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Allelochemical stress induced biochemical changes in the leaves and plant growth regulation in *Ricinus communis* L.

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ABSTRACT

In laboratory experiments, the application of *Piper betle* (L.) leaf extract and eugenol on the castor leaves induced biochemical changes in protein, phenol compounds, sugars and oxidative enzymes in the leaves. The plants treated with *P. betle* leaf extract and eugenol at 20%, 40% and 60% (w/v in acetone) increased the biochemical changes but decreased the enzyme (superoxide dismutase) activity and growth. The allelochemicals application increased the phenolic compounds, protein and sugars contents in treated plants; indicating the changes in metabolism. The allelochemicals at 20, 40 and 60 mg/ml/20 seeds showed considerable impact on shoot growth, which ameliorated with the increasing concentrations.

Key words: Allelochemical stress, eugenol, plant defence, *Piper betle* extract, *Ricinus communis* (L.)

INTRODUCTION

Allelopathy involves plant chemical interactions in both natural and agroecosystems (8). The allelochemical compounds can interfere with the metabolism of other plants. If the effect of these allelochemicals is harmful to plant growth and development, it becomes a biotic stress known as allelochemical stress, which indirectly or directly influences the recipient plant (4). The advantage of working with phytotoxic natural products is that their biological activity is known (16).

Plants are often exposed to the risk of non-targeted post emergence herbicides, which are absorbed through the leaves. Once they have entered the plant, most plants have no way to remove the materials. To protect themselves, plants must either sequester the foreign materials or metabolize them into something less harmful by adding molecules onto them or splitting them into smaller pieces to make them less toxic. On the other hand, if a plant metabolizes a relatively benign substance the result could be different.

Plants are chemically defended in their environments. In certain plants exposure to any stress leads to the qualitative and quantitative changes in the plant biochemicals and enzymes as a part of defence mechanism. These defensive allelochemicals may act as feeding deterrents or alter the physiology and development of the attacking organisms (20). Small organic compounds often have important ecological roles and mediate

interactions between the producing plant and other organisms (20). Allelochemicals can affect many aspects of plant ecology including occurrence, growth, plant succession, and the structure of plant communities, dominance, diversity, and plant productivity (35). Allelopathic inhibition is complex and can involve the interaction of different classes of chemicals (phenolic compounds, flavonoids, terpenoids, alkaloids, steroids, carbohydrates, and amino acids), with mixtures of different compounds [sometimes having greater allelopathic effects than individual compounds alone (31, 37)]. Not much attention has been given to evaluating the physiological processes of the phytotoxic activity of allelochemicals and their impact on the plant development. Like other stress factors, allelochemicals have several molecular targets, and some of their physiological processes or modes of action have been reviewed (36). They include disruption of membrane permeability (14), ion uptake (24), alterations of enzyme activities and inhibition of cell division (4). It has also been postulated that allelopathy stress might lead to an imbalance between antioxidant defenses and the amount of reactive oxygen species (ROS), resulting in oxidative stress (7). Although allelochemicals act as inhibitors of seed germination, their mode of action often remains unclear. Recently, it was proposed that oxidative stress could play a role in phytotoxic phenomenon (36). Many allelochemicals have effects on seed germination too. It has been reported that exposure to certain root extracts reduced stomatal conductance, leaf transpiration and net photosynthesis and significantly increased root peroxidase and superoxide dismutase activities (36). Previous reports are available on herbivore feeding and pathogen infestation stress effects on plants and the plants responses to these strains have been well documented before (20, 27). However, the plant responses to the external application of allelochemicals stress are not given much importance and the available literature is scanty.

This paper aimed to study the impact of allelochemical stress in the model plant *Castor*, that is designed to generate qualitative and quantitative information toward the specific processes involved in the mechanism of action. In our previous studies, we observed that both *P. betle* extract as well as eugenol treatments has some impact on castor plants and showed visible detrimental effects. Therefore we chose these two allelochemical agents as stress factors on castor plants for the study.

Castor (*Ricinus communis* L.) crop is cultivated around the world due to the commercial importance of its oil. India is the world's largest producer of castor seed and meets most of the global demand for castor oil. We hypothesize that the castor plants under the attack of allelochemicals extract may develop certain defence responses. These may be in the form of developing quantitative and qualitative changes in the different enzymes of the plant primary metabolites, nutritive chemicals and production of toxic chemicals as secondary metabolites. The principle defences against the relative molecules and free radicals in plants include detoxifying enzymes catalase, superoxide dismutase. The main objectives of this investigation were (i) to study the castor plant's defensive responses to the application of certain allelochemicals and compare the qualitative and quantitative changes of certain biochemical compounds as well as oxidative enzymes due to this stress, and (ii) to determine the effects of the allelochemicals on plant growth.

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MATERIALS AND METHODS

The allelochemicals used as stress causing agents were (i) acetone extract of the *Piper betle* leaves and (ii) eugenol (99% LR) obtained from Wilson laboratory, Bombay, India. Both the allelochemicals samples, *P. betle* leaf extract and the eugenol were dissolved in acetone (w/v) to prepare the 20%, 40% and 60% dilutions. The samples were stored in the refrigerator at 4°C until use in experiments.

Piper betle L. leaves were purchased from the local market and were air dried at room temperature (28 ±2°C) for 7 days, coarsely ground to a 2 mm particle size and extracted for 18 h in a Soxhlet apparatus using acetone as solvent. The crude extract was concentrated in a rotary evaporator. Leaf material weighing 150 g yielded a dry extract of 5.5 g. Castor seeds (obtained from Directorate of Oil Seed Research, Hyderabad) were washed with two changes of sterile distilled water and sown in plastic pots (15 cm dia, 18 cm height) containing garden mix soil. Plants were kept under controlled conditions in a laboratory glasshouse (28±3°C temperature, 65 to 75% relative humidity and 12 h photoperiod) and protected from pests. Twenty day-old plants were brought to the laboratory in the morning hours and the upper leaves from each plant were selected for the treatment.

The castor plants of 20-day-old (30 cm height with 4 to 5 leaves) were chosen for biochemical studies. Using micropipette, different concentrations of *P. betle* leaf extract and eugenol were applied individually on the leaf surface as a circular spot of 1 cm dia. Each plant received 50 µl of a single concentration of the test chemical and the plants treated with the same amount of acetone alone served as controls. For each treatment there were 10 treated plants and same number for controls. The experiments were repeated three times. Immediately after the application, the treated plants were shifted to the controlled conditions in an environmental chamber and allowed to remain up to 24 h. The leaf material from the treated and control plants were collected, immediately frozen in liquid nitrogen and stored at -80°C and processed for biochemical assays. The material from the treated area was omitted.

Biochemical parameters

The biochemical profile of the castor plants under the stress of allelochemicals application was studied using standard procedures and was compared with that of the normal untreated castor plants. The proteins, phenols and carbohydrates contents were determined. With regard to the possible roles of oxidative stress in phytotoxicity, the changes in the main oxidative enzymes such as catalase, superoxide dismutase and peroxidase were studied.

(i) **Protein content:** Protein content in the enzymatic extracts was determined according to the method described by Lowry *et al.* in 1951 (26). In this method bovine serum albumin was used as calibration standard.

(ii) **Phenolic compounds:** The method reported by Swain and Hillis (34) was used for extraction and quantification of phenols. Fresh leaves (1 g) were extracted in 80% methanol for 90 min at 80 °C. The extract was centrifuged at 14,000 × g for 15 min, and

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100 µl of the extract was diluted to 1 ml with water and mixed with 0.5 ml of 2 M Folin-Ciocalteu reagent as well as 0.5 ml of 1 M Na₂CO₃. The absorbance of the sample solution was measured at 724 nm using spectrophotometer after 1 hr. Concentration of total phenols in the extract was calculated from a standard curve prepared with gallic acid.

The qualitative profile of phenolic compounds was determined by thin layer chromatography using the methods of Harborne (15). In this, *R. communis* leaves were extracted in 80% methanol and the extracts concentrated to the aqueous phase by a vacuum rotary evaporator. Phenols were then partitioned twice with equal volume of ethyl acetate. A single spot of extract (25 µl) was applied onto silica gel (250 mesh), and the TLC plates developed in n-butanol-acetic acid-water (BAW, 4:1:5 v/v/v) and were exposed to iodine vapours for 5 min. The spots became visible.

(iii). **Total carbohydrate content:** For quantitative estimations of carbohydrate content in allelochemicals treated and normal castor leaves the method described by Dubios *et al.* (11). Briefly, to the 0.1 g of leaf 80% of cold ethanol was added and the samples were centrifuged at 5,000 × g for 10 minutes. Resulting extract was filtered using Whatman No 1 filter paper, and to 0.1 ml of plant extract, 0.5 ml of 5% phenol and 2.5 ml of sulfuric acid were added. Stock solution of glucose was prepared and added to all test tubes and a blank was maintained for the series. Samples were incubated at 30 °C for 20 minutes followed by measuring the absorbance at 490 nm.

(iv). **Catalase (CAT) activity:** Catalase activity was determined from the leaf extract prepared according to the method of Aebi (2). All steps of the extraction were carried out at 4°C. Leaves (1 g FW) were homogenized in a cold mortar in 10 ml of 0.1 M potassium phosphate buffer (pH 7.8) containing 2 mM α-dithiothreitol, 0.1 mM EDTA, 1.25 mM PEG 4000, and 0.2 g polyvinylpyrrolidone and mixed for 15 min. The homogenate was centrifuged at 11,000 × g for 15 min, the supernatant was collected, and immediately used for determination of enzyme activities. CAT activity was assayed by measuring the rate of disappearance of H₂O₂ (extinction coefficient of 39.4 mM⁻¹·cm⁻¹) at 240 nm.

(v). **Superoxide dismutase (SOD) activity:** The activity of SOD was measured according to the method described by Krishnan *et al.* (23). This method tests the ability of SOD to inhibit the reduction of nitro blue tetrazolium by the superoxide anion generated photochemically. One milliliter of assay mixture consisted of 50 mM sodium phosphate buffer with pH 7.8, 13 mM Methionine, 75 mM nitro blue tetrazolium, 2 mM riboflavin, 0.1 mM EDTA, and enzyme extract. Riboflavin was added at the end and the samples were placed 30 cm below a light source (4,000 lux), up to 15 min. Switching off the light stopped the reaction. A non-irradiated reaction mixture, which was run in parallel, did not develop colour and served as a control. The absorbance was measured at 560 nm.

(vi). **Peroxidase (POX) activity:** For the quantitative estimation of the peroxidase activity 1 g of plant leaf material was weighed and homogenized with 0.1 M K₂ phosphate buffer (pH 7.0). Sample was centrifuged at 10,000 × g for 20 minutes and to 0.5 ml obtained supernatant (diluted 1:10), 1.5 ml of 0.05 M pyrogallol was added followed by addition of 0.5 ml of 1% H₂O₂ and samples were incubated in water bath at 25°C for 5 minutes. Next

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1 ml of 2.5 N H₂SO₄ was added to stop the reaction. Absorbance was monitored at 420 nm (21).

Germination and growth bioassay

Seeds of *R. communis* were surface-sterilized in 70% ethanol for 10 min then in 2% sodium hypochlorite for 10 min and finally washed several times with distilled water. The sterilized seeds were treated with acetone (control) and test doses of respective allelochemicals (20, 40 and 60 mg/ 20 seeds in 1 ml acetone) separately with each allelochemical and dried for 10 min. Thereafter, 20 seeds of each treatment and control were transferred to 15-cm-dia petri plates containing moist filter paper (No.1) as lining. These were kept in Growth Chamber under controlled conditions (28 ± 2°C and 65 ± 10% relative humidity). Distilled water was added to all the petri plates on alternate days. Final germination (%) and the length of root and shoot were recorded at 10 days after treatment. Each treatment and control was replicated three times. The data were subjected to factorial analysis of variance in a completely randomized manner using Graph pad Software. The inhibition percentage calculated by using following formulae.

$$\text{Inhibition percentage (\%)} = [(\text{control} - \text{treated}) / \text{control}] \times 100$$

Data analysis

Analysis of variance (ANOVA) was used to test the significance of the main effects. When significant difference was found among means, Tukey's HSD test was used to determine whether significant (P<0.05) differences occurred between individual treatments. In germination tests, the difference between two treatments was analyzed by paired t-test. Analyses were carried out using Sigmatat (ver. 3.5) software. Graphs were plotted using origin plot software.

RESULTS AND DISCUSSION

The application of the allelochemicals on *R. communis* plants, increased the proteins and phenolic content in the treated leaves (Fig. 1). All the three test concentrations, significantly (P<0.05) increased the protein contents (Fig. 1A). Phenols generally increases under the stress (12). Eugenol at 40 and 60% concentrations caused maximum increase in phenolics levels (Fig. 1B). The phenolic compounds were elicited due to the application of the allelochemicals and were significantly different (P<0.05, ANOVA). The differences in concentrations of all analyzed compounds among the treatments were observed. The quantitative analysis of phenolic compounds in plant material is major step to determine their biological activity (38). Accumulations of phenolics in plants are strongly associated with the environmental conditions (18), which influence their synthesis and concentrations in the plant tissue and generally enhances the allelochemicals production (19). The active band in UV (R_f 0.29) was observed in all castor leaf extracts both treated with allelochemicals and control (Table 1) and TLC profile (Fig. 2). The bands corresponding to R_f 0.85, and R_f 0.66 were yellow in the visible light and were seen in all treatments. However, interestingly one band of R_f 0.73 was seen only

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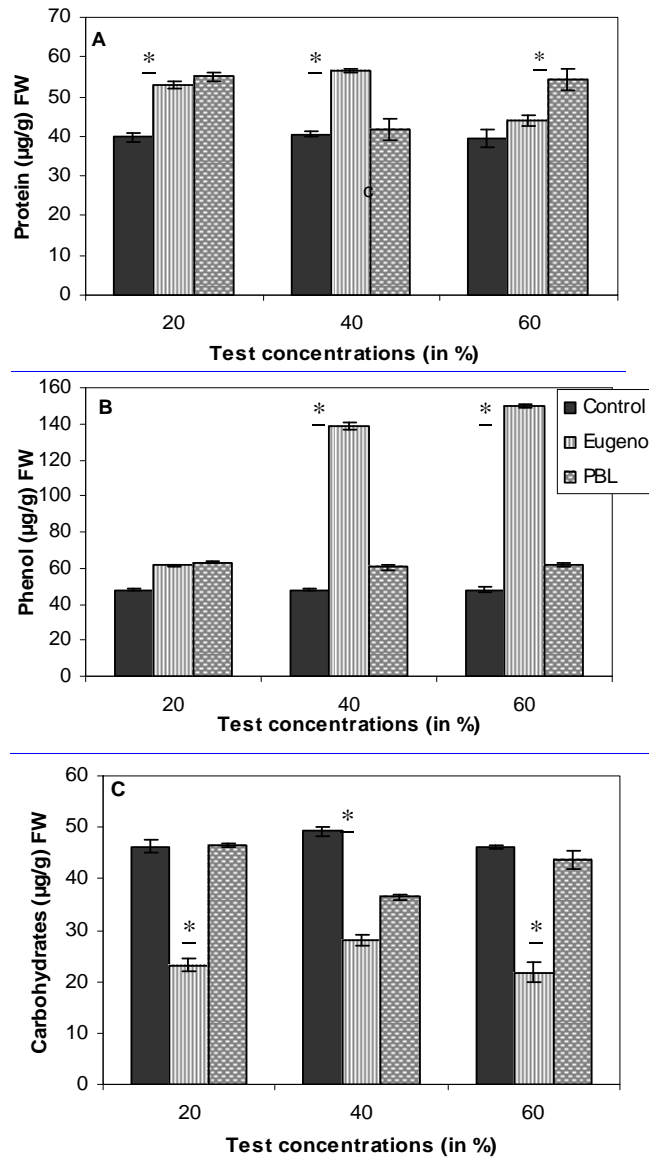


Figure 1. Effects of *Piper betle* extract and eugenol on protein, phenols and carbohydrate content of *R. communis*. In each treatment, bars indicated by "*" are significant differences (Tukey's

Allelochemical stress induced biochemical changes in Ricinus communis L.

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HSD test, $P < 0.05$), (N = one leaf/plant/replicate, 30 replicates per treatment). Lines on the bars indicating SE (standard error).

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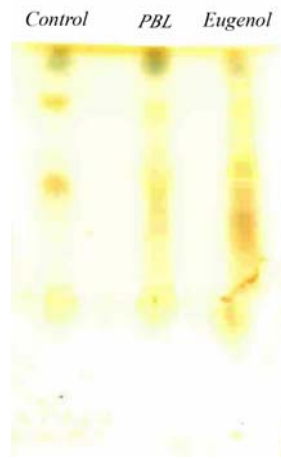
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Table 1. Qualitative analysis (TLC) of phenol extracts from the castor leaves treated with *P. betle* extract and eugenol (24 h after exposure to these two allelochemical factors)

Control	Retention factor (R_f) values		Visible (Iodine)	UV
	Piper treatment	Eugenol treatment		
0.29	0.29	0.29	=	P
0.66	0.66	0.66	Y	
		0.73	Y	
0.85	0.85	0.85	Y	

Colour reaction of phenol after treatment Y - yellow, P - pink.



PBL- Piper betle leaf extract

Figure 2. TLC profile of the phenol extracts from the castor leaves surface treated with two allelochemicals (24 hrs after exposure to *P. betle* and eugenol).

in eugenol treated leaf extracts. Earlier reports showed similar observations with the application of leaf leachate of *Eucalyptus globulus* on seedlings of rice, sorghum and black gram, which increases the phenol content (9). The application of ferulic acid and vanillic acid on soybean also increases the phenolic acid content in the roots (17).

However, the carbohydrates were decreased significantly in treated plants than in control plants (Fig. 1). Previous investigations showed that plant exposure to cinnamic or benzoic acids inhibited the photosynthesis, protein synthesis, enzyme activities and lipid mobilization (5, 30).

The application of allelochemicals on castor leaves changed the activity of redox enzymes (superoxide dismutase, peroxidase and catalase). The difference between the treated and control leaves were significant in peroxidase and catalase enzymes. The application of piper extract and eugenol at 20 and 40% concentration increased the



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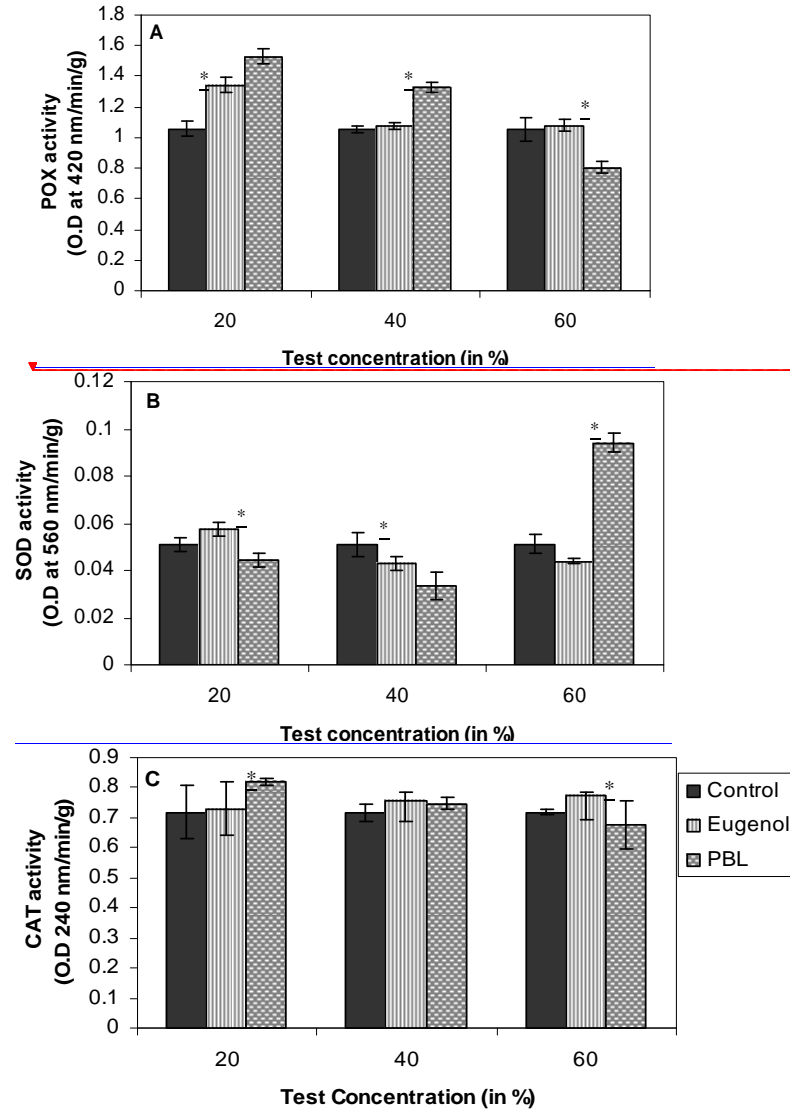


Figure 3. Effects of allelochemical stress, along with control at different concentrations on redox enzymes – (A) peroxidase (POX), (B) superoxide dismutase (SOD) and (C) catalase (CAT) in leaves of *R. communis*. In each treatments stars at the top of the bars show significant differences (Tukey’s HSD test, $P < 0.05$). (N – one leaf/plant/replicate, 30 replicates per treatment). Lines on the bars indicating SE (standard error).

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peroxidase activity (Fig. 3A). The activity of peroxidase decreased with the increase in concentration of *P. betle* extract and was significantly different between treatments and controls ($P < 0.05$).

The SOD activity was increased due to allelochemical stress induced by *P. betle* leaf extract and eugenol treatments than controls (Fig. 3B). The increased activity of peroxidase (POX) due to the application of the allelochemicals, *P. betle* crude extract and eugenol is similar to cucumber, where, the enzyme activities of SOD and POX in the germinating seedlings increased by application of cucumber root extract (39). The catalase (CAT) activity decreased with the increase in concentration of *P. betle* extract, but enhanced in eugenol treated castor plants (Fig. 3C). On the contrary, the rice aqueous extracts blocks the SOD and catalase activity in *Echinochloa crus-galli* (barnyard grass) (25). Herrig *et al.* (17) reported the increased peroxidase activity in the soybean roots when treated with ferulic and vanillic acid, which is similar to our results. Bogatek *et al.* (6) demonstrated that allelochemicals present in leaf extracts of sunflower cause oxidative stress in mustard seedlings through ROS generation and it enhanced the activities of CAT, SOD, and GR (glutathione reductase). It appears that there is a direct physical interaction between the susceptible plant cells and allelochemicals.

The application of allelochemicals significantly affected the seed germination (Figure 4). There was significant ($P < 0.001$) inhibition of seed germination under the influence of piper extract (64%) as well as eugenol (81%) at a concentration 60 mg/ml per 20 seeds.

The piper extract and eugenol significantly ($P < 0.001$) decreased the shoot and root length (Fig. 4A). Similarly, the root growth was also decreased as the concentration increased. This negative impact on the root and shoot growth also decreased the growth of castor seedlings. In tomato seedlings, the allelochemical stress caused by *Sicyos deppei* aqueous leachate inhibited the root growth but not germination, and produced an imbalance in the oxidative status of cells both in ungerminated seeds and in primary roots (32).

Both the allelopathic factors reduced the germination of castor seeds and their seedlings growth. The root and shoot length of castor seedlings, at all the three concentrations were less than seedlings of untreated control (Fig. 4 B and C). Eugenol had more allelopathic effect than piper crude extract (only roots) and reduced the growth of seedlings. Sometimes allelochemicals may not be toxic compounds but they induce toxic responses (36).

Many monoterpenes are potent inhibitors of seed germination and growth in several plant species (13). For example, α - and β -pinene, limonene, and citronelol extracted from leaves of *Citrus aurantium* L. inhibits the growth of *Amaranthus retroflexus* (3); residues of *Eucalyptus globulus* and *Acacia melanoxylon* inhibits the germination and seedling growth of *Lactuca sativa* (33); and essential oils of *Eucalyptus globulus* and *Eucalyptus citriodora* inhibits the germination of *Phaseolus aureus* and *Lens esculentum* (22). The mechanisms of affecting germination by monoterpenes are not known (29). The effects of four monoterpenes (camphor, eucaliptol, limonene, and α -pinene) were investigated on the oxidative metabolism of mitochondria isolated from the maize root (*Zea mays*), on maize seed germination and on primary root growth and found that their interference with mitochondrial respiration inhibited the seed germination and plant growth (14).

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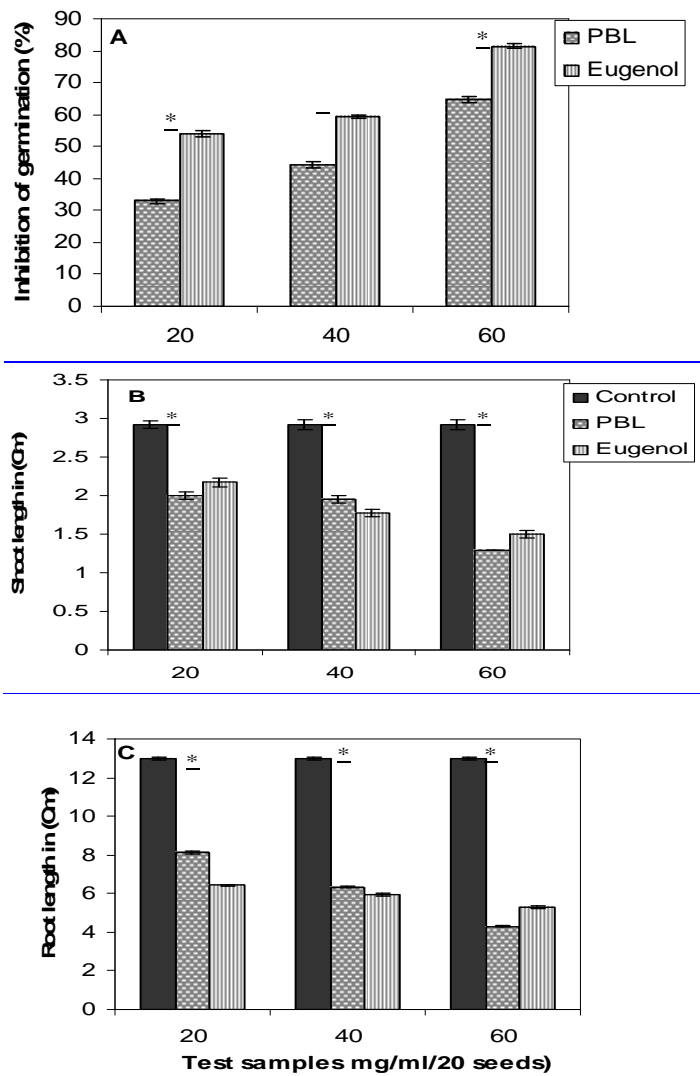


Figure 4. [Effects of *P. betle* leaf extract and eugenol on seed germination and seedling growth of castor seeds.](#) (A) Germination tests; significant differences ($P < 0.001$) are indicated by ‘*’ (paired t-test). (B and C) Seedling growth; bars indicating ‘*’ are significant differences (ANOVA, $P < 0.001$, Tukey’s test). (20 seeds/replicate, 3 replicates per treatment, $N = 60$). Lines on the bars indicating SE (standard error). PBL – *Piper betle* leaf extract.

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We found that allelochemical treatment caused general disorders in the treated area and changes the amounts of biochemical parameters as stress markers. Since modes of action of natural products are different from synthetic herbicides, hence, it is interesting to study the mechanism of phytotoxicity induced in susceptible plants by plant-produced allelochemicals. The phytotoxicity of some allelochemicals is attributed to their ability to disrupt normal metabolic processes in the plant.

CONCLUSIONS

The application of *Piper* leaf extract and eugenol induced changes in the qualitative profile of total soluble phenols and oxidative enzymes in castor by activating the natural defence pathways. The changes in specific phenols and proteins might be the useful biogenetic markers in castor plants. Thus treatment changed the level of phenols in the plant and it varied with the treatment as well as the concentration of the compound. The mode of action of these two allelochemicals will show the way to use these compounds as future herbicides. The study of natural phytotoxins, including allelochemicals, provides an avenue to identify the mechanisms of action that have developed due to interactions among the various organisms and plants over long periods of time.

ACKNOWLEDGEMENTS

Author is grateful to the Department of Science and Technology, New Delhi for the research grant, to Peta Devanand, Y. Jyothsna and K. Sudheshna of Biology and Biotechnology Division, Indian Institute of Chemical Technology, Hyderabad, India for their technical and statistical assistance and to Dr. J. S. Yadav, Director, IICT for the facilities and encouragement.

REFERENCES

1. Abraham, D., Braguini, W.J., Kelmer-Bracht, A.M. and Shii-Iwamoto, E.L. (2000). Effects of four monoterpenes on germination, primary root growth, and mitochondrial respiration of maize. *Journal of Chemical Ecology* 26: 611-624.
2. Aebi, H.U. (1983). Catalase In: *Methods in Enzymatic Analysis* (Eds., H.U. Berg Meyer). Vol. 3: pp 276-286. Academic Press, New York.
3. Alsaadawi, I.S., Arif, M.B. and Alrubeaa, A.J. (1985). Allelopathic effects of *Citrus aurantium* L. II. Isolation, characterization, and biological activities of phytotoxins. *Journal of Chemical Ecology* 11:1527-1534.
4. Anava, A.L. (1999). Allelopathy as a tool in the management of biotic resources in agroecosystems. *Critical Reviews in Plant Science* 18: 697-739.
5. Baleroni, C.R.S., Ferrarese, M.J.L., Braccini A.L., Scapim, C.A. and Ferrarese-Filho, O. (2000). Effects of ferulic and *p*-coumaric acids on canola (*Brassica napus* L.) seed germination. *Seed Science and Technology* 28: 201-207.
6. Bogatek, R., Gniazdowska, A., Zakrzewska, W., Oracz, K. and Gawroński, S.W. (2006). Allelopathic effects of sunflower extracts on mustard seed germination and seedling growth. *Biologia Plantarum* 50: 156-158.

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7. Cruz-Ortega, R., Ayala-Cordero, G. and Anaya, A.L. (2002). Allelochemical stress produced by the Aqueous leachate of *Callicarpa acuminata*: effects on roots of bean, maize and tomato. *Physiologia Plantarum* **116**: 20-27.
8. Dakshini, K.M.M., Foy, C.L. and Inderjit. (1999). Allelopathy: one component in a multifaceted approach in ecology. In: *Principles and Practices in Plant Ecology: Allelochemical Interactions* (Eds. Inderjit, K.M.M. Dakshini and C.L. Foy), pp. 3-14. CRC Press, Boca Raton, Florida.
9. Djanaguiraman, M., Vaidyanathan, R., Sheeba, J.A., Devi, D.D. and Bangarusamy, U. (2005). Physiological responses of *Eucalyptus globulus* leaf leachate on seedling physiology of rice, sorghum and blackgram. *International Journal of Agriculture and Biology* **7**: 35-38.
10. Dubey, P. and Tripathi, S.C. (1987). Studies on antifungal, physico-chemical and phytotoxic properties of the essential oil of piper betle. *Zeitschrift Fuer Pflanzenkrankheiten Und Pflanzenschutz* **94**: 235-241.
11. Dubious, N.K., Giller, K.A., Hamilton, K., Kellers, P.A. and Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Annals of Chemistry* **28**: 350-356.
12. Edwards, P.J. (1992). Resistance and defense: the role of secondary plant substances. In: *Pest and Pathogens*. (Eds. P.G. Ayres), pp. 69-84. Bios. Scientific Publishers, UK.
13. Fischer, N.H. (1991). Plant terpenoids as allelopathic agents. In: *Ecological Chemistry and Biochemistry of Plant Terpenoids* (Eds. J.B. Harborne and F.A. Tomes-Barberan), pp. 377-399. Clarendon Press, Oxford, U.K.
14. Galindo, J.C.G., Hernandez, A., Dayan, F.E., Tellez, M.R., Macias, F.A., Paul, R.N. and Dukes, O. (1999). Dehydrozalaninone, a natural sesquiterpenoid, causes rapid plasma membrane leakage. *Phytochemistry* **52**: 805-813.
15. Harborne, J.B. (1973). *Phytochemical Methods*. Chapman and Hall, London.
16. Henkel, T., Brunne, R.M., Uller, H. and Reichel, F. (1999). Statistical investigation into the structural complementarity of natural products and synthetic compounds. *Angewandte Chemie International Edition* **38**: 643-647.
17. Herrig, V., De Lourdes, M., Ferrarese, M.D.L., Suzuki, L.S., Rodrigues J.D. and Ferrarese, O. (2002). Peroxidase and phenylalanine ammoniylase activities, phenolic acid contents, and allelochemical-inhibited root growth of soybean. *Biological Research* **35**: 59-66.
18. Horner, J.D. (1990). Non linear effects of water deficits on foliar tannin concentration. *Biochemistry Systematics and Ecology* **18**: 211-213.
19. Hura, T., Dubert, F., Dabkowska, T., Stupnicka-Rodzynkiewicz, E., Stoklosa, A. and Lepiarczyk A. (2006). Quantitative analysis of phenolics in selected crop species and biological activity of these compounds evaluated by sensitivity of *Echinochloa crus-galli*. *Acta Physiologiae Plantarum*, **28** (6): 537-545.
20. Karban, R. and Baldwin, I.T. (1997). *Induced Responses to Herbivory* (Interspecific Interactions). University of Chicago Press, Chicago.
21. Kar, M. and Mishra, D. (1976). Catalase, peroxidase, and polyphenoloxidase activities during rice leaf senescence. *Plant Physiology* **57**: 315-319.
22. Kohli, R.K. and Singh, D. (1991). Allelopathic impact of volatile components from *Eucalyptus* on crop plants. *Plant Biology* **33**: 475-483.
23. Krishnan, N., Chattopadhyay, S., Kundu, J.K. and Chaudhuri, A. (2002). Superoxide dismutase activity in haemocytes and haemolymph of bombix mori following bacterial infection. *Current Science* **83**: 321-325.
24. Lehman, M.E. and Blum, U. (1999). Evaluation of ferulic acid uptake as a measurement of allelochemical dose: effective concentration. *Journal of Chemical Ecology* **25**: 2585-2600.
25. Lin, W.X., He, H.Q. and Guo, Y.C. (2001). Rice allelopathy and its physiobiochemical characteristics. *Chinese Journal of Applied Ecology* **12**: 871-875.
26. Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry* **193**: 265-275.
27. Masters, G.J. (1995). The impact of root herbivory on aphid performance: field and laboratory evidence. *Acta Oecologica* **16**:135-142.
28. Panda, S. and Kar, A. (1998). Betel leaf extract can be both antiperoxidative and peroxidative in nature. *Current Science Bangalore* **74**(4): 284-285.
29. Penuelas, J., Ribas-Carbo, M., Giles, L. (1996). Effects of allelochemicals on plant respiration and oxygen isotope fractionation by the alternative oxidase. *Journal of Chemical Ecology* **22**: 801-805.
30. Politycka, B. (1996). Peroxidase activity and lipid peroxidation in roots of cucumber seedlings influenced by derivatives of cinnamic and benzoic acids. *Acta Physiologiae Plantarum* **18**: 365-370.

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31. Rice, E. L. 1984. *Allelopathy*, 2nd edn. Academic press. New York.

32. Romero-Romero, T., Sanchez-Nieto, S., San Juan-Badillo A., Anaya A. L. and Cruz-Ortega, R. (2005). Comparative effects of allelochemical and water stress in roots of *Lycopersicon esculentum* Mill. (Solanaceae). *Plant Science* **168**: 1059-1066.

33. Souto, X.C., Gonzalez, L. and Reigosa, M.J. (1995). Allelopathy in forest environment in Galicia, NW Spain. *Allelopathy Journal* **2**: 67-78.

34. Swain, T.W. and Hillis, E. (1959). The phenolic constituents of *Prunus domestica* I. The quantitative analysis of phenolic constituents. *Journal of the Science of Food Agriculture* **10**: 63-68.

35. Takeuchi, Y., Kawaguchi, S. and Yoneyama K.F. (2001). Inhibitory and promotive allelopathy in rice (*Oryza sativa* L.). *Weed Biology and Management* **1**: 147-156.

36. Weir, T.L., Park, S.W. and Vivanco, J.M. (2004). Biochemical and physiological mechanisms mediated by allelochemicals. *Current Opinion in Plant Biology* **7**: 472-479.

37. Williamson, G.B. (1990). Koch's postulates and the neck riddles. *Allelopathy: Perspectives on Plant Competition*. (Eds., J. B. Grace and D. Tilman), pp.143-162. Academic Press, New York.

38. Wu, H., Pratley, J., Lemerle, D. and Haig T. (1999). Crop cultivars with allelopathic capability. *Weed Research* **39**: 171-180.

39. Yu, J.Q., Ye, S.F., Zhang, M. F. and Hu, W.H. (2003). Effects of root exudates, aqueous root extracts of cucumber (*Cucumis sativus* L.) and allelochemicals on photosynthesis and antioxidant enzymes in cucumber. *Biochemical Systematics and Ecology* **31**:129-139.

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Table 1. Qualitative analysis (TLC) of phenol extracts from the castor leaves treated with *P. betle* extract and eugenol (24 hrs after the exposure to these two allelochemical factors). . . . ¶

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*** For correspondence**

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Allelochemical stress induced biochemical changes in the leaves and plant growth regulation in *Ricinus communis* L.

PATHIPATI USHA RANI

Biology and Biotechnology Division, Indian Institute of Chemical Technology
Tarnaka, Hyderabad-500 007. Andhra Pradesh India.

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In our previous studies we observed that both *P. betel* (*betle*) extract as well as eugenol treatments has some impact on castor plants and showed visible detrimental effects. (Transfer this sentence to Introduction, please)

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Plants

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seeds

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(*Ricinus communis* L).

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were collected

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surface sterilized (sterilized with water? It is impossible)

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Sample preparation

Both (The allelochemicals samples, *P. betle* leaf extract and the oil of eugenol (and what about eugenol? It is not clear) were dissolved in acetone (w/v) to prepare the dilution 20%, 40% and 60% dilution. The samples were stored in the refrigerator at 4°C until the use in experiments.

Treatments and measurements

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(*Ricinus communis* L.)

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Extraction and determination of (iv). cCatalase (CAT) activity:

Catalase activity was determined from the leaf extract prepared according to the method of Aebi (2). All steps of the extraction were carried out at 4°C. Leaves (1 g FW) were homogenized in a cold mortar in 10 ml of 0.1 M potassium phosphate buffer (pH 7.8) containing 2 mM α -dithiothreitol, 0.1 mM EDTA, 1.25 mM PEG 4000, and 0.2 g polyvinylpyrrolidone and mixed for 15 min. The homogenate was centrifuged at 11,000 \times g for 15 min, the supernatant was collected, and immediately used for determination of enzyme activities. CAT activity was assayed by measuring the rate of disappearance of H₂O₂ (extinction coefficient of 39.4 mM⁻¹ cm⁻¹) at 240 nm.

Extraction and determination of (v). sSuperoxide dismutase (SOD) activity:

The activity of SOD was measured according to the method described by Krishnan *et al.* (230). This method tests the ability of SOD to inhibit the reduction of nitro blue tetrazolium by the

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Extraction and determination of		
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was carried out in both treated and healthy (control) castor leaves. (Does it means that other enzymes was not?)		
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The allelochemical effect on seed germination and seedling growth of <i>Ricinus communis</i> plants were assessed examined in the laboratory.		
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The protein content was found to be significantly ($P<0.05$) increased at a		
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Phenols are considered to be the first compounds to be increased on stress		
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was recorded in eugenol treated leaves at the concentrations of 40 and 60%		
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quantitatively in the

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comparison with the

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and the difference was significant

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The carbohydrates are the energy sources that are synthesized by the chloroplasts (truism). The decrease in the carbohydrate content indicates the decreased in photosynthesis tic rates (or increased respiration).

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was affected due to the treatment of the allelochemicals and a significant difference was noted among the treatments as well as between treatments and controls. The germination and seeding growth records were given in (Figure 4). Seed germination is the important stage component (stage) in plant development.		
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differences in shoot and root length were also significant		
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Especially the effect on shoot growth was more evident with the increasing concentrations (really?).		
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The data presented showed that allelochemical treatment caused		
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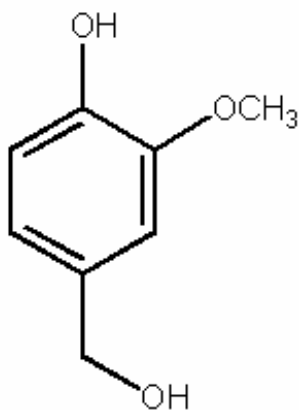
From the above study it may be concluded that the treatments,

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Table 1. Qualitative analysis (TLC)(R_f values) of phenol extracts from the castor leaves treated with *P. betle* extract and eugenol the two-allelo chemicals (24 hrs after the exposure to these two allelochemical factors *P. betel* and eugenol).

Retention factor	(R_f) values	values	UV	visible	Visible
Control	Piper Treatment		treatment	Eugenol treatment	(Iodine)
0.66	0.29	0.29	0.66	0.29	- P
	0.66			0.73	Y
	0.85	0.85		0.85	Y

Colour reaction of phenol after treatment Y - yellow, P - pink,.



AAB - chemical structure of eugenol

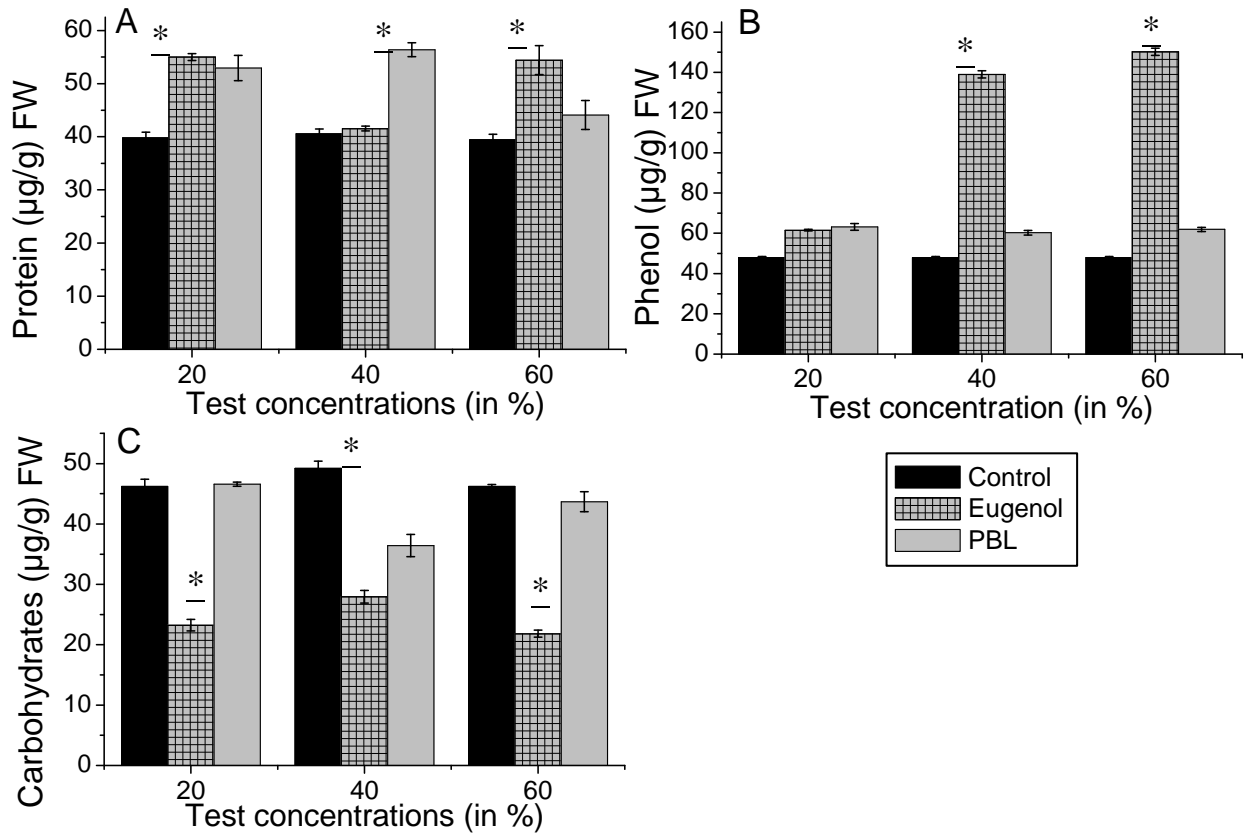


Figure. 12. Effect of *Piper betle* extract and eugenol on protein, phenols and carbohydrate content of *R. communis*. In each treatment, Bbars indicated by '*' are significant differences (ANOVA, $P < 0.05$, Tukey's HSD multiple comparison test, $P < 0.05$), (N = one leaf/plant/replicate, 30 replicates per treatment). Lines on the bars indicating SE (standard error).

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Figure 2. TLC profile of the phenol extracts from the castor leaves surface treated with two allelochemicals (24 hrs after exposure to *P. betelle* and eugenol).

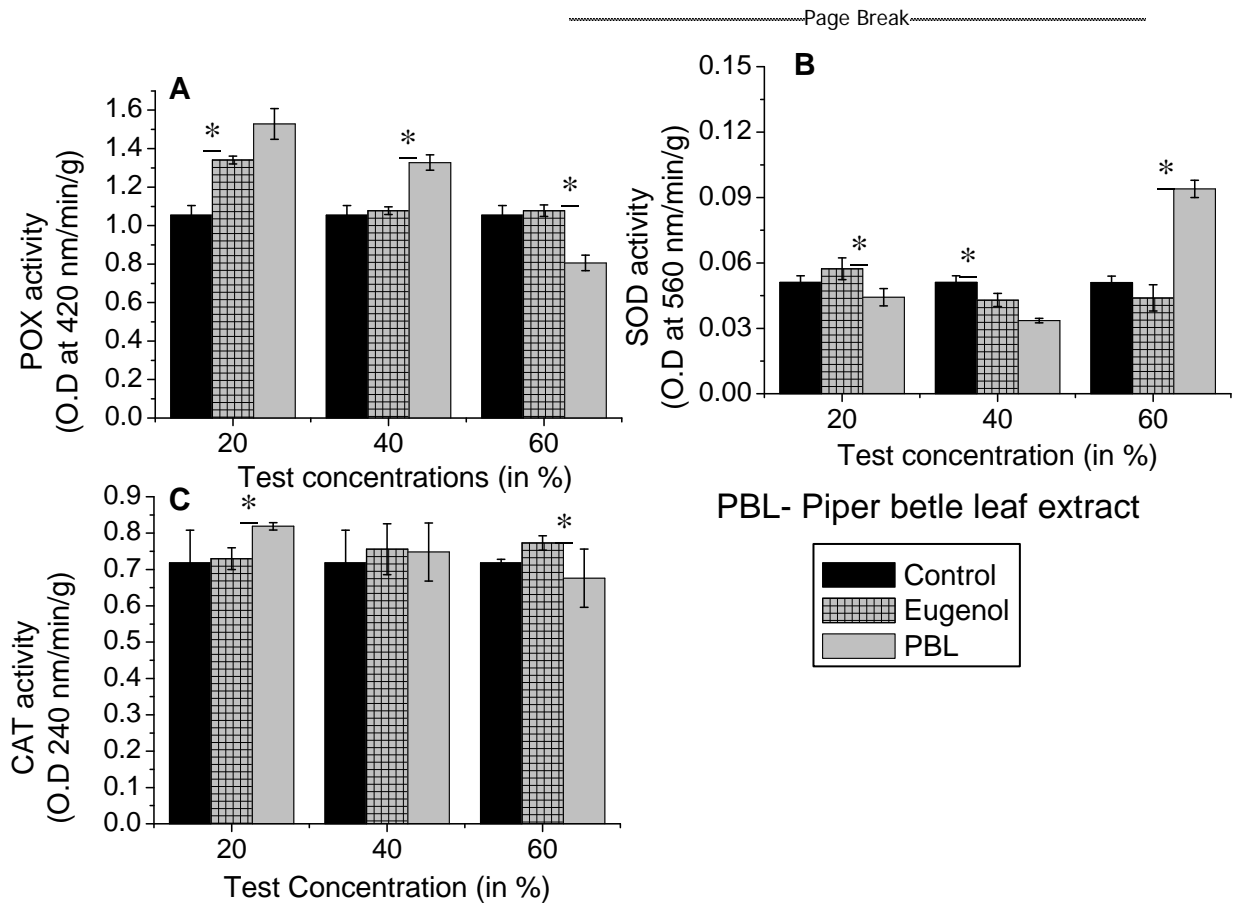


Figure 3. Effect of allelochemical stress, along with control at different concentrations on oxidative (redox) enzymes – (A) peroxidase (POX), superoxide dismutase (SOD), (B) superoxide dismutase (SOD) peroxidase (POX) and (C) catalase (CAT) in leaves of *R. communis*. In each treatments stars at the top of the Bbars indicating showdifferent letters are significant differences (ANOVA, $P < 0.05$, Tukey's HSD multiple comparison test, $P < 0.05$), ($N =$ one leaf/plant/replicate, 30 replicates per treatment). Lines on the bars indicating SE (standard error).

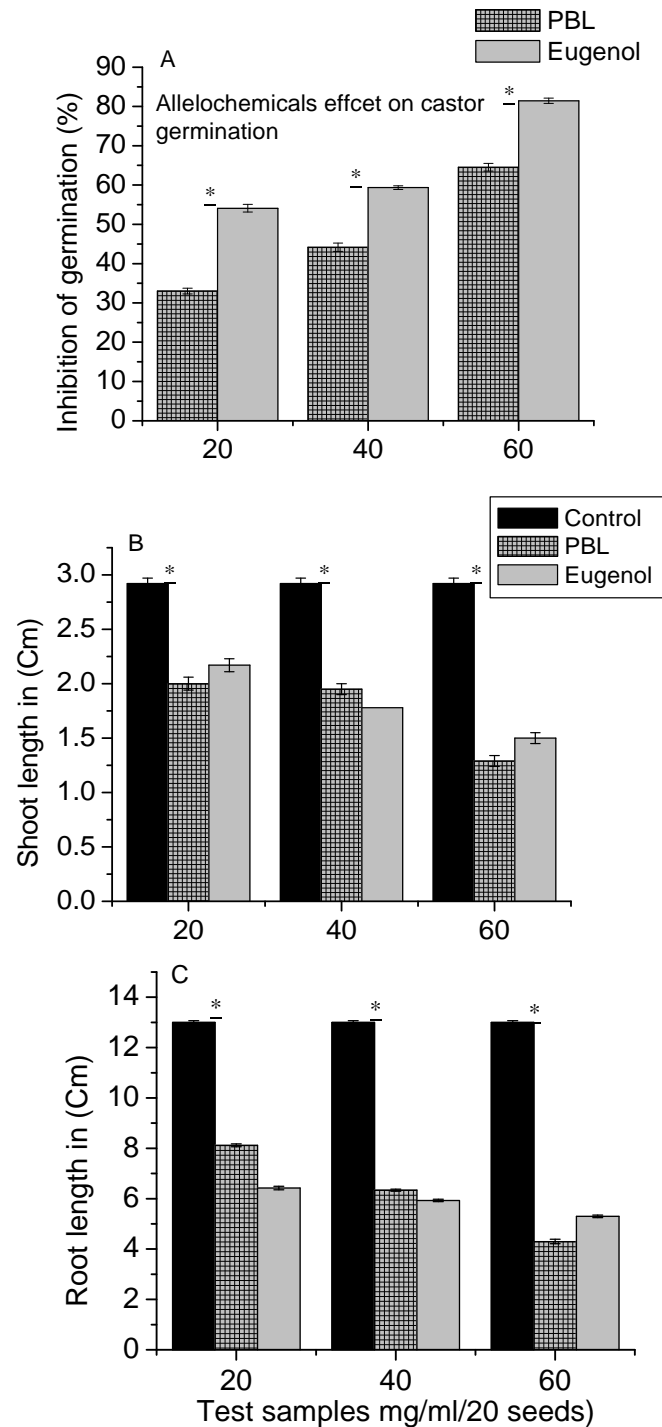


Figure. 4 Effect of *P. betle* leaf extract and eugenol on seed germination and seedling growth of castor seeds. (A) Germination tests; significant differences ($P < 0.001$) are indicated by '*' (paired t-test). (B and C) Seedling growth; bars indicating '*' different letters are significant differences (ANOVA, $P < 0.001$, Tukey's multiple comparison test), (20 seeds/replicate, 3 replicates per treatment, $N = 60$). Lines on the bars indicating SE (standard error). PBL -- *Piper betle* leaf extract.

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Djanaguiraman, M., Vaidyanathan, R., Sheeba, J. A., Devi, D. D., Bangarusamy, U. (2005). Physiological responses of *Eucalyptus globulus* leaf leachate on seedling physiology of rice, sorghum and blackgram. *International Journal of Agriculture and Biology* 7(1): 35-38. (This paper is not in good place)

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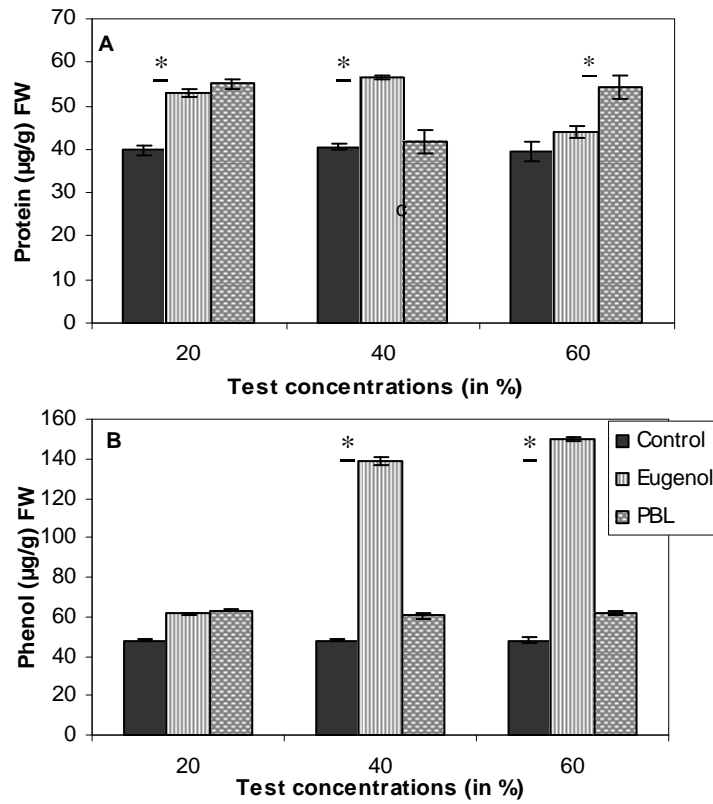
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Table 1. Qualitative analysis (TLC) of phenol extracts from the castor leaves treated with *P. betle* extract and eugenol (24 hrs after the exposure to these two allelochemical factors).

Control	Retention factor (R_f) values		Visible (Iodine)	UV		
	Piper treatment	Eugenol treatment				
	0.29	0.29	0.29		-	P
	0.66	0.66	0.66		Y	
					0.73	
Y	0.85	0.85	0.85		Y	

Colour reaction of phenol after treatment Y - yellow, P - pink.



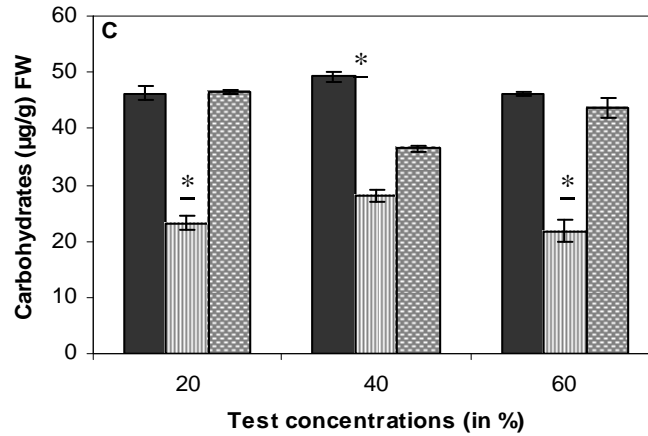


Figure 1. Effects of *Piper betle* extract and eugenol on protein, phenols and carbohydrate content of *R. communis*. In each treatment, bars indicated by '*' are significant differences (Tukey's HSD test, $P < 0.05$), (N = one leaf/plant/replicate, 30 replicates per treatment). Lines on the bars indicating SE (standard error).

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Figure 2. TLC profile of the phenol extracts from the castor leaves surface treated with two allelochemicals (24 hrs after exposure to *P. betle* and eugenol).

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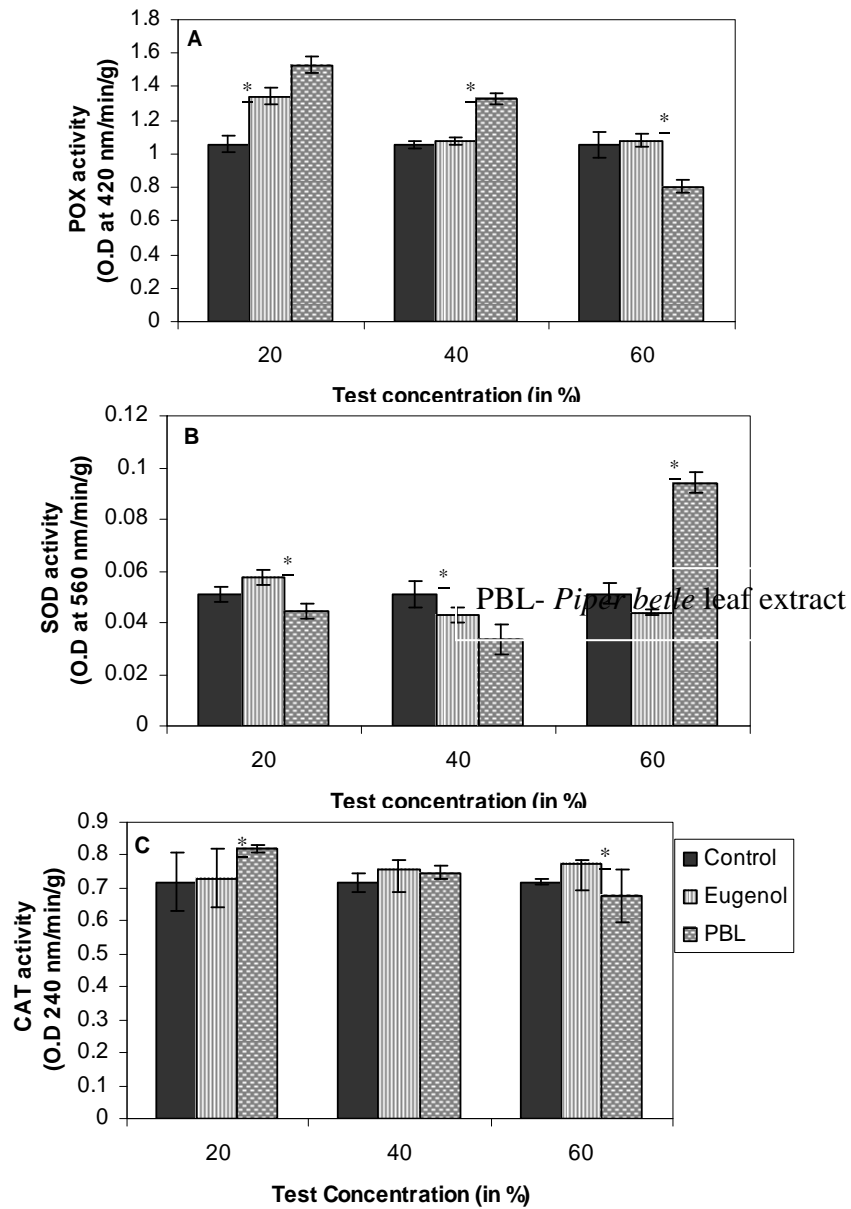


Figure. 3. Effects of allelochemical stress, along with control at different concentrations on redox enzymes – (A) peroxidase (POX), (B) superoxide dismutase (SOD) and (C) catalase (CAT) in leaves of *R. communis*. In each treatments stars at the top of the bars show significant differences (Tukey's HSD test, $P < 0.05$), (N – one leaf/plant/replicate, 30 replicates per treatment). Lines on the bars indicating SE (standard error).

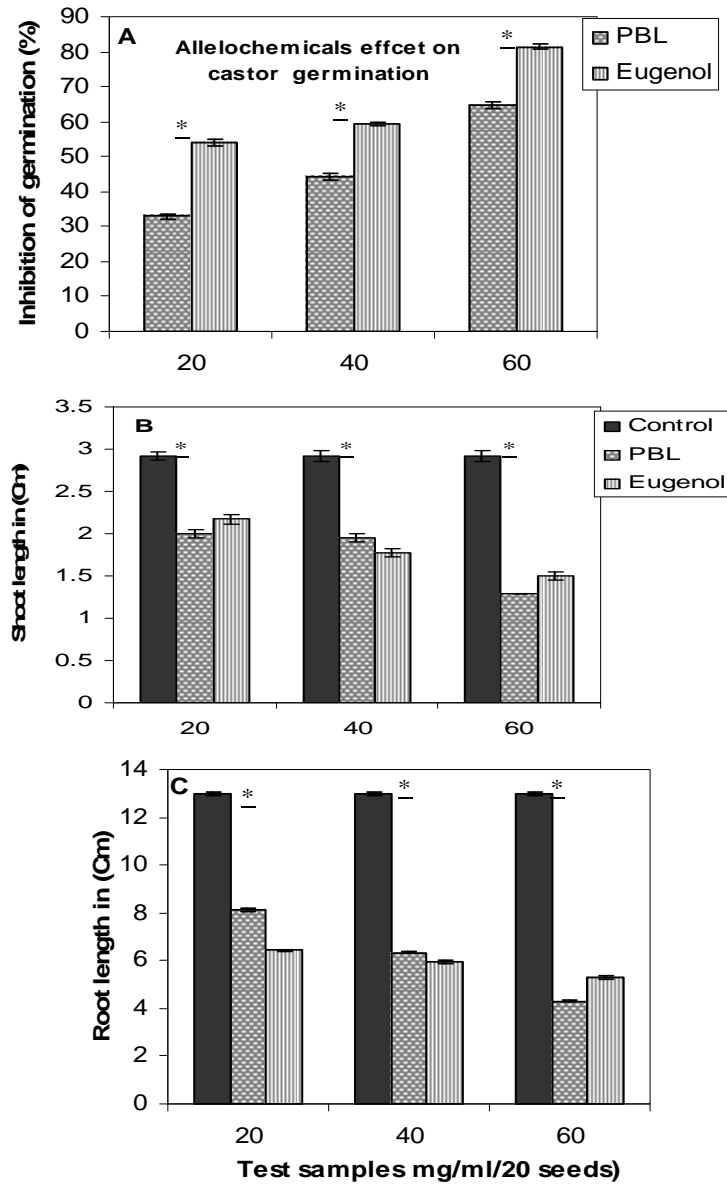


Figure. 4 Effects of *P. betle* leaf extract and eugenol on seed germination and seedling growth of castor seeds. (A) Germination tests; significant differences ($P < 0.001$) are indicated by '*' (paired t-test). (B and C) Seedling growth; bars indicating '*' are significant differences (ANOVA, $P < 0.001$, Tukey's test), (20 seeds/replicate, 3 replicates per treatment, N = 60). Lines on the bars indicating SE (standard error). PBL – *Piper betle* leaf extract