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Evaluation of allelopathic effect of *Tinospora cordifolia* on biochemical activity of some weeds

K.M. Abdul Raoof* **and M. Badruzzaman Siddiqui**

Department of Botany, Aligarh Muslim University, Aligarh, 202002, India

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Abstract A study was conducted to assess the magnitude of suppressing ability of *Tinospora cordifolia* (Willd.) Miers on some selected weeds of Aligarh namely, *Chenopodium album* L., *Chenopodium murale* L., *Cassia tora* L. and *Cassia sophera* L. The research finding was conducted to evaluate the effect of aqueous leaf leachate and organic fractions of donor plant on biochemical activities (carbohydrate content, chlorophyll content and protein content). The result showed that aqueous leachate and organic fractions reduced the level of biochemical activities. Carbohydrate content was increased in treated plants compared to control while chlorophyll content and protein content were reduced as compared to control. Aqueous leachate showed maximum toxicity on weeds than organic fractions. The reduction may be due to the allelochemicals present in *T. cordifolia* leaves.

Key words: Allelochemicals, aqueous leachate, chlorophyll, *Tinospora cordifolia*, toxicity.

Introduction

Weeds are the major enemy to the crop plants and create harmful effects on agricultural crops due to several factors such as competition for space, light and nutrients. Organic chemicals released as leaf leachates, root exudates affect the crop plants. Weeds species are considered as rich source of secondary metabolites (Allelochemicals) and these chemicals improve a certain kind of environmental system on other plants growing in their vicinity and the phenomenon known as allelopathy (Nandal *et al*., 1994). Allelopathy is a potential field of research all over the world. Few researchers consider only the deleterious interactions as allelopathy, while, the latest thinking includes allelopathy to both harmful and beneficial interactions between the plants (Rizvi *et al*., l986). *T. cordifolia* (neem giloy) is a deciduous climbing shrub usually climbs on neem and mango trees. It is found throughout the tropical India. It is a medicinal plant used to cure various diseases.

* **Correspondent author**: K.M. Abdul Raoof; **e-mail**: abdulraoofkm@gmail.com

The present study was to evaluate the aqueous leaf leachate and organic fractions effect on biochemical activity of some weed plants (*Chenopodium album, C. murale, Cassia tora* and *C. sophera*).

Materials and methods

Collection of material

Leaves of *Tinospora cordifolia* were collected locally from AMU campus and Aligarh. Healthy and freshly collected leaves were clearing surface cleaned, dried and powdered. Seeds of the weed species, *Chenopodium album, Chenopodium murale*, *Cassia tora* and *Cassia sophera* were collected from the agricultural field and road sides of the Aligarh Muslim University, Aligarh, U.P., India. Leaves of *T. cordifolia* Collected from the premises of Aligarh Muslim University campus.

Preparation of leachable allelochemical

Based on the methods devised by Kumari *et al.* (1985) healthy and freshly collected leaves of *Tinospora cordifolia* were cut roughly in to pieces after clearing their surface and their dry weight per unit fresh weight were determined by desiccating the tissue in the oven. The weighed amount of fresh leaf pieces of the plant was soaked in requisite amount of pure for a period of 20hrs at room temperature. It was filtered completely through double layer of muslin cloth followed by whatman No. 1 filter paper and the requisite concentration was made with distilled water. One half of this filtrate referred to as the aqueous leachates was used as such, while the other part was chilled and subjected to acid hydrolysis using pre-chilled, 3N HCl. The precipitates so formed were recovered through centrifugation (2000rpm). These were washed 5-6 times with pure water. Every time the recovery was made through mild centrifugation. For experimental purpose, requisite amount of the precipitate was dissolved in a few drop of ethyl alcohol and the final volume was made with pure water. A drop of twenty 20 was added to it, to serve as surfactant. This is referred to as aglycone or aglyconic or organic component of aqueous leachates.

Extraction of organic fraction

The healthy leaves of the donor plant were freshly collected, surface cleaned, then dried under shade and powdered. The powder was immersed in Petroleum ether (60°-80°C) for 20hrs. The liquid was separated from the residue (marc), through mild centrifugation (500rpm for 2 min). From the liquid portion the solvent (Petroleum ether) was recovered on a hot water bath. Requisite amount of the residue so obtain was weighed and a few drops of xylene, a part from a drop of tween- 20 (to act as surfactant) were added to it. Final volume was made with pure water. This was termed Petroleum ether fraction.

The marc (residue from petroleum ether suspension) was suspended in methanol for 20hrs and filtered, from one half of the filtrate, methanol was covered on a hot water bath. The residue, so obtained was dissolved in a drop of methanol and the final volume was made with pure water. It has been called Methanol fraction. From another half of methanol filtrate the solvent was removed and the residue was partitioned between chloroform and water (1:1 V/V). The two layers so formed were separated in a separating funnel. The chloroform was recovered over a hot water bath. To the requisite amount of residue a few drops of methanol were added and the final volume was made with pure water. This has been termed as the chloroform fraction. The water from the aqueous layer after separating chloroform fraction was dried under low pressure on a rotary flash evaporator. The solution made with water has been termed as the Water fraction.

Treatment to mature Plants

For the estimation of various macro-molecular content (carbohydrate contents, protein content and chlorophyll content), 9 plants for each treatment were sprayed with 100 ml, of the treatment solution per plant daily for five days. On the sixth day, the estimation of the carbohydrate content, protein content and chlorophyll content was done from the freshly plucked leaves.

Determination of carbohydrate content

The methodology employed by Loweus (1952) was followed for this purpose. *(a) Water-Soluble Carbohydrates*- To 5 mg dry powdered material was added 5 ml. of pure water. It was kept in boiling water bath for 5 min and centrifuged; the supernatant was used as water soluble fraction (WSF). *(b) Acid Soluble Carbohydrates*-To the residue left as above was added 5 ml of 6 N HCl. This was kept in a boiling water bath for 20 min and centrifuged. The supernatant was used as acid-soluble fraction (ASF).

Estimation of chlorophyll content

The total chlorophyll content from leaves of treated or control plants were extracted in Di-methyl Sulphoxide (DMSO) following the method of Hiscox and Israelstam (1979). Finely cut uniform discs (100 mg fresh weight) were made fully expanded leaves of test plants. Dry weight equivalents of each of the treated samples were determined by keeping 100 mg fresh weight discs in an oven. The weighted material (100 mg. fresh weight leaf disc) was suspended in 10 ml of Di-methyl sulphoxide (DMSO) incubated at 65°C for one hour (the period of incubation was found sufficient for the complete extraction of chlorophyll). The DMSO was recovered by thorough decantation. The final volume was corrected to 10 ml with fresh DMSO. The extinction of chlorophyll thus recovered in DMSO was measured at dual wave –length of 645 and 663 nm on spectro-photometer against DMSO as blank. The extinction values were read and the amount of chlorophyll was calculated according to the equation given by Arnon (1949), with modification by Hiscox and Israelstan (1979).

Estimation of total soluble proteins

The method as given by Lowry *et al*. (1951) was adopted for this purpose.

Statistical Analysis

The data from the experiment samples were analyzed by one-way analysis of variance and mean were separated at $P < 0.05$ by Duncan Multiple Range Test (Duncan, 1955).

Result and discussions

The plant Allelo-chemicals may interfere the growth, development and performance of a plant through their direct or indirect action on metabolism of plants. Many physiological and chemical activities altered by these chemical interference. Nevertheless, it is difficult to determine the primary mechanism involved for the actions of these chemicals. It is believed that a variety of mechanisms of action must exist for different allelo-chemicals or herbicides.

Even a specific compound may affect several metabolic functions and as a result, it is seldom possible to sort out primary effects from the secondary ones.

In addition, the uncertainty in interpreting the observed effects in isolated enzymes to other bio-chemical effects in intact plant system also exists. No doubt, allelo-chemicals or herbicides (natural or synthetic) act on plants though enzymatically controlled reactions.

One thing is very clear from the result of this experiment that *T. cordifolia* leaves exert a very negative influence on the acid soluble and water soluble carbohydrates of weeds, *C. album, C. murale, C. tora* and *C. sophera.* It is very well depicted by an increased amount of carbohydrates content exerts its influence mainly through its aqueous leachates. Effect of different treatments on carbohydrate content in the following order AL>CF>PF>WF>MF (Table. 1). Synthesis of carbohydrates takes place in the living tissue. An increased amount of carbohydrates points out to the fact that the plant is under stress and it is gathering up its energy reserves to meet any conditions of adversity.

Table 1. Effect of aqueous leachates and organic extract fractions derived from the leaves of *T. cordifolia* on total carbohydrate content of the leaves of *Chenopodium album* L., *Chenopodium murale* L., *Cassia tora* L. *and Cassia sophera* L.

Treatment Solutions	*C. album*		*C. murale*		*C. tora*		*C. sophera*	
	Acid soluble carbohydrates (mg/gdry wt.)	Water soluble Carbohydrate (mg/g dry wt.)	Acid soluble carbohydrate (mg/gdry wt.)	Water soluble Carbohydrate (mg/g dry wt.)	Acid soluble carbohydrates (mg/gdry wt.)	Water soluble Carbohydrates (mg/g dry wt.)	Acid soluble carbohydrates (mg/gdry wt.)	Water soluble Carbohydrates (mg/g dry wt.)
Control	51.45 ± 0.65	56.12 ± 0.31	37.63 ±0.18	39.21 ±0.72	54.47 ± 0.36	56.79 ± 0.49	52.83 ± 0.57	67.83 ± 0.33
Aqueous leachates AL (1%g/ml fresh wt.)	77.45 ± 0.12	79.99 ± 0.37	74.33 ±0.67	69.56 ±0.88	73.37 ± 0.61	85.04 ± 0.67	84.37 ± 0.52	87.85 ± 0.56
Petroleum ether Fraction PF(0.09%w/v)	68.34 ± 0.15	70.11 ± 0.15	67.43 ±0.52	71.82 ±0.68	63.96 ± 0.51	78.27 ± 0.31	76.31 ± 2.57	79.84 ± 2.65
Methanolic fraction MF(0.09%w/v)	53.63 ± 0.92	66.52 ± 0.39	69.16 ±0.43	68.80 ±0.76	45.12 ± 0.35	57.66 ± 0.66	68.02 ± 1.53	80.05 ± 1.14
Chloroform fraction CF(0.09%w/v)	76.12 ± 0.29	80.02 ± 0.61	68.12 ±0.93	73.15 ±0.13	71.19 ± 0.92	79.78 ± 0.87	53.32 ± 0.52	79.38 ± 0.43
Water fracrtion WF(0.09%w/v)	65.71 ± 0.54	60.45 ± 0.37	45.13 ±0.24	48.36 ±0.20	64.29 ± 0.38	84.79 ± 0.17	67.21± 0.81	69.67 ± 2.32
LSD at 5%	6.28	6.59	5.92	5.98	5.96	7.10	6.55	7.39
LSD at 1%	8.94	9.38	8.42	8.51	8.48	10.10	9.32	10.51

± represents standard deviation

Recently, there has been increased in research on the role of the demand for photo-assimilates in regulating photosynthesis through changes in carbohydrate partitioning and accumulation under stress condition (Paul and Foyer, 2001; Paul and Driscoll, 1997; Nielsen *et al*., 1998; Osmond *et al*., 1987; Levitt, 1982).

The protein content and chlorophyll content was also reduced as compared to control (Table 2 and 3). Aqueous leachate and organic fraction shows different level of inhibition on different weeds. In *C. album* maximum inhibition was seen in aqueous leachate treatment. The reduction in the chlorophyll content in this experiment may be due to the reason that allelochemicals either inhibit the synthesis of chlorophyll or perhaps they break – down the chlorophyll, molecule by acting on the pyrrolic ring and the phytol chain (Blum *et al*., 1985; Colton and Einhelling, 1980; Yang *et al*., 2006). Hence the allelo-chemicals act by inhibiting the process of photosynthesis which ultimately can lead to the death of plant. Allelopathic effect of *Croton bonplandianum* cause reduction in chlorophyll content was reported by Sarkar and Chakraborty (2010) on *T. aestivam* and *Brassica campestris.* Liu *et al*. (2009) in tomato and Abu-Romman *et al*. (2010) in *Euphorbia hierosolymitana.*

Table 2. Effect of aqueous leachates and organic extract fractions derived from the leaves of *T. cordifolia* on total protein content of the leaves of *Chenopodium album* L., *Chenopodium murale* L., *Cassia tora* L. and *Cassia sophera* L.

Treatment solutions	**Total protein content (mg/g dry wt.)**			
	C. album	*C. murale*	*C. tora*	*C. sophera*
Control	59.17 ± 0.17	44.13 ± 0.56	59.78 ± 2.21	56.94 ± 0.99
Aqueous leachates AL (1% g/ml fresh wt.)	33.44 ± 0.73	37.16 ± 0.13	46.52 ± 1.14	46.69 ± 0.17
Petroleum ether Fraction PF(0.09% w/v)	36.17 ± 0.76	33.03 ± 0.76	43.62 ± 0.65	49.21± 0.65
Methanolic fraction MF (0.09% w/v)	46.24 ± 0.23	32.19 ± 0.45	36.81 ± 0.82	37.27± 0.19
Chloroform fraction CF (0.09% w/v)	48.16 ± 0.31	42.01 ± 0.86	49.4 ± 0.84	38.9± 0.12
Water fraction WF(0.09% w/v)	34.17 ± 0.12	30.14 ± 0.43	40.02 ± 0.32	34.93 ± 0.32
LSD at 5%	3.99	3.45	4.38	4.24
LSD at 1%	5.68	4.90	6.22	6.03

Table 3. Effect of aqueous leachates and organic extract fractions derived from the leaves of *T. cordifolia* on total Chlorophyll content of the leaves of *Chenopodium album* L., *Chenopodium murale* L., *Cassia tora* L. *and Cassia sophera* L.

Treatment solutions	**Total chlorophyll content (µg/mg fresh wt.)**			
	C. album	*C. murale*	*C .tora*	*C. sophera*
Control	3.85± 0.13	4.80 ± 0.07	4.46 ± 0.09	4.26 ± 0.11
Aqueous leachates AL (1% g/ml fresh wt.)	2.83 ± 0.16	3.26 ± 0.15	4.24 ± 0.06	2.34 ± 0.19
Petroleum ether Fraction PF(0.09% w/v)	2.67 ± 0.42	3.53 ± 0.19	3.85 ± 0.04	2.11± 0.37
Methanolic fraction MF (0.09% w/v)	2.72 ± 0.19	2.76 ± 0.32	3.49 ± 0.65	3.26 ± 1.75
Chloroform fraction CF (0.09% w/v)	1.89 ± 0.73	2.17 ± 0.35	2.14 ± 0.03	3.51 ± 0.44
Water fraction WF(0.09% w/v)	2.76 ± 0.49	3.15 ± 0.25	2.10 ± 0.15	3.14 ± 0.92
LSD at 5%	0.270	0.321	0.339	0.290
LSD at 1%	0.384	0.456	0.482	0.412

Conclusion

The plant Allelo-chemicals may interfere the growth, development and performance of a plant through their direct or indirect action on metabolism of plants. Aqueous leachate and organic fractions reduce the level of biochemical activities. Carbohydrate content was increased in treated plants compared to control while chlorophyll content and protein content were reduced as compared to control. Aqueous leachate showed maximum toxicity on weeds than organic fractions. The reduction may be due to the allelochemicals present in *T. cordifolia* leaves.

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