natural populations while ensuring a sustainable supply of valuable bioactive compounds (Ali et al., 2022; Iftkhar et al., 2026). However, its potential allelopathic effects should be carefully considered before large-scale agricultural integration.

Future research should focus on the isolation, identification, and characterization of individual allelochemicals responsible for the observed phytotoxic effects. Advanced analytical techniques such as HPLC, LC–MS, and GC–MS may provide valuable information regarding the chemical constituents involved. Additional field-based investigations are also required to determine whether the inhibitory effects observed under laboratory and greenhouse conditions occur under natural agricultural environments.

Overall, the findings suggest that *Caralluma tuberculata* possesses phytotoxic properties that may be associated with its phenolic and flavonoid constituents. However, because solvent controls also demonstrated inhibitory effects, the results should be interpreted as evidence of potential allelopathic activity rather than definitive proof of allelopathy. Further

chemical and field investigations are necessary to confirm the mechanisms underlying these interactions.

Limitation of the study: Significant phytotoxic effects were also observed in solvent controls (n-hexane, acetone and ethanol), indicating that solvent toxicity may have contributed to some inhibitory responses. Therefore, allelopathic effects of *Caralluma tuberculata* should be interpreted with caution, and future studies should employ solvent-free bioassays and chemical characterization of active allelochemicals.

Conclusion

The allelopathic potential of *Caralluma tuberculata* N.E.Brown was evaluated using sandwich and pot methods. In the sandwich method, 0.05 g and 0.1 g of plant material suppressed radicle and hypocotyl growth of *Raphanus sativus* L., *Spinacia oleracea* L., *Cucumis sativus* L., and *Abelmoschus esculentus* L. in a dose-dependent manner, except for the hypocotyl of *A. esculentus*. Increasing concentrations inhibited germination and growth of all test plants, indicating a strong negative allelopathic effect, making it unsuitable for use with agricultural crops or as a bioherbicide. In the pot method, n-hexane, acetone, and ethanol extracts (1% and 3%) also inhibited growth and morphology of *Lactuca sativa* L., *R. sativus* L., *S. oleracea* L., *C. sativus* L., and *A. esculentus* L., with solvents alone showing some inhibitory effects. Overall, *C. tuberculata* has strong allelopathic activity and could be cultivated for biochemical extraction for herbal drug preparation while conserving wild populations.

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The authors declare that any artificial intelligence (AI)-assisted technologies used during the preparation of this manuscript were employed solely to improve language, grammar, readability, or formatting. All scientific content, including the study design, data collection, analysis, interpretation of results, and conclusions, was conceived, verified, and approved by the authors. The authors accept full responsibility and accountability for the accuracy, originality, and integrity of the manuscript.

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