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EVALUATING THE ALLELOPATHIC INFLUENCE OF MESQUITE (*Prosopis juliflora* DC.) AQUEOUS LEAF EXTRACT ON THE GERMINATION OF RICE (*Oryza sativa* L.) SEEDS USING DIFFERENT GERMINATION INDICES

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ABSTRACT

Prosopis juliflora DC. is well-known for its invasive and allelopathic nature. A laboratory experiment was established to examine the effect of aqueous extract of *P. juliflora* on rice seed germination. The extract stock was prepared from fresh and dried leaves (in the ratio of 1:20), subjected to incubation from 1 to 7 days under standard conditions. Two concentrations viz., 0.1 and 1% were prepared from the stock. Extract was analyzed for total polyphenol content, pH and EC prior to set up of the experiment. Rice seeds were inoculated for germination in the petri plates containing extract moistened (0.1% and 1%) filter papers. Germination was recorded each day up to 10 days and germination indices were statistically compared to the control. Further, the germination was correlated to the levels of polyphenols content, pH and EC of the extracts. Most of the treatments had no effect on germination; however, some treatments showed inhibition and others stimulation. The mature leaf extracts were found to be favourable for the germination. Among all, the 0.1% two days (2D) mature leaves' extracts were particularly stimulatory.

KEYWORDS: Allelopathy, Polyphenols, Germination indices

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INTRODUCTION

A new generation of the flowering plant's life begins through seed. It possesses the embryo, a future plant, which is protected by the seed coat from the outer environment. It contains the food reserves for the growth of the seedling till it starts to synthesize its own food materials through photosynthesis. Germination, which is defined as the water uptake by quiescent dry seeds and the emergence of radical or plumule[1], comprises of various physiological and molecular steps. In nature, both the internal and external factors such as enzymes, dormancy, edaphic factors, pH, and allelochemicals combined together affect the germination. Among them, allelochemicals are the substances which are released into the environment by decaying or damaged tissues/cells and influence the growth and development of the other plants. These can be released from any part of the plant either living or dead/decaying. These exudates affect metabolite production, photosynthesis, respiration, membrane transport, germination, root-shoot growth and cell mortality in plants which are particularly susceptible to it[2,3]. Allelochemicals can be alkaloids, tannins, polyphenols and so on. Many aspects of plant-litter-soil interactions are influenced by polyphenols; such as, regulation of nutrient cycling and organic matter dynamics, amelioration of chemical and physical infertility factors and alteration of soil properties[4]. According to Kuiters[5], seed germination and root growth of the competing vegetation were suppressed by these polyphenols which were believed to play a negative role in soil chemical ecology. Polyphenols were reported to be detrimental to the germination by Chiapusio *et al*.[6] and Muscolo *et al*.[7]. The effect of polyphenols from the different parts of various plants are studied by applying specific isolated allelochemicals, application of aqueous extracts or by using the synthetic chemical compounds which are homologous to the isolated compounds from different allelopathic plants. One among these plants, most studied for this reason (allelopathic effect), is *Prosopis juliflora*. Influence of different plant part extracts of *P. juliflora* on the growth and development of different plants have been studied[8-14]. *P. juliflora* is known to be rich in polyphenols. Some of the known compounds which were isolated from the *P. juliflora* are L-Tryptophan[12,15], syringine and (-)-lariciresinol[16]. There are many studies reporting negative effects of *P. juliflora* on the germination and growth of other plants[17-20]. Sen and Chawan[14], however, reported both the positive and negative effects of the aqueous extracts of *P. juliflora* on germination of *Eucryphia cardifolia*. Although the results were not compared statistically with control, they indicated that the fruit extracts of *P. juliflora* promoted the germination, whereas the extracts from leaves inhibited the germination. Here, we report the results of our study to assess the effect of aqueous extracts of mature leaves of *P. juliflora*, both the fresh and dried, on the germination of Rice. We have made use of four germination indices which were calculated and compared statistically.

MATERIALS AND METHODS

General Experimental procedures

Extracts were prepared using distilled water. Polyphenol content was measured using NaCl, Foline phenol reagent. Visible Spectrophotometer was used for the detection of colour change in the Polyphenol estimation. pH and EC were measured using Digital Soil and Water analysis Kit. For germination assays Watman No. 1 filter paper was used.

Plant Materials

Rice (*Oryza sativa* L.) seeds of variety Kurnool Sona BPT 5204 were used as testing plant material. This rice variety is agronomically important to the farmers of the study area. Mature leaves collected from the *P. juliflora* tree located at the Sri Venkateswara University, Tirupati, Chittoor district, at the longitude and latitudes of 13°40'N and 79°20'E and authentified by Dr. M. Madhava Chetty, Plant Taxonomist (IAAT:357), Dept of Botany, S.V. U. College of Sciences, S.V. University, Tirupati.

Some leaves were shade dried and others used freshly for the extract preparation.

Extract preparation

After shade drying the leaves were powdered and sieved with 2mm sieve. The powder was mixed with distilled water in the ratio of 1:20 (10 grams of fresh leaves in 200ml of distilled water) and kept for 1-7 days in different conical flasks at the temperature of 28±2^{o}C for incubation. The fresh leaves were macerated with the help of the pestle and mortar in the same ratios of 1:20 as dried leaves, and kept for incubation under similar conditions. After completion of incubation, the solutions were filtered with Watman No.1 filter paper and stored at 4^{o}C for further use. These extracts served as stock solutions. Extracts were named as Fresh Mature (FM) and Dry Mature (DM). Based on the Day of the maturation of the extracts, these were labelled as one day mature extracts (1D), two day mature extracts (2D), and so on up to seven days (7D). Polyphenol content of the extracts was estimated according to the method of Dehghan *et al*.[21], and expressed as µg of Gallic Acid Equivalents (GAE) mL^{-1} extract. The pH and the EC values of the extracts were measured with the help of water and soil analysis kit.

Germination assays

Two concentrations i.e. 0.1% (v/v) and 1% (v/v) solutions were prepared from stock solutions by diluting with distilled water. Rice seeds were surface sterilized and inoculated in the petri plates containing filter paper moistened with the extracts. On the first day, 5mL of extract was added to moist the filter paper entirely. From the second day onwards 3mL of the extract was added to each of the petri plates. The extract addition continued for 10 days. In each of the petri plates, 5 seeds were kept and the treatments were quadruplicated. The observations were made up to 10 days by keeping the petri plates with lid (to avoid evaporation) in an incubator with the temperature at 25± 2^{o}C.

Data collection, calculations and Statistical analysis

Germination of seeds was observed for 10 days following the set up, and the number of seeds germinated each day in every treatment was recorded and the germination indices i.e. Total germination (Gt), Speed of Germination (S), Speed of accumulated germination (AS) and Coefficient of the rate of germination (CRG) were calculated according to Chiapusio *et al.*[6]. All the values were statistically compared to the control using Mann-Whitney U Test. The Pearson's Correlation test was used to assess the correlation between different parameters.

RESULTS AND DISCUSSION

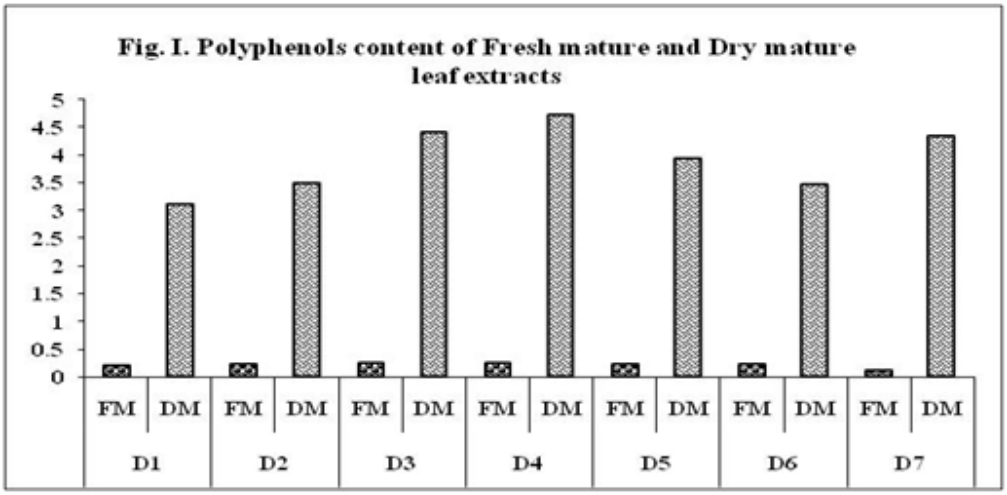
Different extracts influenced the seed germination differently, depending on both, the concentration of the extract, and the type of the extract. Results were mixed; some treatments showed reduction in the germination and others showed a positive response. In FM leaf extracts, Gt was not affected by any of the treatments. 5D 1% extracts reduced AS and S up to 24% and 27% respectively. CRG was negatively affected by 1% treatments of all the extracts except in 7D mature leaves' extract. The reduction was up to 5%. As in FM extracts, Gt was ineffective in all the DM treatments. DM extracts also showed the negative effects of in some treatments. 4D 1% extracts reduced S by 18%. 6D 0.1% extracts reduced CRG by 26% and 7D 1% extract reduced the CRG by 6%. The 2D 0.1% extracts were found to promote germination indices i.e. CRG, AS, and S by 2%, 22%, and 25% respectively (Table I). When the FM and DM extracts were compared, DM extracts acted favourably for the germination of the seeds, especially the 2D matured leaf extracts at 0.1% concentration

Table I

Values of Germination indices of Rice seeds germinated in Fresh Mature and Dry Matures leaves extracts at different concentrations#

Treatments		FM Gt	S	AS	CRG	DM Gt	S	AS	CRG
D1	0.1%	112.5^{ns}	108.89^{ns}	109.73^{ns}	99.31^{ns}	100^{ns}	95.79^{ns}	94.15^{ns}	97.65^{ns}
	1%	118.75^{ns}	99.33^{ns}	101.43^{ns}	95.30*	105.56^{ns}	98.97^{ns}	98.89^{ns}	97.76^{ns}
D2	0.1%	93.75^{ns}	93.33^{ns}	93.43^{ns}	99.75^{ns}	111.11^{ns}	123.71*	120.98*	102.33*
	1%	87.50^{ns}	76.67^{ns}	78.92^{ns}	96.09*	83.33^{ns}	79.38^{ns}	80.28^{ns}	99.91^{ns}
D3	0.1%	106.92^{ns}	104.44^{ns}	104.30^{ns}	99.34^{ns}	94.44^{ns}	93.81^{ns}	93.95^{ns}	99.60^{ns}
	1%	125^{ns}	111.11^{ns}	113.22^{ns}	96.99*	111.11^{ns}	106.19^{ns}	106.51^{ns}	98.69^{ns}
D4	0.1%	100^{ns}	90.00^{ns}	92.05^{ns}	97.72^{ns}	100.00^{ns}	94.23^{ns}	95.03^{ns}	98.21^{ns}
	1%	118.75^{ns}	100.44^{ns}	103.53^{ns}	95.71*	94.44^{ns}	81.44*	83.65^{ns}	96.93^{ns}
D5	0.1%	106.25^{ns}	96.67^{ns}	98.06^{ns}	96.37^{ns}	94.44^{ns}	75.46*	76.76^{ns}	94.10^{ns}
	1%	87.50^{ns}	72.22*	74.94*	95.52*	105.56^{ns}	92.58^{ns}	94.03^{ns}	96.61^{ns}
D6	0.1%	118.75^{ns}	111.11^{ns}	112.33^{ns}	98.16^{ns}	111.11^{ns}	85.77^{ns}	87.86^{ns}	94.30*
	1%	100^{ns}	87.78^{ns}	89.39^{ns}	96.57*	111.11^{ns}	105.15^{ns}	106.36^{ns}	98.62^{ns}
D7	0.1%	112.50^{ns}	92.00^{ns}	94.98^{ns}	94.91*	111.11^{ns}	105.57^{ns}	106.61^{ns}	98.73^{ns}
	1%	100^{ns}	86.67^{ns}	88.82^{ns}	97.00^{ns}	94.44^{ns}	73.20^{ns}	76.86^{ns}	94.47*

On the basis of earlier reports about the inhibitory effect of polyphenols on seed germination and growth of seedlings, we hypothesized that the reasons for the reduction in the germination indices' values could be one of the factors investigated in the present study i.e., polyphenol content of the extract, pH change or change in EC. Polyphenols content was high in 4D extracts of both FM and DM leaf extracts, and low in 7D in FM leaf extracts (Fig. I). However, there was no significant correlation between polyphenols content and the germination indices (Table II and III). It has been reported that under canopy of allelopathic plants has more polyphenol content. The polyphenols play a major role in forming soil humic substances through oxidative polymerization[22,23] and also increase the CEC (Cation Exchange Capacity) of soil organic matter[24]. But our results indicate insignificant correlation between polyphenols content and the germination indices.



*# Values are the mean percentage of control; ns-nonsignificant, *-significant at the level of 0.05 by Mann-Whitney U test*

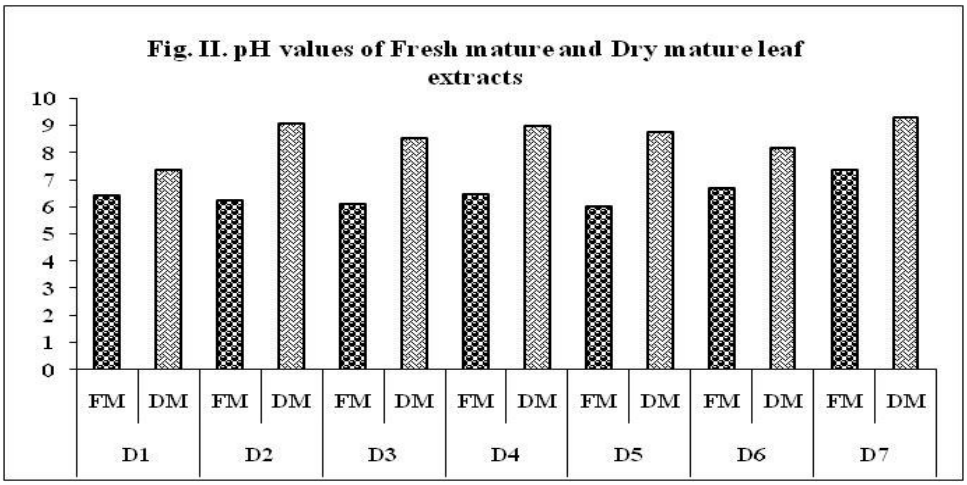
Table II
Pearson's Correlation values of pH, EC, Polyphenol content with germination indices of FM extracts

	pH	EC	Polyphenol content	Gt 0.1%	S 0.1%	AS 0.1%	CRG 0.1%	Gt 1%	S 1%	AS 1%	CRG 1%
pH	1										
EC	0.294	1									
Polyphenol content	-0.886**	-0.198	1								
Gt 0.1%	0.489	0.450	-0.425	1							
S 0.1%	-0.173	0.366	0.199	0.679	1						
AS 0.1%	-0.079	0.389	0.120	0.758*	0.993**	1					
CRG 0.1%	-0.625	-0.114	0.687	-0.323	0.444	0.349	1				
Gt 1%	-0.056	-0.646	0.160	0.157	0.294	0.293	0.325	1			
S 1%	-0.033	-0.570	0.145	0.154	0.316	0.307	0.363	0.983**	1		
AS 1%	-0.043	-0.595	0.155	0.132	0.291	0.282	0.355	0.986**	0.999**	1	
CRG 1%	-0.450	0.276	-0.439	0.242	0.025	0.034	-0.202	0.072	0.230	0.213	1

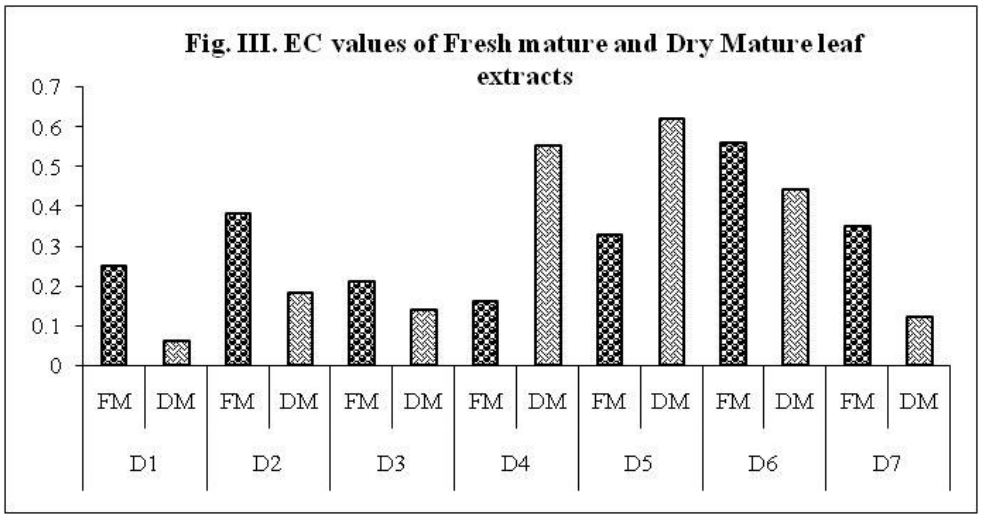
Table III
Pearson's Correlation values of pH, EC, Polyphenol content with germination indices of DM extracts

	pH	EC	Polyphenol content	Gt 0.1%	S 0.1%	AS 0.1%	CRG 0.1%	Gt 1%	S 1%	AS 1%	CRG 1%
pH	1										
EC	0.230	1									
Polyphenol content	0.677	0.296	1								
Gt 0.1%	0.259	-0.253	-0.294	1							
S 0.1%	0.343	-0.626	-0.114	0.591	1						
AS 0.1%	0.393	-0.606	-0.069	0.632	0.996**	1					
CRG 0.1%	0.342	-0.635	0.121	0.231	0.908**	0.894**	1				
Gt 1%	-0.616	0.128	-0.095	-0.484	-0.803*	-0.807*	-0.683	1			
S 1%	-0.734	0.020	-0.327	-0.428	-0.571	-0.597	-0.446	0.886**	1		
AS 1%	-0.712	0.043	-0.292	-0.418	-0.604	-0.625	-0.483	0.910**	0.998**	1	
CRG 1%	-0.322	-0.110	-0.459	0.029	0.264	0.225	0.301	0.015	0.472	0.426	1

pH also plays an important role during germination; slightly acidic to basic pH is more suitable for germination. This could also be related to activity of polyphenols in soil, as the soil pH significantly influences the adsorption of phenolic acids onto the soil particles. Inderjit and Bhowmik[25] stated that at lower pH, phenolic compounds were non-ionized and likely to be adsorbed onto organic matter and clay through weak physical forces. Under slightly acidic to basic conditions, negatively charged phenolic acids can be adsorbed onto positively charged sites of soil colloids. Thus, the polyphenols doesn't remain as such in the environment when released from the source material. In our study pH was more in the D7 of DM extract and less in D5 of FM extracts (Fig. II). The FM extracts were slightly acidic in nature and DM extracts were slightly basic. However, we did not find significant correlation between different germination indices and pH values. Instead, the correlation between pH and the EC of the FM extracts was negatively correlated at $p<0.01$ (Table II).



Results indicated that the D5 and D1 extract of DM leaves had high and low levels of EC respectively; and on D6 and D4 of FM leaves extract had high and low levels of EC respectively (Fig III). Some reports indicated that the germination failure can be due to increase in the EC values; the more the EC, the more reduced the germination of rice[26], soybean[27], pea[28,29] and sudan grass[30] seeds. However, we could not arrive at such conclusions based on our results of correlation between EC and germination indices.



Analysis of the various possible causes for their effect on germination process indicated that the reasons could not be singled out, as many factors interact with one another and influence the development of the seedling. Earlier germination failure has been correlated with the inhibition of enzymes of glycolysis and OPPP pathway, when treated with phenolic compounds[7]. The enhanced membrane deterioration also was reported to inhibit the germination when treated with allelopathic extracts by Leather [31].The promoting effect of germination by 2D 0.1% of DM extracts could be due to the presence of allelopathic stimulators. An earlier report supports it in the sense that, many inhibitory compounds could be stimulatory at small concentrations[32,33]. Rice[34] also reported that some allelopathic compounds are not directly involved in the growth or promoting effects, but, these improved the organic matter which affects the growth positively. In our study, we have used aqueous extract to expose the test plants to more natural conditions. The use of a single compound at a range of concentrations could result in reduction or stimulation of the process of germination, but, such situations cannot be expected in nature where many factors act in

coherence. It's true that at very high concentrations, extracts of many plant species have shown inhibition of germination, but, such situations are found only under the canopy of the source plants and the concentrations will decrease as we move away from the source plant. With reference to the laboratory incubations, an important point often overlooked is the gradual build up of the concentration of the extract. Since, each day the same concentration of the extract (0.1% or 1%) is added to the treatment petri dishes, gradually there is evaporation of water, and the extracts and all the chemicals which are part of it increase in concentration with passage of time. Hence, effectively the actual concentration in the laboratory incubation experiments is not 0.1 or 1% but many times more of that.

CONCLUSION

We conclude that at moderate concentrations, the extracts will have positive/neutral effect and at very high concentrations only, when one or more chemical constituent of the so called allelopathic plant, increases in concentration enormously, will it have negative effect on the growth of the target plant.

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CONFLICT OF INTEREST

Conflict of interest declared none.

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