Express, an International Journal of Multi Disciplinary Research
ISSN: 2348 – 2052 , Vol. 1, Issue 7, July 2014
Available at: www.express-journal.com

Qualitative analysis of the relative amount and composition of the phenolic acids present in the rhizosphere of some trees showing allelopathy

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ABSTRACT

Allelopathy is a phenomenon which involves either useful or harmful and either directs or indirect effect of one plant on another plant through the release of chemicals in the surrounding environment. These chemicals or secondary metabolities, aremostly terpenes, phenolic acids, resins etc., are released in environment either through leachation or by decomposition of litter. Out of various secondary metabolities released, phenolic acids are one of the most commonallelochemic responsible for this phenomenon. In the present experiment the qualitative analysis and comparison of phenolic acids present in the rhizosphere of *Eucalyptus tereticornis & Leucaena leucocephala* (exotic in India) and *Dalbergia sissoo*&*Acacia nilotica* (indigenous in India) was carried out to correlate with their allelopathic potential. It was found that most of the phenolic acids belong to either Benzoic acid derivatives or Cinnamic acid derivatives category. Syringic acid which is a form of Benzoic acid is found to be present on the floor of each of the plantations studied. Maximum phenolic acids -8 were found on the floor of *E. tereticornis* and minimum -4 on the floor of *D. sissoo*.

INTRODUCTION

Allelopathy, which is reported over 2000 years ago in literature with reference to plant interference (Weston, 2003), is exercised by the plants through secretion of secondary metabolites (Kaminsky and Muller,-1977) which are normally the products of primary metabolism. The secondary metabolites are mostly terpenes, phenolic acids, resins etc. These get release into the environment through leachation or upon decomposition. Out of the various secondary metabolites, phenolic acids and alkaloids are credited with the allelopathic properties (Kumar, 2001;Batish *et al.*, 2008; Gao-feng, 2010). Kumar (2001) found that there was a reduction in undergrown vegetation (allelopathic effect) of some tree species due to phenolic acids released by these species. The phenolic acids in soil are further divided into leachable, non-leachable and bound soluble parts (Singh, 1991). Keeping in mind the volume of data only the soluble or free form phenolic acids were studied, in the present experiment to co-relate the allelopathic potential of *Eucalyptus tereticornis & Leucaena leucocephala* (exotic in India) and *Dalbergia sissoo*&*Acacia nilotica* (indigenous in India).

OBSERVATION PARAMETER

The different types of phenolic acids and their relative amounts present in the soil on the floor of monoculture plantations of *E. tereticornis, L. leucocephala,D. sissoo* and *A. nilotica* trees were evaluated.

METHODOLOGY

a) Collection of soil and extraction of soil chemicals

The soilsfrom the floor of monoculture plantations of exotic and indigenous trees mentioned above, were collected. The soil samples were taken from different distances of 1.0, 3.0 and 5.0 m. from the tree trunk. The organic chemicals from these samples were extracted using the formula of Kaminsky and Muller (1977). The various samples of organic chemicals extracted from the soil, collected from one plantation type were pooled for further analysis.

b) Separation and identification of phenolic acids

Each of the samples, dissolved in methanol was subjected to thin layer chromatography (TLC) on plates coated with silica gel, pre- activated at 120^0C. Benzene and ethyl acetate (8:2v/v) formed the mobile phase. The phenolic acids were visualized on plates as bright spots against iodine. The spots from the chromatograms were eluted collectively in methanol and subjected to further identification through High Pressure Liquid Chromatography (HPLC).Pure Phenolic acids of Sigma make served as standards.

RESULTS

In the soils of monoculture plantations of *E. tereticornis, L. leucocephala, D. sissoo* and *A. nilotica*, the number of phenolic acids which could be identified (or remained unidentified but counted) were found to differ. From the list of 13 which could be counted from all these four plantations, only syringic acid and gentisic acid were the ones which could be identified from the plantation floor of each of the tree species (Table 8.1).

Table 8.1: average amount of phenolic acids (relative percent) present in organic chemicals extracted from soils from the floor of various exotic and indigenous tree species (arranged as per their Retention time).

S.No	Phenolic acid	Retantion time (RT)	*E. tereticornis*	*L. leucocephala*	*D. sissoo*	*A. nilotica*
1	Gentisic acid	3.40	4.56%	0.70%	1.08%	11.30%
2	Unidentified I	3.90±0.1	3.95%	-	-	
3	Syringic acid	4.31±0.21	35.18%	63.29%	50.59%	60.80%
4	Unidentified II	4.67+0.01	24.03%	_	21.26%	_
5	Vanilic acid	4.93	13.84%	-		
6	Caffeic acid	7.80±0.03	5.74%	_	-	_
7	Unidentified III	13.81	_	0.98%	_	_

8	Unidentified IV	14.57	_	0.98%	_	_
9	Salicylic acid	18.01±0.2	_	_	27.05%	13.17%
10	Cinnamic acid	18.81	1.05%	1.92%	-	1.12%
11	Ferulic acid	19.21±0.51	11.61%	-	-	-
12	Unidentified V	21.78	-	-	-	8.95%
13	Unidentified VI	23.12	-	25.3%	-	-

From the 13 phenolic acids only seven could be identified and for the rest six, due to non-availability of standards identification could not be made. Therefore, these have been referred to as Unidentified I-VI. From among the seven identified phenolic acids four happen to be derivatives of benzoic acid while rest three that of cinnamic acid (Table 8.2). The identified phenolic acids include gentisic acid, syringic acid, vanillic acid, salicylic acid, cinnamic acid and ferulic acid.

From among the four plantation soil maximum (eight) types of phenolic acid could be found from the soils of *E. tereticornis* and the least (four types) from *D. sissoo*. The soils of *L. leucocephala* and *A. nilotica* were having six types of phenolic acids with Gentisic acid, syringic and cinnamic acid as the common (Table 8.1).

Table 8.2: Chemical nature and name of different identified phenolic acids

Phenolic acid	**Chemical nature**	**Chemical name**
1. Gentisic acid	Benzoic acid derivative	2,5-Dihydroxy benzoic acid
2. Syringic acid	Benzoic acid derivative	4- hydroxy 3,5- dimethoxy benzoic acid
3. Vanillic acid	Benzoic acid derivative	4-Hydroxy –3 methoxy benzoic acid
4. Caffeic acid	Cinnamic acid derivative	3D (3,4-Dihydroxy phenyl)-2 propanoic acid
5. Salicylic acid	Benzoic acid derivative	2-Hydroxybenzoic acid
6. Cinnamic acid	Cinnamic acid derivative	3- Phenyl 2- propanoic acid
7.Ferulic acid	Cinnamic acid derivative	3- (4-Hydroxy –3methoxy-phenyl) –2 propanoic acid

In the soil of *E. tereticornis* except for salicylic acid all the other identified (six) were found. In addition, two unidentified marked as Unidentified I and Unidentified II with retention times (RT) 3.90+ 0.1 and 4.67+ 0.01 and relative amount of 3.95% and 24.03% respectively were also noticed (Table 8.1). With respect to RT value no apparent relationship as regards the amount in respective phenolic acids could be seen. However, it was noticed that with increasing molecular weights of identified phenolic acids, the relative amounts were seen to increase (Table 8.3). Thus, in comparison to the amounts of 1.05% of cinnamic acid with molecular weight of 148 the

syringic acid contents of molecular weight of 198 were seen to be maximum (38.15%) in terms of relative percentage. Exception was, however, in case of vanillic acid in this regard (Table 8.3).

The soil of *L. leucocephala* was seen to contain syringic acid (63.29%), Cinnamic acid (1.92%) and gentisic acid (0.706%). In addition to these, three unidentified marked as unidentified III, IV and VI with RT values of 13.81, 14.57 and 23.12 respectively were also seen. Among the unidentified samples, the contents of the samples increased with increasing retention time (Table 8.1).

Table 8.3: Percentage of identified phenolic acids with reference to their respective molecular weight (arranged as per molecular weight).

Phenolic acid	**Mol.Wt.**	***E. tereticornis***	***L.leucocephala***	***D. sissoo***	***A. nilotica***
Salicylic acid	138	-	-	27.05	13.17
Cinnamic acid	148	1.05	1.92	-	-
Gentisic acid	154	4.56	0.706	1.08	-
Vanillic acid	168	13.84	-	-	-
Caffeic acid	180	5.74	-	-	4.63
Ferulic acid	194	11.61	-	-	-
Syringic acid	198	35.18	63.18	50.59	60.80

The soil of *D. sissoo* was seen to have only four types of phenolic acids, of which one marked as unidentified II with RT value of 4.67 could not be identified (Table 8.1). Syringic acid with relative value of 50.59% followed by salicylic acid with relative value of 27.05% and gentisic acid having relative value of 1.08% could also be noticed (Table 8.1) Each of the identified phenolic acids belong to the group of benzoic acid derivatives (Table 8.2).

Three identified and three unidentified phenolic acids were recorded from the soil collected under the plantation of *A. nilotica*. Like in other, maximum relative content (60.80%) was that of syringic acid, while the minimum in cinnamic acid (1.12%) (Table 8.1). Unidentified V was present only in *A. nilotica* while Unidentified III, IV and VI could be found only in the soils of *L. leucocephala* (Table 8.1). On the other hand, gentisic acid was found to be present under each of the plantation studied except *A. nilotica*, and cinnamic acid in all but *D. sissoo* (Table 8.3).

DISCUSSION

Phenolic acids and their derivatives form a major part of phytotoxins released by plants which show allelopathic properties (Blum *et al*., 1984; Li *et al*, 2010). The phytotoxic potential of the phytotoxins ororganic chemicals released by the plants depend upon the composition and relative amount various phenolic acids (Lehmann *et al*., 1987).

It is explicit from the results that phenolic acids either in their benzoic acid or cinnamic acid derivative form occur in the soil. Syringic acid, a form of benzoic acid is found to be present on the floor of each of the plantations studied. Except for syringic acid most of the phenolic acids like Caffeic acid, cinnamic acid, gentisic acid, vanillic acid are polar in nature. During thestudies on phytotoxins present on the floor of *E. tereticornis,* Singh (1991) also found that most of phenolic acids present in these phytotoxins were polar in nature.

The phenolics may be released as such or during microbial decomposition of lignin polymers. Vanillic acid, P-hydroxybenzoic acid, protocatechuic acid, syringic acid were released upon bacterial decomposition (Cheng *et al*.,1982). Various workers (Rice, 1984; Whitehead *et al*., 1983; Singh, 1991; Biswas *et. al*., 2013) had also identified the presence of phenolic acid in different soil systems. Cinnamic acid derivatives compared to the benzoic acid derivatives were more reactive in soil ecosystems (Lehmann *et al*., 1987; Zuo *et al*., 2002) and toxic to plants (Blum *et al*, 1984). A highly toxic nature of cinnamic acid and its derivatives as compared to benzoic acid derivatives may be the possible reason of relatively less amount of cinnamic acid and its derivatives in the soil chemics, as was found in the present experiment.

From the present set of observations, it can be concluded that the various exotic and indigenous tree species release certain chemicals including phenolic acids in the extractable amount which may result in their allelopathic potential. In other words tree like *E. tereticornis, L. leucocephala, A. nilotica, D.sissoo* exerts their allelopathic influence by releasing compounds including phenolic acids. The relative phytotoxicity depends upon the type and amount of phenolic acids present in their organic compounds. The *D. sissoo*, as held relatively least number of phenolic acids that too benzoic acid derivatives, has the least phytotoxic influence on undergrown vegetation as compared to other plantations. Further studies can be carried out to co-relate these observations with the allelopathic potential of studied tree species by studyingthe undergrown vegetation.

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