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An Investigation on Allelopathic Effect of *Melaleuca leucadendron* L. on Horse Gram Seeds

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A B S T R A C T

Melaleuca leucadendron L. plant was analysed to evaluate the existence of allelopathic effect using fully viable seeds of horse gram (*Dolichos biflorus* L.) as the bioassay material. Different concentrations (1:2 and 1:4) of bark extracts and leachates of *Melaleuca* reduced the percentage of seed germination as well as increased the T_{50} hours for all the different seed pretreatment hours. TTC stainability was significantly reduced in bark extract and bark leachate pretreated seed sample. Amino acids level was rapidly increased in the leachates of seeds pretreated with bark extracts and leachates of the *Melaleuca* plant. Insoluble carbohydrate levels as well as activities of dehydrogenase and peroxidase enzymes were significantly reduced in seed samples pretreated with bark extracts and leachates. Inhibitory action was more prominent in bark extracts than the bark leachates.

***Key words*:** Allelopathy, Amino acids, Bark extracts and leachates, Enzymes, Insoluble carbohydrates, Macromolecules, *Melaleuca leucadendron*, TTC-stainability

Allelopathy refers to biochemical interaction among plants. It is the effect of one plant upon another occurring under natural conditions and exerted by chemical means other than nutritional ones. Allelopathy is different from competition and implies that the effect depends on a chemical constituent escaping into the environment. Since 1960, there has been a spurt in publications dealing with this phenomenon [1-7]. It is an expression of the ecological phenomena which are normal constituents of the environment of the terrestrial plants [8-9].

There are some common indices for assessing allelopathic action of plants or plant parts. These include among others seed germination behaviour (percentage and T_{50} of seed germination), field emergence capacity of seeds, seedling growth (root length, shoot length, leaf area) and metabolism. Moreover, the allelopathic potential of a plant particularly of exotic one, may turn it aggressive and invasive which in course of time can discourage and displace other co-existing biodiversity thriving the same habitat [10]. With this background, the present investigation is an attempt to evaluate the allelopathic potential of *Melaleuca leucadendron*, an exotic tree of India growing abundantly in the coastal belt of Digha in West Bengal. For precise screening of allelopathic action of this plant some select physiological and biochemical parameters were analysed using horse gram seed as bioassay material.

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MATERIALS AND METHODS

Fresh and mature 150 g bark of *Melaleuca leucadendron* L. (Family – Myrtaceae), collected from the coastal belt of Digha, Purba Medinipur, West Bengal were thoroughly homogenized using 150 ml distilled water. The homogenates were strained using a fine cloth and then centrifuged at 5000 g for 15 minutes. The supernatant was then made up to 300 ml using distilled water and this was considered as bark extract of 1:2 (w/v) concentration and from this solution 1:4 concentration grade was made by using distilled water. Another lot of fresh and mature 150 g of bark sample of this plant species was immersed in 200 ml distilled water in 500 ml beaker for 72 hours and the leachate was then decanted in a separate beaker (500 ml). The total volume of the leachate was then made up to 300 ml using distilled water and this was taken as the bark leachate of 1:2 (w/v) concentration and from this solution 1:4 concentration grade was made by using the distilled water. These two concentration grades each of extract and leachate were used for allelopathic analysis.

Fully viable 200 g horse gram (*Dolichos biflorus* L.) seeds were surface sterilized with 0.1% $HgCl_2$ solution for 90 seconds. The seeds were equally divided into five lots, and then separately presoaked in the bark extracts and leachates in different concentrations (1:2 and 1:4) or distilled water for 4, 8, 12 and 16 hours. Thus, the presoaked seed lots were thoroughly sun dried till the original moisture level was achieved. Thereafter, the seeds were kept in room temperature (30 ± 2°C) and thus allowed the seeds for experiments. Data on seed germination behaviour (percentage and T_{50} of seed germination), TTC-stainability, leaching of amino acid and

insoluble carbohydrate levels as well as peroxidase and dehydrogenase activities of seeds were analysed after 4, 8, 12 and 16 hours of seed pretreatment.

To analyse percentage germination, three groups of 100 seeds i.e., 300 seeds of each treatment were transferred to separate Petridishes containing filter paper moistened with 10 ml distilled water. Germination data were recorded up to 120 h of seed soaking following the International Rules for Seed Testing [11]. The time for 50% germination (T_{50}) was determined following the method described by Coolbear *et al*. [12].

For analysing TTC stainability three 100-seed samples of dehusked horse gram seeds of each treatment hours (4, 8, 12 and 16 hours) were allowed to imbibe in 0.5% TTC (2, 3, 5-triphenyl tetrazolium chloride) solution (w/v) in Petri dishes for 16 hours in dark condition. Percentage TTC stainability were recorded taking samples from the embryonal axes of the horse gram seeds.

Amino acids level was analysed from the extract and leachate -soaked seeds obtained after immersing 1 g seeds in 10 ml deionized distilled water for 16 hours as per the method of Moor and Stein [13]. Insoluble carbohydrate level was analysed from the seed kernels following the method of McCready *et al*. [14].

For analysing dehydrogenase activity, the TTC (2, 3, 5-triphenyl tetrazolium chloride)-stained (formazan formed) embryonal axes of the seeds of each treatment was extracted with 5 ml 2-methoxyethanol, and O.D. values of the solutions were recorded at 520 nm. This method was adopted after Rudrapal and Basu [15] with slight modifications.

Extraction and estimation of the enzyme peroxidase was done as per the method described by Kar and Mishra (16). For the assay of this enzyme the blank was taken as zero-time control. The activity of this enzyme was expressed as $[(\Delta A \times Tv) / (t \times v)]$, where ΔA is the absorbance of the sample after incubation minus the absorbance of the zero-time control, Tv is the total volume of the filtrate, t is the time (minutes) of incubation with the substrate and v is the volume of the filtrate taken for incubation [17].

All the data were statistically analysed at the treatment and replication levels; the least significant difference (LSD) was calculated at 95% confidence limits [18].

RESULTS AND DISCUSSION

Effect on percentage germination

Data revealed that percentage seed germination gradually declined with the duration of seed pretreatment periods of each treatment. Percentage seed germination was inhibited by the bark extracts and leachates of *Melaleuca* plant in horse gram seeds. This inhibitory effect was found to be drastic in seeds which experienced pretreatments for 16 hours and bark extracts exerted stronger inhibition than bark leachates pretreatment (Table 1).

Table 1 Effect of seed pretreatment of different hours (4, 8, 12 and 16 hours) with bark extracts and leachates of *Melaleuca* on percentage germination of horse gram seeds

Treatments	Germination (%)			
	Seed pretreatment hours after intervals			
	4 hours	8 hours	12 hours	16 hours
Control	100	100	100	100
Bark extract (1:2)	96	89	83	80
Bark extract (1:4)	97	91	86	84
Bark leachate (1:2)	97	90	82	83
Bark leachate (1:4)	99	96	88	86
LSD (*P*=0.05)	NS	5.11	4.89	4.33

Table 2 Effect of seed pretreatment of different hours (4, 8, 12 and 16 hours) with bark extracts and leachates of *Melaleuca* on time (h) to 50% germination (T_{50}) of horse gram seeds

Treatments	T_{50} hours			
	Seed pretreatment hours after intervals			
	4 hours	8 hours	12 hours	16 hours
Control	24.2	24.3	24.5	24.4
Bark extract (1:2)	28.4	29.6	32.5	36.1
Bark extract (1:4)	26.4	26.9	28.4	31.9
Bark leachate (1:2)	25.3	25.9	29.8	32.4
Bark leachate (1:4)	24.1	25.6	27.7	31.3
LSD (*P*=0.05)	1.13	1.24	1.65	2.03

Effect on T_{50} values of germination

Time required for 50% seed germination was found to be significantly high in the bark extract and bark leachate-pretreated samples. However, all the cases 50% germination was achieved when data were recorded upto 16 hours of seed pretreatment (Table 2).

Effect on TTC stainability

TTC stainability was not affected by the bark extracts and bark leachates of *Melaleuca* except 16 hours pretreated seeds (Table 3).

Effect on leaching of amino acids

Various hours of seed pretreatment enhanced leaching of amino acids in horse gram seeds and the extent of leaching was found to be strictly pretreatment period dependent. bark extract and leachate pretreatment significantly induced rapid increase of amino acid levels (Table 4).

Effect on insoluble carbohydrate levels in seed kernels

Seed pretreatments reduced *insoluble carbohydrate* levels in horse gram seed and magnitude of reduction was found to be significantly high in seed samples pretreated with bark extracts than bark leachates of *Melaleuca* plant. Again, the inhibitory effects of the bark extracts pretreatment were found to be high in seed lots which received 16 hours of pretreated seed (Table 5).

Table 3 Effect of seed pretreatment of different hours (4, 8, 12 and 16 hours) with bark extracts and leachates of *Melaleuca* on percentage TTC-stained of horse gram seeds

Treatments	TTC-stainability (%)			
	Seed pretreatment hours after intervals			
	4 hours	8 hours	12 hours	16 hours
Control	100	100	100	100
Bark extract (1:2)	98	96	95	93
Bark extract (1:4)	99	97	95	94
Bark leachate (1:2)	100	97	95	95
Bark leachate (1:4)	99	97	95	95
LSD (*P*=0.05)	NS	NS	NS	0.78

Table 4 Effect of seed pretreatment of different hours (4, 8, 12 and 16 hours) with bark extracts and leachates of *Melaleuca* on leaching of amino acids from horse gram seeds

Treatments	Amino acids (mg/g/10 ml)			
	Seed pretreatment hours after intervals			
	4 hours	8 hours	12 hours	16 hours
Control	1.52	1.65	2.21	2.88
Bark extract (1:2)	1.71	2.49	3.65	4.20
Bark extract (1:4)	1.65	2.10	2.86	3.47
Bark leachate (1:2)	1.70	1.86	2.50	3.30
Bark leachate (1:4)	1.55	1.80	2.41	3.19
LSD (*P*=0.05)	NS	0.18	0.23	0.29

Table 5 Effect of seed pretreatment of different hours (4, 8, 12 and 16 hours) with bark extracts and leachates of *Melaleuca* on insoluble carbohydrates level in kernels of horse gram seeds

Treatments	Insoluble carbohydrates (mg/g/fresh weight)			
	Seed pretreatment hours after intervals			
	4 hours	8 hours	12 hours	16 hours
Control	482.2	474.4	466.5	451.9
Bark extract (1:2)	478.6	426.1	388.7	367.7
Bark extract (1:4)	480.3	435.4	403.8	397.1
Bark leachate (1:2)	480.2	457.6	440.5	401.5
Bark leachate (1:4)	481.8	464.4	455.4	412.3
LSD (*P*=0.05)	NS	21.1	16.6	11.7

Each bar is mean value of 3 replicates and the vertical lines on the bar represent the standard errors of the mean

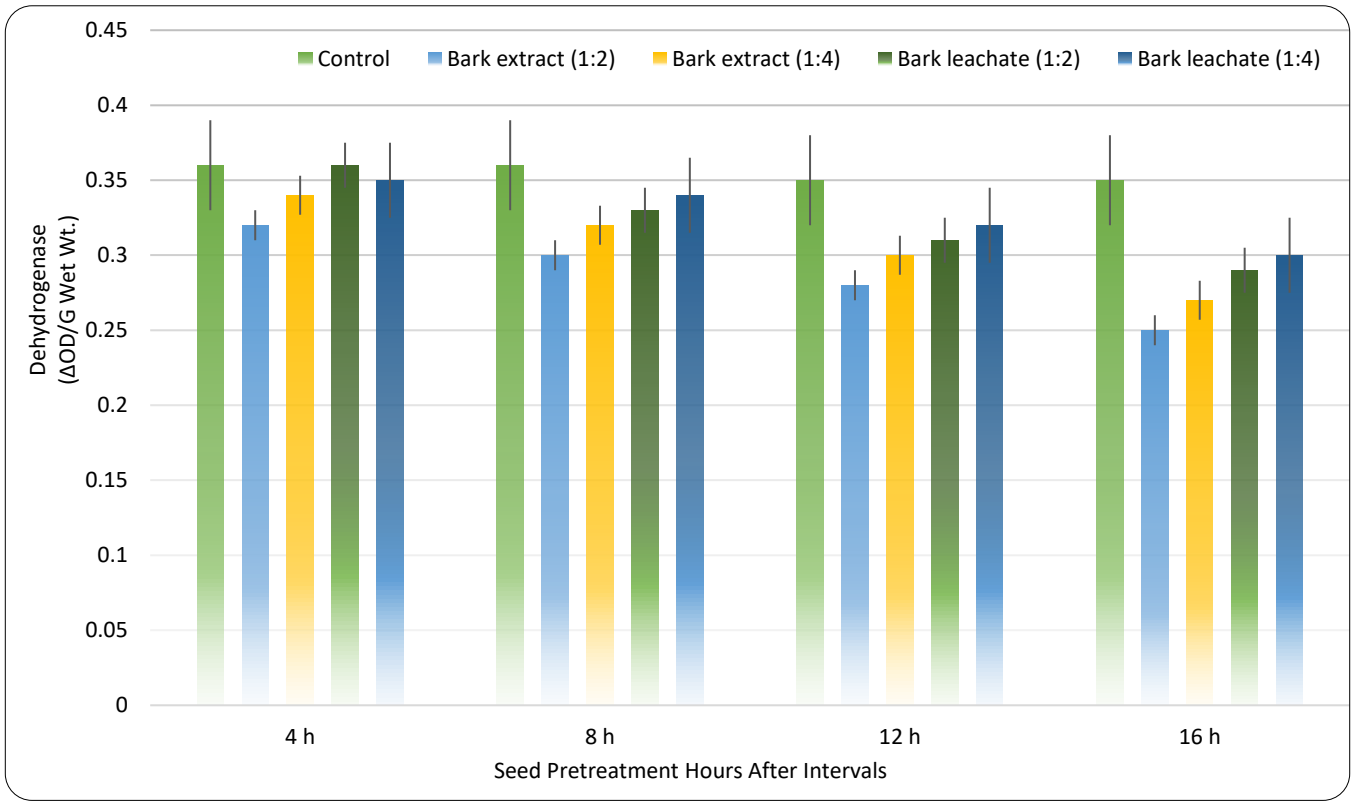

Fig 2 Effect of seed pretreatment of different hours (4, 8, 12 and 16 hours) with bark extracts and leachates of *Melaleuca* on the activity of peroxidase enzyme in the kernels of horse gram seeds

Effect on dehydrogenase activity in seed kernels

Seed pretreatment period induced loss of dehydrogenase activity was gradually increased by seed pretreatment with bark extracts and bark leachates in the seed sample. Here also, the inhibitory action was best exerted by bark extract pretreatment and the magnitude of inhibition was more significant in 16 hours pretreated seed sample (Fig 1).

Effect on peroxidase enzyme activity in seed kernels

As regards the changes of peroxidase enzyme under different pretreatment periods, activity of the enzyme declined progressively with the advancement of pretreatment period duration. The pretreating bark extracts and leachates significantly increased the pretreatment periods induced loss of peroxidase activity (Fig 2).

Each bar is mean value of 3 replicates and the vertical lines on the bar represent the standard errors of the mean

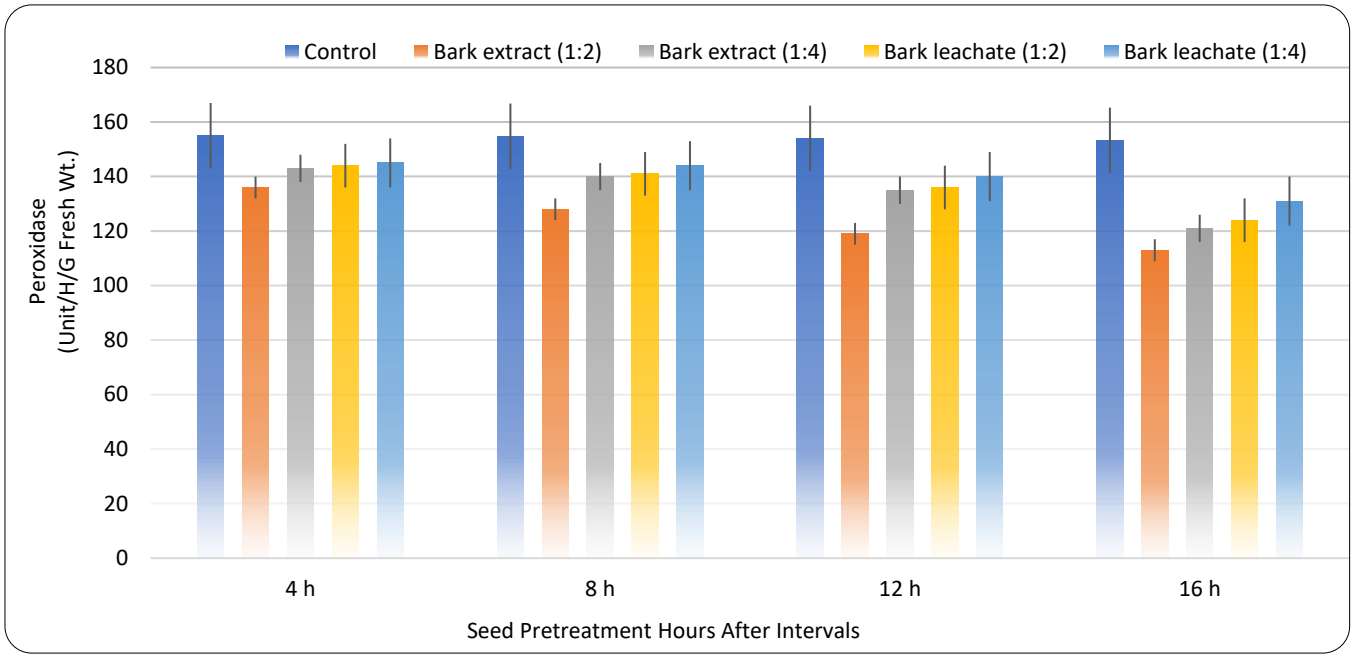

Fig 2 Effect of seed pretreatment of different hours (4, 8, 12 and 16 hours) with bark extracts and leachates of *Melaleuca* on the activity of peroxidase enzyme in the kernels of horse gram seeds

The present study shows that different hours of seed pretreatment of horse gram with different concentrations (1:2 and 1:4) of bark extracts and leachates of *Melaleuca* reduced percentage germination (Table 1), increase T_{50} hours (Table 2) and reduced TTC stainability of seeds (Table 3), increased leaching of amino acids (Table 4), decreased insoluble carbohydrate levels (Table 5) as well as dehydrogenase (Fig 1) and peroxidase (Fig 2) activities.

Reduced seed germinability is the important effect of allelopathic action of plants and such action is chiefly exerted by a number of inhibitors of diverse chemical nature [4], [19], [24-25]. In this investigation different concentrations (1:2 and 1:4) of bark extracts and leachates-induced inhibition of percentage and T_{50} hours of seed germination is clear indicative of the allelopathic action of the test material. Relatively high allelopathic potential of bark extract and leachate was recorded from its stronger germination inhibition capacity, significant increase T_{50} hours as well as significant reduction of TTC stainability of the seeds. Allelopathic action of *Melaleuca* plant can also be substantiated from the profuse leakage of amino acids which is indirect indication of the damage of seed membrane. Membrane is the most important site of a seed which appears to be affected first by treatment with plant extracts having strong allelopathic action [5-6]. The results of the study is thus in conformity with some reported observations [4], [6-7], [19-23].

Allelopathic potential of *Melaleuca* plant can further be corroborated from the present data on the bark extracts and leachates-induced reduction of insoluble carbohydrates level as well as activities of dehydrogenase and peroxidase enzymes. Various inhibitors present in plants having allelopathic property reduce the overall metabolism of plants or plant parts, and particularly anabolic activities are reported to be strongly impaired [4-6]. Results, therefore, point out that bark extracts and bark leachates of *Melaleuca* plant possess some chemicals (essential oil, cajeputol which is identical with eucalyptol) which efficiently render allelopathic action on the bioassay material (horse gram) of this study.

CONCLUSION

Thus, a conclusion can be made from this investigation using a number of physiological and biochemical indices that different concentrations (1:2 and 1:4) of bark extract and bark leachate of *Melaleuca* exert strong allelopathic effect on the test material. Longer periods of seed pretreatment (16 hours) experiment strengthen this effect. From the overall observations, bark extract seems to be more effective than that of leachate with respect to exhibiting allelopathic action on the bioassay material of this experiment.

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