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Allelopathic inhibition of teak leaf extract: A potential pre-emergent herbicide

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ABSTRACT

The effect of teak leaf extracts was tested on germination of rice and two weeds: junglerice and sedge. The methanol extract of deciduous leaves exhibited sustained inhibitory action (GI ≈ 56-61%) on junglerice whereas water extract inhibited sedge germination by 25-45%. No extract exhibited significant inhibition on rice. The activity of methanol extract on junglerice was enhanced by 1.7-2.1 times by column chromatography. The chloroform fraction was the most effective one exhibiting 100% inhibition on junglerice. Out of 5-11 compounds present in methanol extract and its column fractions, four phenolic acids (viz. salicylic acid, p-hydroxy benzoic acid, chlorogenic acid and tannic acid) were identified and quantified by HPTLC. Multiple Regression followed by Principal Component Analysis indicated the dependency of observed activity on salicylic acid content in various combinations with benzoic acid, tannic acid and chlorogenic acid in the plant. Further exploration of the methanol extract of the deciduous leaves was suggested for development of a potential pre-emergent herbicide.

Nomenclature: Junglerice (Echinochloa colona (L.)); sedge (Cyperus difformis L.); rice (Oryza sativa L) Teak (Tectona grandis L.)

Key words: Botanical herbicide, germination inhibition, phenolic acids, HPTLC.

Allelopathy refers to 'any process involving secondary metabolites produced by plants, algae, bacteria and fungi that influence the growth and development of agricultural and biological system' which can play an important role in crop productivity, conservation of genetic diversity, and maintenance of ecosystems stability (Anaya 1999; Khalid *et al.* 2002). Allelochemicals from plants are released into the environment by root exudates, leaching from aboveground parts and volatilization and/or by decomposition of plant material (Rice 1984). Various crop and weed species with allelopathic activity are known (Batish *et al.* 2006; Batish *et al.* 2007; Inderjit *et al.* 1999; Weston 1996). Putnam and Duke (1974) first explored the possibility of utilizing allelopathic crops to inhibit weed growth in agricultural site. The allelopathic properties of plants can be exploited successfully as a tool for pathogen and weed reduction (Xuan *et al.* 2005). The plants like saururaceae (*Houttuynia cordata* Thunb), dwarf lilyturf (*Ophiopogon japonicus* K.) have been reported to control various weed species including *Echinochloa, Cyperus, Monocharia*, etc. in rice field (Lin *et al.* 2006). Plant-derived natural products through allelopathic competitiveness inhibit the growth of neighbouring plants (Field *et al.* 2006). In earlier studies, the specific compounds were seldom identified as allelopathic agents (Duke *et al.* 2001) due to lack of definitive tests (Baldwin *et al.* 2003). However, a number of experiments have revealed phenolic acids as the major allelochemicals (Batish *et al.* 2006; Lin *et al.* 2004) responsible for inhibitory effect on the germination of other plants (Chave et al. 1997). The most significant phytotoxic compounds isolated from the aqueous extracts of *Acacia confusa* were identified as phenolic acids (Chou *et al.* 1998). Weed control mediated by allelopathy either as natural herbicide or through the release of allelopathic compounds from a living cultivar or from plant residues is assumed to be biodegradable and less pollutant than traditional herbicides (Nilsson *et el.* 1993). Numerous growth inhibitors identified from allelopathic plants may be a useful source for the future development of bio-herbicides and pesticides (Xuan *et el.* 2005).

Teak, a large deciduous forest tree is native to the south and south-east Asia. In India it is mostly confined to central and southern regions propagated artificially in the Indo-Gangetic plains and the foothills from Bengal in the east to Haryana and Punjab in the north. Under ex-situ condition teak leaf litter decomposed rapidly as compared with eucalyptus litter. However, no significant addition of organic carbon to soil from decomposing litters was evident (Sankaran 1993). An extract from teak bark inhibited *Listeria monocytogenes* and methicillin resistant *Staphylococcus aureus* (Neamatallah *et al.* 2005) due to the presence of 5-hydroxy-1,4- naphthalenedione (Juglone). The extract of the root heart wood of teak exhibited cytotoxic effect due to lapachol and 5-hydroxylapachol (Khan and Mlungwana 1999). It is reported to possess allelopathic properties in its root, shoot and leaf exudates. The teak leaf extracts exhibited inhibitory effects on germination and/or on seedling growth of *Casuarina equisetifolia* (Balasubramanian *et al.* 1996), *Oryza sativa*, *Vigna unguiculata* (Jadhav *et al.* 1994; Mandal *et al.* 1998) and sorghum (Channal *et al.* 2002). The inhibitory effect on the germination and growth of wheat

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seedlings increased with an increase in leachate concentration of teak (Patil *et al.* 2003). Some of the phenolic acids viz., salicylic acid, *p*-hydroxy benzoic acid, chlorogenic acid, tannic acid, caffeic acid, vanillic acid have been reported to occur in teak as inhibitory or stimulatory bioactive allelochemicals (Mandal *et al.* 1998; Tripathi *et al.* 1999).

To explore further, the teak leaf extracts were tested on germination of rice and two weed species. Attempts have also been made to purify the active extracts along with the identification and quantification of active constituents with the objective to have a potential pre-emergent bio-herbicide for weed control in rice.

MATERIALS AND METHODS

Collection of plant materials

Both green (TG) and the deciduous (TD) leaves of teak were collected separately from the Central Research Farm, Bidhan Chandra Krishi Viswavidyalaya (BCKV), Gayeshpur, Dist-Nadia, West Bengal. The leaves were air dried and powdered using a grinder. The powdered materials were extracted to evaluate the allelopathic activity on the germination of rice and weed seeds. Seeds of two weed species viz. junglerice (F: Poaceae) and sedge (F: Cyperaceae) commonly growing in rice fields were collected from the Weed Science Laboratory, Department of Agronomy, BCKV, Mohanpur, Dist-Nadia, West Bengal. Processed rice seeds (variety-IET 4094) were collected from the Gontra Samobaya Samiti, Gontra, Dist. Nadia, West Bengal. All the collected seeds were sun dried time to time and stored in desiccator using $CaCl_2$ as hygroscopic agent.

Extraction of teak leaves

The powdered teak leaves (150 g) were extracted in a soxhlet apparatus using four solvents viz. hexane (Hex), chloroform (Chl), methanol (MeOH) and water (W) separately for 10 hours. The solvent extracts were evaporated at 45°C using a rotary vacuum evaporator and water extract was evaporated over water bath using porcelain basin. The residual extracts were dissolved in respective solvents to prepare a stock solution (10%, w/v w.r.t dry powder weight) and were diluted to 1% (w/v) for herbicidal assay.

Procurement of phenolic acids

The following phenolic acids were procured: *p*-Hydroxy benzoic acid, BA (purity 98.0%, Loba Cheme, India); Salicylic acid, SA (purity 98.0%, E. Merck, India); Tannic acid, TA (purity 99.0%, Rankem, India); Vanillic acid, VA (purity 98.5%, Acros Organics, USA); Chlorogenic acid, ChA (purity 99.0%, Acros Organics, USA); Caffeic acid, CfA (purity 98.0%, Koch Light Laboratories Limited, UK). Standard solutions (1000 $\mu g\ ml^{-1}$) of the phenolic acids were prepared in MeOH and diluted suitably for analysis.

Pre-emergent herbicidal bioassay

The leaf extracts and the phenolic acids were tested on germination of rice and two weed species. Germination test was carried out by plate technique (Dongre et al. 2004). Filter papers (Whatman No.1) were cut to fit into the Petriplates (diameter: 9 cm) to act as the media for testing the seed germination. A total number of 25 seeds for rice and junglerice and 50 seeds for sedge were evenly distributed on each petriplate. A measured volume (1 ml) of the extracts (1%, w/v) and solution of phenolic acids (0.01 and 0.1%) were added uniformly to each petriplate to form a treatment. The solvents (viz. hexane, chloroform, methanol and water) were also applied as the respective control set. The petriplates were incubated in growth chamber at 28 ± 2 °C and relative humidity 80% in artificial light for 20 days. The moist condition of filter papers was maintained by periodic addition of measured amount of distilled water. Each treated and untreated plates (with respective solvent control sets) were replicated thrice. Observations on the number of seeds germinated were recorded up to 20 days after sowing (DAS) as shown in Figure 1-4. However, time required to obtain the maximum seed germination was ascertained when radicle and shoot were longer than 5 mm for weeds (20 DAS) and 15 mm for rice (5 DAS).

Germination Inhibition (GI)

The pre-emergence herbicidal activity of a treatment (extracts or pure compounds) was evaluated in terms of % Germination Inhibition (GI) using the following formula (Giaveno *et al.*, 2007):

$$\% \text{ Germination Inhibition (GI)} = [(X - Y)/ X] \times 100$$

where, X = Maximum number of seed germinated in control set

Y = Maximum number of seed germinated in treated set

Purification of extracts

The active plant extract (TDMeOH) was fractionated by column chromatography on silica gel (BDH 60-120 mesh) using various eluting solvents with increasing polarity and monitored by thin layer chromatography (TLC). Based on the TLC behavior, five fractions of the column elute F1, F2, F3, F4 and F5 using Hexane, 20% Benzene in Hexane, Benzene, Chloroform and Methanol) were prepared (1% w/v) for further herbicidal bioassay.

The most active column fraction F4 was further subjected to column chromatography to produce the re-chromatographic fractions designated as P1 using Hexane:Benzene (3:7), P2 [Benzene:Chloroform (1:1)], P3 [Chloroform: Methanol (9:1)] and P4 [Methanol].

The water extract of *Teak* deciduous leaf (TDW), however, was subjected to liquid-liquid partitioning in a separatory funnel (1L) using solvents of increasing polarity viz. hexane, benzene, chloroform and ethyl acetate in succession.

Partitioning was done thrice with the solvents (200 + 100 + 100 ml) in each case and the combined fraction was evaporated in a rotary vacuum evaporator. The partitioning fractions (1%, w/v) were subjected to herbicidal bioassay.

Estimation of phenolic acids by HPTLC

The analytical method for identification and estimation of the phenolic acids was standardized by High Performance Thin Layer Chromatography, HPTLC. The method has some unique advantages over other methods. Sample treatment is simple because of single use of the layer and simultaneous chromatography of samples and standards under identical conditions on the same layer leads to results with accuracy and precision. All solvents used for this were HPLC grade (E-Merck, India) and HPTLC aluminium plates pre-coated with silica gel ($60GF_{254}$) 20x10 cm, layer thickness 0.2 mm was obtained from Camag (Multenz, Switzerland).

Chromatographic procedure

Methanolic solutions of standard compounds of known concentrations were applied to the plates as 6 mm wide bands by Camag 100 µl syringe (Hamilton, Bonaduz, Switzerland) positioned 15 mm above the bottom and 20 mm from the side of the plate, using an automated Camag applicator (Linomat 5) with nitrogen flow providing a delivery speed @ 150 nL/sec). The space between two bands was 11 cm. The application parameters were identical for all the analyses performed.

Development of the HPTLC plates in mobile phase

HPTLC plates after application were developed in a Camag twin trough glass tank (20x10 cm) pre-saturated with the mobile phase. The TLC runs were performed under laboratory conditions of 25 ± 2° C and 65% relative humidity. After development (about 8 cm run) the plates were removed and dried with a hand drier and visualized in Camag UV chamber.

Detection and quantification of phenolic acids

The phenolic acids were quantified using a Camag TLC scanner Model 3 equipped with Camag Wincats software. Scanning was conducted under absorption-reflection scan mode at wavelengths (λ_{max}) 250 and 300 nm, slit width: 5×0.45 mm with scanning speed 5 mms^{-1}. Stock solutions (1-20 µl) of *p*-Hydroxy benzoic acid, Salicylic acid, Tannic acid, Vanillic acid, Chlorogenic acid, Caffeic acid (1000 µg ml^{-1}) were analysed by HPTLC as described above. The Wincats software controlling the scanner produces a calibration graph relating the concentration of standard and scan area and different amounts of phenolic acids present in the extract were automatically interpolated from the calibration curve.

Preparation of mobile phases

Different compositions of the mobile phase for HPTLC analysis of phenolic acids were tested in order to obtain high resolution, symmetrical and reproducible peaks. The desired mobile phase as well as R_f and the wavelength for higher sensitivity of the peak for the phenolic acids were as follows: Ethyl acetate: MeOH: Formic acid :: 80:19:1 for p-Hydroxy benzoic acid and Salicylic acid; Isopropanol:Ethyl acetate:Water :: 70:20:10 for Tannic acid (λ_{max} : 250 nm for all) with the corresponding R_f values at 0.45, 0.55 and 0.37. Chloroform:Ethyl acetate:Formic acid :: 5:4:1 for Vanillic acid and Chlorogenic acid; and Ethyl acetate:Formic acid:Acetic acid:Water :: 200:11:11:27 for Caffeic acid (λ_{max} : 300 nm for all) with the corresponding R_f values at 0.37, 0.27 and 0.29. The TDMeOH extract and its fractions were also analysed by HPTLC to encounter the maximum number of constituents using various solvent mixtures as follows: Chloroform : MeOH :: 9:1 for TDMeOH and F5; Benzene:Chloroform :: 1:1 for F1 and F2; Benzene:Ethylacetate :: 1:1 for F3 and F4. The crude extracts and various purified fractions of teak were tested for the selected phenolic acids and quantified in comparison to the standard phenolic acids using the mobile phases as described above. Solutions of sample and standard phenolic acid of known concentration were applied to HPTLC plates and analysed following the method as mentioned above. The identification of phenolic acid in the extract and fractions was confirmed by super-imposable UV spectra of sample and standard within the same R_f window. The analysis was repeated thrice for quantitative determination of phenolic acid content in teak leaf extracts.

Statistical Analysis

The % GI values obtained for extracts and phenolic acids were subjected to non-parametric analysis of variance technique *i.e.* Kruskal-Wallis test (χ2-asymptotically) for significance. The data on activity of the extracts and the phenolic acid content were further subjected to multiple regression analysis using backward elimination and full model techniques followed by principal component analysis.

RESULTS AND DISCUSSION

Effect of crude leaf extracts on germination

There was no significant effect observed on germination of rice by the extracts of green or deciduous leaves of teak. The observed inhibitory activity of green leaf extracts on the germination of two weeds are presented in Fig. 1-2. The maximum GI (15-26%) was recorded in water extract (TGW) followed by chloroform extract (TGChl, GI = 17-18%) on junglerice (Fig. 1). In contrast, a significant stimulation (GI = - 58.04%) was observed on sedge germination (Fig. 2) by hexane extract (TGHex). The stimulatory effect of hexane extract was reduced in case of junglerice with time and about 10-12% inhibition was observed on 15-20 DAS.The methanol extract (TDMeOH) of deciduous leaf exhibited significant inhibitory action (55.6-60.9%) on junglerice germination over 5-20 days of observation (Fig. 3). Besides, the hexane extract (TDHex) inhibited the germination of junglerice by about 35% followed by chloroform extract (TDChl) by 16% on 20 DAS.

Table 1: Phenolic acid profile and herbicidal activity of methanol extract of Teak (TDMeOH)

Treatment type	Treatment code	% inhibition on 20 DAS	Phenolic acid content (µg/ml)			
			Salicylic	Benzoic	Tannic	Chlorogenic
Phenolic acids (T_1 = 100 µg/ml)	SA T_1	22.98	100	0	0	
	BA T_1	12.17	0	100	0	0
	TA T_1	27.04	0	0	100	0
	ChA T_1	8.12	0	0	0	100
Phenolic acids (T_2 = 1000 µg/ml)	SA T_2	63.52	1000	0	0	0
	BA T_2	24.33	0	1000	0	0
	TA T_2	28.39	0	0	1000	0
	ChA T_2	16.23	0	0	0	1000
Extract	TDMeOH	60.86	135.45	1198.70	862.79	80.82
Fractions of TDMeOH	F1	0	0	0	0	0
	F2	95.56	112.78	203.90	125.70	0
	F3	77.78	174.67	153.10	137.06	0
	F4	100.00	995.21	1588.80	593.00	40.19
	F5	95.56	157.43	1368.54	1149.90	64.38
Fractions of F4	P1	5.55	0	0	426.99	4.42
	P2	65.28	0	8.40	316.09	10.07
	P3	16.67	0	19.82	691.89	1.41
	P4	25.00	0	0	301.13	2.08

However, all the extracts initially exhibited stimulatory action on germination of sedge (Fig. 4) which reduced with time. The stimulatory effect of MeOH extract (GI = - 209.8%) was prominent followed by Hex (GI = - 120.09%) extract. The water extract (TDW), on the other hand, initially induced a stimulatory effect on germination of sedge (GI = - 60%) but with time inhibited the germination ranging from 25-45% (Fig. 4). A comparison of the activities of the extracts revealed that the TDMeOH exhibited the highest inhibitory activity on germination of junglerice followed by TDHex, TGW and TDChl. On the other hand, inhibition in sedge germination (Fig. 4) was the highest in TDW. Based on these observations the TDMeOH and TDW extracts were selected for further investigation.

Activity of the fractions of TDMeOH and TDW extracts

The activity of the fractions on 20 DAS are shown in table 1. Fractionation of the TDMeOH extract by column chromatography remarkably enhanced the herbicidal potency in the fractions F2 – F5 (about 1.3-1.6 times) on junglerice, while the activity reduced drastically in the hexane fraction (F1). The chloroform fraction (F4) produced 100% inhibition followed by F5 and F2 (> 95%). However, re-chromatography of the most active fraction F4 (to produce P1– P4) was not effective to further enhance the inhibitory activity which might be due to disintegration of the possible herbicidal allelochemicals in the re-chromatographic fractions. Moreover, the fraction P2 exhibited a considerable inhibition of rice (43-68%), about 5 times that observed in F4 (9-12%, data not shown). Based on these findings, the chloroform fraction of methanol extract of teak deciduous leaves appeared as a potential pre-emergent allelopathic herbicide for controlling the junglerice in rice field. The activity of the TDW extract (Fig. 4) on sedge, however, could not be improved by liquid-liquid partitioning (Table 1). Therefore, liquid-liquid partitioning method was not effective to enrich the herbicidal activity of TDW extract.

Herbicidal activity of phenolic acids

Results of herbicidal activity of the selected phenolic acids (0.01% = T_1 and 0.1% = T_2) on germination of junglerice on 20 DAS are presented in table 1. None of the compound exhibited significant effect on rice germination even at higher (0.1%) concentration. The maximum inhibitory activity was exhibited by salicylic acid (27%) followed by tannic acid (23%) at 0.01%. The activity of salicylic acid remarkably increased (by about three times) with increase in concentration level.

HPTLC finger print and estimation of phenolic acids

HPTLC analysis of the active extract (TDMeOH) and its five column fractions (F1-F5)

produced the finger print of at least 5-11 chemical constituents. Out of six phenolic acids tested, the presence of four compounds (viz. salicylic acid, *p*-hydroxy benzoic acid, tannic acid and chlorogenic acid) was identified in the extracts and its fractions (Table 1). The concentration of the identified phenolic acids was quantified by comparing the area of respective chromatographic peaks of the sample with the corresponding standards in the range of 60.9-1198.7 μg ml^{-1} in the active extract (TDMeOH).

Fig. 1: Germination inhibition of teak green leaf extracts on junglerice

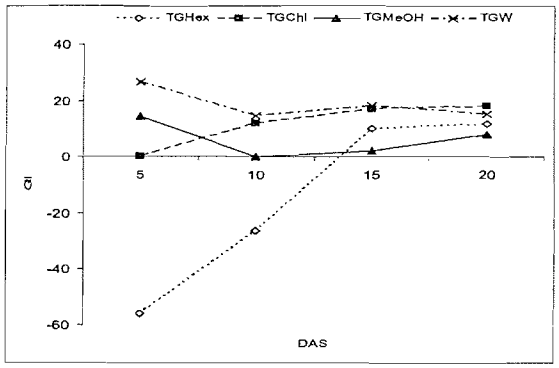


Fig. 2: Germination inhibition of teak green leaf extracts on sedge

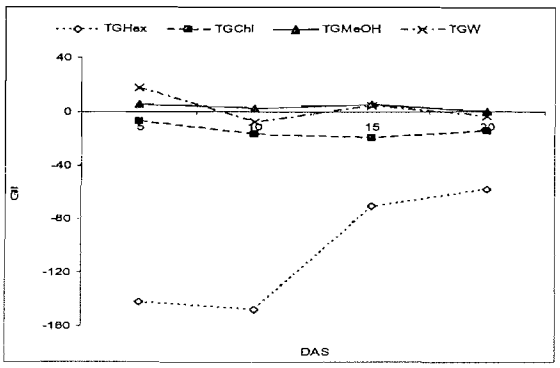


Fig. 3: Germination inhibition of teak deciduous leaf extracts on junglerice

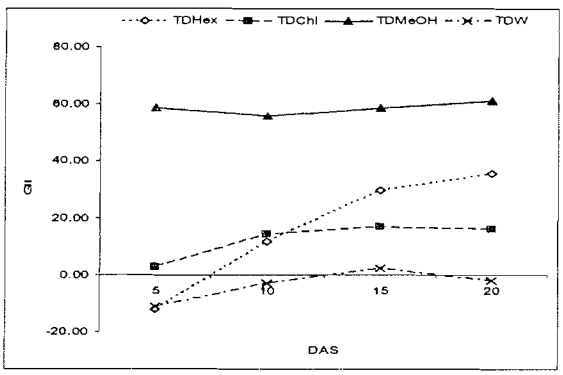
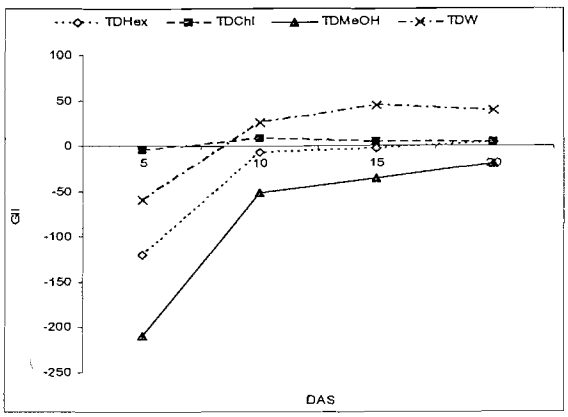


Fig. 4: Germination inhibition of teak deciduous leaf extracts on sedge



Among the phenolic acids, the concentration of salicylic acid in the fractions (F3-F5) has been increased (157.4-995.2 μg/ml) considerably in comparison to the TDMeOH extract. The increase in salicylic acid content was remarkable in F4 (by about 7.3 times), Benzoic acid in F4 and F5 (by about 14-32%), while tannic acid content was increased only in F5 (by about 33%). However, chlorogenic acid content in TDMeOH was decreased in the column chromatographic fractions. None of the acids could be detected in F1 (hexane elution of TDMeOH) possessing insignificant activity (Table 1), while F4 completely inhibited the germination of junglerice indicating possible role of phenolic acids for the observed activity.None of the re-chromatographic fractions (P1-P4) was detected for salicylic acid while benzoic acid was detected only in P2 and P3 fractions at very low levels (8.4-19.8 μg/ml). The content of tannic acid in the purified fractions reduced by about 20-65% in comparison to F4 and chlorogenic acid content reduced drastically by about 88-98%. Therefore, re-chromatography of F4 was not effective in improving the phenolic acid content nor it could enhance the pre-emergent herbicidal action (Table 1).

Relationship between the activity and phenolic acid content. To understand the role of the phenolic acids influencing the herbicidal action of the extract/fractions, the data on activity of the extracts and the phenolic acid content (Table 1) were further subjected to Multiple Regression Analysis followed by Principal Component Analysis. The data on activity exhibited by the pure phenolic acids were also included for better statistical interpretation. However, the data on caffeic acid and vanillic acid were not included as they could not produce any significant activity on the seed germination, neither the occurrence of these two compounds was detected in the plant extract/fractions. The data matrix subjected to statistical analysis is presented in table 2.

Fig. 5: Multiple regression equations of the phenolic acid content and activity relationship

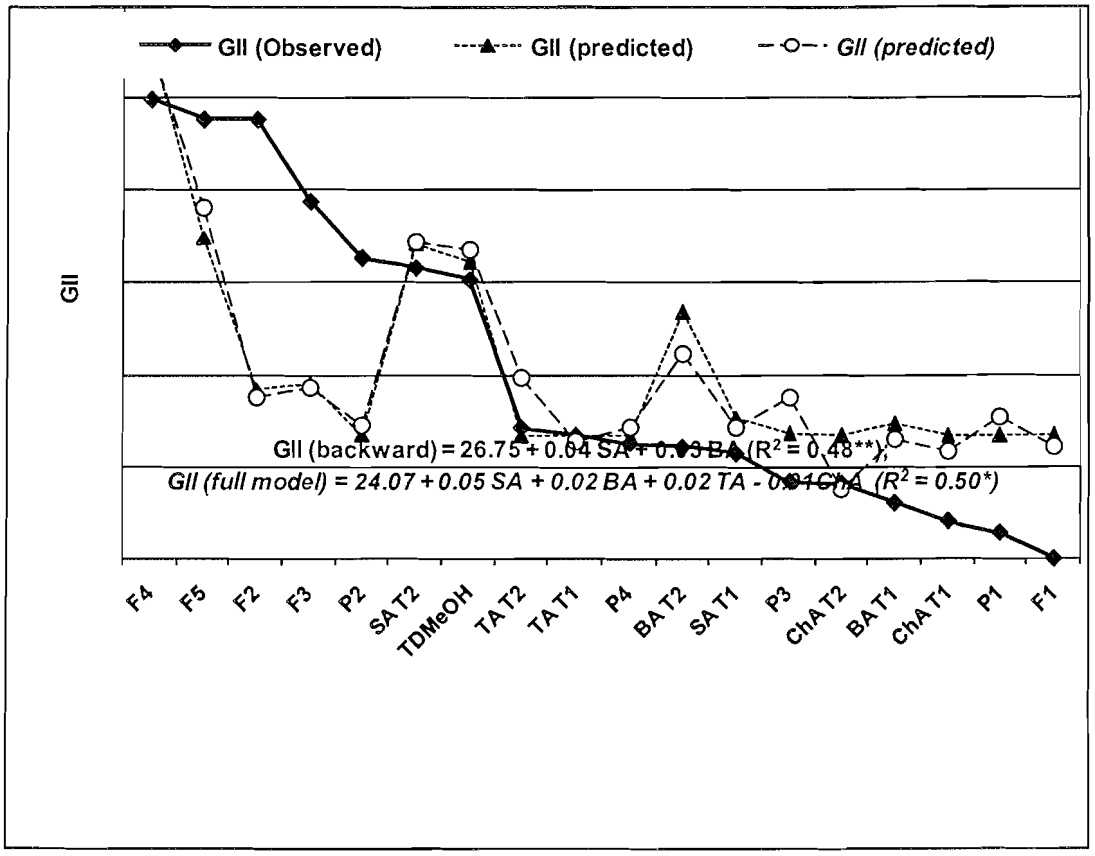
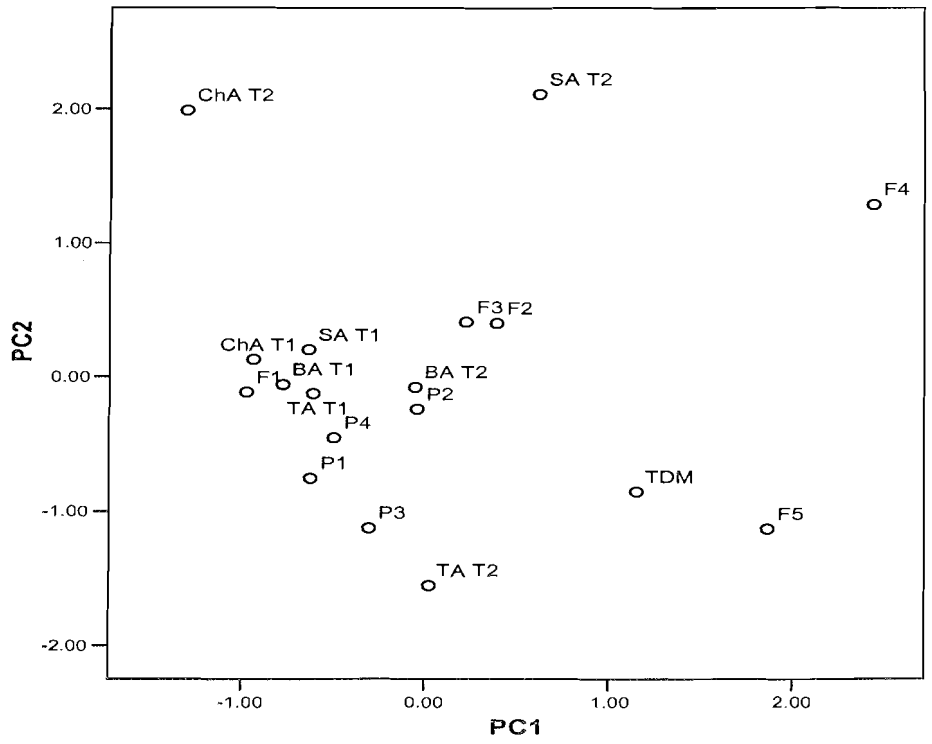


Fig. 6: Scattered diagram of the Principal Components (PC) influencing the herbicidal activity



The multiple regression eequations obtained following Backward elimination and full model technique have been compared with the observed GI values (Fig. 6). Both the regression equations were significant indicating the positive dependency of the inhibitory action of the treatments on salicylic acid and benzoic acid content and to a lesser extent on tannic acid, but not on chlorogenic acid content. The experimentally observed GI values, however, markedly varied from the predicted equations in case of F2, F3, P2 and to some extent in case of F5 fractions indicating the involvement of other constituents for activity. The observed activity was in well agreement with the predicted equations for F4, TDMeOH and salicylic acid. Therefore, the role of salicylic acid in germination inhibition of F4 and TDMeOH extract is definitely indicated by the multiple regression equations.

Table 2: Component matrix indicating the influence of phenolic acids on the germination inhibition of junglerice seeds

Phenolic acids	Principal Components (PC)	
	1	2
SA	0.66	0.60
BA	0.83	-0.05
TA	0.61	-0.62
ChA	-0.27	0.48
GI	0.86	0.19
Eigen value	2.32	1.02
% of Variance	46.35	20.34
Cumulative %	46.35	66.68

Principal Component Analysis of the data (Table 1) extracted two component matrices consisting of the phenolic acids governing the activity relationship (Table 2). The activity relationship in the form of regression factor scores of all treatments (viz. pure phenolic acids, extract and fractions) have also been presented in the scattered diagram (Fig. 7) showing the contribution of two components along the two axis. The Principal Component-1 (along the X-Axis in Fig. 6) could explain about 46% of the variation in inhibitory activity of the extracts and also indicated the activity might be due to the presence of salicylic acid, benzoic acid and tannic acid (Table 1). Accordingly, the activities of the extract/fractions appeared in the order of F4 > F5 > TDMeOH (Fig. 6).The Principal Component-2 (along the Y-Axis) could explain about 20% of the variation in inhibitory activity of the extracts and also indicated that the activity might be due to the presence of salicylic acid and chlorogenic acid. The activities of the extract/fractions appeared in the order of F4 > F3 > F2 (Fig. 6).

From the results it may be concluded that F4 was the most active column fraction and its activity might be attributed to the occurrence of the combination of the phenolic acids with their relative importance in the order of salicylic acid > *p*-hydroxy benzoic acid > tannic acid > chlorogenic acid. The activities of F5 was attributed to the combination of salicylic acid > benzoic acid > tannic acid. But the activities in F3 and F2 fractions were less influenced by the presence of these acids, rather the observed activity may be due to some other unidentified compounds in F2, F3 and also in P2.

The phenolic compounds have been reported to possess inhibitory effect on the germination of the seeds of other plants (Chaves *et al.* 1997) due to their physiological effect on membrane functions, membrane potential, mineral absorption and plant-water relations (Barkosky *et al.* 1993, Einhellig 1995, Harper *et al.* 1981). Phenolic acids are considered as one of the major allelochemicals responsible for herbicidal bio-activity (Batish *et al.* 2006, Lin *et al.* 2004) in many plants. But, allelopathic inhibition typically results from the combined action of a group of allelochemicals which, collectively, interfere with several physiological processes through joint action of allelochemicals (Einhellig 1996). A variety of experiments have established that the combination of allelochemicals that act additively or synergistically to inhibit growth is especially important because the concentration of a single compound in field situations is generally below its inhibition threshold. *p*-hydroxy benzoic acid derivatives at 500 µM inhibited seedling growth of velvetleaf (*Abutilon theophrasti* Medic.) in the greenhouse, yet an equimolar mixture of 50 µM each of these ten compounds was just as inhibitory (Einhellig 1989).

Therefore, the herbicidal bio-activity observed in the leaf extract of teak and its various fractions was attributed to the occurrence of the combination of phenolic acids (salicylic acid, *p*-hydroxy benzoic acid, tannic acid and chlorogenic acid) which might have been at the optimum level in F4 and disintegrated due to further fractionation (P1-P4). The ratio of these phenolic acids present in the active fraction may be useful for future development of bio-herbicide.

Based on these findings the chloroform fraction of methanol extract of teak deciduous leaves may be considered as a potential pre-emergent allelopathic herbicide for controlling the weed junglerice in rice field. The allelopathic herbicidal activity was assigned due to the presence of phenolic acids in various combinations. However, the possibility of other bio-active compounds was also indicated in the plant for the observed inhibitory activity on germination. Further exploration of the

methanol extract of the deciduous leaves of teak with hexane clean up was suggested for development of a potential pre-emergent herbicide.

ABBREVIATIONS USED:

GI = Germination inhibition index; DAS = days after sowing; TG = Teak (Tectona grandis) Green Leaf; TD = Teak (Tectona grandis) Deciduous Leaf; Hex = Hexane; W = Water; Chl = Chloroform; MeOH = Methanol; BA = p-hydroxy benzoic acid; CfA = Caffeic acid; ChA = Chlorogenic acid; SA = Salicylic acid; TA = Tannic acid; VA = Vanillic acid; TLC = Thin layer chromatography; HPTLC = High performance thin layer chromatography.

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