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ALLELOPATHIC EFFECT OF *EUPHORBIA HIRTA* (PIG WEED) EXTRACTS AND POWDER ON SEEDLING GROWTH, CHLOROPHYLL AND PROTEIN CONTENT OF *CICER ARIETINUM* (BLACK GRAM) IN PAKISTAN

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Abstract

The present study was planned to describe the allelopathic effect of *Euphorbia hirta (Pig weed)* on *Cicer arietinum (black gram)* The effect of weed extract and powder on different growth parameters were studied on seed germination, velocity of germination, seedling growth, plant height, fresh and dry weight, chlorophyll and protein contents of *Cicer arietinum.* In Petri plate experiments extract of different concentrations were used such as 3, 5 and 7% and distilled water used as a control with their five replicates. The 7% extract of *E. hirta* showed highest inhibitory effect on germination, velocity of germination, seedling growth and plumule length was more inhibited than the radicle length of test crop. In Pot experiments weed powder were taken in different amount such as 10, 15 and 20 g, mixed with uniform amount of soil (500 g) and control pots were filled only with soil, with their five replicates. Weed powder has non-significant effect on plant height of *C. arietinum*. Fresh and dry weight also indicates inhibitory effect was proportional to the amount of weed powder. Chlorophyll contents and carotene were gradually decreased in treated plants as compared to the control. The highest % inhibition was observed in 20g. The inhibition may be due to the allelochemicals present in *E. hirta*.

Key words: Black gram, C*icer arietinum, Euphorbia hirta*, Seedling growth.

Introduction

Euphorbia hirta is a pantropical weed, commonly known as asthma plant. The name *hirta* has referred to peculiar hairy conditionof plant. *Euphorbia* belonging to the family Euphorbiaceae and consist of about 2160 species. It is native of tropical America, now widely spread throughout the world. It is one of the most diverse genera in the plant kingdom. Weeds growing among crop plants adversely affect yield, quality of the harvest and increase production costs, resulting in high economic losses (Alam, 1991); (Qureshi *et al*., 2021). It is widely used as a medicinal herb.

Cicer arietinum commonly known as black gram. It is major food Legume. It was originated from south-eastern Turkey. It can be grown in tropical, sub-tropical and temperate regions. It is grown an area of 1.081 m ha with a production of 0.741 m tons in Pakistan (Anon., 2009). The main producers are India, Australia, Pakistan, Turkey, Myanmar, Ethiopia, Iran, USA and Canada. It is Major Rabi crop in Pakistan. Black gram is cholesterol free. It is a good source of dietary fibre; vitamins and minerals. Seeds are the excellent source of nutrition contains 20.6% protein, 2.2% fat and 61.2% carbohydrates (Gupta, 1987); (Qureshi *et al*., 2014).

Allelopathy is a mechanism in which chemicals produced by weed plants may increase or decrease the associated plant growth. It was firstly introduced by a German scientist Molisch in (1937) to describe the biochemical interactions of the plants which are harmful and beneficial for the growth and development of neighboring plants. Plants interfere with neighbouring plants by releasing water soluble chemicals into the soil that inhibits the seed germination, plant growth chlorophyll and protein contents. These chemicals affect the plants at seed emergence and seedling levels (Mohamadi & Rajaie, 2009). The allelopathic potentialities present in the plant can inhibit the crops growth which is growing in that environment told by Kadiolgue *et al*., (2005); Salam *et al*., (2018). *E. hierosolymitana* had allelopathic effect on seed germination, seedling growth, chlorophyll and protein on wheat reported by Abu-Romman *et al*., (2010); Qureshi *et al*., 2015.

Allelochemicals leaching from plants with Phenolic property may partially block the biosynthetic pathway of chlorophyll and reduce photosynthesis process. Allelopathic effect of *C. bonplandianum* cause reduction of chlorophyll content on *Triticium aestivam* Sarkar and Chakrborty, (2010). Allelochemical directly effect on seed germination and seedling growth (Hussain *et al*., 2007) and (Naseem *et al*., 2009). Allelochemicals also inhibt the amount of protein in seedling growth. *Acaccia nilotica* extracts had significant decrease the level of proteins in legume crops (Duhan & Lakshinarayana, 1995); (Waqas *et al*., 2020). Present study focused on the production of protein content in the test species as it is influenced by weed powder, plants released allelochemicals they could be sensitive to the other plants. They reduced the percent germination, seedling growth, plant height, chlorophyll and protein contents of *C. arientinum*. It was noticed that extract of *E. hirta* inhibit the seedling growth of *C. arietinum*. On the other hand it was confirmed that *E. hirta* (powder) has no significant effect the plant height of *C. arietinum*. Thats why present study was design to evaluate the possible effects of *Euphorbia hirta* on physiological parameters of crops.

Materials and Methods

The experimental work was conducted during at research laboratory of Botany department (Federal Urdu University of Arts, Sciences and Technology). *Euphorbia hirta* were collected from different areas of Karachi. All the glass wares were sterilized in autoclave at 121 C with 15 Lb pressure for 30 minutes. Collected weeds were air dried at room temperature during summer season and ground in the willey mill for the preparation of extract. 3, 5 and 7 g *E. hirta* powder were weighed and kept into their marked conical flasks with 100 ml distilled water in each conical flask and left these suspensions for 24 hours, after 24 hours these suspensions were centrifuged by centrifuge machine and then finally filtered through Whattman No. 1 filter paper. Seeds of test crop were surface sterilized by 0.1% $HgCl_2$. Petri plates were marked as control, 3, 5 and 7% respectively, in each Petri plate kept Whattman No.1 filter paper and ten sterilized seed was placed in each Petri plate, along their 5 replicates. Now pour 2ml of 3, 5 and 7% whole plant extracts in their marked Petri plates and 2 ml distilled water in control at alternate days. The Petri plate of *C. arietinum* was placed at optimum temperature (15-20°C) Now seeds germination was observed every day, radicle and plumule length was observed in alternate days. The trail was terminated after 12 days, before terminating the trail photographs were taken with the help of digital camera. For the preparation of soil we used sandy loam soil with natural humus fertilizer 8:2. All the pots were marked as control, 10, 15 and 2og whole plant powder with their five replicates. Five hundred gram soil was weight and mixed with whole plant powder of *E. hirta* in different quantity such as5, 10, and 20g. In control pots were only filled with 500g of soil. Ten surface sterilized seeds of test crop were sown in their marked pots. The pots were kept in green house for 3-4 days and were irrigated properly. Germination record was taken per day. Seed germination completed, the plants were thinned and 3 plants were left in each pot. After that the plant height were recorded weekly. The experiments were terminated after one month and photographs were taken with the help of digital camera. At the end of the trail, plants were uprooted from each replicates for taken fresh and dry weight. Chlorophyll is extracted in 80% acetone and the absorption at 645 and 663 nm are read in a spectrophotometer. Using the absorption coefficients, the amount of chlorophyll is calculated by Arnon, 1949. 5g of fresh leaves were collected from each treatment, now 20ml of 80% acetone added and make the supernatant with pestle and mortar. It can be store in the dark room. Centrifuged for 5 minutes and then transferred in a cuvette. Calculate of chlorophyll a, b and carotene by direct determination of absorbance at different wavelength, using a standard spectrophotometer (JENWAY 6310). Data were analyzed and subjected to analysis of variance (ANOVA) depending upon the experimental design (Gomez & Gomez, 1984).

Total protein content estimation: Protein content of whole plant was measured as follows:

- The fresh uprooted plant was washed with sterilized distilled deionized water and crushed with mortar and pestle in the presence of liquid nitrogen.

Protein profiling by native polyacrylamide gel electrophoresis: The soluble plant extracts obtained from were used for the analysis of protein profile in all tests and control groups. The gel electrophoresis was performed using 12% separating and 4% stacking gel (Bio-Rad Mini Protean3 System, Bio-Rad Laboratories, Hercules, CA, USA) for about 2 hours at 30V per centimeter.

Statistical Analysis

Chlorophyll content can be calculated from the following formula (Arnon, 1949).

Chlorophyll a (mg/g) = $12.7A_{663}$- $2.69A_{645}$ × V/1000 x W
Chlorophyll b (mg/g) = $22.9A_{645}$- $4.68A_{663}$ × V/1000 x W
Total chlorophyll (mg/g) = $20.2\ A_{645}$+ $8.02A_{663}$ × V/1000 x W

Result and Discussion

The results of the present study showed that *E. hirta* extract had inhibitory effects on percent germination, velocity of germination, S.V.I, radicle and plumule length, plant height, fresh and dry weight, chlorophyll and protein contents of black gram were slightly affected by powder. The present results are strongly supported with the finding of Quasem (1995), who said that *Parthenium* leaf extracts have allelopathic effect on plant growth of wheat. The primary phase of seed germination considered emerging of radical was seen out the seed. The result showed in (Table 1) (Fig. 1) and (Fig. 6) the percent germination was decreased as the concentration of extracts increased as compared to control. Our result indicates that the different extracts concentration had inhibitory effect on germination. Our finding agrees with the views of Kumbhar & Dabgar, (2012), who said that *Chrozopara tinctoria* had Allelopathic effect on seed germination of Chickpea. Maximum percent inhibition was seen in 7% extract while minimum percent inhibition was observed in 3 % extract. These results also supports with the findings of Mishra *et al.*, (2001); Xiangxiang *et al.*, (2009), who reported that increasing concentration of *Asphodelus tenuifolius* and *Hemistepta lyrata* extract adversely affect the germination of wheat, mustard, lentil, Chickpea and *Sorghum* respectively. The velocity of germination was highly affected by the increase of extract concentrations. Highest velocity of germination was recorded in control whereas the lowest velocity of germination was observed in 7 % extract. Our results also correlated with the findings of Jabeen *et al.*, (2013), who said that allelopathic effect of *E. hirta* extract reduced the germination and speed of germination in wheat. Our results also resembles with the finding of Bora *et al.*, (1999), who told that the inhibitory effect of leaf extract of *Acacia auriculiformis* on germination of some agricultural crops was proportional to the concentration of extract.

Table 2. Effect of *Euphorbia hirta* on the percent germination, plant height, and chlorophyll contents, carotene, fresh and dry weight of *Cicer arietinum*.

Treatments	% Germination Mean ± S.E.	% Inhibition	Velocity of germination	Plant height Mean (cm) ± S.E.	% Inhibition in plant height	Fresh weight (g)	Dry weight (g)	Chlorophyll (a) mg g -1	Chlorophyll (b) mg g -1	Total chlorophyll mg g -1	Carotene
Control	74 ± 6	0	80 %	11.8 ± 0.9a	0	2.7 ± 0.2	1.4 ± 0.1	15.79	14.69	32.465	0.950
10 g	60 ± 5.4	18.9	70 %	10.8 ± 1a	8.4	2.4 ± 0.1	1 ± 0.1	14.87	13.56	28.419	0.747
15 g	52 ± 2	29.7	61 %	10.3 ± 0.3a	12.7	1.8 ± 0.2	0.8 ± 0.2	12.65	5.09	17.741	0.178
20 g	50 ± 3.1	32.4	50 %	10 ± 0.4a	15.2	1.4 ± 0.1	0.5 ± 0.3	11.56	4.87	16.439	0.120

Table 1. Effect of *Euphorbia hirta* on the percent germination, radicle and plumule length of *Cicer arietinum*.

Treatments	% Germination Mean ± S. E.	% Inhibition	Velocity of germination	S. V. I.	Plumule length Mean (cm) ± S. E	% Inhibition in plumule length	Radicle length Mean (cm) ± S.E.	% Inhibition in radicle length
Control	100 ± 0	0	89 %	1510	4.1 ± 0.8a	0	11 ± 1.6a	0
3 %	98 ± 2	2	83 %	1391.6	3.5 ± 0.6a	14.7	10.7 ± 1.7a	2.8
5 %	72 ± 8	28	47 %	468	1.7 ± 0.6b	58.6	4.8 ± 1.2b	56.4
7 %	62 ± 3.7	38	45 %	241.8	1.1 ± 0.4b	73.2	2.8 ± 0.9b	74.5

The data of seedling vigor index is measure the quality of seed and the viability of the seed. When the strength of extract was increased the S.V.I. value was gradually decreased. Our results support with the finding of Rehman *et al*., (1991), who said that S.V.I. decrease as the concentration increases because of presence of water soluble inhibitors.

The data of radicle and plumule length is used as an indicator of test crops reported by Burnes & Putnam, (1987). The radicle and plumule length (p<0.05) decrease as the concentration of extracts were increased (Table 1; Figs. 2, 3 & 6). The maximum values of radicle and plumule length were examined in control whereas minimum radicle length was observed in 7% extract as compared to 3 and 5% extracts. These results are correlated with the findings of Kil and Yun, (1992), who reported that extract of *E. dracunculoides* showed that significant reduction in plumule and radicle length of Chickpea. Our results also support with the findings of Donger *et al.*, (2004), who reported that leaf extracts of *Parthenium hysterophorus* had greatest inhibition of seedling growth in black gram. These results similar to previous finding of Bhatt *et al.*, (1994), who stated that extract of *Quercus glauca* and *Q. leucotricophora* significantly, reduced the germination, radicle and plumule length of *Triticum* sp. Extract treatment result showed inhibitory effects on radicle and plumule length of black gram, this might be due to direct contact with the extracts containing inhibitory allelochemical. Lowest % inhibition in radical and plumule length were observed in 3% extract while highest % inhibition was observed in 7% extract. Our results support with the findings of Chaturvedi & Jha (1992), who explained that inhibition in seedling growth is due to the presence of allelochemical.

Weed powder has non-significant effect on plant height of *Cicer arietinum* are showed in (Table 1; Figs. 4; 5 and 7) the plant height was (p>0.05) effected in treatment. The highest inhibition was found in 20 g (15.2%) while in remaining pots when the amount of weed powder increased the plant height moderately decreased, in 15 g, 10 g as compared to control. The present findings agree with reports of Iqbal *et al.*, (2003), who said that allelochemicals can be present in different parts of plant had exhibiting inhibitory effect on plant height and growth of many crops.

Fresh and dry weight was decreased with increased the amount of weed powder as compared to control. Minimum fresh and dry weight was recorded in 20g.According to Shaukat & Siddique, (2001), the toxicity progressively increased with the increasing amount of weed material which reduced the fresh and dry weight of test species. Our results also correlate with the findings of Hussain *et al.*, (2007), who reported that soil infested with *Imperata cylindrical* reduced the early growth, reduction in fresh and dry weight of wheat and lentil.

Chlorophyll contents are the most important components of pigment system. Chlorophyll molecules play a major role in photosynthesis. The chlorophyll a and b decreased with increasing the amount of weed powder

whereas carotene also increased at lower concentration and decreased at higher concentration of weed powder. These results indicate that the chlorophyll contents were affected in treatments as compared to control. Our results support with the findings of Al-Saadawi *et al.*, (1986), who said that chlorophyll contents and ion uptake reduced significantly by allelochemicals. As well as the effects of allelochemicals on chlorophyll contents and photosynthesis process. Our observations correlate with the findings of Batish *et al.*, (2007), who observed a marked reduction in chlorophyll contents of chickpea plants by *Chenopodium murale*. Treatment with different extracts concentration caused a significant reduction in protein contents.

Total protein estimation of plants showed reduction in total protein content with the increased in the concentration of *Euphorbia hirta* extracts (Table 2 and Fig. 8). For Native PAGE analysis of molecular weight of plant proteins, bovine serum albumin (BSA) had been used as marker protein. Imaging and analysis of parameters was performed using Gel doc system (Bio-Rad). Few bands in the range of 280-200 KDa and some protein bands in the range of 200- 100 KDa have been observed. Highest intensity of 243 KDa band has been observed among all bands in the black gram plant.

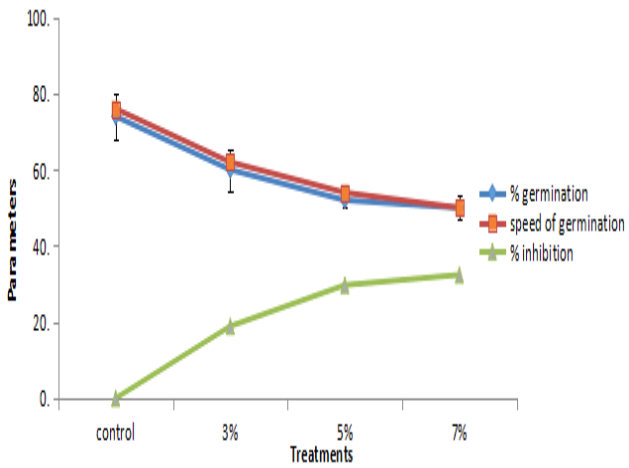

Fig. 1. Effect of *E. hirta* on germination, speed of germination and % inhibition of *cicer arietinum.*

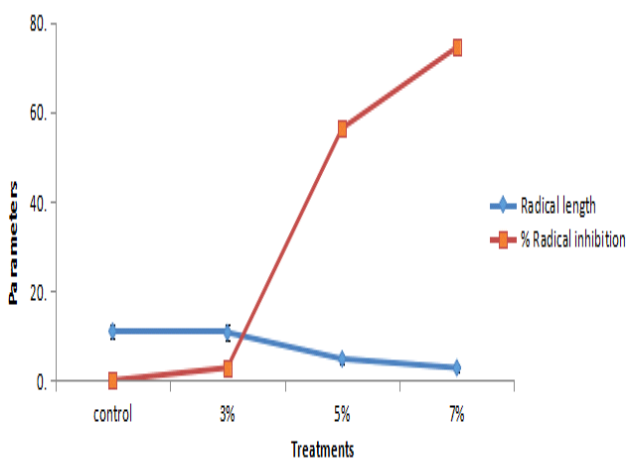

Fig. 2. Effect of *E. hirta* on radical lengths and % inhibition of *cicer arietinum.*

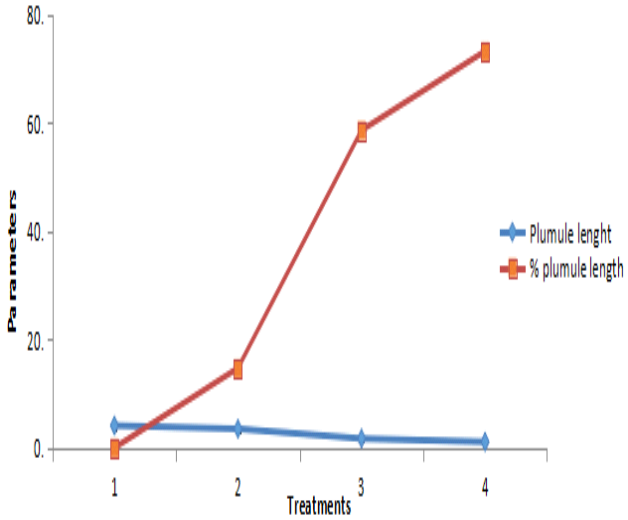

Fig. 3. Effect of *E. hirta* on plumule length and % inhibition of *cicer arietinum.*

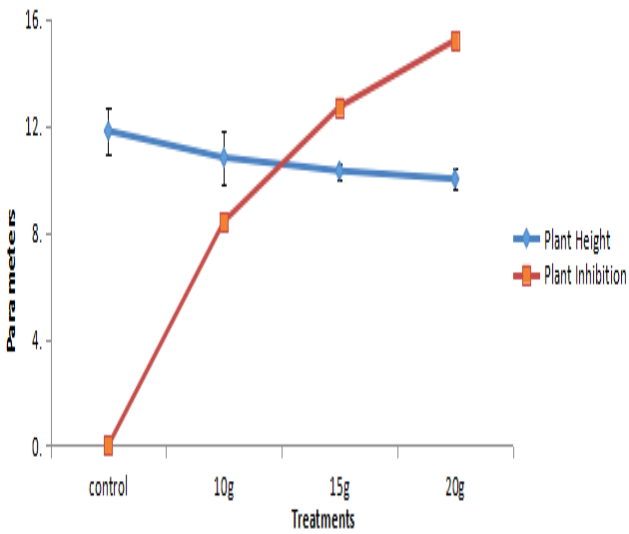

Fig. 4. Effect of *E. hirta* on plant height and % inhibition of *cicer arietinum.*

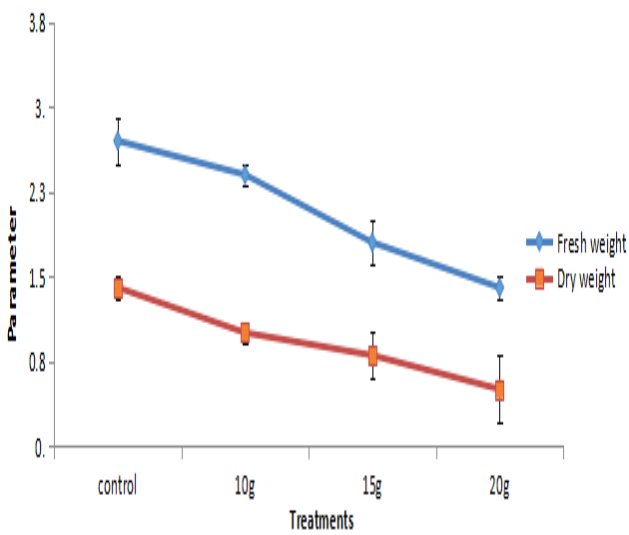

Fig. 5. Effect of *E. hirta* on fresh and dry weight of *Cicer arietinum.*

Control **3 %** **5 %** **7%**

Fig. 6. Effect of different extract concentrations of *E. hirta* on radicle and plumule length of *Cicer arietinum.*

It has been reported that germination of chickpea seed had been inhibited by extracts of *Solanum nigrum* L., *Chenopodium album* L., and *Matricaria chamomilla* L. (10%, 20% and 22.5%, respectively) observed on a period of 21 days Kadiolgue *et al*., (2005) similarly decrease in chick pea seed germination and seedling growth at higher concentration of *E. hirta* (1:1% leaf extract with chick pea soil) whereas lower concentrations (1:4%) showed growth stimulation effects. The findings from current research show that higher concentrations of *E. hirta* (10gm, 15gm and 20gm) resulted in stunted growth of chick peas. However, in the light of previous literature it is suggested that lower concentration of *E. hirta* be tested for allelopathic effects so that an established link be reported on the co-cultivation of these two plants species. These first results regarding protein turn over during sesame growth suggest that the morphological changes observed in treated sesame seedlings are related to a few changes in protein synthesis. The investigation of allelopathic effects on protein synthesis opens the perspective of a better knowledge of allelochemical action at biochemical level.

Our results, indicates that higher amount of *E. hirta* effects the percent germination, velocity of germination, plant height, chlorophyll contents, fresh and dry weight whereas lower amount has less inhibitory effect on growth parameters. Our findings support the results of Arshad & Frankenberger, (1998), who told that many plants secr *et al* lelochemicals which are highly affected on germination, growth and development of plant even at low concentration.

Plants released allelochemicals they could be sensitive to the other plants. They reduce the percent germination, seedling growth, and plant height and chlorophyll and protein contents of *C. arietinum.* The results showed that extract of *E. hirta* was inhibit the seedling growth of *C. arietinum*. On the other hand it was observed that *E. hirta* (powder) has no significant effect the plant height of *C. arietinum.*

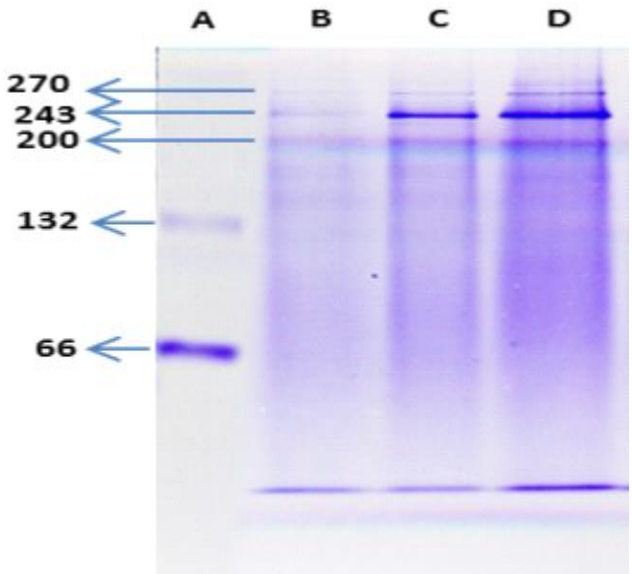

Fig. 8. Effect of different amount of *E. hirta* on protein of *Cicer arietinum.*

Fig. 7. Effect of different amount of *E. hirta* on plant height of *Cicer arietinum*.

Conclusion

On the basis our investigations, we concluded that the aqueous extract of *E. hirta* had inhibitory effect on percent germination, velocity of germination, seedling growth while in pot experiments percent germination, fresh and dry weight, chlorophyll and protein contents were also markedly affected in test crop. This study revealed that allelopathic effects of *E. hirta* are due to the presence of allelochemicals. Therefore, *E. hirta* should be removed from fields at early stage to save test crops from harmful effects. Further studies are recommended to investigate, the possible physiological changes related to allelopathic effect on valuable crops.

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