

## A BIOASSAY OF PROTEINS AND EVALUATION OF PHYSIOLOGICAL ACTIVITY OF *CICER ARIETINUM* L. SEEDLINGS UNDER THE INFLUENCE OF ALLELOPATHIC WEED *EUPHORBIA HIRTA* L.

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### Abstract

Physiological and metabolic actions are the key elements of plant growth and these activities are consistently effected by many environmental factors including allelopathy. Present study describes the allelopathic effect of *Euphorbia hirta* L. on physical and physiological aspects of *Cicer arietinum* L. Weed powder have significantly affected plant height, fresh and dry mass whereas, chlorophyll (a and b), carotene and protein contents are gradually decreased as the amount of weed increased up to a maximal of 20g of weed powder. Highest inhibition has observed in 20g. Protein contents have been greatly disrupted by allelopathic influence as well as chlorophyll contents while carotenes have been synthesized in greater amount with the increase in manure concentration. PAGE analysis has produced highest bands of proteins in control sample whereas, 20 gram weed powder showed lowest number of bands. MANOVA computation released highly significant ( $p < 0.001$ ) differences among the samples. The soil samples show significant ( $p < 0.01$ ) effectiveness of pH that can be a factor for allelopathic response from soil and plant growth. It can be concluded that *E. hirta* has produced inhibitory effects on plant growth, specifically responsible for protein damage and deactivation of chlorophyll content.

**Key words:** Inhibition, Protein, MANOVA, PAGE, Fresh mass, Chlorophyll.

### Introduction

Plant's growth is entirely dependent upon the physiological factors that are being disturbed by many stresses such as disrupted environmental conditions, presence of pathogens, leaching of exudates, poor water quality used for irrigation and emergence of allelopathic weeds in an agriculture field. Allelopathy is mainly concerned with the interference of plant species in an agricultural field by the production of certain phenolic compounds that could possess either detrimental or beneficial effects on growth, physiology and survival rates of neighbouring plants (Oraon & Mondal, 2020). Secondary biochemicals (allelochemicals) are the major constituents of this phenomenon that directly enter-course with the cell of recipient species and activated the process of ex-osmosis that leads to plasmolysis of the cell, this may cause physical as well as physiological alteration in plant's cell (Wu *et al.*, 2003). There is a huge contribution of allelochemicals in influencing the biochemical and nutritional aspects of recessive plant however, Pawlowski *et al.*, (2012) observed that terpenoides play a vital role in the enhancement of mitotic abnormalities that cause reduction in root size. Chlorophyll contents are the main components of photosynthesis, a little hindrance in these pigments could bring a great change in all physiological aspects of a plant, however, various phenolic acids including cinnamic acid, produced harmful effects by altering the cellular structure of chloroplast (Wu *et al.*, 2004). Antioxidants have significant importance in the life cycle of any plant as it boosts the whole plant mechanism against stress

condition, their activities and amount were chiefly retarded due to presence of caffeic acid that changes the activities of peroxidase (Batish *et al.*, 2008). There is a wide range of allelochemicals that inhibit protein synthesis i.e., ferrulic acid and cinnamic acid could break the long chain of amino acids thus creating hurdles in transferring them into proteins.

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

*Cicer arietinum* L. commonly known as black gram. It is a major food legume, known for its protein rich nutritional quality. Black gram is a cholesterol free crop. 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). Biochemicals produced by allelopathy have a great influence on the physical and physiological properties of recessive plants. However, the current study is focused on the exploration of common physiological and nutritional factors of *C. arietinum* that have been predominantly affected by *E. hirta*. This will evaluate the relative destructive or constructive effects featuring biochemical activities and nutritional value of the crop.

## Material and Methods

**Experimental design and site:** The experimental work was conducted in the month of February 2018 by following control randomized block design at research field in Department of Botany, Federal Urdu University. *Euphorbia hirta* plants were collected from different areas of Karachi which were air dried and ground in the wiley mill for the preparation of fine powder. Five-hundred-gram soil was weighed and mixed with the obtained plant powder of *E. hirta* to prepare 10, 5 and 20g of soil samples, placed in their respectively marked pots along with the replicates. Soil texture was composed of sandy loam soil with natural humus fertilizer in 8:2 ratio. Control pots were filled with 500g of soil only. Ten surface sterilized seeds of test crop were sown in their respective pots. The pots were kept in green house for 3-4 days and were irrigated properly. Germination record was taken per day while plant height was recorded weekly. Experimental duration covered 8 weeks period. Soil parameters were detected after harvest of plants using Multiparameter (EUTECH INSTRUMENTS PC-650).

**Chlorophyll contents:** Treated plants from each pot were uprooted for chlorophyll estimation. The estimations were carried by following Arnon (1949) method. However, absorbed wavelengths were recorded at 645 and 663 nm by the help of spectrophotometer (JENWAY 6310).

Formula:

$$\text{Chl a (mg g}^{-1}\text{)} = \frac{12.7D_{663} - 2.69D_{645}}{W} \times V$$

$$\text{Chl b (mg g}^{-1}\text{)} = \frac{22.9D_{645} - 4.68D_{663}}{W} \times V$$

$$C_{x+c} = \frac{7.6D_{480} - 2.63D_{510}}{W} \times V \quad (x = \text{xanthophylls and carotenes})$$

**Total protein content estimation:** 1g of treated plant leaves were crushed to make extracts under 80% acetone. These extracts were centrifuged and the obtained liquid was analyzed for its total protein content estimation followed by Lowry *et al.*, (1951).

$$\text{Protein concentration} = 1.55 A_{280} - 0.76 A_{260}$$

**Protein profiling by native polyacrylamide gel electrophoresis (PAGE):** The soluble plant extracts of each concentration were further examined for protein profiling. For this purpose, gel electrophoresis method 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. Gel was stained later by R-250 Coomassie brilliant blue (CBB) and de-stained with methanol-water mixture followed by Laemmli, (1970).

## Statistical Analysis

Multiple analysis of means was computed for investigation of differences in means and sum of squared means of the physical and biochemical multiple parameters followed by Andrews & Herzberg, (1985) and Rencher, (2002). Data was analyzed by using SPSS, MANOVA was performed for comparison of means in each sample and replicates involved following equation, where SSE used for simple ANOVA and SPE for multiple parameters explaining within group and between group mean differences.

$$SSE_{11} = \sum_{i=1}^k \sum_{j=1}^n (y_{ij1} - y_{i.1})^2$$

$$SPE_{23} = \sum_{i=1}^k \sum_{j=1}^n (y_{ij2} - y_{i.2})(y_{ij3} - y_{i.3})$$

## Result and Discussion

It has been elucidated from the analysis that *E. hirta* weed powder produced inhibitory effects on germination, velocity of germination, plant height, fresh and dry weight, pigments (chlorophyll and carotene) and protein contents of black gram. The weed powder has adversely affected the physical as well as biochemical parameters of *Cicer arietinum*, consequently distorted the soil structure in treated samples. Inhibition in stem elongation took place in the order of Control > 10gm > 15gm > 20gm, this indicated that as the concentration increased, inhibition have also increased showing an inversely proportional relationship between plant height and manure concentration (Table 1 and Fig. 1). Current findings validated the hypothesis that allelochemicals can be present in different parts of a plant that can exhibit inhibitory effects on plant height and growth of other plants (Iqbal *et al.*, 2003). Fresh and dry weights can also be decreased by increasing concentration of weed powder. Lowest fresh and dry weight was recorded in 20g samples. According to Shaukat & Siddiqui (2001) the toxicity progressively increased with the increasing amount of weed material which reduced the fresh and dry weight of test species. Gradually decreased weights in current findings correlated with Hussain *et al.*, (2007) in which soil infested with *Imperata cylindrical* green manure that have reduced the early growth *i.e.*, fresh and dry weight of wheat and lentils.

**Table 1. Preliminary growth and biochemical analysis of treatments.**

Samples	Germination %	VOG%	SL (cm)	TM (g)	CHLA mg g <sup>-1</sup>	CHLB mg g <sup>-1</sup>	CRTN mg g <sup>-1</sup>	PTN mg g <sup>-1</sup>
Control	74 ± 6	80	21.93 ± 0.9	14.26 ± 0.2	26.17	20.16	10.950	15.5
10 g	60 ± 5.4	70	20.09 ± 1	13.13 ± 0.1	23.87	18.23	8.747	11.45
15 g	52 ± 2	61	17.23 ± 0.3	11.53 ± 0.2	20.65	16.09	6.178	9.87
20 g	50 ± 3.1	50	13.11 ± 0.4	9.61 ± 0.1	18.56	8.87	5.120	6.84

Where, VOG = Velocity of germination, SL = Shoot length, TM= Total mass, CHLA = Chlorophyll a, CHLB = Chlorophyll b, CTN = Carotene, PTN = Proteins

**Table 2. MANOVA outputs among treatments and control samples.**

Wilk's $\lambda$	p value	F	df1	df2
0.286	( $p < 0.001$ )	3.741	21	144.1

In addition to physical growth parameters, biochemical synthesis in plants have greatly influenced by the allelopathic flux imposed over the test crop. Chlorophyll contents are the most important components of pigment system, moreover, chlorophyll molecules play a major role in photosynthesis. Chlorophyll a and b decreased with the increasing amount of weed powder whereas carotene decreased at lower concentration and increased at higher concentration of weed powder (Table 1 and Fig. 1). Predefined results indicated that among pigments, chlorophyll contents were severely affected by the addition of weed powder in all the given ranges. Al-Saadawi *et al.*, (1986) found reduction in chlorophyll contents and ion uptake due to the hindrance created by allelochemicals in the course of their metabolism. Similar outcomes reported by Stupnicka-Rodyznkiewicz *et al.*, (2006) while investigating the effects of allelochemicals on chlorophyll contents and photosynthesis. Batish *et al.*, (2007) observed a marked reduction in chlorophyll content of chickpea plants prompted by *Chenopodium murale* allelopathic activity, that have strongly correlated with the current investigations.

While examining the variance of the treated samples, MANOVA produced a highly significant ( $p < 0.001$ ) difference among the treatments and they're within group variables (Table 2).

A colorogram was obtained to elaborate the relationship between physical and biochemical variables with their respective treatments (Fig. 2). Shoot length and fresh weights of treatments have dominantly produced responses over the manure applications whereas among biochemical parameters, there were only carotenes that have produced highly significant response in all concentrations. However, chlorophyll b has also appeared to be non-significant but a little contrast can be observed in case of other pigments. The poorest condition was found in proteins that were drastically reduced from the treatments (Fig. 2). Due to the extensively poor protein condition, the protein test was conducted to examine the current proportion of protein content and figure out the sustainability marks, to check if there was some possibility existed to recover.

Weed manure have caused remarkable reduction in protein contents of *C. arietinum* at different concentrations. Total protein estimation of plants showed reduction in protein content with the increase in concentration of *Euphorbia hirta* extract (Fig. 3). For Native PAGE analysis of molecular weight of plant proteins, bovine serum albumin (BSA) has 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. 3a and b).

Differences in protein bands intensity can be clearly seen in lane B, C, D as compared to lane E (control plant). Protein bands of 243 KDa has drastically reduced with the increase in *Euphorbia hirta* concentration. The band of 270 KDa has remained persistent as compared to other proteins with the increase in concentration of *E. hirta*. Concentration of 200

KDa protein appeared to be directly related with the increased *E. hirta* concentration, while there were thin bands in control as compared to test groups. Side by side a dendrogram was also produced to elucidate the relative production of proteins and their similarities with other treated groups (Fig. 3a). It was observed that control samples have separated apart while 10 and 15gm samples have formed two closed groups. However, some similarities in lower production of proteins were complimentary to 20gm samples that indicated a prominent disturbance in proteinaceous compounds of the crop. The denaturation of pre-existing proteins or poor protein production can be caused even at lower concentration in some cases showing few probabilities (Mamone *et al.*, 2019) while the same outcome has also been recorded at higher concentration in the current findings.

The investigation of allelopathic effects on protein synthesis has opened the perspective of a better knowledge of allelochemical action at biochemical level. A great potential of allelopathic weeds have explored in various crops responsible for growth suppression by undertaking alterations in cellular metabolism (Fragosso *et al.*, 2013). The structure and shape of cell organelles and permeability of membranes have known to be increased by various allelochemicals through increase in lipid peroxidation (Lin *et al.*, 2000). The increased permeability hinders the uptake of nutrients in plants that may produce suppression in metabolic activities, protein and enzyme synthesis. Many previous studies concluded, chlorogenic acid, caffeic acid and catechol as the most commonly found allelochemicals in many weeds (Rice, 1984; Einhellig, 1995) that have produced inhibition to  $\lambda$ -phosphorylase (a seed germinating enzyme) while gallic acid and other phenols also inhibited cinnamic acid-4-hydroxylase and phenylalanine ammonialyase (PAL) (Lin *et al.*, 2001; Zhou *et al.*, 2010; Khan *et al.*, 2018). Reduction in enzymes have brought irregularities in total protein content of plant. Similarly, inhibition of hydrogenase enzyme has brought destruction in photosynthetic machinery hence, ATP synthetase also affected directly or indirectly (Wu *et al.*, 2004). Replacement of  $Q_A$  by  $Q_B$  from PSI to PSII during photosynthesis damaged  $D_1$  protein as an effect of pyrogallol (Shao *et al.*, 2009). Ferrulic acid and cinnamic acid alongwith other alkaloids and phenols prevented DNA and RNA integration and protein biosynthesis (Wink & Latzbruning, 1995). Protein synthesis have affected drastically from allelochemicals that ultimately retarded germination and growth in plants. In the present observations, plant pigments predominantly chlorophyll and proteins were distinctively suppressed biochemicals in which proteins were in the worst condition.

Current findings confirmed that higher amount of *E. hirta* produced negative effects on percent germination, velocity of germination, plant height, chlorophyll contents, fresh and dry weight whereas lower amount persuaded less inhibitory effects on growth parameters. Proteins have existed in the most vulnerable condition that can be recovered at lower levels. In case of higher concentrations, the loss of proteins and related bio-products can occur in the plant causing damage of crop by damaging fundamental physiological activities. Hence, it is a point of concern that the crop being protein rich however, it has been severely affected in its protein contents by the allelopathic bio-stimulants. Therefore, the nutritional value is endangered of this important crop.

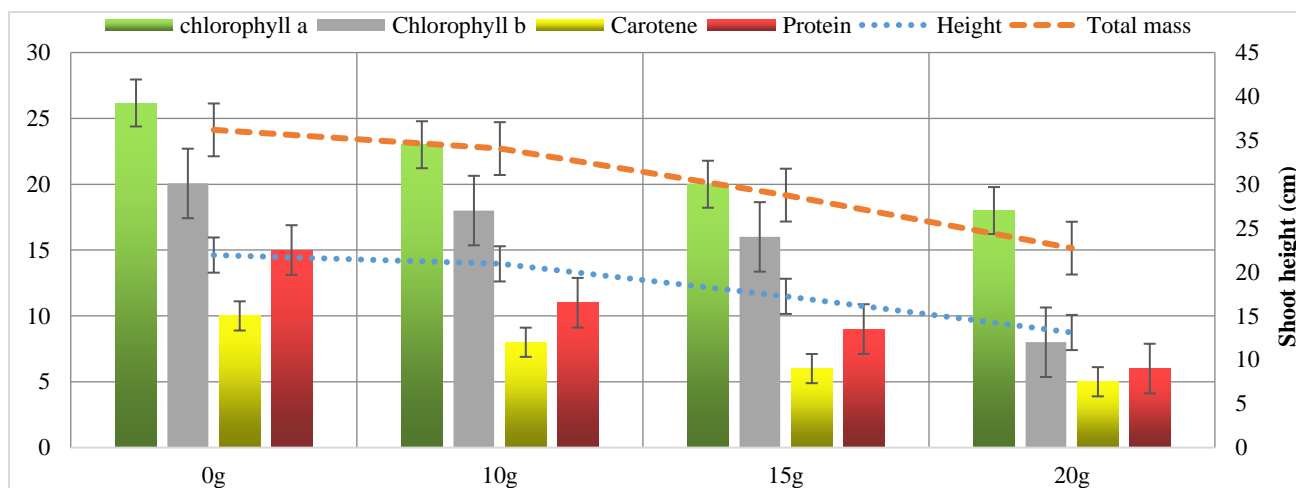


Fig. 1. Effect of *E. hirta* on germination, plant height, biomass, pigment content and carotene of *Cicer arietinum*.



Fig. 2. Relationships of physical, biochemical and edaphic variables with their corresponding treatments and concentrations. Where, 1= Shoot length, 2= Fresh weight, 3= Dry weight, 4= Chlorophyll a, 5= Chlorophyll b, 6= Proteins, 7= Carotene, 8= pH, 9= Total dissolved salts (TDS), 10= Conductivity.

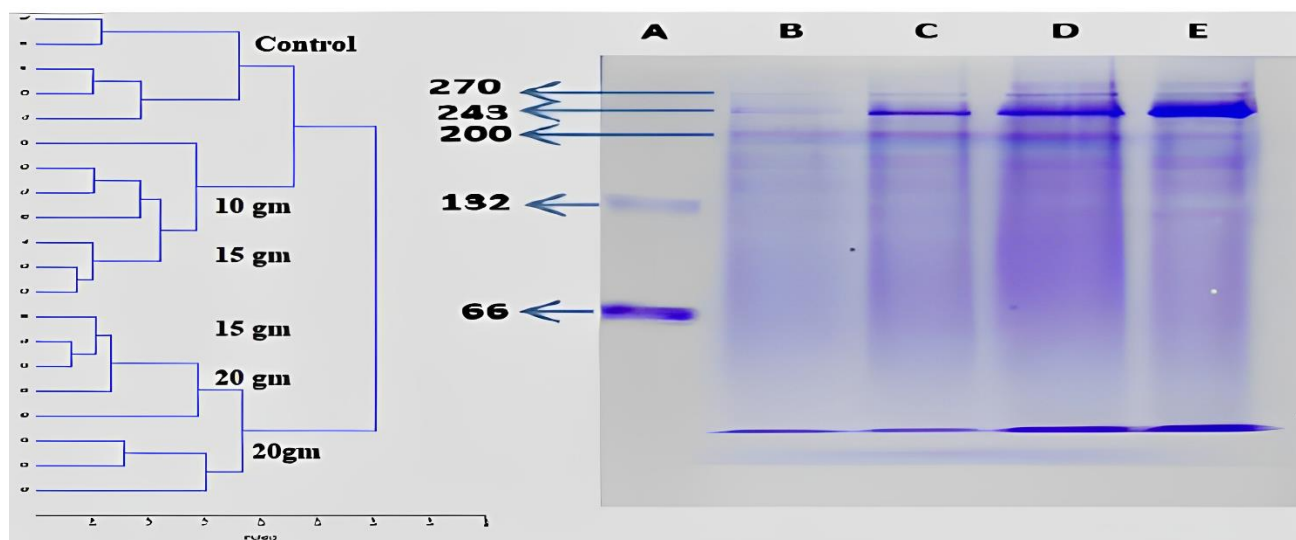


Fig. 3. (a) Protein dendrogram showing relative production probability at different concentrations (b) Protein bands printed from HPLC detector.

## Conclusion

This study revealed that allelopathic effects of *E. hirta* have drastically inhibited the efficiency of important biochemicals in *C. arietinum* seedlings. Since, seedling stage recommends plant's vigour and require appropriate living conditions to enhance plant growth and metabolism. Therefore, *E. hirta* should be removed from fields at early stage to save the crop from harmful effects. Further studies are recommended to investigate, the possible physiological changes related to allelopathic effect on valuable crops and chemical bioassay of *E. hirta* in different ecological conditions.

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