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Inhibition of Seed Germination and Growth of Blackgram (*Vigna mungo (L.) Hepper)* due to Oxidative Damage as Induced by Phenolic Compunds

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ABSTRACT. *Three phenolic compounds were tested for their effects on seed germination, seedling growth, membrane damage and antioxidant enzymes activity in blackgram (Vigna mungo (L.) Hepper) under laboratory conditions. The effects of gallic, p-coumaric and p-hydroxy benzoic acids were tested in four concentrations (0.5, 1, 2 and 4 mM). At 4 mM concentration, all the phenolic compounds exerted most significant reduction in germination and growth processes than other concentrations. A similar trend has been observed in chlorophyll content, soluble protein content, and nitrate reductase (NR) enzyme activity. Enhanced contents of thiobarbituric acid reacting substances (TBARS), hydrogen peroxide and lipoxygenase enzyme activity were observed in all concentrations when compared to control without phenolic compounds. The same trend was also observed in activity of different antioxidant enzymes viz., superoxide dismutase, catalase and peroxidase. Seedlings received phenolic acid treatments accumulated more proline and total free amino acids than control plants. All the phenolic compounds at higher concentration significantly reduced the growth, NR activity and antioxidant enzymes activity over control. Among the three phenolic acids tested, p-hydroxy benzoic acid was found to be highly inhibitory than p-coumaric and gallic acids. The susceptibility at higher concentrations may be attributed to reduced antioxidant enzymes activity and more membrane damage.*

INTRODUCTION

Biologically active substances causing growth inhibition or promotion are released from plants and microorganisms to the environment through leakage, leaching, exudation or decomposition of biomass. Secondary metabolites, which are released into the environment, can positively or negatively affect the growth and development of vegetation. One of the main groups of these substances is phenolic compounds, derivatives of benzoic and cinnamic acids and some of them have been identified: ferulic, p-hydroxy benzoic, p-coumaric, protocatechuic, salicylic, syringic and vanillic acids. The phenolic acid derivatives produced by higher plants have been frequently implicated in allelopathy. Allelochemicals are found to accumulate and persist for a considerable time in soil, thus, significantly interfering with the growth of neighboring weeds and plants. Our earlier studies revealed that leaf leachates of *Eucalyptus globules* had allelopathic effects on seedling growth of greengram, blackgram, cowpea, rice and sorghum. Maximum inhibition of growth was observed in blackgram (Djanaguiraman *et al.* 2005). The allelopathic potential of *Eucalyptus globules* has been attributed to the production of phenolic acids such as gallic, p-coumaric, p-hydroxy benzoic acid, gentistic, vanillic acids and catechol (Narwal, 2004). Among these gallic, p-coumaric, p-hydroxy benzoic acids were found abundantly in the environment

(Sasikumar *et al.*, 2002). Hence, there is a need to appraise the effect of above phenolic compounds on germination and growth of sensitive species such as blackgram.

Many investigations of allelopathic interactions have demonstrated the influence of phenolic compounds on various physiological processes. The negative influence of phenols on physiological processes of plants is well known, as they inhibit growth, cellular expansion, membrane permeability, protein synthesis, enzymatic activity, photosynthesis, respiration, water relations and ion uptake (Politycka, 2002). Membrane perturbations are probably a primary action of many phenolic allelochemicals. Our preliminary tests and others results (Djanaguiraman *et al.* 2005) showed that the leachates of *Eucalyptus globules* have more phenol content, and the seedlings treated with leachates accumulated more proline and free amino acids. Therefore, we hypothesised that growth inhibition under *Eucalyptus globules* leachates may be due to oxidative stress caused by phenolic acids. Hence, the present investigation was aimed to elucidate the negative influence of selected phenolics on seed germination, growth, membrane damage, oxidant production and antioxidant enzymes activity of blackgram.

MATERIALS AND METHODS

Plant material and germination experiment

The study was conducted at Coimbatore, South India, during 2003-2004. Blackgram (*Vigna mungo*) var KM2 seeds were cold treated (+4°C) for 3 days to break dormancy and synchronize germination. The phenolic compounds p-coumaric acid, gallic acid and p-hydroxy benzoic acid (Sigma Chemical Company) were dissolved in methanol: water (20:80 V/V). Control was prepared with distilled water and methanol (80:20 V/V). The methanol was then evaporated in a rotary vaporizer and the solution adjusted to a concentration of 10 mM (Reigosa *et al.*, 1999). These stock solutions were diluted with water to 0.5, 1, 2 and 4 mM. The pH of all the phenolic solutions and control were adjusted to 6.0 with NaOH (Blum and Shafer, 1988). Solutions were bioassayed on seeds of blackgram. Twenty five seeds of blackgram was treated with 0.1% mercuric chloride, washed thrice with distilled water and dried on an absorbent to eliminate fungal attack. The seeds were germinated on filter paper soaked in different phenolic compound solutions of different concentrations in a germination chamber with the following germination conditions: light - 9 hours: 28 °C, dark – 15 hours, 20 °C and a relative humidity of 80% and photon flux density of 400 $\mu M\ m^{-2}\ s^{-2}$. The Petri plates of 14.0 cm diameter were supplied with 5 ml of different phenolic compounds on alternate days. The experiment was arranged in a completely randomized design with five replicates. The effects of the phenolic compounds were determined by germination percentage and vigour index by measuring the root and shoot length as this is the most widely used test (Inderjit and Dakshini, 1995).

Chlorophyll and soluble protein contents and nitrate reductase enzyme activity

Germination, vigour index, chlorophyll *a* and *b* contents, soluble protein content and nitrate reductase enzyme activity were recorded at 10 days after sowing (DAS). Chlorophyll pigments were estimated according to Arnon (1949), by extracting in 80% acetone and expressed as mg g^{-1} FW. Soluble protein was quantified by the method proposed by Lowry *et al.* (1951), and expressed as mg g^{-1} FW. Nitrate reductase activity was quantified according to Nicholas *et al.* (1976) and expressed as $\mu M\ NO_2\ g^{-1}\ h^{-1}$ FW.

Hydrogen peroxide and lipid peroxidation levels and lipoxygenase enzyme activity

At 10 DAS lipid peroxidation was determined by the amount of malondialdehyde (MDA) produced by thiobarbituric acid (TBA) reaction at low pH as described by Behra *et al.* (1999). The pink chromogen was measured at 532 and 600 nm for the correction of blank. Lipid peroxidation was expressed as malondialdehyde content in μM g^{-1} DW. The H_2O_2 level was colorimetrically measured as described by Okuda *et al.* (1991). The H_2O_2 was extracted by homogenizing 0.5 g of tissue with 4ml of perchloric acid (200 nM). The homogenate was centrifuged at 12,000×*g* for 10 min. Oxidation of ferrous ions to ferric ions by H_2O_2 in acidic pH was monitored and stable complex of ferric ions with xylenol orange dye at 560 nm was measured. The H_2O_2 level was expressed as nM g^{-1} DW. Lipoxygenase (LOX) was measured spectrophotometrically at 234 nm (Gallego *et al.*, 1996) and expressed as increase in absorbance per mg of protein per minute.

Antioxidant enzymes assay

For assay of superoxide dismutase, catalase and peroxidase enzymes, frozen tissue was homogenized in ice-cold 0.1M Tris–HCl buffer at pH 7.8 containing 1mM EDTA, 1mM dithiotreitol and 5ml of 4% polyvinyl pyrrolidone per gram fresh weight. The homogenate was filtered through a nylon mesh and centrifuged at 20,000x*g* at 4°C. The supernatant was used for measuring antioxidant enzyme activity. Superoxide dismutase (SOD) was determined by nitroblue tetrazolium (NBT) method proposed by Beyer and Fridovich (1987), by measuring the photoreduction of NBT at 560 nm. One unit of SOD activity equaled to the amount required to inhibit photoreduction of NBT by 50%. Catalase (CAT) was estimated according to Teranishi *et al.* (1974) and expressed as μM H_2O_2 reduced min^{-1} mg^{-1} protein. Peroxidase (POX) activity was determined in the homogenates by measuring the increase in absorption at 470 nm due to formation of tetraguaiacol in a reaction mixture that contained enzyme extract, 50 mM potassium-phosphate buffer (pH 7.0), 0.1mM EDTA, 10mM guaiacol and 10mM H_2O_2 (Racusen and Foote, 1965).

Proline and total free amino acids content

Proline was extracted in 3% sulfosalicylic acid, estimated by using acid ninhydrin reagent, measured by the absorbance of the toluene chromophore at 520 nm (Bates *et al.*, 1973), and expressed as μM g^{-1} FW. Total free amino acids were quantified using ninhydrin reagent as per the methodology proposed by Sadasivam and Manickam (1996) and expressed as μM g^{-1} FW.

Statistical analysis

Statistical analysis was performed using SAS programme for PCs. The mean of three independent samples were taken to represent the result of each replicate (n = 15). The data were analysed statistically using a general linear model for analysis of variance. Significance between control and treatment was determined using Duncan's multiple range tests.

RESULTS AND DISCUSSION

Growth and vigour

Exposure of blackgram seeds to various phenolic acid concentrations for 10 days caused a strong reduction in seed germination and vigour index (Table 1). Phenolic acids affected germination percentage more than the vigour index, and at 4 mM concentration the germination percentage was reduced by 27, 36 and 44%, for gallic, p-coumaric and p-hydroxy benzoic acid, respectively, when compared to the control. Seedling growth was limited increases in concentration of phenolic acids. The inhibition of germination by the phenolic compounds may be due to the entry of this compound into the seed which affected the physiological process such as water uptake and synthesis of α-amylase enzyme (Politycka, 2002).

Pigments, protein and nitrate reductase activity

In the present investigation, chlorophyll *a* and *b* contents, soluble protein content and nitrate reductase enzyme activity decreased progressively with increasing phenolic acid concentrations (Table 1). Among the various phenolic acids tested, p-hydroxy benzoic acid exerted the most significant effect when compared to p-coumaric and gallic acid. At 4 mM concentration, seedlings treated with p-hydroxy benzoic acid had 0.62 mg of chlorophyll g^{-1} fresh weight (FW) and 5.34 mg of soluble proteins g^{-1} FW. Whereas, p-coumaric and gallic acid treated plants had 0.88 mg g^{-1} FW and 1.05 mg g^{-1} FW of chlorophyll *a* and 6.73 mg g^{-1} FW and 7.12 mg g^{-1} FW of soluble protein contents, respectively. The chlorophyll *b* content followed a similar trend. The nitrate reductase enzyme activity decreased significantly at 4 mM concentration, reaching values of 0.073, 0.069 and 0.06 μM NO_2g^{-1} h^{-1}, for gallic acid, p-coumaric and p-hydroxy benzoic acid, respectively, when compared to the control plants. The reduction in chlorophyll contents observed in all the phenolic compounds at all concentrations might be due to enhanced degradation of chlorophyll pigments or reduction in their biosynthesis (Tripathi *et al.*, 2000). The higher reduction of chlorophyll *b* than chlorophyll *a* in various phenolic acids suggests that it is more sensitive than chlorophyll *a* or conversion of chlorophyll *b* to chlorophyll *a* is under stress (Djanaguiraman *et al.*, 2003). Hence, the less decrease in chlorophyll *b* content in gallic acid-treated seedlings than those treated with p-coumaric and p-hydroxy benzoic acid indicates that the former is less toxic. Reduction in chlorophyll content may decrease the photosynthetic process, subsequently decreasing all the metabolites *viz.*, total sugars, proteins and amino acids (Singh and Rao, 2003). Hence, the decrease in soluble protein content may be due to reduced chlorophyll content. Perhaps, the phenolic compounds may have decreased the protein biosynthetic process, which in turn soluble protein contents. This was also observed by Singh and Rao (2003) in rice. The reduction in nitrate reductase activity by phenolic compounds may be due to that both phenolic acids and their oxidation products can inhibit the activity of the enzyme due to the formation of insoluble protein–phenolic complexes or by direct inhibition of enzyme through modification of its active site. Moreover, final products of lipid peroxidation, especially aldehydes may react with thiol and amine groups of proteins and thereby decrease the activity of enzyme (Bartosz, 1995). Hence, among the phenolic compounds assayed, p-hydroxy benzoic acid was found to be highly inhibitory to the enzyme activity when compared to p-coumaric and gallic acid.

Hydrogen peroxide, lipoxygenase and TBARS content

The results presented in Table 2 seem to suggest that phenolic acids tested were able to cause membrane damage by increased lipoxygenase enzyme activity, hydrogen peroxide and TBARS contents. Increasing concentrations of phenolic acids from 0.5 to 4 mM progressively increased lipoxygenase activity, hydrogen peroxide and TBARS contents. Among the three phenolic acids tested, p-hydroxy benzoic acids showed its maximum inhibitory effect through enhanced lipoxygenase activity, hydrogen peroxide content and TBARS content. At 4 mM concentration it recorded an increase of 196, 260 and 240%, respectively, over control. However, gallic and p-coumaric acid showed an increase in lipoxygenase activity, hydrogen peroxide and TBARS content at the rate of 144, 153 and 184 and 185, 206 and 211 per cent over control, respectively. Production of reactive oxygen species (ROS) as well as peroxidation of membrane lipids under stress conditions may cause deleterious effects in plants. In the absence of catalyzing metals or oxide-reducing enzymes, H_2O_2 displays a small reactivity and can easily move in the cell, rapidly infiltrating through plasma membranes. The generation of hydrogen peroxide by NADH–dependent peroxidase is stimulated by phenolic acids and Mn^{2+} ion. This complex reaction liberates superoxide radical, which is dismutated to produce hydrogen peroxide. The increase in H_2O_2 content depends on concentration of phenolic acids, with much decrease at higher concentrations. The hydrogen peroxide formed by the phenolic compounds may have many cellular targets, such as proteins and DNA to denature and enhance lipid peroxidation (Bartosz, 1995). From the results of the present study it can be hypothesized that the activation pathway is induced by hydrogen peroxide, leading to lipid peroxidation through enhanced activity of lipoxygenase.

The higher TBARS content at higher concentrations of phenolic acids may be due to participation of lipoxygenase enzyme (Kacperska, 1995). Ethylene responds to a stress by functional destabilization of membranes as it results in leakage of ions (Politycka, 1997). Ethylene synthesis may occur due to spontaneous decomposition of fatty acid peroxides produced in free radical reactions (Yang and Hoffman, 1984) in which lipoxygenase can participate. A similar phenomenon might have happened under higher concentrations of phenolic acids (4 mM), as it reveals higher membrane damage, and the effect of p-hydroxy benzoic acid was stronger than that of p-coumaric and gallic acid.

Antioxidant enzymes

The activity of antioxidant enzymes *viz.*, superoxide dismutase, catalase and peroxidase activity was also enhanced with increasing phenolic acid concentration. At 4 mM concentration p-hydroxy benzoic acid showed a strong increase in SOD, CAT and POX activity (+114, 114 and 155% when compared to control plants, respectively). However, in p-coumaric and gallic acid treatments, the increase was only 99, 102, 134 and 89, 90 and 127 per cent, respectively (Table 2). Under normal conditions the levels of ROS in cellular compartments is determined by the interplay between the multiple ROS producing pathways and scavenging mechanisms which essentially constitutes the basic ROS cycle. Phenolic acids are known to produce ROS namely superoxide radical and hydrogen peroxides during its oxidation to quinine or semiquinone (Datta *et al.*, 1986).

Table 1. Effect of phenolic acids on germination, vigour index, chlorophyll *a* content, chlorophyll *b* content, soluble protein content, nitrate reductase enzyme activity on black gram

Phenolic compounds	Concentration (mM)				
	0.0	0.5	1	2	4
Germination percentage					
Gallic acid	97.5[a]	88.5[b]	80.5[c]	76.3[c]	70.4[d]
p-coumaric acid		84.4[b]	75.2[c]	70.4[c]	62.3[d]
p-hydroxy benzoic acid		79.9[b]	70.3[c]	65.6[c]	53.8[d]
Vigour Index					
Gallic acid	1887[a]	1650[b]	1561[c]	1515[c]	1350[d]
p-coumaric acid		1529[b]	1414[c]	1400[c]	1241[d]
p-hydroxy benzoic acid		1422[b]	1420[b]	1291[c]	1052[d]
Chlorophyll *a* content (mg g^{-1} FW)					
Gallic acid	1.63[a]	1.55[b]	1.48[c]	1.39[d]	1.34[e]
p-coumaric acid		1.53[b]	1.42[c]	1.35[d]	1.25[e]
p-hydroxy benzoic acid		1.44[b]	1.33[c]	1.25[d]	1.18[e]
Chlorophyll *b* content (mg g^{-1} FW)					
Gallic acid	0.987[a]	0.856[b]	0.714[c]	0.592[d]	0.501[e]
p-coumaric acid		0.721[b]	0.581[c]	0.490[d]	0.400[e]
p-hydroxy benzoic acid		0.601[b]	0.431[c]	0.373[c]	0.310[d]
Soluble protein content (mg g^{-1} FW)					
Gallic acid	11.24[a]	10.21[b]	9.43[c]	7.66[d]	7.12[d]
p-coumaric acid		9.25[b]	8.13[c]	6.80[d]	6.73[d]
p-hydroxy benzoic acid		8.13[b]	7.54[c]	6.27[d]	5.34[e]
Nitrate reductase enzyme activity (µM NO_2 g^{-1} h^{-1} FW)					
Gallic acid	0.18[a]	0.158[b]	0.134[c]	0.112[d]	0.073[e]
p-coumaric acid		0.154[b]	0.126[c]	0.099[d]	0.069[e]
p-hydroxy benzoic acid		0.143[b]	0.125[c]	0.088[d]	0.060[e]

Within a row, means followed by a same letter are not significantly different by the Duncan's Multiple range Test at p=0.05.

The combined action of superoxide dismutase and catalase or peroxidase is critical in mitigating the effects of oxidative stress, since the former merely acts on the superoxide anion converting it to another reactive intermediate (H_2O_2) and the latter acts on H_2O_2 converting it to water and oxygen. In the present study, it was seen that catalase and peroxidase actively participated in H_2O_2 reduction in all the plants which received phenolic acid treatment. Superoxide dismutase was also active in scavenging the superoxide produced by phenolic acids. If the superoxide radicals are not scavenged properly it will inhibit most of the enzymes (Behrà *et al.*, 1999). Therefore, enhanced activity of superoxide dismutase at higher concentrations indicates its need. The increase in superoxide dismutase activity at higher concentrations might have increased the peroxidase and catalase activity by providing substrate namely H_2O_2. The key enzyme scavenging H_2O_2 is catalase and it has a high reaction rate but a low affinity for H_2O_2 (Orendi *et al.*, 2001). The enhanced catalase activity under p-hydroxy benzoic acid treatment, than gallic and p-coumaric acids clearly indicates that p-hydroxy benzoic acid exerted a high inhibitory effect than p-coumaric and gallic acid. The maintenance of this enzyme activity at high level prevents an increase in cytosolic H_2O_2 that

can create toxic conditions in the plant cells leading to oxidative stress (Srivalli and Chopra, 2001). Peroxidase was more efficient in destroying H_2O_2 than was catalase under all phenolic acids. The reason for this could be that unlike catalase which is present only in the peroxisome and has low substrate affinities (it requires simultaneous access of two molecules of H_2O_2), whereas, peroxidase is present through out the cell and has higher substrate affinity (Willekens *et al.*, 1995). Among the phenolics bioassayed, p-hydroxy benzoic acid recorded a higher activity than p-coumaric and gallic acid. High levels of H_2O_2 can also accelerate the process like Haber-Weiss reaction, resulting in the formation of hydroxyl radicals that can also cause lipid peroxidation. The increase of peroxidase activity in lower concentration (0.5 mM) over control may be to scavenge low concentrations of H_2O_2 produced as the enzyme has a high affinity to H_2O_2. All the phenolic acids at 0.5 mM concentration had lower H_2O_2 content and lipid peroxidation, which indicates that peroxidase have scavenged the produced H_2O_2 in an efficient manner. However, peroxidative activity of peroxidase in the presence of phenolic acids also cannot be ruled out.

Total free amino acid and proline content

Total free amino acids content was enhanced with increasing phenolic acid concentration showing a strong relationship between these two parameters. However, there was a gradual decrease in proline content from 0.5 mM to 4 mM (Table 2). Among the three phenolic acids tested, maximum accumulation of proline was observed in gallic followed by p-coumaric acid and p-hydroxy benzoic acid treatment. However, an inverse trend was observed for total free amino acid contents. The inhibition of respiratory process by the phenolic acids was documented by Moreland and Novitzky (1985). The TCA cycle might be inhibited because electron transport associated with the oxidation of intermediates of TCA cycle and NADH was inhibited (Abrahim *et al.*, 2003). Glycolysis and TCA cycle produce numerous plant metabolites including amino acids which are not oxidized to CO_2 in the presence of phenolic acids (Moreland and Novitzky, 1985). Seedlings in phenolic acid treatment accumulated more free amino acids than the control seedlings, which indicate that their respiratory pathways have been hampered. During germination, storage proteins disappears and there is rapid formation of protein and accumulation of amino acids and amides will occur and the process of amino acid utilization might be inhibited due to presence of phenolic compounds or degradation of protein into amino acids by enhanced protease activity (Tang *et al.*, 1995). This may be the reason for enhanced accumulation of free amino acids at higher concentration of all phenolic acids.

The increased proline accumulation at lower concentrations (0.5 mM) may be attributed to increased synthesis from glutamate. Besides, in germinating seeds increased proteolysis can also lead to increase in free proline along with other amino acids (Djanaguiraman *et al.*, 2005). Proline protects proteins from denaturation by maintaining the hydration level. As proline is involved in the stabilization of protein it has a role as a protector of enzymes concerned in plant metabolism. It also plays a role in osmoregulation and provides a store of nitrogen and carbon for subsequent utilization during post stress recovery. Hence, the increased content of proline indicates the protective role of it under oxidative stress.

Table 2. **Effect of phenolic acids on hydrogen peroxide content, lipoxygenase enzyme activity and malondialdehyde (TBARS) content, superoxide dismutase enzyme activity, catalase enzyme activity and peroxidase enzyme activity, total free amino acids and proline content on black gram.**

Phenolic compounds	Concentration (mM)				
	0.0	0.5	1	2	4
H_2O_2 content (nM g^{-1} DW)					
Gallic acid	0.15^a	0.21^b	0.26^c	0.32^d	0.38^e
p-coumaric acid		0.29^b	0.35^c	0.40^d	0.46^e
p-hydroxy benzoic acid		0.34^b	0.43^c	0.49^d	0.54^e
Lipoxygenase enzyme activity (Δ OD mg protein^{-1} min^{-1})					
Gallic acid	3.14^a	4.26^b	5.81^c	6.79^c	7.68^d
p-coumaric acid		5.34^b	6.73^c	7.86^c	8.96^d
p-hydroxy benzoic acid		5.97^b	6.75^c	8.33^c	9.34^d
TBARS contents (μM g^{-1} DW)					
Gallic acid	18.5^a	29.7^b	37.6^c	46.3^d	52.7^e
p-coumaric acid		38.0^b	43.2^c	50.8^d	57.6^e
p-hydroxy benzoic acid		45.2^b	53.3^c	58.3^d	63.7^e
Superoxide dismutase enzyme activity (Enzyme Units)					
Gallic acid	1.22^a	1.88^b	1.92^b	2.18^c	2.31^d
p-coumaric acid		2.07^b	2.24^c	2.41^d	2.62^e
p-hydroxy benzoic acid		1.96^b	2.12^c	2.29^d	2.43^e
Catalase enzyme activity (μM H_2O_2 reduced min^{-1} mg^{-1} protein)					
Gallic acid	4.25^a	4.90^b	6.10^c	7.11^d	8.62^e
p-coumaric acid		4.81^b	5.52^c	6.83^d	8.13^e
p-hydroxy benzoic acid		5.40^b	6.62^c	7.43^d	9.12^e
Peroxidase enzyme activity (nM tetra guaiacol min^{-1} mg^{-1} protein)					
Gallic acid	2.95^a	4.87^b	5.85^c	6.27^d	6.71^e
p-coumaric acid		5.18^b	5.88^c	6.36^d	6.92^e
p-hydroxy benzoic acid		5.14^b	6.05^c	6.82^d	7.54^e
Total free amino acid content (μM g^{-1} FW)					
Gallic acid	37.5^a	44.5^b	52.1^c	62.7^d	73.6^e
p-coumaric acid		50.3^b	63.7^c	74.2^d	81.9^e
p-hydroxy benzoic acid		62.1^b	75.4^c	82.5^d	92.2^e
Proline content (μM g^{-1} FW)					
Gallic acid	11.8^a	33.5^b	29.2^c	25.7^d	22.3^e
p-coumaric acid		29.7^b	25.4^c	23.0^d	20.4^e
p-hydroxy benzoic acid		29.1^b	25.2^c	22.0^d	18.7^e

Within a row, means followed by a same letter are not significantly different by the Duncan's Multiple range Test at p=0.05.

CONCLUSIONS

The results of the present experiment clearly demonstrated the negative effects of gallic, p-coumaric and p-hydroxy benzoic acid on germination and seedling vigour of blackgram. The inhibition of germination and vigour index under higher concentrations may

be attributed to increased H_2O_2 and TBARS contents and lipoxygenase activity, coupled with reduced antioxidant enzymes and proline content.

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