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ALLELOPATHIC POTENTIAL OF *Euphorbia dracunculoides* ROOT AQUEOUS EXTRACT ON SEED GERMINATION AND EARLY SEEDLING GROWTH OF CHICKPEA

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ABSTRACT

The present investigation was carried out to determine allelopathic potential of Euphorbia dracunculoides aqueous root extract (EARE) on seed germination and early seedling growth of Chickpea. The dry root powder was extracted in autoclaved distilled water to make 12.5%, 6.25%, 3.125%, 1.56% and 0.78% extracts. The EARE was a good source of natural phenolics and was applied as seed soaking to Chickpea seeds prior to sowing in pots. The EARE did not significantly affect seed germination indices such as seed germination (%), germination index (GI) and germination rate index (GRI) of Chickpea. The EARE at 12.5% concentration significantly increased ($p<0.05$) shoot fresh weight, root area, root length, chlorophyll a, total soluble phenolics content of root and shoot of chickpea. The carotenoids content was higher at 3.25% EARE. It is inferred from findings of the present investigation that EARE exhibited stimulatory effects on growth of Chickpea at early seedling stage. Further investigation of the plant may lead to the formulation of some novel compounds, applicable as plant growth regulators.

Key words: Allelopathy, *Euphorbia dracunculoides,* phenolics, photosynthetic pigments.

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INTRODUCTION

Plants can synthesize certain phytotoxic chemicals which can affect the germination, growth and development of other plants. This effect of one plant on the germination and growth of another plant is known as phytotoxicity (Ahmad *et al.,* 2011). Various types of phytotoxic chemicals such as tannins, phenolic acids, lignins, alkaloids,

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flavonoids, coumarins and terpenoids can have phytotoxic activity and are considered as phytotoxic chemicals (Abu-Romman *et al.,* 2010). Phenolic acid on one hand regulate the function of various enzymes, it however is also considered to have phytotoxic activity (Capasso *et al*., 1992). It is an active antifungal compound and helps in detoxification of reactive oxygen species (Capasso *et al.,* 1997). They are usually released when some part of plant body is decomposed. However, they have also been found in free form, in the root zone of some phytotoxic plants (Zhao *et al.,* 2010).

Phytotoxicity can serve as a mechanism through which plant species interact with each other resulting in the establishments of a new community (Ridenour and Callaway 2001).The extract of phytotoxic plants can be applied as the most practical mechanism to control weeds (Dhima *et al*., 2006).

A lot of phytotoxic compounds can be found in cells and tissues of plants (Ashrafi *et al.,* 2008; Gilani *et al*., 2007). Phenols are regarded as the most effective phytotoxic compounds which can affect the germination, seedling growth and cell division (Khan *et al*., 2011). These phytochemicals can be applied for the welfare of human being (Tsakala *et al.*, 2006; Hussain *et al*., 2010; Afridi and Khan, 2015). The phytochemicals present in extracts of allelopathic plants inhibit the photosynthetic machinery of target plant species by reducing the content of photosynthetic pigments (Narwal, 2004). Recent scientific studies have shown that higher phenolic contents can negatively affect seed germination (%) and seedling growth of target plants (Ullah *et al.,* 2014).The present investigation was aimed to determine allelopathic potential of aqueous root extract of *Euphorbia dracunculoides* on seed germination and seedling establishment of Chickpea.

MATERIALS AND METHODS

Fresh roots of *Euphorbia dracunculoides* were dried under shade at room temperature. The dried roots were grinded into fine powder. The powdered material (12.5 g) was soaked in 100 ml of distilled water for 48 hours. The mixture was filtered through a filter paper (Whatman No. 1) to obtain aqueous extracts. A stock solution (12.5g/100ml of distilled water) for root extract was thus prepared. The stock solutions was further diluted to make 12.5%, 6.25%, 3.125%, 1.56% and 0.78% solutions through serial dilution.

Determination of Total phenolic content of *Euphorbia dracunculoides* extracts

Folin Ciocalteau method was used for estimation of total soluble phenolics content of EARE (Chun *et al*., 2003). The 0.5 ml of the EARE was added to Folin Ciocalteau reagent. The mixture thus obtained was

incubated at room temperature (25 ± 1°C) for 5 minutes. The solution was added with aqueous sodium carbonate (7.5%). The mixture was incubated at room temperature (25 ± 1°C) for 2h and absorbance of the mixture was measured at 765nm by using a spectrophotometer (Hitachi's U-510 Tokyo Japan). Various concentrations of gallic acid were applied to obtain standard curve for of measurements. Total phenolic content was expressed as mg gallic acid equivalents / g extract.

Bioassay

Pot experiment was conducted for the assessment of phytotoxic effect of EARE on Chickpea in a glass house in the Department of Botany, University of Science and Technology, Bannu, KP, Pakistan. Seeds of chickpea were surface sterilized by rinsing them in a 0.2% solution of mercuric chloride and were subsequently washed with sterilized distilled water. The seeds were soaked in various concentrations of the EARE for 4hours, provided with proper aeration (Khattak *et al.,* 2015). The treatments were control (seeds soaked in distilled water), seeds soaked in 12.5 % EARE, seeds soaked in 6.25% EARE, seeds soaked in 3.125% EARE, seeds soaked in 1.56% EARE, seeds soaked in 0.78 % EARE.

The seeds were sown in plastic pots measuring 11x8 cm^2 filled with sand and clay (1:1). The experimental design was complete randomized design (CRD). Germinated seeds in all treatments were counted on daily basis for eight consecutive days. After 21 days of the experiment, the seedlings were harvested for further analysis. Seed germination (%) was determined as:

Seed germination (%) = (Germinated seeds /Total number of seeds grown) x 100

Germination index (GI) was determined by standard formula as given by Association of Official seed Analysts (Anon 1983).

Germination index (GI) = (Seeds which were germinated at first count + Number of seeds which were germinated at last count) / (Days of first count + days of final count)

The germination rate index (GRI) was calculated as follow:

GRI = Germination index / germination percentage

The shoot fresh and dry weight was determined using an electric balance. Root Law Software (Washington State Research Foundation USA) was applied for the determination of root architecture.

Determination of photosynthetic pigments

The method of Arnon (1949) was used for the extraction and quantification of photosynthetic pigments.

Determination of total soluble phenolics content of Chickpea leaves and roots

Freshly collected leaves or roots (0.01 g) of the target plant were extracted in 10 ml of methyl alcohol. The 50 µL of the methanolic extract was added to 0.25mL of diluted Folin-Ciocalteau reagent. After 5 minutes of incubation, aqueous solution of 7.5% sodium carbonate was added and the whole mixture was incubated at room temperature (25±1^{o}C) for 2 hours to complete the reaction. The absorbance of mixture was measured at 765nm using a spectrophotometer (Hitachi's U-510 Tokyo Japan). Various concentrations of gallic acid solutions were used to obtain standard curve. The concentration of total soluble phenolic contents was represented in mg gallic acid equivalents / g fresh weight (Wolfe *et al*., 2003).

Statistical analysis

The data was analyzed for significance using one way ANOVA. The number of replicates for each treatment was three. Means values of treatments were compared ($p<0.05$) using least significant differences (LSD) test (Steel and Torrie 1984).

RESULTS AND DISCUSSION

The various concentrations of EARE were characterized for total soluble phenolics content**.** Higher total soluble phenolics content was recorded in 12.5 % EARE (259.77 mg Gallic acid eq. /gram extract) followed by 6.25% EARE (219.24 mg Gallic acid eq. /gram extract), 3.125% EARE (146.41 mg Gallic acid eq. /gram extract), 1.56% EARE (71.67 mg Gallic acid eq. /gram extract) and 0.78% EARE (43.09 mg Gallic acid eq. /gram extract) as shown in Fig. 1. Previous studies have reported that aqueous plant extracts were sources of natural phenolics which exhibited either stimulatory or inhibitory effects on target plant species (Sharma *et al,.* 2009; Ullah *et al*., 2014).

Results presented in Fig. 2 (a, b & c) showed that seed germination indices of chickpea like seed germination (%), germination index and germination rate index were not significantly affected by EARE at all the concentrations.

The seedling weight of Chickpea was significantly increased by EARE at $p<0.05$ as indicated in Fig. 3 (a & b). The stimulatory effect of EARE on seedling fresh weight was significantly higher at 12.5% concentration; whereas, stimulatory effect of EARE on seedling dry weight was significantly more pronounced at 1.56% concentration.

The seed soaking with EARE significantly increased root area and root length of chickpea plants at $p>0.05$ (Fig. 3c, 3d). The beneficial effect of all the concentrations EARE on root area was statistically at par. The increase in root length was significantly higher by application of EARE at 12.5% and 1.56% concentration.

During present studies EARE proved its effectiveness as biostimulator for Chickpea. The beneficial effects of EARE on Chickpea plants were related to its stimulatory role on photosynthetic pigments and exceptional changes in root surface area. The higher photosynthetic activity might have led to the accumulation of dry matter as was evident by the production of higher shoot fresh weight and dry weight. This might be because that EARE was a good source of beneficial allelochemicals. Earlier workers have find out that allelochemicals can have either stimulatory or inhibitory effects on target plant species (Khattak *et al.,* 2015).

All the concentrations of EARE significantly increased chlorophyll *a* content of Chickpea as compared to control. However the stimulatory effect of EARE was significantly more pronounced at 12.5% concentration as clear from Fig. 4a. There was found significant reduction in chlorophyll *b* content of Chickpea by application of EARE at 6.25%, 3.125%, 1.56% and 0.78%. The application of EARE at 12.5% did not significantly affect chlorophyll *b* content as compared to control as indicated by Fig. 4b. The effect of EARE on chlorophyll a/b ratio of Chickpea was stimulatory and significant at $p<0.05$. The beneficial effect of the EARE on chlorophyll a/b ratio was higher at 6.25% and 12.5% concentration as shown in Figure 4c. Results presented in Fig 4d indicate that the leaf carotenoids content of Chickpea was not significantly affected by EARE at 12.5%, 6.25%, 1.56% and 0.78%. However, the application EARE at 3.125% concentration significantly increased carotenoids content as compared to control.

Seed soaking with EARE caused accumulation of soluble phenolics in roots and leaves of chickpea (Fig. 5 a, b). The accumulation of phenolics was higher by application of higher concentration of EARE (12.5% and 6.26 %). The results further revealed that accumulation of phenolics was higher in roots as compared to leaves. The presence of allelochemicals is reported in different plant parts such as leaves, flower and root etc (Ashrafi *et al.,* 2008; Gilani *et al.,* 2007). Recent researches have shown that phenols were the most effective substances on germination, seedling growth and cell division (Khan *et al.,* 2011). During present studies seed soaking with EARE caused accumulation of soluble phenolics in root and leaf of Chickpea. These phenols exhibited physiological action and improved growth of Chickpea. These results are in agreement with previous studies that cucumber plants supplemented with phenolics exhibited higher content of endogenous phenolics (Muzaffar *et al.,* 2012)

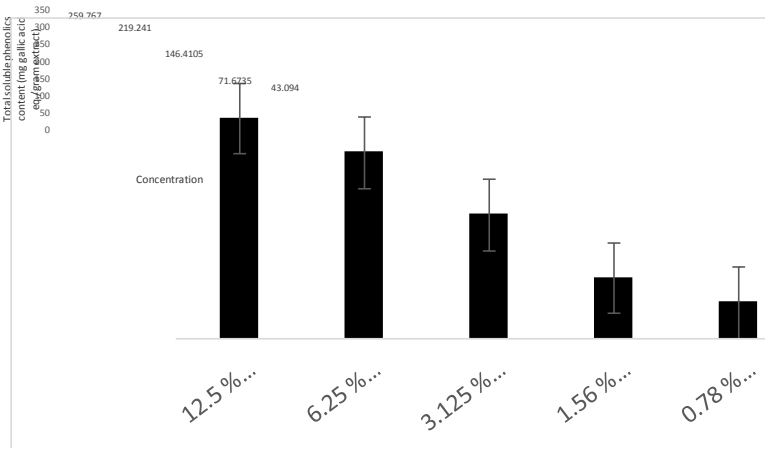
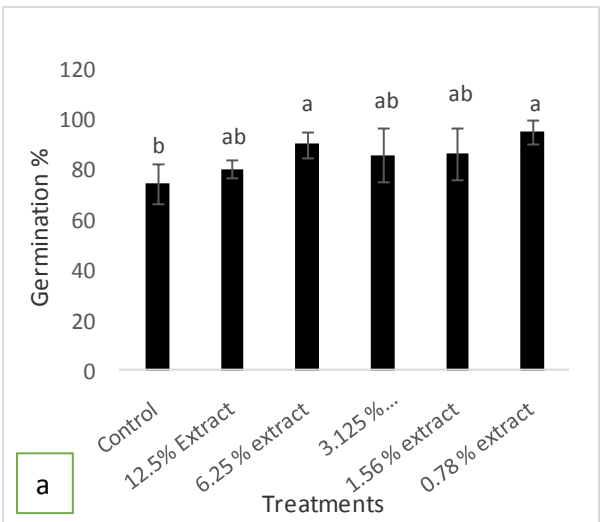
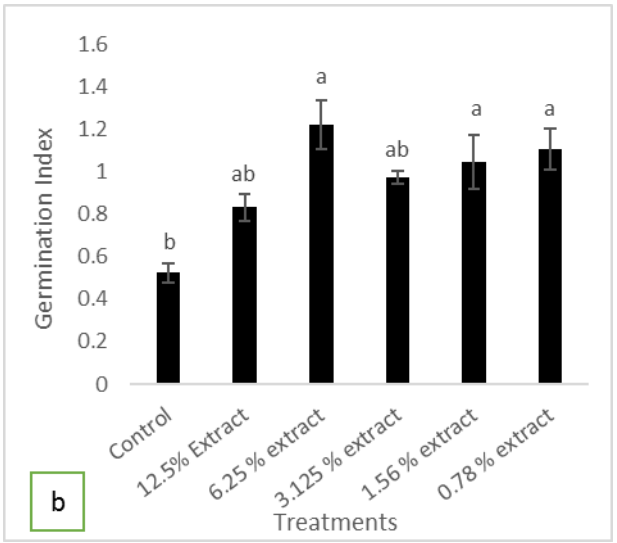


Figure 1. Total soluble phenolics content of EARE





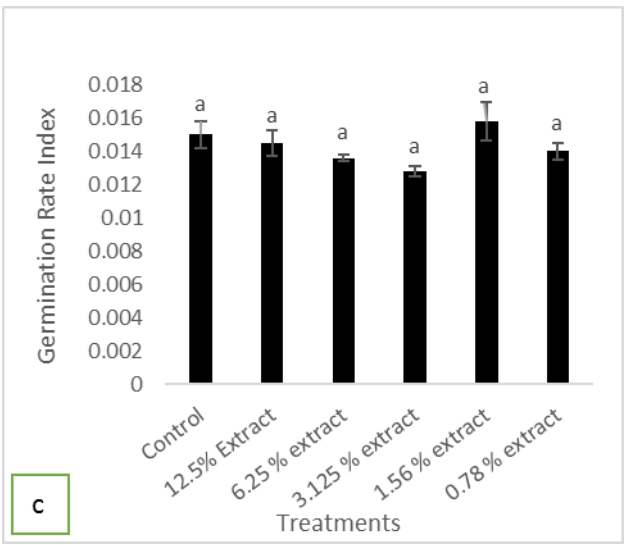
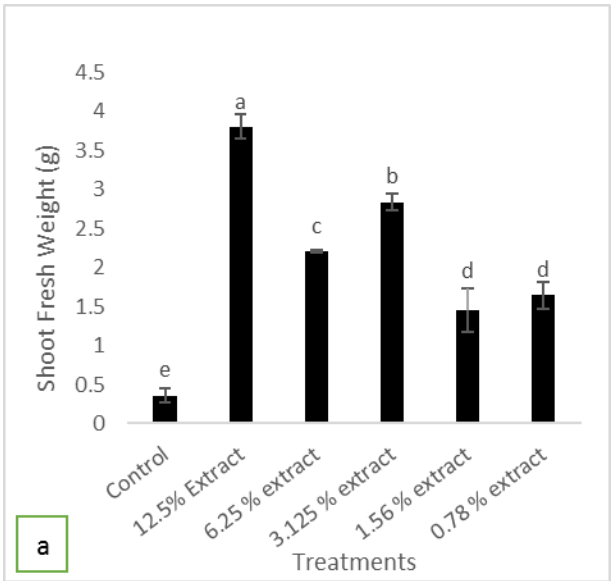
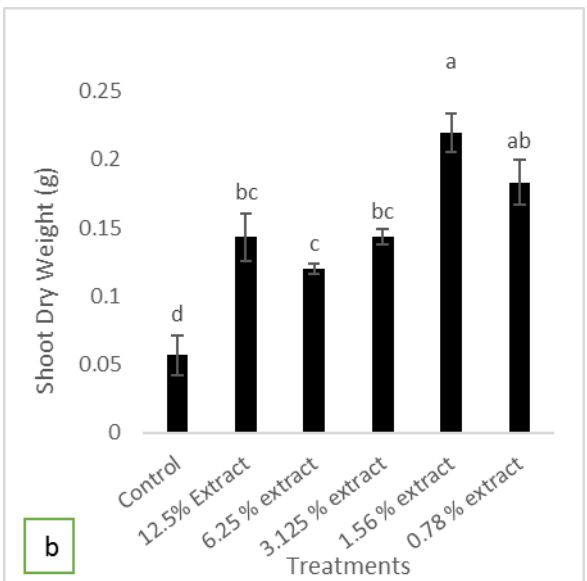
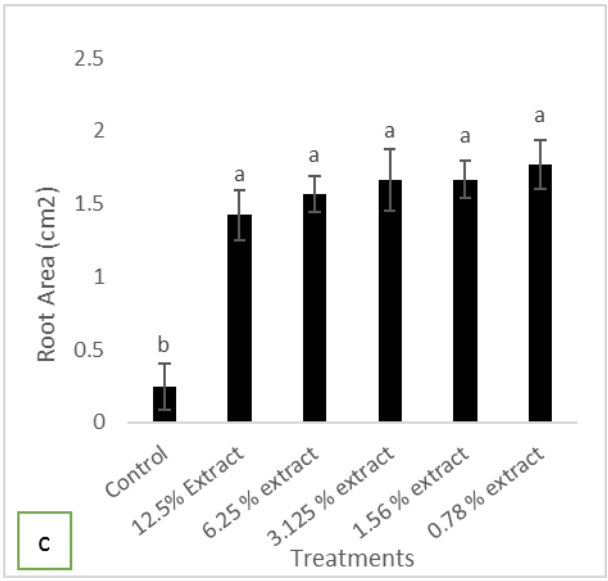


Figure 2. Effect of *Euphorbia dracunculoides* root extract on (a) Germination % LSD: 15.632 (b) Germination index LSD: 0.5099 (c) Germination rate index LSD: 0.006. Means sharing common English letters are statistically similar.


4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
Shoot Fresh Weight (g)
a
b
c
d
d
e
Control
12.5% Extract
6.25 % extract
3.125 % extract
1.56 % extract
0.78 % extract
Treatments
a


0.25
0.2
0.15
0.1
0.05
0
Shoot Dry Weight (g)
a
ab
bc
bc
c
d
Control
12.5% Extract
6.25 % extract
3.125 % extract
1.56 % extract
0.78 % extract
Treatments
b



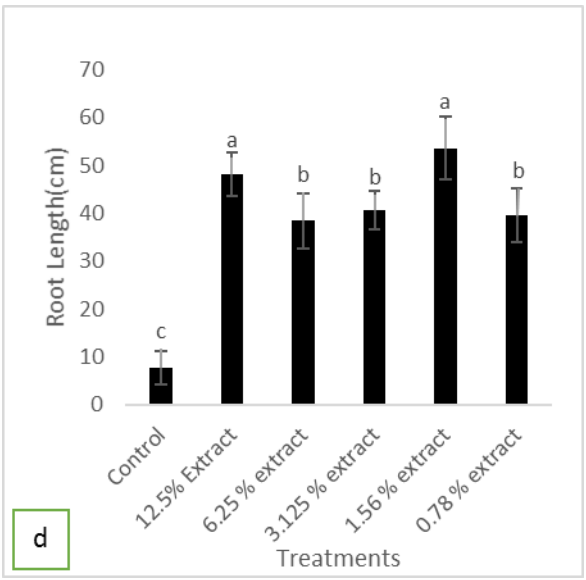
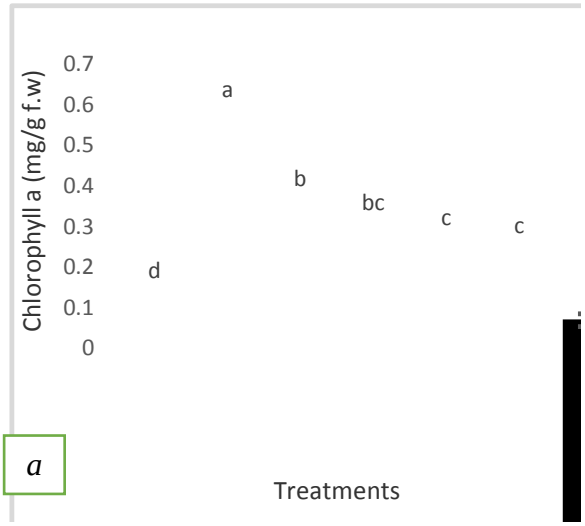
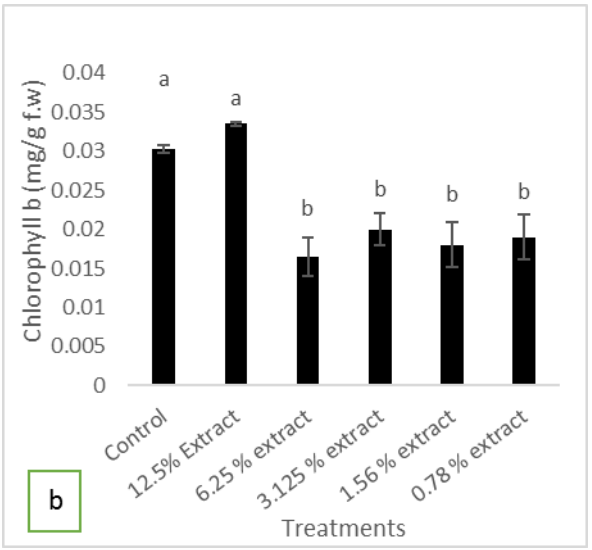
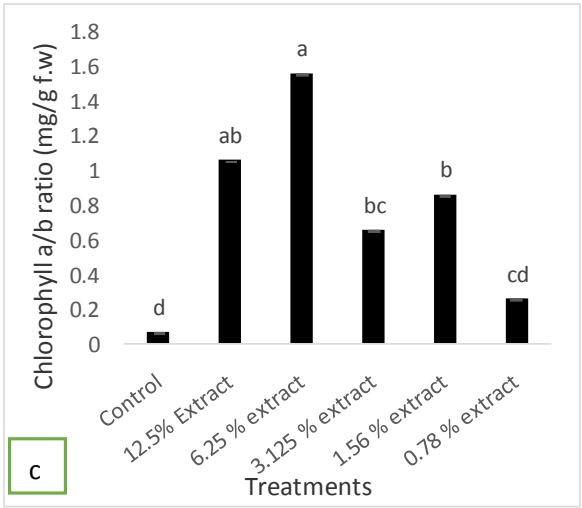


Figure 3: Effect of *Euphorbia dracunculoides* Root extract on (a) Shoot fresh weight LSD: 0.4446 (b) Shoot dry weight LSD: 0.0622 (c) Root area LSD: 0.5930 (d) Root length LSD: 6.2456.Means sharing common English letters are statistically similar.


Chlorophyll a (mg/g f.w)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
a
b
bc
c
c
d
a
Treatments


Chlorophyll b (mg/g f.w)
0.04
0.035
0.03
0.025
0.02
0.015
0.01
0.005
0
a
a
b
b
b
b
Control
12.5% Extract
6.25 % extract
3.125 % extract
1.56 % extract
0.78 % extract
Treatments
b



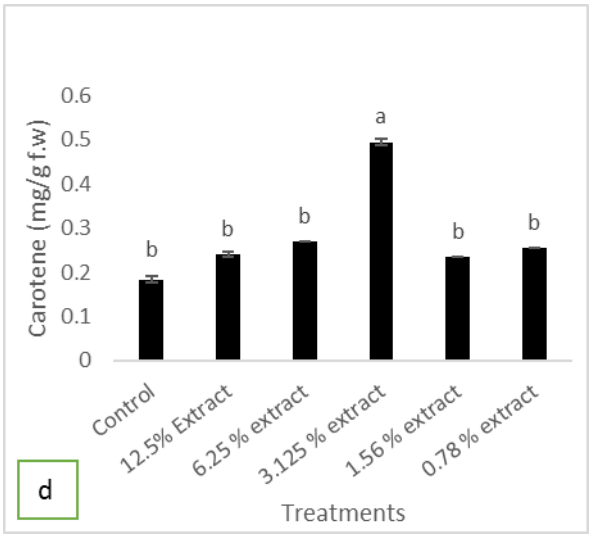


Figure 4. Effect of *Euphorbia dracunculoides* Root extract on (a) Chlorophyll a LSD: 0.0725 (b) Chlorophyll b LSD: (c) Chlorophyll a/b ratio (d) Carotene LSD: 0.1915.Means sharing common English letters are statistically similar.

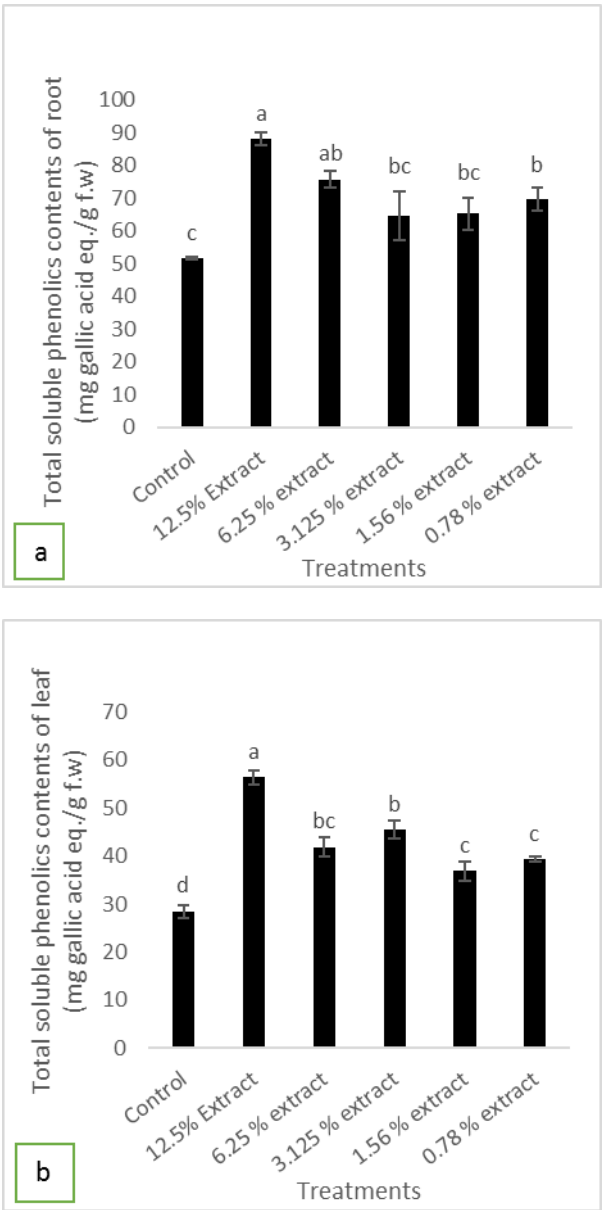


Figure 5. Effect of *Euphorbia dracunculoides* root extract on (a) Phenolics root LSD: 13.696 (b) Phenolics leaf LSD: 5.6019.Means sharing common English letters are statistically similar.

CONCLUSION

The EARE was a good source of natural phenolics which exhibited stimulatory effect on growth attributes of Chickpea. The effectiveness of EARE was higher at 12.5 % concentration. It is inferred from findings of the present investigation that EARE can be a potential bioregulator for improving growth of Chickpea. Further investigation on other crop species can lead to the formulation of some novel bioregulators.

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