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Research Article
Effect of Lantana Leaf Extract on Growth, Biochemical Aspects and Yield of Chickpea Plants

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Abstract

Background and Objective: An effective technique for reducing chemical fertilizer used is allelopathy technique which improves production of different crops as well as weed control. So, this investigation was carried out to evaluate the allelopathic effect of lantana aqueous leaf extract on growth, some physiological aspects and yield of chickpea plant. **Materials and Methods:** A pot experiment was done at two winter successive seasons at the wire house of National Research Centre using various concentrations (5, 10, 15, 20 and 25% w/v) of aqueous leaf extract of lantana as seed soaking treatment on chickpea plant with factorial design experiment and different growth parameters such as number of pods/plant, weight of pods/plant (g), weight of seeds/plant (g) and 100 grains weight (g), proteins (%), carbohydrates (%), phenolics and flavonoids contents etc., were estimated. **Results:** Chemical composition of aqueous leaf extract showed higher contents of phenolics, flavonoids, carbohydrates and tannins contents. Soaking chickpea seeds in different concentrations of lantana aqueous leaf extract caused significant increases in different growth criteria. These effects were proportional to the extract concentration. Moreover, all different concentrations caused significant increases in photosynthetic pigments. Overall the growth parameters and photosynthetic pigments were increased with the increase in leaf extract concentration till 15% then different growth characters showed decline, but still greater than control plants so the most effective treatment was 15% as it gave the greatest increases in the studied growth parameters as compared with control plants. **Conclusion:** So, it is concluded that the maximum growth parameters, yields attributes and nutritional value of chickpea seeds were obtained in response to 15% of aqueous leaf extract of lantana.

Key words: Allelopathy, chickpea, growth parameters, lantana, pods/plant, allelopathic effect, weed control

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Reducing the amount of used chemicals in agriculture is of great importance in production of different crops. This reduction could be achieved by different techniques such as using bio-fertilizers by introducing recent biological and ecological methods. Allelopathy, is one of these used techniques[1,2]. The term 'allelopathy' signified the interactions between plants might lead to either stimulation or inhibition of growth. Different groups of plants like, algae, lichens, crops and annual and perennial weeds have wide known allelopathic interactions[3,4]. Allelopathy is the phenomenon where natural compounds are released from the root, shoot, leaves or flower of the plant to influence other plants[5]. Also, it is used to refer the potentiality of secondary metabolites produced by plants including micro-organisms which effect on growth, productivity of another plant[6]. Chemicals that inhibited the growth of some species at certain concentrations can stimulate the growth of the same or different species at lower concentrations. Hence, it is expected that due to the perceived ambiguous nature of allelopathy, the phenomenon is sometimes hesitantly accepted or even refuted, as an important factor in crop production. A significant portion of the agricultural and in developing countries in the tropics is heavily infested by various native and alien (invasive) weeds[7] and controlling weeds is a big challenge to farmers. There is much evidence that allelo chemicals liberated from certain weeds into the soil reduce crop growth. Crop weed interactions were referred to as plant competition, i.e., crop-weed competition, although without adequate evidences to indicate whether such effects were owing to competition alone, allelopathy or both[5]. These effects may be occurred via formation of allelochemicals which are biochemical compounds which extracted from different parts of living or decomposing plant materials. Phenolic compounds, flavonoids, terpenoids, alkaloids, steroids, carbohydrates and amino acids among the main allelochemical formed by plant. Sometimes mixture of various compounds might have a higher allelopathic effect than every compound alone[6]. *Lantana camara* L. is called lantana weed and wild sage. It is a big shrub evergreen scrambling plant. Different parts of lantana weed have various allelochemicals such as; carbohydrates, flavonoids and tannins[8]. Such chemicals or compounds enhance or reduce growth and yield of different crops. This effect depends on allelochemicals concentrations and specific to different species[9]. In addition, lantana plant and its extract have a wide range of antioxidant activity and antioxidant compounds[10].

Chickpea plant (*Cicer arietinum* L.) is a popular crop in arid and semiarid regions and one of the most important legumes cultivated in marginal area and under different a biotic stress environments[11,12]. Chickpea plant is particularly sensitive to salt stress[13]. As well as chickpea plant is the third largest food legume crop in the world as it is highly nutritional value because it contains high amount of protein so, it is considered an important source of proteins for consumption of human in several developing countries[14].

So, this study was an attempt to assess the potentiality of using aqueous leaf extract of eucalyptus plant to induce promotive effect on growth, some biochemical aspects, yield and nutritional value of chickpea plant.

MATERIALS AND METHODS

Preparation of aqueous leaf extract: Healthy *Lantana camara* leaves were brought to remove dust and other unwanted compounds these leaves washed thoroughly by tap water, then dried in air and grinded to fine powder. Tap water was used as extract solvent to enhance natural allelochemicals to extract. So, the powdered leaves were soaked in distilled water to make different concentrations (5, 10, 15, 20 and 25% w/v) and keeping them in a shaker for 24 h. The extract was filtered through cheese cloth and the solid was discarded. The phenolic compounds, flavonoids, total carbohydrate and tannins were estimated at different concentrations of aqueous extract.

Experimental procedure: At the green house of the National Research Centre, Dokki, Egypt, a pot experiment was done at two successive winter seasons, 2014/2015 and 2015/2016 to investigate the role of presowing seed treatment of chickpea plant with lantana leaf extract. Chickpea (*Cicer arietinum* L.) cv. Giza 195 was used for this experiment.

Chickpea seeds were divided into 6 groups and soaked for 24 h, the first group were soaked in dist. water (ALE1), the remained groups were soaked in different concentrations on leaf extract 5, 10, 15, 20 and 25% w/v, respectively (named as ALE2, ALE3, ALE4, ALE5 and ALE6) and left to dry for approximately 1 h at room temperature. At 30 mm depth plastic pots, 10 chickpea soaked seeds were sown at center row in 7 November in the two seasons. Mixed clay and yellow sand with a ratio 3:1 (v/) were used as soil used in each pot to enhance compaction and decrease compaction. Seedlings were thinned to 5 seedlings per pot after 10 days of sowing. Different fertilizers were used as usual.

The experiment was in a complete randomized arrangement. Plant samples were taken after 60 days from sowing for the estimation of growth characters as plant height (cm), branches and leaves number/plant, shoot fresh and dry weight (g/plant). Plant samples were taken for chemical analysis. Chemical analysis was photosynthetic pigments. At harvest (on first week of April) the following characters were recorded: Number of pods/plant, weight of pods/plant (g), weight of seeds/plant (g) and 100 grains weight (g). Some chemical parameters were measured in the yielded seeds as proteins (%), carbohydrates (%), phenolics and flavonoids contents.

Measurements

Phenolic contents: A known weight of the fresh samples was taken and extracted with 85% cold methanol (v/v) for three times at 0°C. The combined extracts were collected and made up to a known volume with cold methanol and then 0.5 mL of the extraction was added to 0.5 mL Folin reagent, shaked and allowed to stand for 3 min. Then 1 mL of saturated sodium carbonate was added to each tube followed by distilled water shaken and allowed to stand for 60 min. The optical density was determined at wave length of 725 nm using spectrophotometer[15].

Flavonoids: Flavonoid content of crude extract was determined by the aluminium chloride colorimetric method[16]. In brief, 50 µL of crude extract (1 mg mL^{-1} ethanol) was made up to 1 mL with methanol, mixed with 4 mL of distilled water and then 0.3 mL of 5% $NaNO_2$ solution, 0.3 mL of 10% $AlCl_3$ solution was added after 5 min of incubation and the mixture was allowed to stand for 6 min. Then, 2 mL of 1 mol L^{-1} NaOH solution were added and the final volume of the mixture was brought to 10 mL with double-distilled water. The mixture was allowed to stand for 15 min and absorbance was measured at 510 nm. The total flavonoid content was calculated from a calibration curve and the result was expressed as mg rutin equivalent per gram dry weight.

Total carbohydrates: Determination of total carbohydrates were carried out according Hebert *et al.*[17]. A known mass (0.2-0.5 g) of dried tissue was placed in a test tube and then 10 mL of sulphuric acid (1 N) was added. The tube was sealed and placed overnight in an oven at 100°C. The solution was then filtered into a measuring flask (100 mL) and completed to the mark with distilled water. The total sugars were determined colorimetrically according to the method of DuBois *et al.*[18] as follows: An aliquot of 1 mL of sugar solution was transferred into test tube and treated with 1 mL of 5% aqueous phenol solution followed by 5.0 mL of concentrated sulphuric acid. The tubes were thoroughly shaken for 10 min then placed in a water bath at 23-30°C for 20 min. The optical density of the developed color was measured at 490 nm using Shimadzu spectrophotometer model UV 1201.

Nitrogen and crude protein: Nitrogen and protein contents were determined with micro Kjeldahl's apparatus according to the method of Miller and Houghton[19]. Crude protein was determined according to multiplying nitrogen contents by 5.75.

Tannins: Tannin concentrations were determined by a modified version of a method reported by Maxson and Rooney[20] and the modified by Lin and Tang[21]. Samples were mixed with 5 mL vanillin-HCl (8% conc. aq. HCl and 4% vanillin in methanol). Absorbance at 500 nm was read after 20 min. Catechin was used as the standard. The condensed tannin concentration is expressed as catechin equivalents in mg per gram of extract.

Photosynthetic pigments: Total chlorophyll a and b and carotenoids contents in fresh leaves were estimated using the method of Lichtenthaler and Buschmann[22]. The fresh tissue was ground in a mortar and pestles using 80% acetone. The optical density (OD) of the solution was recorded at 662 and 645 nm (for chlorophyll a and b, respectively) and 470 nm (for carotenoids) using a spectrophotometer (Shimadzu UV-1700, Tokyo, Japan). The values of photosynthetic pigments were expressed in mg g^{-1} FW.

Statistical analysis: The analysis of variance procedure of one way factorial design according to Snedecor and Cochran[23], treatments means were compared using multiple range[24] test at 5% of probability. Combined analysis of the two growing seasons was carried out.

RESULTS AND DISCUSSION

Changes in allelochemicals of aqueous leaf extract: Different allelochemicals compounds such as phenolics, flavonoids, carbohydrates and tannins contents in different concentrations of aqueous leaf extract of lantana plant are presented in Fig. 1a-d. Results clearly showed that increasing leaf extract concentrations from 5-25% increased phenolics percentages from 5.49-8.59%, flavonoids percentages from 1.28-1.81%, carbohydrates percentages

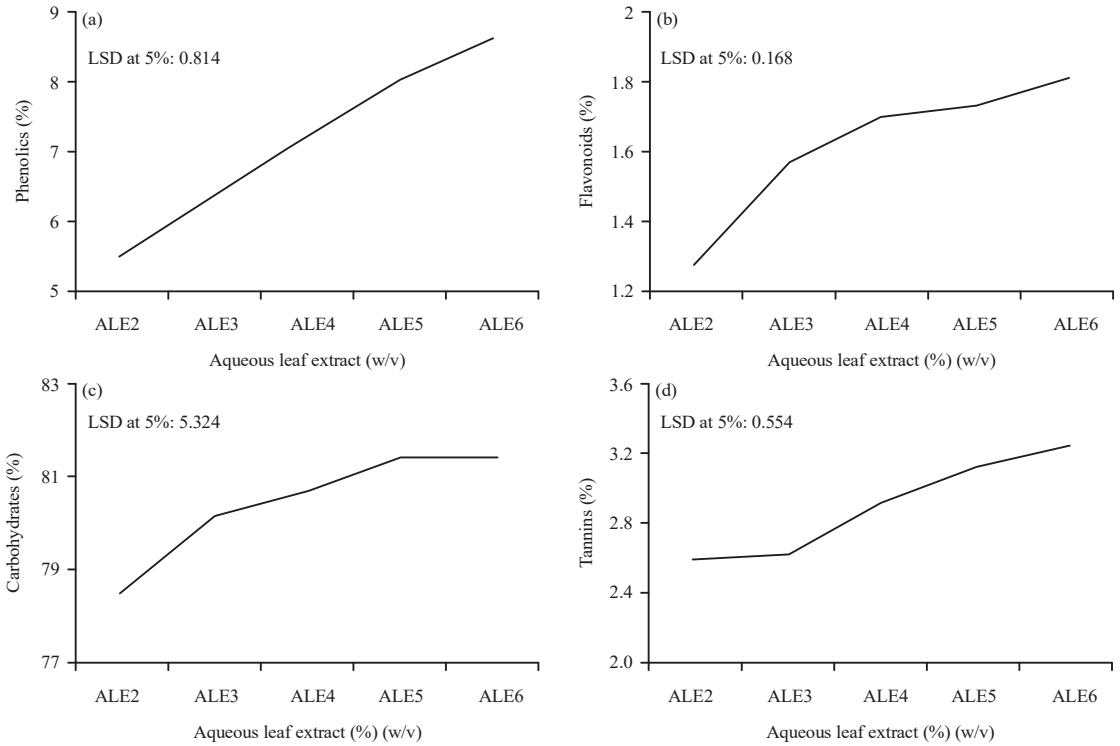

Fig. 1(a-d): Chemical composition (a) Phenolics, (b) Flavonoids (%), (c) Carbohydrates (%) and (d) Tannins (%) of aqueous leaf extract of lantana

Table 1: Effect of aqueous leaf extract of lantana leaf on growth parameters of chickpea plant (Data are means of two seasons)

Aqueous leaf extract (%) (w/v)	Plant height (cm)	Branches No./plant	Leaves No./plant	Plant fresh wt. (g)	Plant dry wt. (g)
ALE 1	44.33	1.33	30.67	4.64	0.88
ALE 2	47.33	2.00	36.67	5.08	0.98
ALE 3	48.50	3.00	44.67	5.61	1.16
ALE 4	52.33	3.33	46.67	5.86	1.24
ALE 5	50.67	3.00	43.33	4.71	1.09
ALE 6	47.33	2.33	42.67	4.30	0.98
LSD at 5%	2.243	0.245	1.542	0.354	0.014

from 78.52-81.42% and tannins from 2.59-3.24%. It was mentioned earlier that, allelochemicals compounds were distributed in different parts of lantana plant with various concentrations, whereas lantana leaves are the most potent source of different allelochemicals. The most prominence components of these allelochemicals that presented in lantana leaf extract were phenols, carbohydrates, tannins and flavonoids[8].

Effect of aqueous lantana leaf extract on growth parameters: The data in Table 1 clarified the effect of soaking chickpea seeds in lantana leaf extract with different concentrations on growth parameters of chickpea plant. Increasing leaf extract concentrations caused significant increases in growth parameters (shoot length, number of branches and leaves/plant, fresh and dry weight of plant) as compared with untreated control plant. These were increased gradual with increasing leaf extract concentration till 15% then different growth characters showed declines, but they still greater than control plants. The most effective treatment was 15% as it gave the greatest increases in the studied growth parameters as compared with control plants (Table 1). Generally, these increases in different growth parameters in response to various concentrations of aqueous lantana leaf extract is referred to the allelochemicals which present in leaf extract especially the contents of phenolic compounds[25]. The stimulatory effect of phenolic compounds on chickpea plant growth could be attributed to their stimulatory effect on photosynthesizing tissue[26]. The increases in dry weights of chickpea plant might be attributed to the increase in number

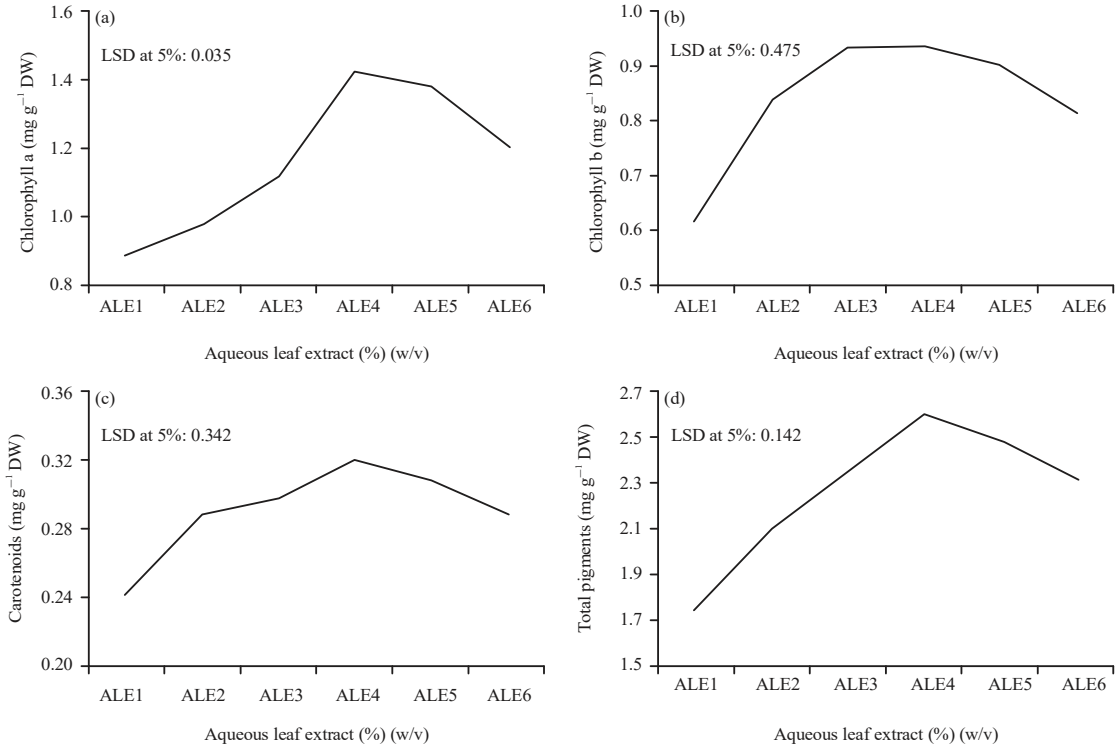

Fig. 2(a-d): Effect of aqueous leaf extract of lantana leaf on photosynthetic pigments (a) Chlorophyll a, (b) Chlorophyll b, (c) Carotenoids and (d) Total pigments as mg g^{-1} dry wt. of chickpea plant
(Data are means of two seasons)

of branches and leaves leading to increase in photosynthetic process. Moreover, the promotive effect of leaf extract phenolic contents might be resulted from increased ion uptake, cell division, cell differentiation, cell elongation enzymatic activities, photosynthetic activities, protein synthesis, sink/source regulation and plant antioxidant capacity which resulted via the bioregulator role on these physiological processes in plant cells[27].

Changes in photosynthetic pigments: The results in Fig. 2a-d showed that soaking chickpea seeds in different concentrations of lantana leaf extracts had a positive effect on photosynthetic pigments of chickpea plant. Different photosynthetic pigments increased with direct proportional to the extract concentrations. It was cleared that the lowest increase obtained by soaking chickpea seeds in 5% concentration, but the highest increase for all photosynthetic pigments were obtained with 15% concentration after this different photosynthetic pigments showed decline, but they still greater than control plants so the most effective treatment was 15%. Under field condition, addition of *lantana camara* to the soil enhanced photosynthetic pigments of rice plant and this reflected on yield of grains[28]. Mona and Hanan[6] showed that treated lupine plants with different concentrations of lantana leaf extract increased photosynthetic pigments of lupine leaves. The promotive effect of allelochemicals which present in aqueous leaf extract such as phenolic compounds might be due to the enhancement effect of allelochemicals especially phenolic compounds on the net photosynthetic rate[29].

Changes in yield and yield attributes: Yield and yield attributes of chickpea plant as affected by different concentrations of aqueous extract of lantana are presented in Table 2. The obtained data showed that all applied treatments increased significantly the examined parameters (pods No./plant, pods wt./plant, seeds wt./plant and 100 seeds wt.). These increments were directly proportional to the extract concentrations till 15 where 15% aqueous leaf extract of lantana was the most effective treatment as it gave the highest significant increases in all studied parameters in comparison with untreated plant. Those obtained results are in accordance with previous study[30] on rice plant. Moreover; Mona and Hanan[6] obtained higher increases in yield components of lupine plant as affected by aqueous extract of lantana plant.

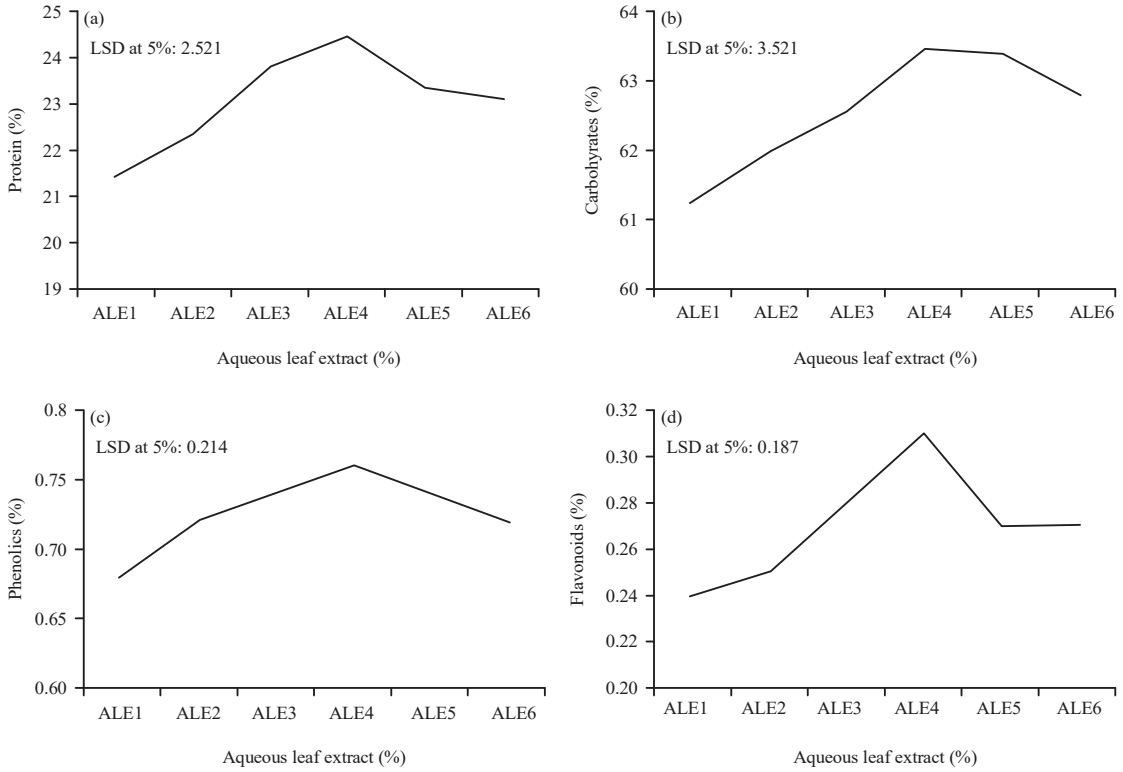

Fig. 3(a-d): Effect of aqueous leaf extract of lantana leaf on chemical constituents the yielded seeds of chickpea plant ALE1 (a) Protein (%), (b) Carbohydrate (%), (c) Phenolics (%) and (d) Flavonoids (%)
(Data are means of two seasons)

Table 2: Effect of aqueous leaf extract of lantana leaf on yield of chickpea plant (Data are means of two seasons)

Aqueous leaf extract of lantana (w/v)	Pods No./plant	Pods wt./plant (g)	Seeds wt./plant (g)	100 seeds wt. (g)
ALE 1	3.67	4.29	3.86	26.33
ALE 2	5.33	4.79	4.09	28.62
ALE 3	6.00	5.66	4.26	30.19
ALE 4	7.00	6.23	4.47	31.15
ALE 5	7.67	5.74	4.15	29.04
ALE 6	6.33	5.50	3.65	28.87
LSD at 5%	0.241	0.342	0.152	1.542

Changes in nutritional value of the yielded chickpea seeds: The chemical composition of the yielded seeds of chickpea plant had a special attention (Fig. 3a-d). Data clearly showed that, soaking chickpea seeds in different concentrations of lantana aqueous leaf extract led to significant increases in protein, carbohydrates and phenolic contents in the yielded seeds. Increasing aqueous leaf extract increased gradually the studied parameters till 15% then the increases decrease, but still more than the untreated control plants. The allelochemicals present in the aqueous leaf extract especially phenolic compounds had a stimulatory effect on different plants growth and yield. Phenolic compounds affect on enzymes and phytohormones activity and mineral content[31]. An increase in protein synthesis in different plant species were obtained[32,33].

CONCLUSION

So, the findings of current study clearly showed the positive role of external treatment of aqueous leaf extract of lantana plant as seed soaking on increasing growth and yield of chickpea plant as well as some nutritional values of the resulting seeds. Finally, it could be concluded, aqueous leaf extract of lantana with 15% concentration is more effective for all growth parameters.

SIGNIFICANCE STATEMENT

This study discovered the allelopathic effect of lantana aqueous leaf extract on growth, some physiological aspects and yield of chickpea plant and observed that maximum growth parameters, yields attributes and nutritional value of chickpea seeds were attained at 15% of lantana leaf extract that can be beneficial for the production of chickpea and this study will help the researchers to increase the nutritional value of chickpea seeds that many researchers were not able to explore.

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