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ORIGINAL ARTICLE

ALLELOPATHIC EFFECT OF *LANTANA CAMARA* L. ON THREE WEED SPECIES OF NORTH 24 PARGANAS, WEST BENGAL

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Abstract*: An experimental study was conducted to observe the effect of the aqueous leaf extract of the plant Lantana camara L. (Family: Verbenaceae) on germination and growth of three of its non-associated weeds: Cassia sophera, Cassia tora and Crotalaria pallida var pallida. It was observed that all growth parameters of these three weeds were seriously affected upon treatment with various concentrations of Lantana camara leaf extract as compared to their corresponding controls. Germination, root length, hypocotyls length, fresh and dry weight as well as chlorophyll content was inhibited at higher level with higher concentrations of the extract. It was observed that, with 1:5 (w/v) concentration of extract, the root length of Cassia tora was reduced to 40% and its hypocotyl length to 50%. The same extraction causes 30% and 50% reduction in case of Cassia sophera and 30% and 30% reduction in case of Crotalaria pallida, when root length and hypocotyl length were considered. Almost similar result was also observed in case of Cassia tora, Cassia sophera and Crotalaria pallida with other concentrations of Lantana leaf extract. The amount of chlorophyll content was found to be much lower in all the three weeds when treated with higher concentrations of extract. Chemical analysis indicated the presence of phenols in the leaf extract of Lantana camara may be the cause of such inhibition suggesting a possibility of biological control of these three unwanted weeds in crop fields, gardens and other areas.*
Overall findings showed maximum inhibition of Cassia sophera followed by Cassia tora and Crotalaria pallida.

Key words: Allelopathy, allelochemicals, *Lantana camara*, germination, chlorophyll content, weeds.

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1. INTRODUCTION

Allelopathy describes the reciprocal harmful biochemical interaction that is believed to occur in nature between all classes of plants including microorganisms. The invasive nature of weeds may be due to their

allelopathic property which is regulated by the presence of various chemicals commonly known as allelochemicals. These chemicals are secondary metabolites of plants and are specific in their action [1]. They are liberated from plants by abscission and litter fall, leaching of foliage by rain, volatilization by foliage and root exudation. Allelochemicals are generally produced at a later stage of development of a plant species and once released, influences the growth and reproduction of ecologically associated plants growing in same plant community. These chemicals are present in almost all plant parts such as root, stem, leaves, flowers, seeds and buds [2], but the concentration and effect vary in different parts. This is due to the water-soluble nature of allelochemicals [3]. In 1984, Rice [4] suggested the influence of weeds through this mechanism of plant interference can be positive (stimulatory) or negative (inhibitory). It can be called a chemical warfare between plants and is believed to have the involvement in many natural and manipulated ecosystem and plays a role in the evolution of plant communities [5-7]. Many of these allelochemical compounds are phytotoxic and have potential as herbicide or templates for new herbicide classes. Therefore, these chemicals are current areas of research for development of new pesticides, herbicides, insecticides, nematicides or fungicides.

Lantana camara L. (hereafter referred to as *Lantana*) belongs to the family Verbenaceae and has two main forms: a cultivated and non-thorny compact form planted on gardens and a weedy fast growing shrubby variety with thorny stem. This variety is native to tropical and subtropical areas of South and Central America but has spread to other countries including India [8]. Initial seedling growth is slow but when the roots become established, close stems intertwine and begin to form thickets. *Lantana* can grow in a wide range of climate condition and on variety of soil types. It generally grows invasively in open unshaded situations such as waste lands, roadside, railway tracks, canals, fence lines, agricultural fields and is a serious threat to biodiversity [9]. Allelopathy, non-palatability and out competing for soil nutrients may be the reason for its successful invasion. There is report about the aromatic phenols and alkaloids in *Lantana* inhibiting seed germination and growth of many plant species [10]; flower, stem and seed extract inhibiting the germination, fresh and dry weight of *Dalbergia sissoo* [11] and other vegetable species [12]. Leaf extract and leachates of *Lantana* showed reduction in germination of forest crops [13] and agricultural crops like *Brassica*, *Phaseolus* and Vigna [14].

Despite the mentioned inhibitory activity of *Lantana*, information about its interference on growth and establishment of weeds is scanty in the state of West Bengal. In beginning of 21st century, Cheema and Khaliq [15] observed the allelopathic behavior of *Sorghum* for controlling weeds in wheat crop field. Hence, the present study was carried out to observe the allelopathic potential of *Lantana* as a mean to control three very common leguminous weeds: *Cassia sophera*, *Cassia tora* and *Crotallaria pallida* growing profusely near the fields of experimental region of Gangetic West Bengal, where 60% population are depended on agriculture and farming.

2. MATERIALS AND METHODS

A. Sampling

Mature leaves of *Lantana* were collected from the surrounding of cultivated field areas of Habra, situated near India- Bangladesh border of North 24 Parganas. They were washed thoroughly under tap water and air dried at room temperature (26±2^{o} C) for 96 h.

B. Preparation of aqueous solution

Twenty grams of dried leaves were chopped into 1 cm long pieces and soaked in a corked conical flask containing 100 ml distilled water. The flasks were then kept on mechanical shaker for 24 h and filtered through Whatman No.1 filter paper. This filtrate served as a stock inhibitor solution of 1:5 (w/v) concentration. Dilutions 1:10 and 1:20 extract solutions (w/v) were prepared from the stock solution with Distilled water for bioassay.

C. Determination of percentage of germination, root length and hypocotyls length

Seeds of all three weeds were scarified with concentrated H_2SO_4 for 20 min and washed thoroughly with distilled water. 10 seeds of each experimental weed were placed on 9 cm diameter Petri dish containing a double layer of Whatman filter paper (No. 1) and 10 ml of 1:5, 1:10 and 1:20 concentration of *Lantana* leaf extract. Dishes containing distilled water served as control. The experimental setup with four replication per set was kept in the laboratory at room temperature (26 $\pm 2^{O}$ C). Percentage of seed germination, root and hypocotyl lengths were noted on the 8^{th} day of germination. Emergence of root approximately 1 mm in diameter was taken as the index of germination.

D. Determination of fresh weight (FW), dry weight (DW) and chlorophyll content of seedlings

For the determination of growth activities of treated seedlings, 20 cm pots were filled 300 g of soil collected from the experimental area and homogenized properly. Ten surface sterilized (2% sodium hypochlorite solution for 5 min) and thoroughly washed seeds of each weed were sown approximately 5 mm deep in each pot. The pots were divided into four sets. The first three sets received a daily dose of 50 ml aqueous extract of *Lantana* (1:5, 1:10 and 1:20 concentration) poured directly into the soil. The fourth set received the same amount of tap water and served as control.

To determine the fresh and dry weight, 15 days old seedlings were uprooted keeping the root system intact, washed under slow flowing stream of tap water to remove soil particles and dried with paper towel. After noting the fresh weight (FW) in digital electronic balance, seedlings were kept in the oven at 70^{o}C for 24 h and their dry weight (DW) was determined.

Estimation of chlorophyll content was done by collecting the green leaf portion of 10 days old seedling from the pots, washed twice with 0.1 % $HgCl_2$ followed by several washings with distilled water for surface decontamination. They were extracted twice with 80% acetone and centrifuged for 10 min at 5,000 rpm. The supernatant was collected, a definite volume maintained and chlorophyll estimation was done according to Mackinney [16]. Optical density values were recorded with a Beckman spectrophotometer at 663 nm and 645 nm and using the following equation:

$$C = 0.0202\ D_{645} + 0.0080\ D_{663}$$

Where C= Total chlorophyll content in g/L, D_{645} and D_{663}=density values at 645 and 663 nm respectively.

E. Chemical analysis of Lantana leaves

Fresh leaves of *Lantana* were collected, washed thoroughly under tap water, air dried for 6 h and kept in the oven at 28^{o}C for 72 h. The dried sample was then crushed in a mixer to make a powder. This powdered sample was analyzed for the presence of phenols and ketones.

Firstly, 1 gm sample was taken in a test tube containing 5 ml ethanol and shaken for 2 min before the addition of 5-6 drops of neutral $FeCl_3$. The change in colouration was observed for the presence of phenols. In the same way to ascertain the presence of ketones, 1 g sample was added to 5 ml of ethanol in a test tube and shaken for 2 min. 2,4- Dinitrophenyl hydrazine (Brady's reagent) was added to it and allowed to react for 3-4 min until the appearance of coloured precipitate.

*F. **Statistical analysis***

Using standard procedure of statistical data analysis (including the software Biostat 2009, version 5.7, 8.1, and the inbuilt mathematical functions of Microsoft Exel 2007), the effects of aqueous leaf extract of *Lantana* were correlated with the rate of germination, root length, hypocotyls length, FW, DW and chlorophyll content of *Cassia sophera, C. tora* and *Crotallaria pallida*.

3. RESULTS

It is quite evident from the result obtained that aqueous leaf extract of *Lantana* exerts a negative influence on the growth performance of *Cassia sophera, Cassia tora* and *Crotallaria pallida*. Germination of seeds was affected with higher concentration of the extract (Figure 1). In case of *Cassia sophera* and *C. tora*, percentage of germination was reduced by almost 60% and 40% respectively with 1:5 concentration, while it was reduced by 20% in case of *Crotallaria pallida* with the same concentration as compared to control. Among the three weeds *Cassia sophera* seed germination was affected most as compared to the other two weeds.

The length of root and hypocotyl of 5-day-old treated seedlings were much lower in comparison to those of control. Root length (Figure 2) and hypocotyls length (Figure 3) of *Cassia sophera* and *C. tora* showed little change with 1:20 concentration but a drastic reduction with 1:5 concentration. In case of *Crotalaria pallida*, a gradual decline in both root and hypocotyl's length was observed with 1:20, 1:10 and 1:5 concentration. In case of root length, it was 8.4 mm, 7.0 mm and 3.5 mm respectively with the three concentration levels, as compared to 9.8 mm of control set. Similarly, in case of hypocotyl length, these had become 6.8 mm, 5.0 mm and 4.5 mm respectively for the three concentration levels as compared to 7.2 mm of control set.

Fresh weight of *Cassia sophera* was reduced to 4.20g ± 0.05, *Cassia tora* to 9.20 g ± 0.55 and *Crotalaria pallida* to 8.85 g ± 0.62 as compared to 8.45g ± 0.30, 12.50 g ± 0.55 and 12.0 g ± 0.31 of the controls respectively with 1:5 concentration. Similar pattern followed with other concentrations also. There was marked reduction in dry weight with higher concentrations. With 1:5 concentration dry weight of *Cassia sophera* seedlings was 0.35 g ± 0.33, *Cassia tora* was 4.30 g ± 0.55 and *Crotalaria pallida* was 5.0 g ±0.05 as that of 1.95g ± 0.05, 7.31g ± 0.27 and 7.00g ± 0.33 of control respectively (Table 1 - 2).

Chlorophyll molecules are the core component of pigment protein complex embedded in the photosynthetic membrane and play a major role in photosynthesis. The total chlorophyll content of the three weed seedlings showed a gradual decrease with increasing concentration of the extracts (Figure 4). Seedlings turned yellowish while still in pots when the concentration was increased from 1:20 to 1:5. In all the three weeds, chlorophyll content was brought down to almost one-third as that of control. In this case *Cassia sophera* and *C. tora* was more sensitive than *Crotalaria*. The reduction was concentration dependent and the maximum reduction was observed in *Cassia tora* than other weeds.

Laboratory analysis of *Lantana* leaf extract confirmed the presence of phenols when a bluish – green colour developed during ferric chloride test. A bright orange red precipitate with Brady's reagent showed that there was a significant number of ketones present in the leaf extract. In comparative analysis, when all the relative

effect of the studied parameters were considered, the inhibitory effect of *Cassia sophera* was the highest, followed by *Cassia tora* and *Crotalaria pallida*. Regarding the correlation analysis, the correlation coefficient of each observation was determined to be significant ($P \leq 0.05$).

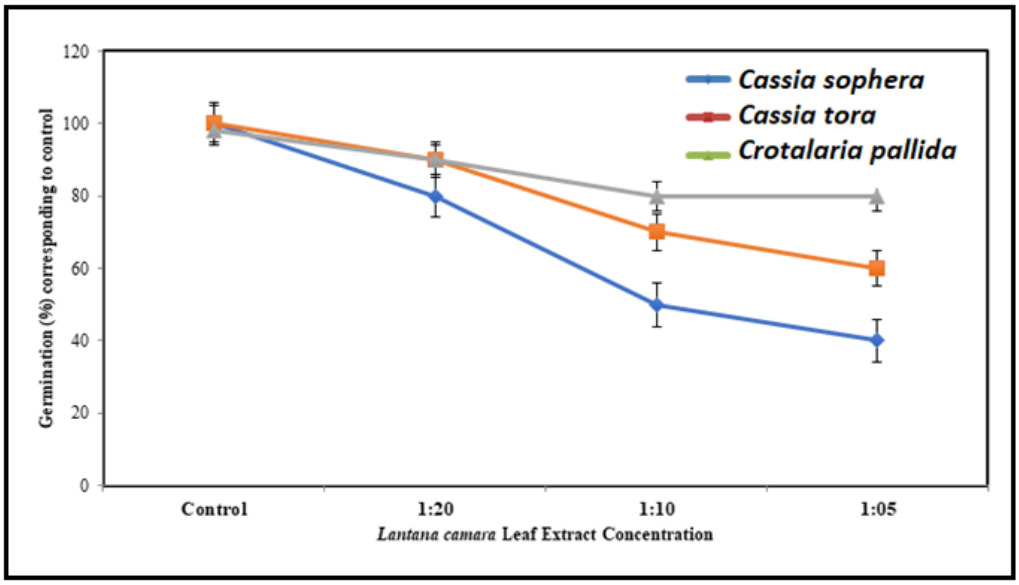

Figure 1 The effect of increasing concentration of *Lantana camara* leaf extract on the germination of weeds (n=10). The bars indicate standard deviation.

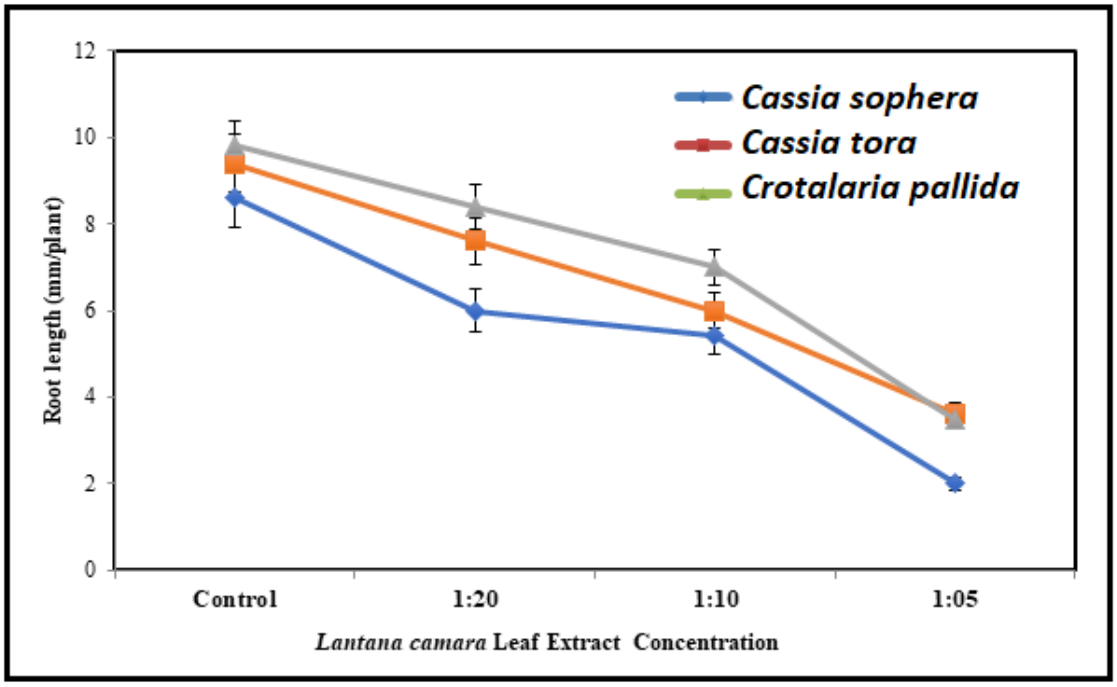

Figure 2. The effect of increasing concentration of *Lantana camara* leaf extract on the root length of weeds (n=10). The bars indicate standard deviation.

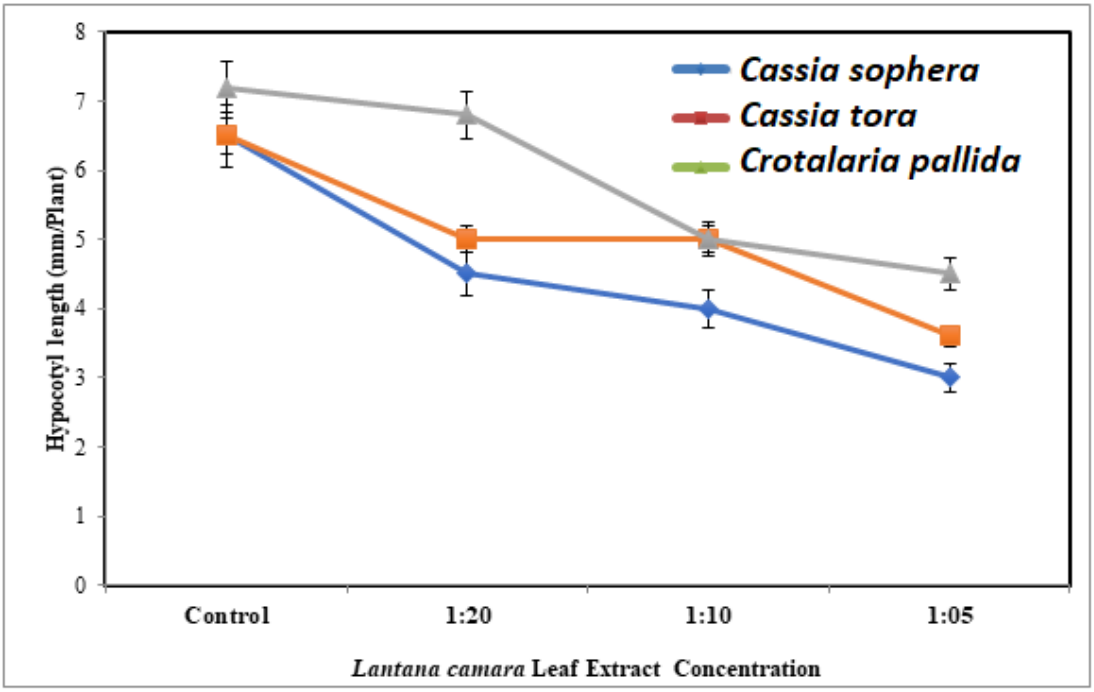

Figure 3. The effect of increasing concentration of *Lantana camara* leaf extract on the hypocotyl length of weeds (n=10). The bars indicate standard deviation.

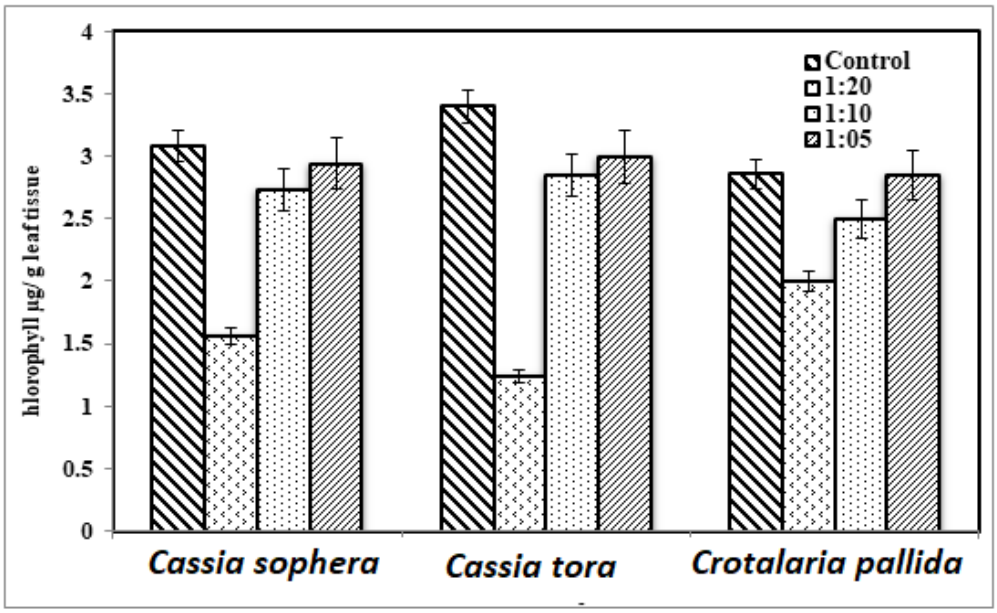

Figure 4. The effect of increasing concentration of *Lantana camara* leaf extract on the Chlorophyll content of weed species (n=10) of *Cassia sophera, Cassia tora* and *Crotalaria pallida*. The bars indicate standard deviation.

Table 1. Effect of *Lantana camara* leaf extract concentrations on fresh weight of weeds

Plant Species	Control	1:20 Concentration	1:10 Concentration	1:5 Concentration
Cassia sophera	8.45 ±0.31	7.00 ±0.30	5.60 ±0.62	4.20 ±0.05
Cassia tora	12.50 ±0.55	12.00 ±0.08	10.50 ±0.03	9.20 ±0.55
Crotalaria pallida	12.00 ±0.31	10.50 ±0.62	9.00 ±0.05	8.85 ±0.62

Note: t- value significant at p = 0.05*, 0.01** and 0.001***; ± represents standard deviation.

Table 2. Effect of *Lantana camara* leaf extract concentrations on fresh weight of weeds

Plant Species	Control	1:20 Concentration	1:10 Concentration	1:5 Concentration
Cassia sophera	1.96 ±0.50	1.40 ±0.32	0.65 ±0.75	0.35 ±0.33
Cassia tora	7.31 ±0.27	7.00 ±0.30	7.00 ±0.30	4.30 ±0.55
Crotalaria pallida	7.00 ±0.33	6.80 ±0.25	6.00 ±0.05	5.00 ±0.05

Note: t- value significant at p = 0.05*, 0.01** and 0.001***; ± represents standard deviation.

4. DISCUSSION

The present study suggests that leaves of *Lantana camara* have strong allelopathic effect on the germination and growth of various test species used. The results agree with other similar studies who reported differential allelopathy of plants and differential response of test species to same extract [17-19]. Plants containing allelochemicals can affect germination and growth of other plants on concentration depended manner and the effect of these chemicals are selective, which can vary with different plant species [20-22]. Seed germination of weeds showed negative response to the increasing concentration of aqueous *Lantana* leaf extract reflecting allelopathic potential of the plant. Allelochemicals suppressed the mitotic activity of young cells and brings about decrease in cell number or decrease in cell elongation or both, resulting in the inhibition of cell germination.

Allelochemicals have direct effect on the biochemical and physiological processes of plant growth and metabolism. The ion exchange is greatly influenced by the presence of such chemicals. As compared to control the inhibition of root length was more than that of hypocotyls. This may be due to the contact of the roots with the filter paper leading to constant absorption of the extract solution [23]. Ye *et al*. [24] showed that allelochemical stress can cause oxidation damage and increased degree of membrane lipid peroxidation. Similar findings reported about significant effect of *Lantana* leaf extract on germination and growth of *Melilotus alba* [25]. Present findings are also corroborative with those of previous workers [26-

27]. The reduction in root and radical length as morphologically observed may be due to the reduction in cell size or cell elongation [28]. Nishida *et al*. [29] stated that the permeability of allelochemicals into the root tissue is higher than the shoot tissue. This may be because hypocotyl growth of seedlings largely depends on cell expansion which is relatively insensitive to allelochemicals, whereas root growth requires not only cell expansion, but also cell proliferation, which is sensitive to the allelochemicals and therefore, the root growth exerts higher inhibition than the hypocotyls growth.

Measurement of root and hypocotyls length are sometimes complicated when allowed to grow in Petri dishes due to curling. To compare the allelopathic effect tested in the laboratory with what happens actually in the field, the fresh and dry weight of seedlings were recorded by growing them in pots containing field soil irrigated with inhibitor extract. The concentrated extract of *Lantana* suppressed FW as well as DW of the three experimental weeds. FW was reduced more in *Cassia sophera* and DW in *Cassia tora*. The reduction of dry matter production indicates interference by the toxic substances from the extract with cell division, nutrient uptake, and transport. Studies of UV absorption, pH, electroconductivity and osmotic potential showed the contribution of chemicals towards inhibitory activity on growth and length of root and shoot [30]. Reduction of macro and micro nutrient absorption as well as IAA oxidase in plant root cells is inhibited by various allele chemicals [31]. In a previous study on barley and wheat, the elongation, DW of seedlings were reported to be reduced by the walnut allelochemicals juglone (5- hydroxyl-1-4-naphtha quinone) in a similar pattern [32]. Additionally, the allelochemicals' higher salt and chemical concentration in the extract might possibly cause an osmotic stress during seedling growth. In addition to allelochemical release, soil surface residue may also prevent seedling emergence [33]. The result showed a marked reduction in FW and DW of weed seedlings on the 8^{th} day of germination. This inhibition may also be attributed to the alteration of enzyme activity, which affects the mobilization of storage compounds during growth. When potted weeds were treated with different concentrations of *Lantana* leaf extract, chlorophyll pigment content of three test species exhibited significant difference from control. This reduction may be because of the allelochemicals which either inhibit the biosynthesis of chlorophyll or stimulates the chlorophyll degrading substances or both [34]. Sarkar *et al*. [35] observed reduction in chlorophyll concentration in leaves of mustard and rice plant following treatment with *Cassia tora* plant extract. Higher concentration of extract caused mosaic chlorosis, resulting in the yellowing of leaves of potted weed seedlings thereby affecting chlorophyll content. Patterson (1981) found suppression in concentration of chlorophyll in leaves of soyabean plant following treatment with several allelopathic compounds [36]. Einhellig *et al*. [37] reported that allelochemicals caused marked reduction in the chlorophyll pigment of test plants through their effect on the biosynthesis and denaturation of chlorophyll molecules.

As documents by previous workers, allelochemicals of *Lantana* include mono and sesquiterpenes, flavonoids, iridoid glycoside, furano naphoquiones, triterpenes and diterpenes [38]. Yi *et al*. [39] reported the presence of several phenolic compounds in *Lantana* leaf extract identified by HPLC as salicylic, gentisic, B-resorcyclic acid, vanillic, caffeic, ferulic, coumarin and 6-methyl coumarin. Begum *et al*. [40] isolated lantanilic acid, pomolic acid and lantoic acid from *Lantana* which was responsible for suppression of many weeds. Laboratory analysis of *Lantana* leaves in the present study showed the presence of phenols and ketones responsible for the reduction of weed growth parameters. Essential oils extracted from the leaves of *Lantana* was found to possess significant insecticidal, antifeedant, antimicrobial and exhibited fungicidal and insecticidal activity as found by Mishra [41].

5. CONCLUSION

The present investigation shows that aqueous leaf extract of *Lantana camara* has an inhibitory effect on seed germination and establishment of *Cassia sophera*, *Cassia tora* and *Crotalaria pallida*. This confirms the presence of allelochemicals and allelopathic potential of the studied plant. The detrimental effect may also be due to the interaction of chemicals present in the target weed with those of test weed plants. This harmful allelopathic effect of *Lantana* on its non-associated weeds can be considered 'beneficial' as it can be exploited to be ecofriendly, economic and effective green herbicide, which is easily available and can be used to ward off unwanted weeds surrounding the agricultural fields. Further research is necessary to confirm the activity of a single chemical(s) present in the weed responsible for inhibition of test crops.

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