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Assessment of allelopathic potential of *Tinospora cordifolia* (Willd.) Hook. f. and Thoms leaf extract on growth and biochemical constituents of *Brassica oleracea* var. capitata

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Abstract

By releasing phytochemicals during the breakdown of its litter, *Tinospora cordifolia* (Willd.) Hook. f. and Thoms (Ait). R.Br. is known to have an impact on the growth of nearby plants. The goal of this study is to evaluate the allelopathic effects of *Tinospora cordifolia* (Willd.) Hook. f. and Thoms aqueous leaf extraction on seed germination rate, primary growth and biochemical alterations of *Brassica oleracea* var. capitate. Dry mature leaves of the *Tinospora cordifolia* converted into aqueous leaf extracts at concentrations of 0.1%, 0.2%, 0.3%, 0.4%, and 0.5% for the study, with tap water serving as the control. *Brassica oleracea* var. capitate seeds were incubated in various leaf extract concentrations of *Tinospora cordifolia* R. BR, and germination data was recorded after 15 days, 30 days, and 45 days of extract exposure. The seedlings were collected, and their biochemical features, such as their carbohydrate, chlorophyll, proline, and phenol contents, were measured, along with their fresh weights of root and shoot. The extraction T1, T2, and T3 treatments had no detrimental effects on germination or phenotypic outcomes. Chlorophyll, and total carbohydrate content levels were all observed to be stimulated in T3. Stress-relieving substances like total phenol and free proline decreased at lower concentrations (T3) that seemed suited for *Brassica oleracea* var. capitata. Growth that was optimal and healthy was seen in T3-treated plants. The findings point to a potential use of *Tinospora cordifolia* aqueous leaf extract for growth enhancement of *Brassica oleracea* var. capitata.

Key words : Allelopathy, Biochemical constituents, *Tinospora cordifolia, Brassica oleracea* var. capitata.

Crop plants are continuously in competition with weeds, which significantly reduces their output[6]. Weeds can reduce crop output by an average[6] of 34%. The following essential crops could have their yields reduced by weeds: wheat 23%, soybeans 37%, rice 37%, maize 40%, cotton 36%, and potatoes[6] 30%. For the agricultural plants, they serve as a barrier against the elements of nutrients, space, light, and moisture. Thus, the presence of weeds has a deleterious impact on crop growth and physiological activity[14]. Additionally, they impair crop quality, block streams, harm people's health, and are ugly in amenity areas like gardens, parks, footpaths, and pavements, among other things[16]. Weeds affect crop production and are unsightly, but they also pose a fire risk[15,18]. Insects and viruses live permanently on weeds, making it more difficult to control them[15]. Consequently, weeds have been recognised as significant plant pests from antiquity[20]. Since crop plants have been domesticated, weeds have always been a problem, necessitating the use of weed management methods[12,20]. The list of traditional weed-control techniques includes hand-pulling, chopping, and physically covering weeds [12,18]. A member of the Asclepiadaceae family and usually referred to as "Aakawa," *Tinospora cordifolia.* (Willd.) Hook. f. and Thoms is an erect perennial shrub found in tropical and subtropical areas all over the world. The Aligarh District of Uttar Pradesh, India, has a wide ecological distribution in many of its sections, which primarily demonstrates its high prevalence and intrusion from around farming land and fields.

Brassica oleracea var. capitata were purchased from a local market in the Aligarh District of Uttar Pradesh, India. They were then carefully washed with deionized water before being surface sterilized with a 0.1% $HgCl_2$ solution for 1 minute with continuous shaking.

Fresh *Tinospora cordifolia* leaves were gathered from a male tree in Aligarh. The leaves were shade dried for 10 days before being ground into a fine powder in a lab mill. For future use, the powder was kept in an airtight container. *Tinospora cordifolia* leaves weighing 0.1, 0.2, 0.3, 0.4, and 0.5 grammes each were dissolved in 100 cc of water, heated for 24 hours at 70 degrees Celsius, cooled, and filtered before being utilised for further processing. Tap water served as the standard (C).

A piece of filter paper was placed on top of a set of clean, disinfected Petri dishes. *Brassica oleracea* var. capitata seeds were counted and placed on the filter sheets of each petri dish, making sure that the seeds did not touch. The filter paper in the Petri dishes was wet by the various concentrations of the *Tinospora cordifolia* filtrate. Then, in order to prevent drying out and infection, parafilm was placed over each petri dish. Plants were grown in pots using tap water as the control. On alternating days, the filtrate was applied to the pots (T1-T5) to reach field capacity. For the purpose of measuring various morphological and biochemical traits, plant samples were taken on days 15, 30, and 45. Three replications of each treatment, including the control, were carried out, including germination. The samples were examined for morphological characteristics such as the percentage of germination, shoot and root length, and shoot and root weight. Anthrone and sulfuric acid were used to estimate the carbohydrate content. For the analysis of total carbs, a sample weighing 0.5 g was hydrolyzed in $4NHCl_2$ for two hours in a water bath. Additionally, two hours were spent extracting 0.5 g of dry material using distilled water. The Anthrone sulphuric acid technique was used to filter and complete both extracts to a specific volume. The data were computed using a calibration curve created using pure glucose. Chlorophyll from leaves was extracted and quantified according to Arnon's method from 1949. Using the Folin-Ciocalteau reagent, the total water-soluble phenolics were calculated in accordance with the procedure. The Ninhydrin technique was used to measure the free proline content in leaves. One-way analysis of variance (ANOVA) and Duncan's multiple range test were used in the statistical study (DMRT). The results are shown as the mean (average deviation) for 3 samples in each category. P values 0.05 and lower were regarded as significant.

In the current study, the allelopathic potential of *Tinospora cordifolia* leaf extracts against Brassica oleracea var. capitate was examined at various concentrations. Both the allelopathy impact and seedling growth stages were examined. *Tinospora cordifolia* leaf extracts were found to have polyphenols that closely correlated with scavenging and overall antioxidant activity[17]. Variation in seed germination rate was noted. As extract concentration increased, the rate of seed germination dropped (Table-1). Similar to our findings, charlock with *Glaucium* species, soybean and chive with ginger, sesame, corn, sorghum, and sunflower with mungbean were all associated with inhibition of seed germination[10]. T4 and T5 had the lowest germination rates in comparison to the control, and the result was determined to be significant at p 0.001. Reigosa *et al.*[15] assumed that allelopathic chemicals have concentration-dependent effects on a variety of physiological systems at once. High plant extract concentrations also slowed down seed germination time. This is clear from the results, which show that 100% of the seeds in the control, T2 and T3 sprouted on the second day itself, whereas in treatments, seed germination was significantly delayed as plant extract concentration increased. (Table-1). This conclusion is consistent with those of Khaliq *et al.*,[8], who noted delayed seed germination in rice treated with sorghum, sunflower, and brassica crop wastes.

Brassica oleracea var. capitata's root and shoot experienced a stimulatory response from treatment with aqueous *Tinospora cordifolia* leaf extract at a lower concentration of (0.3%). When compared to the corresponding control at each growth stage, the level of promotion in all growth parameters was more pronounced in the shoot than the root (Table 2). However, contrasted to the control, raising the level of T5 to 0.5% resulted in a considerable reduction in shoot and root length while only mildly stimulating the length, fresh weight, and dry weight of the shoot of 15, 30, and 45-day-old plants (Table-2). The findings of Jayakumar *et al.*, 1990, who demonstrated that irrigation of peanuts and maize with 5, 10, 15, and 20% water extract of abscised Eucalyptus globulus leaf significantly reduced plant height, are in agreement with this finding. By increasing the incorporation rate of Acacia nilotica leaf residue from 0.25 to 0.5% (w/w), Abu El-Soud *et al.*[1] showed stimulation in pea growth parameters, but gradual inhibition from 1.5 to 2% (w/w). Reigosa *et al.*,[15] assumed that allelopathic chemicals have concentration-dependent effects on a variety of physiological systems at once. The amount of carbohydrates at T3 concentration was considerably impacted by the allelopathy therapies (Figure 1). At T3, the carbohydrate content rise was at its highest. Beyond this point, the quantity of carbohydrates frequently reduced (Figure 1). El-Darier *et al.*, 2002 obtained the same results. At T5, there was a lower minimum carbohydrate content than in the control. A notable increase in chlorophyll content was seen in the current investigation at lower dosages of aqueous *Tinospora cordifolia* leaf extract administered (Figure 2). The leaf's chlorophyll concentration exhibited a similar pattern to that of carbohydrates. With the application of minerals in barley, Johnson *et al.*[7] showed a considerable increase in photosynthetic pigment, particularly chlorophyll a and carotenoids. When *Brassica oleracea* var. capitate seedlings were exposed to T4 and T5 concentrations of *Tinospora cordifolia* aqueous leaf extract, respectively, the chlorophyll content was decreased by 41% and 48%. (Fig. 2).

Based on the findings of this study, we may hypothesise that at T2 and T3, the capacity of the plants to accumulate chlorophyll, which is a crucial step in the production of food through photosynthesis, increased root and shoot length, fresh and dry weight, and other traits in both examined species. Lower dosages of *Tinospora cordifolia* leaf extract (0.3%) that appeared to be suitable for P. aureus were administered in the current study, however these levels resulted in a decrease in stress-preventing component free proline at the optimal concentration (T3). Acceptor plants' known reaction to allelochemical stress is metabolic alterations that result in an increase in the manufacture of phenolic compounds[13]. The lowest free proline concentrations of P. aureus were found at T3 and decreased by 13.15 and 71.92% over the control, respectively, showing that the beneficiary plant is not under stress. Higher extract application rates (0.4 & 0.5%) led to higher free proline concentrations (Figure 5). According to Tolulope *et al.* (2016), treatment with shoot extract at higher concentrations caused *V. unguiculata* proline levels to rise noticeably. Additionally, T. diversifolia leaf extract was found to stimulate the formation of phenolic compounds in rice plants[4]. The release and potency of allelochemicals, as well as biosynthesis, may all be impacted by environmental factors[5].

Table-1. Effect of aqueous *Tinospora cordifolia* (Willd.) Hook. f. and Thoms leaf extract on Seed Germination of *Brassica oleracea* var. capitata

Treatment (n=40)	Seeds germinated	Seeds not germinated
Control	26	8
T1	24	7
T2	25	7
T3	27	6
T4	23	9
T5	22	10

Insights into the mechanisms may help to reduce the use of synthetic pesticides and herbicides by allowing for the use of some

Table-2. The effect of aqueous *Tinospora cordifolia* (Willd.) Hook. f. and Thoms leaf extract on morphological parameters of *Brassica oleracea* var. capitata

			Fresh	Dry	Fresh	Dry
Control	13.4±0.57	10.7±0.28	0.08±0.39	0.007±0.003	0.16±0.02	0.02±0.03
0.1%	13.1±0.54	10.1±0.21	0.095±0.70	0.005±0.004	0.17±0.04	0.03±0.05
0.2%	13.2±0.53	10.4±0.32	0.12±0.45	0.007±0.07	0.17±0.06	0.03±0.02
15 0.3%	15.4±0.75	11.5±0.18	0.15±0.041	0.01±0.09	0.2±0.04	0.04±0.04
0.4%	12.3±0.23	10.8±0.27	0.13±0.043	0.008±0.002	0.16±0.09	0.02±0.04
0.5%	12.1±0.22	10.6±0.23	0.11±0.32	0.006±0.06	0.14±0.08	0.02±0.04
Control	4.9±0.30	16.6±0.23	0.38±0.04	0.17±0.02	0.4±0.06	0.08±0.04
0.1%	4.8±0.28	16.8±0.34	0.41±0.09	0.18±0.05	0.43±0.12	0.085±0.05
0.2%	5.3±0.26	17.1±0.31	0.44±0.08	0.18±0.02	0.44±0.04	0.089±0.1
30 0.3%	5.8±0.29	17.9±0.29	0.5±0.02	0.2±0.04	0.5±0.04	0.11±0.07
0.4%	5.2±0.38	17.2±0.26	0.46±0.09	0.16±0.01	0.39±0.11	0.078±0.11
0.5%	5.1±0.34	16.9±0.22	0.42±0.03	0.14±0.02	0.37±0.07	0.07±0..09
Control	5.7±0.37	25.6±0.21	0.71±0.09	0.24±0.02	1.07±0.19	0.26±0.03
0.1%	5.1±0.29	26.2±0.25	0.72±0.08	0.26±0.07	1.09±0.22	0.27±0.05
0.2%	5.6±0.27	26.6±0.32	0.76±0.09	0.27±0.05	1.16±0.31	0.27±0.08
45 0.3%	6.8±0.32	27.0±0.26	0.8±0.11	0.3±0.04	1.2±0.14	0.31±0.03
0.4%	5.4±0.43	26.4±0.31	0.75±0.18	0.25±0.09	1.07±0.21	0.25±0.14
0.5%	5.3±0.31	26.2±0.34	0.73±0.21	0.23±0.10	1.05±0.27	0.23±0.11

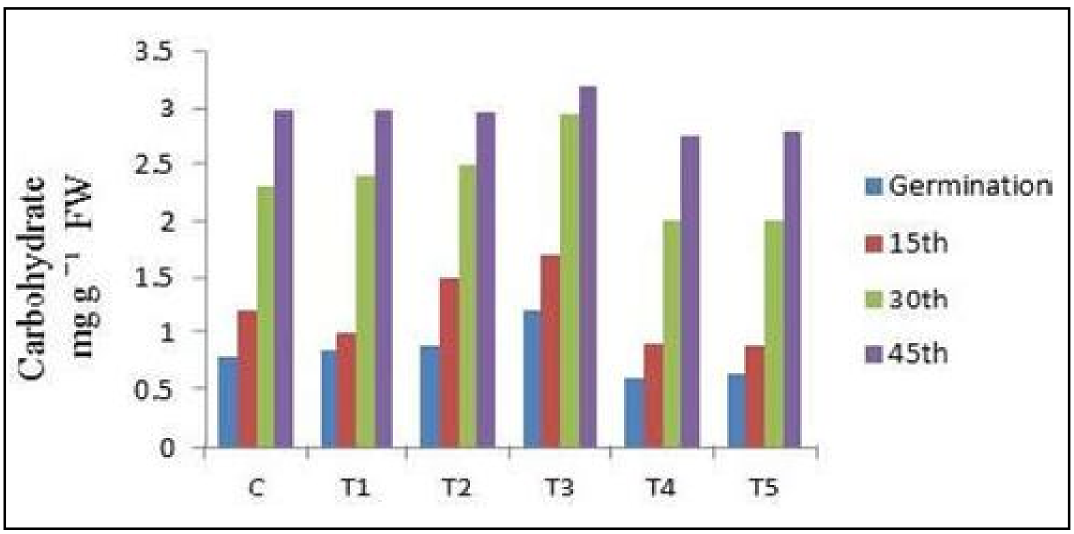

Fig. 1. Effect of *Tinospora cordifolia* (Willd.) Hook. f. and Thoms leaf extract on carbohyderate of *Brassica oleracea* var. capitata.

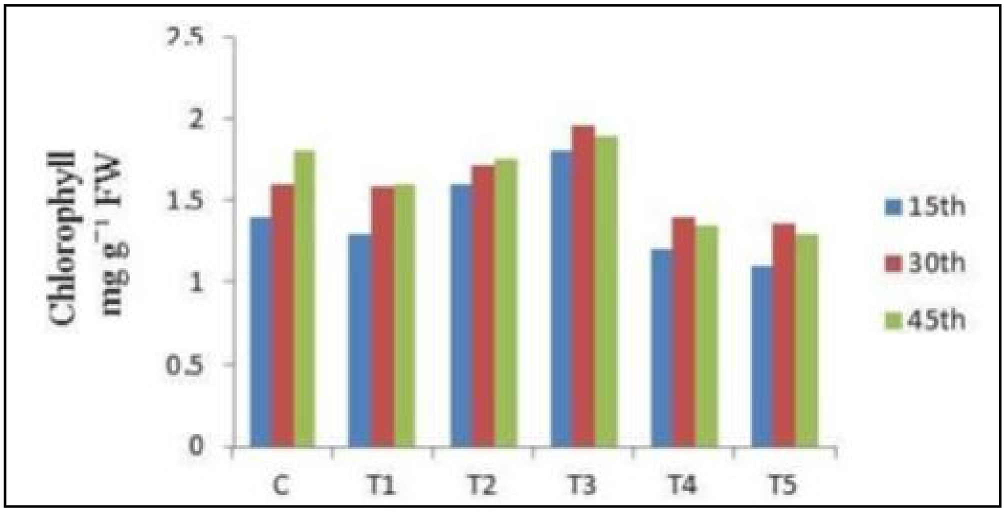

Fig. 2. Effect of *Tinospora cordifolia* (Willd.) Hook. f. and Thoms leaf extract on chlorophyll of *Brassica oleracea* var. capitata

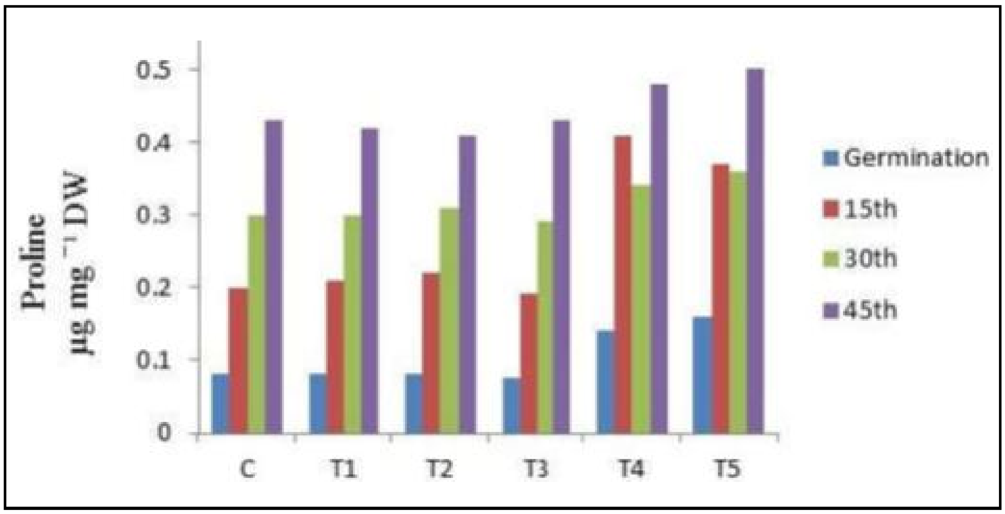

Fig. 3. Effect of *Tinospora cordifolia* (Willd.) Hook. f. and Thoms leaf extract on proline of *Brassica oleracea* var. capitata

plant chemicals as natural pesticides, the use of plant residues with allelopathic qualities, or cultivation with under plantings[9]. Aziz Ahmed Ujjan *et al.*[2] conducted a thorough study on the insecticidal activity of *Tinospora cordifolia* leaf extract against mustard aphid. The current study's findings unmistakably show that *Brassica oleracea* var. capitata plant seedlings respond differently to varied concentrations of *Tinospora cordifolia* leaf extract. The plant, however, showed good development at lower T3 concentrations, as shown by the morphological and biochemical traits.

The allelochemicals in *Tinospora cordifolia* leaf extract had a substantial impact on Phaseolus aureus growth, *Brassica oleracea* var. capitata biochemical parameters, and seed germination. Further investigation into the use of *Tinospora cordifolia* leaf extract as natural insecticides without harming the growth of the beneficiary plants may be based on the discovery that the plants are pest resistant, which is a cost-effective process.

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