

Original Research Article

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The Influence of *Pterocladia capillacea* Extract on Growth and Antioxidant System of *Cicer arietinum*

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ABSTRACT

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The chickpea, *Cicer arietinum* L. (Family: Fabaceae), is one of the most important legume crop in the world because it has high nutritional value due to high protein content and dietary fiber. Seaweeds are marine algae and they are rich source of highly bioactive secondary metabolites. Therefore, chickpea seedlings were treated with *Pterocladia capillacea* (S.G. Gmelin) Bornet (Rhodophyta) extract to find out whether this extract can affect growth and defense system of chickpea. The root length, shoot length, number of lateral roots and number of lateral shoots increased under treatment with different algal extracts. The non-antioxidant compounds including reduced glutathione (GSH), ascorbate, and proline of chickpea seedlings increased under the treatment. Also, the activities of antioxidant enzymes superoxide dismutase (EC: 1.15.1.1), catalase (EC: 1.11.1.6) and ascorbate peroxidase (EC: 1.11.1.11) increased with different rates. It was observed that the total protein and total carbohydrate contents of *Cicer* seedlings increased. The rate of increase in the antioxidant system was dependent on extract concentration.

Introduction

Seaweeds are important marine resources and used as animal feed, human food, and raw materials for several industries (Anisimov *et al.*, 2013; Kolanjinathan *et al.*, 2014).

Seaweeds contain various vitamins, minerals, proteins, trace elements, iodine, bromine and bioactive substances. They are also source of many useful products in medicines (Raghukumar, 2011).

Seaweed extracts are used as fertilizers in the ecological farming (Zhang and Ervin, 2007; Kumari *et al.*, 2011). Also, they are

applied as soil amendment (Gandhlyappan and Perumal, 2001), plant disease management and in pests control (Jayaraj *et al.*, 2008).

Seaweeds are exposed to a combination of oxygen and light and this leads to the production of free radicals (Zubia *et al.*, 2007). Seaweeds protect themselves against free radicals by developing antioxidant defense system, including enzymatic and non-enzymatic (Halliwell and Gutteridge, 2001; Burtin, 2003; Ashraf and Foolad, 2007; Piotrowska-Niczyporuk and Bajguz, 2013).

Chickpea (*Cicer arietinum* L.) is cultivated throughout the world (Segev *et al.*, 2011). Chickpeas are characterized by their nutritional value because of high protein content and dietary fiber. It has appreciable antioxidant activity (Heiras-Palazuelos *et al.*, 2013). Chickpea has been used for treating many diseases: asthenic, hair loss, abortifacient, diarrhea, useful in cold pains, used in indigestion, anti-leukemic and dysentery (Ahmed *et al.*, 2009).

Thus, the present paper aimed to investigate the influence of *Pterocladia capillacea* extract on the growth criteria and the antioxidant system of *Cicer arietinum*.

Materials and Methods

Collection of Algal Material

The seaweed was collected from the submerged rocks on the coastal of Abu-Qir Alexandria – North Egypt during October 2014, representing autumn. It was chosen because it was present in large quantities in the collection sites. Samples were brought to laboratory in plastic bags containing sea water for preventing evaporation.

Preparation of Algal Samples

Seaweed was cleaned up from epiphytes and extraneous by running tap water and rinsed several times in distilled water. The samples were then spread and allowed to dry in air at room temperature. The air-dried samples were ground and stored at room temperature (Abu El-Wafa, 2005). The algal sample was identified following Aleem (1993). It was *Pterocladia capillacea* (S.G. Gmelin) Bornet (Rhodophyta).

Preparation of Algal Extract

The aqueous extract was prepared as described by El-Shora *et al.* (2015d) from dried powder of *Pterocladia capillacea*.

Five grams of powder was soaked in 100 ml of distilled H₂O for 24 hr at room temperature, and filtered through filter paper to obtain 5% w/v. The filtrate was then centrifuged at 3500 rpm for 25 min and the resultant supernatant was used for chemical tests.

Plant Materials

The experimental plant used in this investigation was *Cicer arietinum* L (chickpea, family Fabaceae). The pure strain of seeds was obtained from Egyptian Ministry of Agriculture.

Seeds Sterilization

Growth of the tested plants was carried out according to the method of El-Shora and Abd El-Gawad, (2014a). Chickpea seeds were surface sterilized in 0.5% (v/v) sodium hypochlorite for 15 min and then rinsed several times with distilled water.

Treatment with Algal Extract

The sterilized seeds were placed in 90 cm sterile Petri dishes over Whatman No. 1 filter paper. The seeds were allowed to germinate in the dark at 25 ± 1 °C for 5 days. The 5-day old seedlings with uniform length were divided into two groups each containing 20 seedlings. Each group was transferred to plastic pot and supplemented with Hoagland's nutrient solution (Hoagland and Arnon, 1950) for 7 days. After 7 days, group 1 was treated with aqueous extract of *Pterocladia capillacea* (red alga) and group 2 was left without treatment (control). All the pots were put in a growth chamber under the following conditions: a 16-h light / 8-h dark photoperiod, 22 ± 1 °C, day/night temperature, and 350 $\mu\text{mol m}^{-2} \text{s}^{-1}$ photon flux density. The solution was aerated continuously with an air pump and

renewed every 24 h. All plants were harvested after 7 days of treatment for analysis.

Estimation of Growth Parameters

Plant growth was measured on the basis of: shoot length (cm), root length (cm), number of lateral roots and number of leaves.

Total Glutathione

GSH was estimated according El-Shora and Abd El-Gawad (2014b). The reaction mixture contained 1 ml of 150 mM Na-phosphate buffer (pH 7.5) with 0.2 ml of 10mM 5,5-dithio-bis-(2-nitrobenzoic acid) (DTNB), 0.2 ml of 10 mM EDTA, 0.1 ml of 5mM NADPH and 0.5 ml glutathione reductase (GR). The change in absorbance was measured at 412 nm spectrophotometrically. For determination of GSSG content, 2-vinylpyridine was added to the extract and GSH content was calculated by subtracting the GSSG content from the total glutathione content.

Proline Content

The proline content was determined using the acid ninhydrin according to the method of Bates *et al.* (1973). A sample (5gm) of the leaf tissue was homogenized with 5 ml of 3 % (w/v) sulfosalicylic acid and the resulting homogenate was filtered through Whatman No. 1 filter paper. Two ml of the filtered extract was mixed with 2 ml acid ninhydrin and 2 ml of glacial acetic acid. The reaction mixture was incubated for 1 h in a boiling water bath and the reaction was terminated in an ice bath. Toluene (4 ml) was added to the reaction mixture and the organic phase was extracted, in which a toluene soluble reddish chromophore was obtained, which was read spectrophotometrically at 520 nm using

toluene as blank by UV-visible spectrophotometer.

Preparation of Enzyme Extract

Fresh leaves (2gm) were homogenized with mortar and pestle under chilled conditions with 50 mM phosphate buffer (pH 7.0) containing 1% polyvinylpyrrolidone. The homogenate was filtered through muslin cloth and centrifuged at 6000g for 10 min at 4°C. The resulting supernatant was used for assaying the antioxidant enzymes including superoxide dismutase, catalase and ascorbate peroxidase.

Assay of Antioxidant Enzymes

Superoxide Dismutase (SOD)

Superoxide dismutase (SOD, EC 1.15.1.1) activity was determined by the method of Beauchamp and Fridovich (1971) through measuring the photo reduction of nitroblue tetrazolium (NBT) at 650 nm. The reaction mixture (3 ml) contained 50 mM sodium phosphate buffer (pH 7.8), 75 μ M NBT, 13mM methionine, 2 mM riboflavin, 10 μ M EDTA and enzyme extract (100 μ l). The reaction was started by placing the tubes below two 15-W fluorescent lamps for 10 min and then stopped by switching off the light. The absorbance was measured spectrophotometrically at 650 nm. One unit of SOD was defined as the quantity of enzyme that produced 5 % inhibition of NBT reaction under assay conditions.

Catalase (CAT)

The activity of catalase enzyme was determined according to the method of Aebi (1984). The assay mixture (3 ml) contained 100 μ l H₂O₂ (100 mM), 100 μ l enzyme extract and 2.8 ml 50 mM phosphate buffer with 2 mM EDTA (pH 7.0). CAT activity

was assayed by measuring the decrease in the absorbance at 240 nm.

Ascorbate Peroxidase (APX)

The activity of ascorbate peroxidase was assayed according to the method of Nakano and Asada (1981). The assay medium contained 50 mM sodium phosphate buffer (pH 7.0), 0.1 mM H₂O₂, 0.5 mM ascorbic acid, 0.1 mM EDTA, and 0.2 ml enzyme extract. The decrease in absorbance was measured at 290 nm for 1 min.

Estimation of Protein

The soluble protein content was determined as described by Bradford (1976). The concentration of protein was calculated from standard curve using bovine serum albumin (BSA) as standard.

All the data in the present study are expressed as mean \pm SE obtained from three measurements.

Results and Discussion

Treatment of *Cicer* seedlings with *Pterocladia* extract resulted in an enhancement of root length, shoot length (Fig. 1), number of lateral roots and number of leaves (Fig. 2). In support, Erulan *et al.* (2009) reported that seaweed liquid fertilizer enhanced the growth parameters including root length, shoot length, leaf area, fresh weight and dry weight.

Crouch *et al.* (1990) observed that seaweed extracts improved the nutrient uptake by roots with improved water and nutrient efficiency (Kumari *et al.*, 2011). It was also reported that seaweed products promoted root growth and plant development (Jeannin *et al.*, 1991). Seaweed extracts are important source of plant nutrition for sustainable agriculture production (Khan *et al.*, 2009) as they contain vitamins, various trace

elements as well as amino acids benefiting the plant growth and development.

Seaweed extracts are biostimulant mainly due to the presence of plant hormones in seaweed extracts including: cytokinins, auxins, abscisic acid, gibberellins, salicylic acid, ethylene and some other bioactive compounds such as betaines (El-Miniawy *et al.*, 2014).

Abdel Aziz *et al.* (2011) found that seaweeds extract contain a high cytokine in content, which could be responsible for plant growth. Also, they reported that seaweeds extracts contain active chemical constituents which can regulate shoot elongation, cell division, protein synthesis, leaf aging and senescence, enzyme formation and fruit set. In addition, cytokinins in seaweed include trans-zeatin, trans-zeatin riboside dihydro derivatives (Stirk and Van Staden, 1997). These cytokinins play essential role in transport of nutrients and inhibit aging (Lewak and Kopcewicz, 2009) and they are involved in regulation of cell division.

Gibberellins initiate seed germination as well as growth regulation and gibberellic acid is the most important growth stimulating substance for cell division and cell elongation (Mahmoody and Noori, 2014).

Abscisic acid as an auxin participates in regulation of seed germination. Seaweed extract increased the defense system of chickpea. Similar results were recorded for *Cajanus* *Cajanus* by Padina extract (Mohan *et al.*, 1994).

Yokoya *et al.* (2010) and Devi and Paul (2014) reported that phytohormones are found in seaweed extracts in various concentrations and they can stimulate the growth or abscission and senescence

according to their concentration.

Also, Ramya *et al.*, (2010) reported that seaweed extracts contain glycine betaine which improved the growth yield of *Cyamopsistetra gonolaba*. Glycine betaine acts as osmoregulator, act as active oxygen scavenger and provide protein stabilization (Ashraf and Foolad, 2007; Mohamood *et al.*, 2009).

Glutathione exists in two forms reduced glutathione (GSH) and oxidized glutathione

(GSSG). Reduced glutathione (GSH) content increased but the oxidized glutathione (GSSG) decreased in *Cicer* leaves (Fig. 3) after treatment with pterocladia extract. GSH is abundant thiol in eukaryotes including plants. The reduction potential of glutathione depends on the intercellular GSH/GSSG ratio. Changes in the redox ratio of glutathione mainly depend on the total GSH concentration, GSH biosynthesis, GSH catabolism and pH (Mullineaux and Rausch, 2005).

Fig.1 Root length and Shoot Length of *C. arietinum* treated with *P. capillacea* Extract

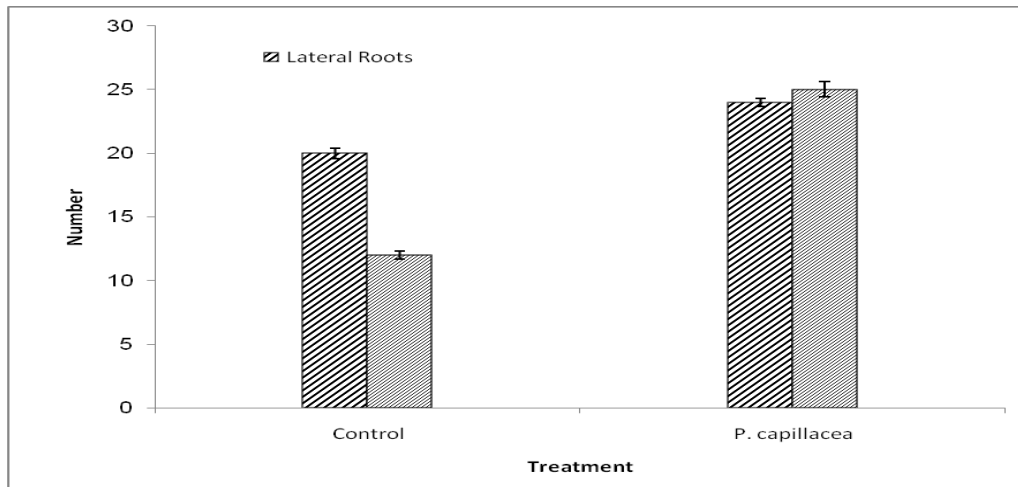


Fig.2 Number of Lateral Roots and Number of Leaves of *C. arietinum* treated with *P. capillacea* Extract

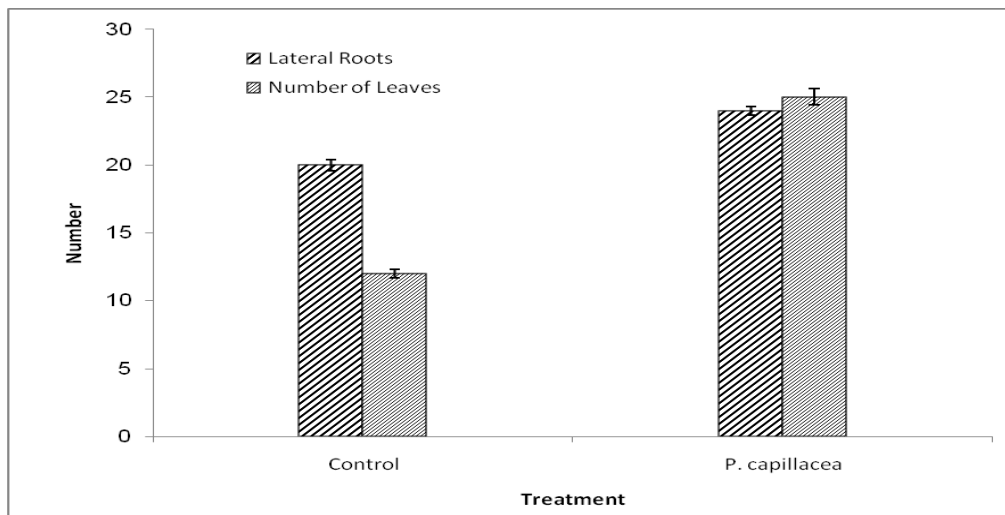


Fig.3 Effect of *P. capillacea* Extract on GSH and GSSG in *C. arietinum*

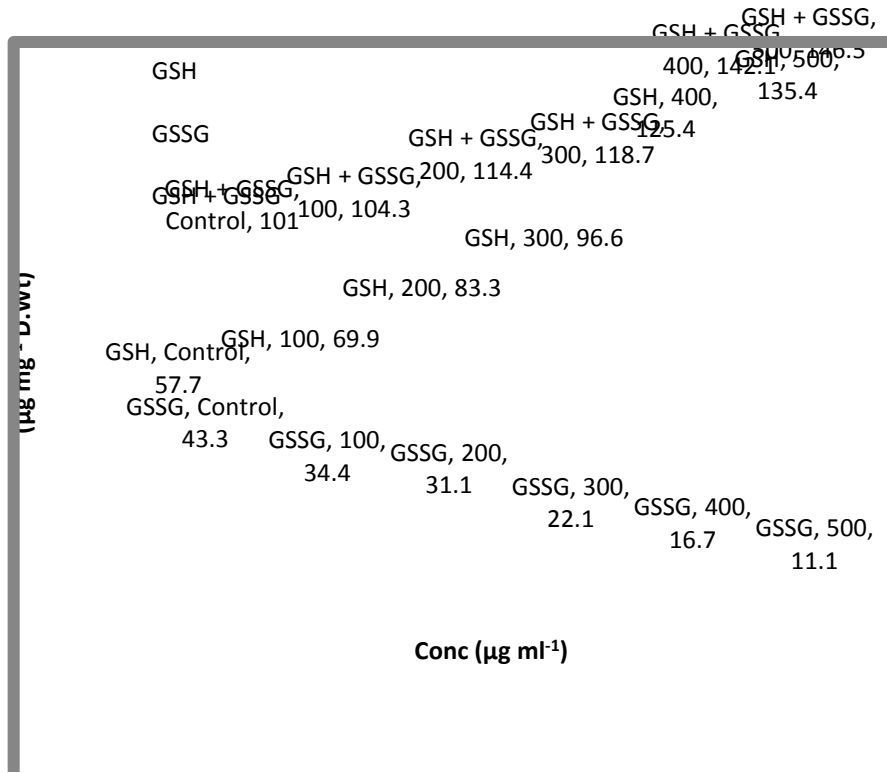


Fig.4 Effect of *P. capillacea* Extract on Ascorbate Content in *C. arietinum*

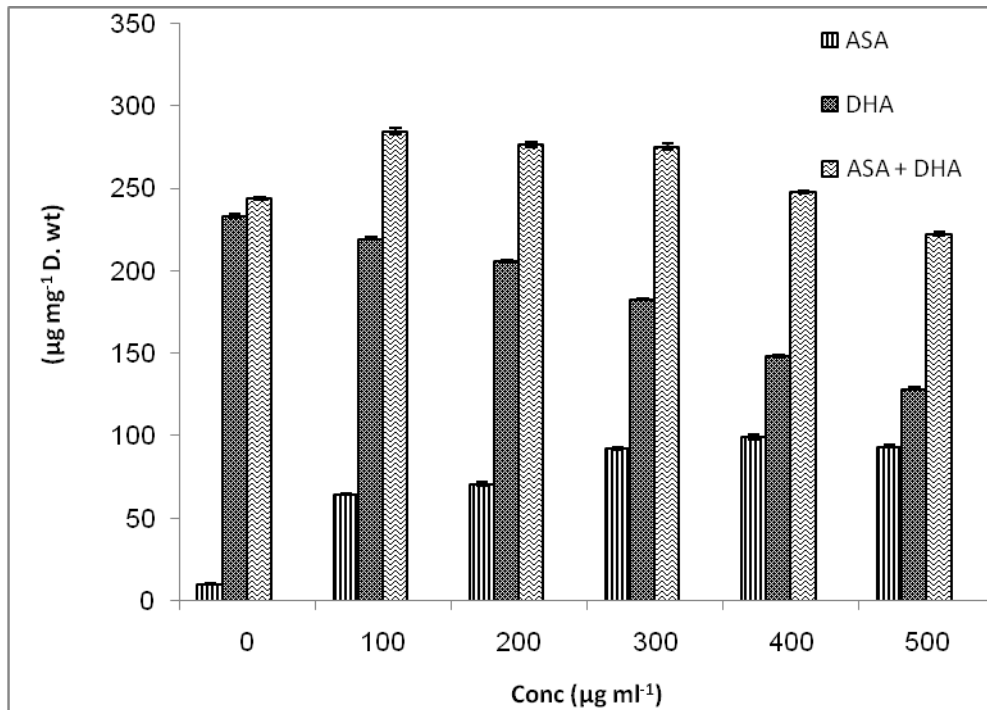


Fig.5 Effect of *P.capillacea* Extract on Proline Content in *C. arietinum*

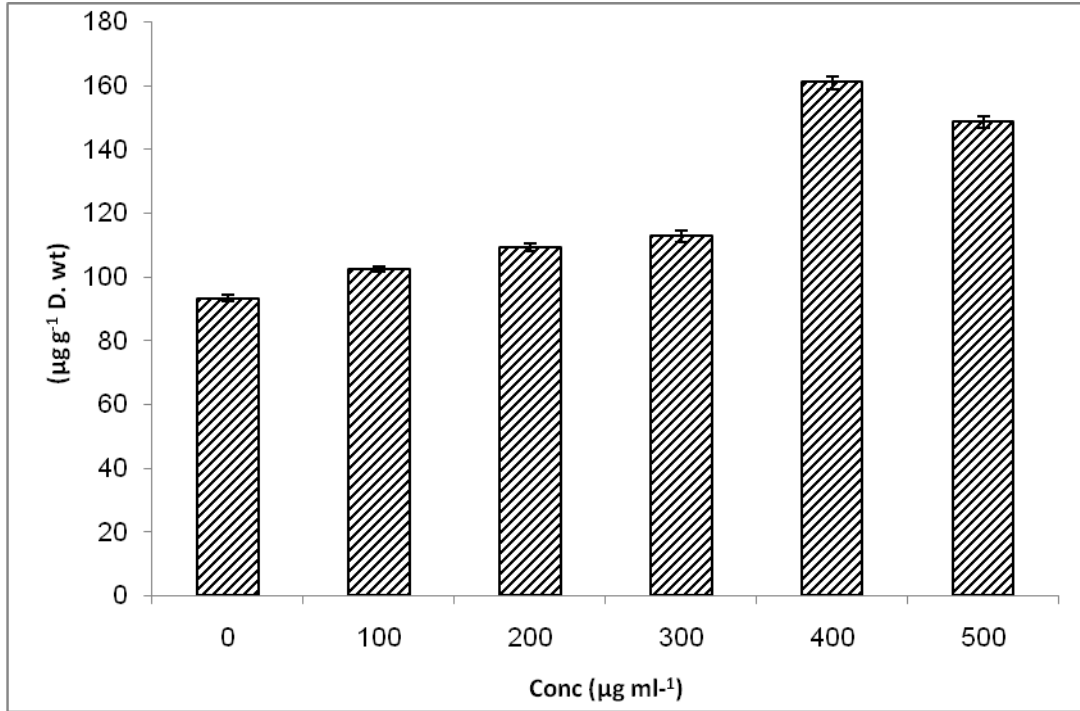


Fig.6 Effect of *P. capillacea* Extract on the Activities of Antioxidant Enzymes in *C. arietinum*

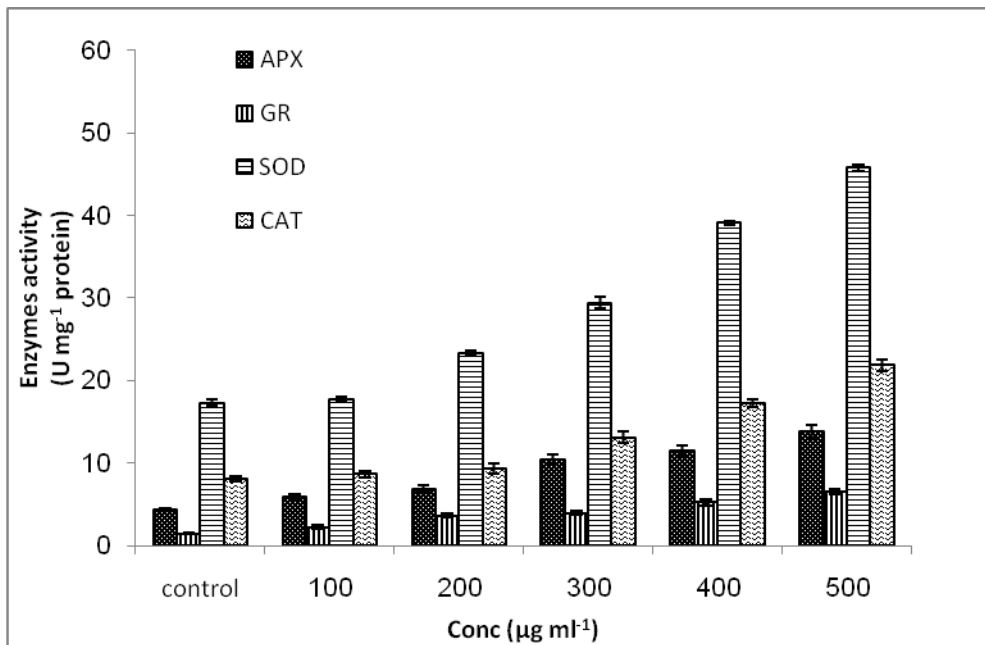


Fig.7 Effect of *P. capillacea* Extract on Total Protein Content in *C. arietinum*

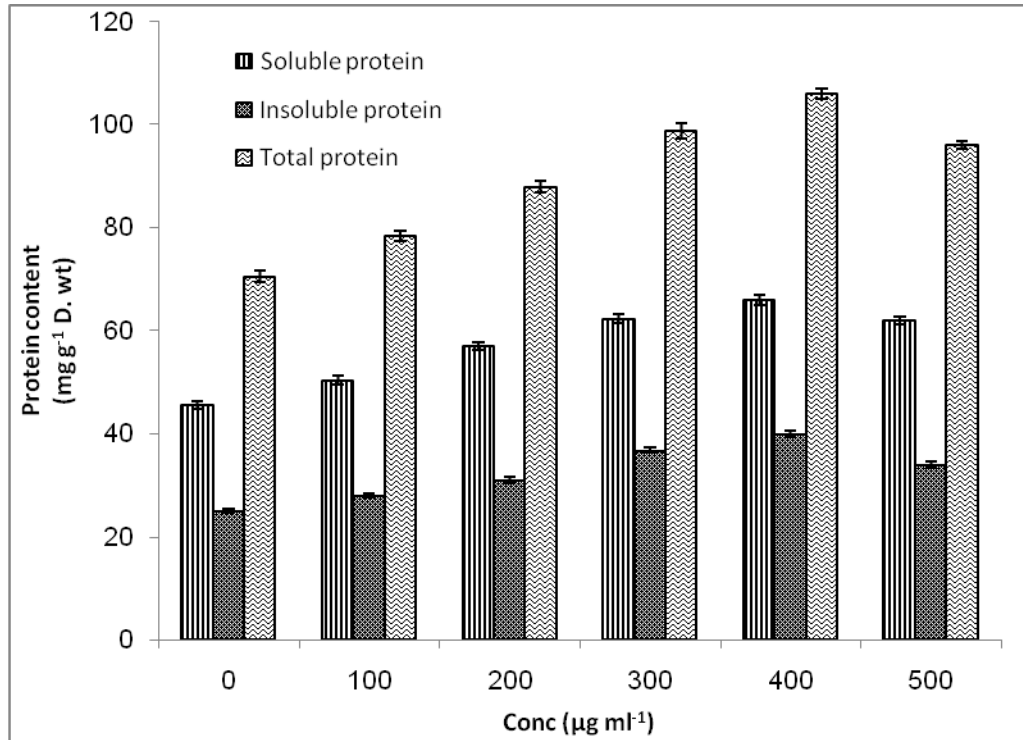
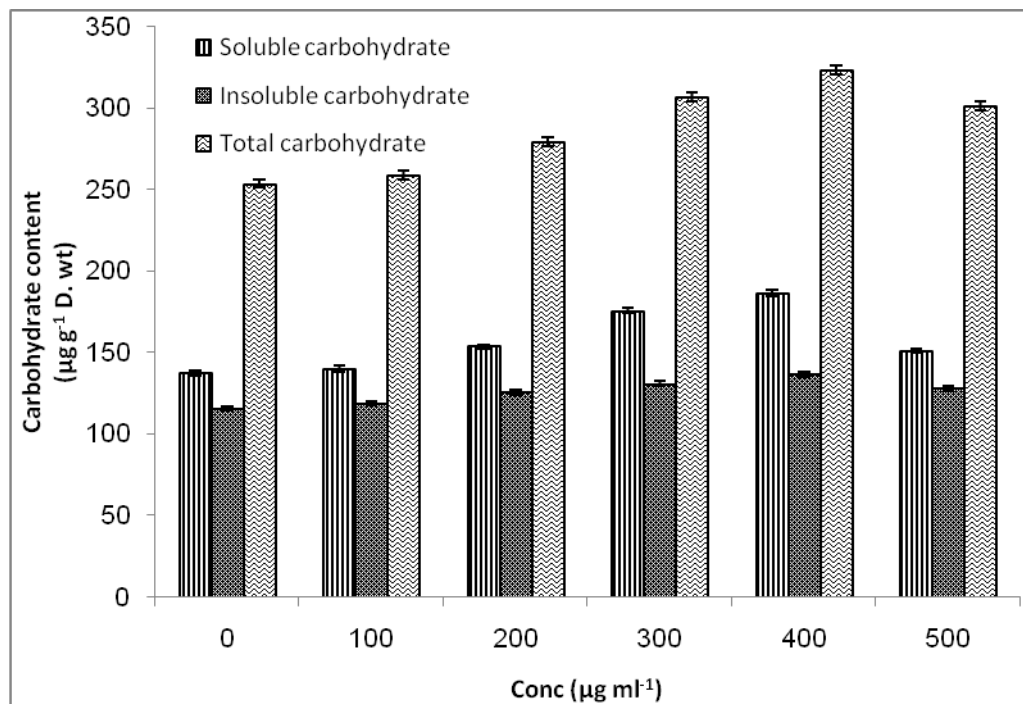


Fig.8 Effect of *P. capillacea* Extract on Carbohydrate Content in *C. arietinum*



The increase in GSH by seaweed extracts may be attributed to the role of auxins of seaweed extract in reducing of lipid peroxidation by stimulation of ascorbate and glutathione thus regulating the homeostasis of reactive oxygen species (Piotrowska-Niczyporuk and Bajguz, 2013).

Ascorbate is one of the most important non-enzymatic antioxidant (El-Shora *et al.*, 2015d) increased with increasing concentration of seaweed extract (Fig 4). The ratio of ASA/DHA is considered as an important indicator for the degree of oxidative stress and the redox state of the cell (Asada, 1994).

Treatment of *Cicer* seedlings with seaweeds induced proline content (Fig. 5). Rhodes and Hanson (1993) observed an increase in proline concentration after seaweed treatment suggesting that seaweed extract could be applied to stressed-plants to gain tolerance against stress. Proline acts as a free radical scavenger and important in overcoming stress than acting as an osmolyte. Proline accumulation in *Cicer* leaves by seaweed extract may be due to activation of proline biosynthesis and inactivation of proline degradation. Proline and betaines are suggested to play a pivotal role in cytoplasmic adjustment in response to osmotic stress.

Treatment of *Cicer* seedlings with seaweed extract increased the activities of tested antioxidant enzymes (Fig. 6) including SOD, CAT, and APX. SOD is the first line of defense against ROS that convert O₂ to O₂ and H₂O₂ (El-Shora, 2003; Nasir *et al.*, 2015). CAT and APX convert H₂O₂ to O₂ and H₂O (El-Shora *et al.*, 2004). CAT activity was higher than APX revealing that removing H₂O₂ occurs mainly by CAT. The increase in the enzymes activities may be due to the effect of auxins in seaweed extract which may induce the antioxidant

enzymes to scavenge ROS (Piotrowska-Niczyporuk and Bajguz, 2013).

The soluble protein (Fig. 7) increased under treatment with *Pterocladia* extract. This increase could be attributed to retardation of proteolysis, the increase in protein synthesis, availability of amino acids and activation of enzymes of protein synthesis. The increase in protein content of *Cicer* leaves under treatment with seaweed extract suggests that *Pterocladia* extract may have substances responsible for such increase. It has been reported that seaweeds extracts have amino acids and zinc. Zinc is present in various enzymes of all six classes of enzymes (Broadley *et al.*, 2012).

The soluble carbohydrate content increased (Fig. 8) after treatment with *Pterocladia* extract. Sridhar and Rengasamy (2010) reported that the inducible effects of seaweed application are correlated with the increase of total soluble carbohydrate in *Tagetes erecta*. Thus, the increase of carbohydrates in the present results could be due to the improvement effect of seaweed extract on photosynthetic efficiency and this might be related to cytokinins, auxins, macro- and micro-nutrients in the seaweed extracts (Abdel Aziz *et al.*, 2011).

In conclusion, the present results show the beneficial effect of seaweed extract application on the growth and antioxidant system of chickpeas crop plant. The exhibited effects are a result of many components in the extracts that may work synergistically at different concentrations, although the mode of action still remains unknown.

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