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MITIGATING EFFECT OF ACTIVATED CHARCOAL AGAINST ALLELOPATHIC STRESS

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ABSTRACT

The aim of the present study was to investigate the mitigating potential of activated charcoal (AC) on growth and metabolism of *Pisum sativum* L. in presence of benzoic acid (BA). BA in concentrations of 0.5, 1.0 and 1.5 mM was added. Activated charcoal at 20g/pot was used. 21 days old seedlings were used for biophysical and biochemical analyses. BA exhibited adverse effect on all parameters. AC significantly ($p \leq 0.001$) improved all parameters in combined treatment. Gradual decrease in growth parameters was recorded in BA treatment. Pigment, protein and sugar contents and nitrate reductase activity significantly decreased under different concentrations of BA. Activities of antioxidant enzymes viz. SOD, CAT and POX significantly enhanced under allelochemical stress to avoid the oxidative damage. AC in combination with BA caused maximum growth as compared with BA treatment. AC mitigated the allelopathic stress and altered the nutrient availability and plant growth in the presence of allelopathic stress. AC buttressed the defense system of pea seedlings in presence of BA.

Key words : Activated charcoal, antioxidants, benzoic acid, oxidative stress, *Pisum sativum*.

INTRODUCTION

In natural and agro-ecosystems, interaction and interference among plants are common phenomena (Singh et al., 2010). Through the release of secondary metabolites, plants may positively and negatively affect the growth and productivity of plants in one cropping system or plants of next generation (Singh et al., 2010). This phenomenon is called allelopathy and secondary metabolites involved are known as allelochemicals (Rice, 1984). Allelochemicals influence the growth and development of recipient plants (Inderjit and Duke, 2003). Allelochemicals belong to various chemical groups and have different sites and mode of action (Singh et al., 2012). Allelochemicals cause a biotic stress in recipient plant (Cruz-Ortega et al., 2002). Allelochemicals are capable of changing the various physiological and biochemical processes in plants (Inderjit and Duke, 2003; Singh et al., 2012). A biotic stress caused by allelochemical affects the antioxidant enzyme activities that ameliorate oxidative stress. The impairment of various metabolic activities finally affects the crop yield (Singh et al., 2012). In plant and soil in the presence of BA and its derivatives are reported (Baziramakenga et al. 1995; Vaughan and Ord 1991; Rice 1984). Seed germination and seedling growth decrease under excess amount of BA (Maffei et al., 1999). It also decreases respiration and photosynthesis by inhibiting electron transport (Zhou et al., 2006), affects chlorophyll biosynthesis and protein content (Baziramakenga et al., 1994), and interferes with

sugar metabolism and nitrate reductase (NR) activity in crop plants (Naguib 1965; Robert et al., 1982). Induction of electrolyte leakage and lipid peroxidation under allelochemical stress causes membrane damage (Baziramakenga et al., 1995). Increased activities of several antioxidant enzymes under BA stress are evident (Baziramakenga et al., 1995). BA exhibits inhibitory effects on crop plants.

In the field of allelopathy, use of activated charcoal is a new technique to minimize the allelopathic stress (Lau et al., 2007). Previously, AC was used to test the presence or absence of allelochemicals in various studies (Mahall and Callaway, 1992; Inderjit and Callaway, 2003; Lau et al., 2007). Huge surface area and pore volume is the charactertistic feature of AC. AC has remarkable adsorptive capacity and complex chemical and physical properties due to its polarity (Lau et al., 2007). To observe the activity of AC various techniques like adsorption, mechanical filtration, ion exchange and surface oxidation (Lau et al., 2007; Cheremisinoff and Morresi, 1978) were used. AC acts as an adsorbent for several organic compounds (Cheremisinoff and Morresi, 1978; Lau et al., 2007). AC easily mixed in soil (Callaway and Aschehoug, 2000; Al Hamdi et al., 2001; Lau et al., 2007). The use of AC provides an idea to investigate the existence of phenomenon of allelopathy (Lau et al., 2007). The present study was undertaken to examine the role of activated charcoal on growth and metabolism of plants grown under BA stress condition. The pea was used as test crop.

MATERIALS AND METHODS

Plant material and treatment:
The certified seeds of *Pisum sativum* L. cv. rachana were procured from seed agency in Allahabad, Uttar Pradesh, India. The pea seeds were surface sterilized with 0.01% (w/v) $HgCl_2$ solution for 3 min and washed extensively with distilled water. Doses of BA were decided before the experiment. 1.0 mM concentration caused 50% inhibition of seed germination (SG). Graded concentrations 0.5, 1.0, and 1.5 mM of BA, prepared in double distilled water were used for the study.

The plastic pots were filled with sterilized garden soil at the rate of 300gm/pot with 20gm activated charcoal. The pots were divided in two sets. In one set sterilized seeds soaked in distilled water were sown with 5 seeds in each pot (AC). The pots were irrigated with 20mL of distilled water. In other set the seeds soaked in varying concentration of BA were sown and the pots were treated with 20mL of respective concentration of BA and referred to as BA_1+AC, BA_2+AC and BA_3+AC. In other sets of pot containing 300gm soil/pot without AC the seeds soaked in varying concentration of BA were sown with seeds in each pot and treated with 20mL of 5 respective concentration of BA and are referred to as BA_1, BA_2 and BA_3 treatments. The seeds soaked in distilled water for 3 hrs were sown (5 seeds/pot) filled with garden soil (300gm soil/pot) referred to as control (C).

The experiment was done in triplicate. The experiment was conducted in a culture room at a temperature 28 ± 2ºC, photoperiod 18/6h, humidity 61±5% and photon flux density 240 µmol m^{-2} s^{-1}. 21 days old seedlings were harvested for biophysical and biochemical analyses.

Measurements of biophysical parameters:
In pea seedlings, root and shoot length was measured with a metric scale and expressed in centimeters. The samples were oven dried at 70^{o}C for 72 h and then weighed independently for dry weight (DW) determination. DW of the seedlings was recorded on an electronic balance.

Estimation of pigment and protein contents:
Chlorophyll of experimental plants was extracted with 80% acetone. The amount of photosynthetic pigments was determined as per Lichtenthaler (1987). Fresh leaf (10mg) was homogenized in 10 mL of 80% acetone and centrifuged. Supernatant was taken and optical density was measured at 663nm, 645nm and 470nm. Protein content was determined following the method of Lowry et al. (1951).

The amount of protein was calculated with reference to standard curve obtained from bovine serum albumin.

Nitrate reductase activity:
Nitrate reductase (EC 1.6.6.1) activity was assayed by modified procedure of Jaworski (1971) based on incubation of fresh tissue (0.25 g) in 4.5 mL medium containing 100 mM phosphate buffer (pH 7.5), 3% KNO_3 and 5% propanol. About 0.4 mL aliquot was treated with 0.3 mL 3% sulphanilamide in 3 N HCL and 0.3 mL 0.02% N-1-naphthyl ethylene diamine dihydrochloride (NEDD). The absorbance was measured at 540 nm. NR activity was calculated with a standard curve prepared from $NaNO_2$ and expressed as µ mol NO_2 g^{-1} FW h^{-1}.

Sugar content:
Sugar content was estimated following Hedge and Hofreiter (1962). Leaf sample (0.25g) was homogenized in 2.5 mL of 95% ethanol. After centrifugation, the sugar content was determined in the supernatant. The supernatant (1mL) was mixed with 4 mL of anthrone reagent and heated on boiling water bath for 8 min. Absorbance was taken at 620 nm after rapid cooling. Standard curve was prepared from glucose.

Antioxidant enzymes assay:
Enzyme extract was prepared by homogenizing 500 mg leaves in 10 mL of 0.1 M sodium phosphate buffer (pH 7.0). The homogenate was filtered and centrifuged at 15000 g at 4^o C for 30 min. The supernant was collected and used for analyses of superoxide dismutase (EC 1.15.11), catalase (EC 1.11.1.6) and peroxidase (EC 1.11.1.7).

Superoxide dismutase (SOD) activity was determined by the nitroblue tetrazolium (NBT) photochemical assay method following Beyer and Fridovich (1987). The reaction mixture (4 mL) contained 63 µM NBT, 13 mM methionine, 0.1 mM ethylene diamintetra acetic acid (EDTA), 13 µM riboflavin, 0.5 M sodium carbonate and 0.5 mL clear supernatant. Test tubes were placed under fluorescent lamps for 30 min and absorbance was recorded at 560 nm. One unit of enzyme was defined as the amount of enzyme which caused 50% inhibition of NBT reduction.

Catalase (CAT) activity was assayed as per the method Cakmak and Marschner (1992). The reaction mixture (2 mL) contained 25 mM sodium phosphate buffer (pH 7.0), 10 mM H_2O_2 and 0.2 mL enzyme extract. The activity was determined by measuring the rate of disappearance of H_2O_2 for 1 min at 240 nm and calculated using extinction coefficient of 39.4 mM^{-1} cm^{-1} and expressed as enzyme unit g^{-1} fresh weight. One unit of CAT was defined as the amount of enzyme required to oxidize 1 µM H_2O_2 $min^{-1.}$

Peroxidase (POX) activity was assayed following McCune and Galston (1959). Reaction mixture contained 2 mL enzyme extract, 2 mL sodium phosphate buffer, 1 mL 0.1 N pyrogallol and 0.2 mL 0.02% H_2O_2 and determined spectrophotometrically at 430 nm. One unit of enzyme activity was defined as the amount which produced an increase of 0.1 OD per minute.

Statistical analysis:
Standard errors of means were calculated in triplicates. In addition, analysis of variance was carried out for all the data generated from this experiment, employing one way ANOVA test using GPIS software 3.0 (GRAPHPAD California USA).

RESULTS

Growth and metabolism of *Pisum sativum* was adversely affected under BA stress. BA, a phenolic compound is produced as byproduct of main metabolic pathway. Allelochemical significantly decreased the growth in dose dependent manner. The decrease in root and shoot growth corresponded to an increased concentration of BA. Root and shoot growth increased to maximum in seedlings under AC treatment. In combined treatments (BA+AC) both root and shoot growth was better in comparison to that of BA treatments. DW of seedlings decreased significantly ($p \leq 0.001$) in BA treatment in comparison to control with

Table-1: Effect of benzoic acid and activated charcoal on root and shoot length and dry weight of pea seedlings.

Treatments	Root length (cm.)	Shoot length (cm.)	DW (gm/ plant)
C	14.35±0.663	26.15±0.779	1.186±0.001
AC	15.2±1.558	27.35±0.259	1.2785±0.314
B_1	8.95±0.721[a]	17.4±1.212[a]	0.724±0.018[b]
B_2	8.2±0.057[a]	14.45±1.241[a]	0.659±0.027[a]
B_3	5.95±0.317[a]	13.1±0.346[a]	0.564±0.004[a]
B_1+AC	10.1±0.346[a]	19.45±0.952[a]	0.819±0.021[c]
B_2+AC	9.05±0.202[a]	18.25±1.587[a]	0.752±0.009[b]
B_3+AC	7.9±0.346[a]	16.1±0.230[a]	0.616±0.031[a]

Data are mean of three replicates ± SEM. [a] p<0.001, [b]p<0.01, [c]p<0.05 versus C. C= control; AC= activated charcoal (20g/pot), B_1=0.5, B_2=1.0 and B_3=1.5mM concentrations of BA.

Table-2: Effect of benzoic acid and activated charcoal on photosynthetic pigment contents of pea seedlings.

Treatments	Chl a (mg/g FW)	Chl b (mg/g FW)	Total Chl (mg/g FW)	Carotenoids (mg/g FW)
C	1.594±0.249	0.967±0.029	2.562±0.219	1.127±0.028
AC	1.633±0.252	1.029±0.033	1.575±0.164[b]	1.207±0.013[c]
B_1	0.939±0.019[a]	0.635±0.184[c]	1.272±0.084[a]	0.791±0.028[a]
B_2	0.748±0.003[a]	0.523±0.081[a]	0.987±0.077[a]	0.728±0.016[a]
B_3	0.575±0.004[a]	0.412±0.073[a]	2.662±0.219	0.634±0.021[a]
B_1+AC	0.997±0.026[b]	0.687±0.145	1.684±0.118[c]	0.844±0.027[a]
B_2+AC	0.827±0.064[a]	0.572±0.034[b]	1.399±0.099[b]	0.799±0.024[a]
B_3+AC	0.659±0.135[a]	0.490±0.038[a]	1.15±0.096[a]	0.671±0.014[a]

Data are mean of three replicates ± SEM. [a] p<0.001, [b]p<0.01, [c]p<0.05 versus C. C= control; AC= activated charcoal (20g/pot), B_1=0.5, B_2=1.0 and B_3=1.5mM concentrations of BA.

maximum decrease in BA_3. AC enhanced DW of the seedlings (Table 1).

The allelochemical stress drastically decreased the photosynthetic pigment content. A significant reduction in pigment content was recorded under the influence of BA with a maximum in BA_3. The amount of pigment increased in AC. Maximum 34.47, 41.63 and 55.12% decrease in chl a, chl b and carotenoids were observed in highest dose of BA, respectively. Pigment content remarkably enhanced in all combined treatments (BA+AC) (Table 2).

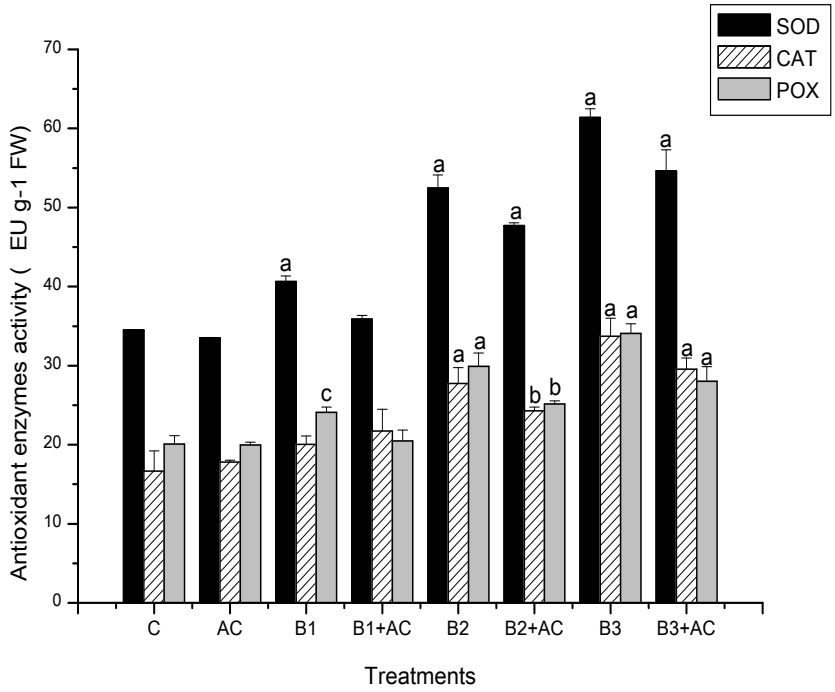
The sugar content of seedlings was variously affected in all treatments. Significant decrease was observed in sugar content of pea seedlings under BA treatments with maximum 14.92% in BA_3 as compared to control. Sugar content increased in the seedlings treated with AC. AC alleviated the effect of BA by increasing the sugar content of seedlings in combined treatments. BA caused significant reduction in

Table-3: Effect of benzoic acid and activated charcoal on protein and sugar content and nitrate reductase activity of pea seedlings.

Treatments	Protein (mg/g FW)	Sugar (mg/g FW)	NR (µmol NO_2 g^{-1} FW h^{-1})
C	45.26±1.991	78.49±1.715	23.05±0.634
AC	48.16±0.433	86.03±2.877[c]	24.77±0.902
B_1	39.51±0.981[b]	63.68±1.468[a]	12.08±0.447[a]
B_2	28.41±1.443[a]	56.33±3.449[a]	9.65±0.403[a]
B_3	21.01±0.286[a]	49.89±1.170[a]	7.26±0.036[a]
B_1+AC	44.76±0.547	71.84±2.594[c]	14.39±1.263[a]
B_2+AC	36.47±2.641[a]	67.67±1.141[a]	13.83±1.947[a]
B_3+AC	27.35±1.616[a]	55.76±0.840[a]	10.43±0.448[a]

Data are mean of three replicates ± SEM. [a] p<0.001, [b]p<0.01, [c]p<0.05 versus C. C= control; AC= activated charcoal (20g/pot), B_1=0.5, B_2=1.0 and B_3=1.5mM concentrations of BA

Figure-1:. Effect of benzoic acid and activated charcoal on antioxidant enzyme activities of pea seedlings.



Data are mean of three replicates ± SEM. [a] p<0.001, [b]p<0.01, [c]p<0.05 versus C. C= control; AC= activated charcoal (20g/pot), B_1=0.5, B_2=1.0 and B_3=1.5mM concentrations of BA.

protein content. The decline in protein was dose dependent. Higher amount of protein in AC treatment was recorded as compared with control. Decline in protein content was concentration dependent under allelochemical stress. Maximum 2.15 folds decrease in protein was recorded in highest concentration of BA. Protein content increased in combined treatment (BA+AC) in comparison with that of BA. Nitrate reductase (NR) activity significantly ($p \leq 0.001$) altered under allelochemical stress. Highest NR activity was recorded in AC-treated plants followed by control. A graded reduction in NR activity was seen in seedlings treated with

various concentrations of BA. AC in combined treatments (BA+AC) significantly ($p \leq 0.001$) improved the NR activity (Table 3).

The antioxidant enzymes viz. superoxide dismutase (SOD), catalase (CAT) and peroxidase (POX) were activated in response to allelochemical stress. SOD activity significantly ($p \leq 0.001$) increased in BA treatments to avoid the oxidative damage caused by reactive oxygen species (ROS). Lowest SOD activity was observed in control. SOD activity was improved under combined treatment. A significant ($p \leq 0.001$) increase in CAT activity was observed in BA_2 and BA_3 as compared with control and AC. CAT activity gradually increased under BA stress to detoxify the ROS. Activity of POX significantly ($p \leq 0.001$) enhanced under BA treatments. Maximum enhancement was observed in BA_2 and BA_3 as compared with control and AC. AC in combined treatment improved the antioxidant enzyme activities under allelochemical stress and minimize the oxidative damage. AC neutralizes the adverse effect of allelochemical (Figure 1).

DISCUSSION

Allelochemicals, as secondary metabolites, released in the surrounding. Leaching, mulching, volatilization, exudation and residue decomposition are the usual phenomena involved in release of allelochemicals (Inderjit and Keating, 1999; Singh et al., 2009). BA as common allelochemical produced by plants accumulates in the soil (Vaughan and Ord, 1991) and adversely affects the growth and metabolism of other plants of same or different species and plants of next generation. Allelopathic stress is the result of environmental change which unfavourably affects growth and development of plants. Allelochemicals altere the metabolic processes of plant (Cruz-Ortega et al., 2002) and cause oxidative damage which induces antioxidant enzymes (Weir et al., 2004). This decrease in dry weight, root and shoot length was the manifestation of impaired metabolic activities due to various allelochemicals (Singh et al., 2010). Positive effect of AC on plant growth under allelopathic stress was previously studied by Lau et al. (2007). Lajayer et al. (2012) also reported increased dry weight of the seedlings grown in soil mixed with AC.

Allelopathic stress caused by BA significantly decreased the pigment content. The plant dry matter is directly related to chlorophyll content (Buttery and Buzzell, 1977). The lower chlorophyll content caused limited net photosynthesis and thus reduced plant growth. The allelochemicals cause degradation of chlorophyll or inhibition of chlorophyll biosynthesis. (Kanchan and Jayachandra, 1980; Singh et al., 2012). AC plays protective role against BA stress in the pea seedlings by increasing nutrients availability to plants (Lau et al., 2007 and Shakuntala Devi, 2013). Cheremisinoff and Ellerbusch (1978) reported that AC has a weak affinity for inorganic nutrients in the form of electrolytes and a strong affinity for organically bound compounds.

Allelochemicals inhibit the protein synthesis by inhibiting the biosynthesis and/ or increasing the degradation of protein (Singh et al., 2010). Under BA stress sugar and protein content gradually decreased (Singh et al., 2012). BA decreased the NR activity while AC elevated the activity which is probably due to adsorption of the allelochemical by AC. The NR activity declined due to starvation (Kaiser and Huber, 2001) or low availability of nitrate (Lin et al., 1994). Addition of AC in growth media increased nitrate availability in plants (Kulmatiski et al., 2006).

Allelochemicals caused oxidative damage which induced antioxidant enzymes (Weir et al. 2004). In abiotic stress condition, the cellular homeostasis is disturbed causing oxidative stress and accumulation of ROS (Asada, 2006). The increased activity of SOD, CAT and POX evinced the fact that plants were able to tolerate the oxidative stress caused by allelochemical. The plants adapted to allelopathic manifestation increased activities of antioxidant enzymes. Thus energy of the seedlings in allelopathic stress is directed towards the biosynthesis of antioxidants to cope with adverse environmental conditions. This diversion of energy is responsible for

decreased growth of plants adapted to allelopathic stress.

CONCLUSION

The pea seedlings successfully tolerate BA stress when supplied with AC. *Pisum sativum* showed improved growth and development in combined treatments (BA+AC). AC minimized the oxidative damage caused by BA stress by inducing antioxidant enzymes. AC ameliorates the phytotoxic effect of BA and activates the defense system of the plant for better survival.

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REFERENCES

1. **Al Hamdi, B., Inderjit, Olofsdotter, M and Streibig, J.C. 2001.** Laboratory bioassay for phytotoxicity: an example from wheat straw. Agronomy Journal 93: 43–48.
2. **Baziramakenga, R., Leroux, G.D and Simard, R.R.** 1995. Effects of benzoic and cinnamic acids on membrane permeability of soybean roots. J Chem Ecol. 21:1271_85.
3. **Baziramakenga, R., Simard, R.R. and Leroux, G.D**. 1994. Effects of benzoic and cinnamic acids on growth, mineral composition and chlorophyll content of soybean. J. Chem. Ecol. 20: 2821–2833.
4. **Beyer, W.F. and Fridovich, I.** 1987. Assaying for superoxide dismutase activity: some large consequences of minor changes in conditions. Anal. Biochem. 161: 559-566.
5. **Buttery, B.R. and Buzzell, R.I.** 1977. The relationship between chlorophyll content and rate of photosynthesis in soybean. Can J Plant Sci. 57:1-5.
6. **Cakmak, I and Marschner, H**.1992. Magnesium deficiency and highlight intensity enhance activities of superoxide dismutase ascorbate peroxidase, and glutathione reductase in bean leaves. Plant Physiol. 98:1222–1227.
7. **Callaway, R.M and Aschehoug, E.T. 2000.** Invasive plants versus their new and old neighbors: a mechanism for exotic invasion. Science 290: 521–523.
8. **Cheremisinoff, P.N and Morresi, A.C. 1978.** Carbon adsorption applications. In: Cheremisinoff PN, Ellerbusch F, eds. Carbon adsorption handbook. Ann Arbor, MI, USA: Science Publishers, Inc., 1–53.
9. **Cruz-Ortega, R., Ayala-Cordero, G and Anaya, A.L.** 2002. Allelochemical stress produced by the aqueous leachates of Calicarpa acuminate: effects on roots of bean, maize and tomato. Physiol Plant. 116:20-7.
10. **Foyer, C.H and Noctor G.** 2005. Oxidant and antioxidant signalling in plants: Is evaluation of the concept of oxidative stress in a physiological context. Plant Cell Environ. 28: 1056–1071
11. **Hedge, J.E and Hofreiter, B.T.** 1962. Estimation of carbohydrate. In: Whistler, R.L. and Be Miller, J.N. (ed.), Methods in carbohydrate chemistry. Academic Press, New York, pp. 17-22.
12. **Inderjit and Duke, S.O.** 2003. Ecophysiological aspects of allelopathy. Planta. 217:529-39.
13. **Inderjit and Callaway R.M. 2003.** Experimental designs for the study of allelopathy. Plant and Soil 256: 1–11.
14. **Jaworski, E.** 1971. Nitrate reductase assay in intact plant tissue. Biochem. Biophys. Res. Commun. 430: 1274–1279.
15. **Kanchan, S.D., and Jayachandra.** 1980. Pollen allelopathy: a new phenomenon. New Phytol. 84:739-46
16. **Lajayer, H. M., Esmaielpour, B and Chamani, E.** 2011**.** Hinokitiol and activated charcoal influence the microtuberization and growth of potato (solanum tuberasum cv. Agria) plantlets in vitro. Australian j of crop sciences. 5(11):1481-1485.
17. **Lichtenthaler, H.K.** 1987. Chlorophyll and carotenoids: pigments of photosynthetic bio-membranes. In: Packer L, Douce R, editors. Methods Enzymology. Academic Press, Sandiego, pp. 350-382.
18. **Lowry, O.H., Rosenbrough, R.J., Farr, A.L., and Randall, R.J.** 1951. Protein

measurement with Folin phenol reagent. J. Biol. Chem. 193: 265–275.
19. **Maffei, M., Bertea, C.M., Garneri, F and Scannererini, S.** 1999. Effect of benzoic acid hydroxyl and methoxy ring substuents during cucumber (Cucumis sativus L.) germination. I. Isocitrate lyase and catalase activity. Plant Sci. 141:139-47.
20. **Mahall, B.E and Callaway, R.M. 1992.** Root communication mechanisms and intracommunity distributions of two Mojave Desert shrubs. Ecology 73: 2145–2151.
21. **Mc Cune, D.C., and Galston, A.W.** 1959. Inverse effects of gibberellin on peroxidase activity and growth in dwarf strains of peas and corn. Plant Physiol. 34: 416–418.
22. **Naguib, M.I. 1965**. Effect of benzoic acid and its hydroxyderivatives on the carbohydrate-metabolism of starved and of sucrose-fed etiolated barley leaves. Planta. 64:20-7.
23. **Rice, E.L.** 1984. Allelopathy. II ed. Academic Press, New York. 424 pp.
24. **Robert, W.H., John, H.Y and George, K.** 1982. Effect of several pesticides on the growth and nitrogen assimilation of the Azolla-Anabaena symbiosis. Weed Sci. 30:54-8.
25. **Shakuntala Devi and K. Suhasini. 2013.** Is Castor A Suitable Alternate Under Tank Irrigation? A Case Study In Mahbubnagar District Of Andhra Pradesh. Biolife. 1(4) 235-241.
26. **Singh, N.B., Singh, A., and Singh, D.** 2010. Autotoxicity of maize and its mitigation by plant growth promoting rhizobacterium *Paenibacillus polymyxa*. Allelopathy J. 195-204.
27. **Singh, N.B., Singh, D., and Singh, A.** 2010. Allelochemicals enhance the severe effects of water stress in seedlings of *Phaseolus mungo.* Allelopathy J. 185-194.
28. **Singh, N.B., Yadav, K., and Amist, N**. 2012. Positive effects of nitric oxide on *Solanum lycopersicum*. J. of Plant Inter. doi.org/10.1080/17429145.2012.748937.
29. **Vaughan, D., and Ord, B.G.** 1991. Extraction of potential allelochemicals and their effects on root morphology and nutrient content. In: Atkinson D, editor. Plant root growth, an ecological perspective. London: Blackwell Scientific, p. 399-421.
30. **Weir, T.L., Park, S., and Vivanco, J.M.** 2004. Biochemical and physiological mechanisms mediated by allelochemicals. Curr. Opin. Plant Biol. 7:472-9.
31. **Zhou, Y.H., Yu, J.Q., Mao, W.H., Huang, L.F., Song, X.S and Nogue´s, S.** 2006. Genotypicvariation on Rubisco expression, photosyntheticelec tron flow and antioxidant metabolism in the chloroplasts of chill-exposed cucumber plants. Plant Cell Physiol. 47:192.

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