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Allelopathic Effect of Aqueous Leaf Extract of *Melia dubia* on Seed Germination and Growth of *Zea mays*

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ABSTRACT
Melia dubia is a multipurpose tree species and its wood is used for various purposes like pulpwood, plywood, packing cases, ceiling planks, agricultural implements, fuel wood etc. It is also planted in agroforestry systems; hence, it is imperative to assess its allelopathic effect on growth of understorey crops. Therefore, in present study, allelopathic effect of different concentrations (0, 1, 2,3,4,5 and 6%) of aqueous leaf extract of M. dubia on seed germination and seedling growth of Zea mays (maize) was assessed. The results indicated that 1 and 2%of aqueous leaf extract of M. dubia had no allelopathic effect on seed germination, as 100% germination was recorded at these concentrations. But, at higher concentrations, germination decreased and minimum germination (86%) was recorded at 5 and 6% concentrations of the extract. All the concentrations of leaf extract had negative allelopathic effect on growth parameters of maize, barring few exceptions. The study revealed that maximum inhibitory effect on germination, growth parameters and vigour index of maize was indicated by 5 and 6% aqueous leaf extract.
Keywords: *Allelopathic effect, maize, Melia dubia, shoot and root weight, vigour index*

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INTRODUCTION

Melia dubia Cav. (Family-Meliaceae) is a fast growing and short rotation tree species having industrial importance [1,2]. It is indigenous to the Western Ghats of Southern India and common in moist deciduous forests of Kerala [3]. Its wood is used for various purposes such as for pulpwood, plywood, pencils, splints and packing cases etc. [4]. Its wood is having resistance to termites and fungus, and thereby considered suitable for furniture, agricultural implements and house construction [5]. Due to multifarious uses of *M. dubia*, it is being promoted as agroforestry tree species in different regions of the country. This species is also under study in agroforestry system at experimental farm of ICAR-Central Agroforestry Research Institute (CAFRI), Jhansi.

Trees and crops in agroforestry systems interact with each other and either inhibit or stimulate the growth and yield of understorey crops through direct or indirect allelopathic interactions [6]. Allelopathy refers to any direct or indirect effect of plants on other plants through the release of chemicals [7, 8] and it plays a significant role under both natural and managed ecosystems [9]. The allelo-chemicals produced by trees may decrease or increase the germination and growth of understorey crops. Allelopathic effects of tree species on the understorey crops have been reported by many researchers [10-12], but level of toxicity varied from one species to another [13]. Keeping in view the importance and increasing popularity of *M. dubia* in agroforestry, the present investigation was conducted to study the effect of aqueous leaf extract on seed germination and growth of maize.

MATERIALS AND METHODS

Preparation of aqueous leaf extracts: Insect-free, disease-free leaves of *M. dubia* were collected from the experimental farm of ICAR-CAFRI, Jhansi (24° 11' N latitude and 78° 17' E longitude) where these trees are grown in an agroforestry system. The collected leaves were washed thoroughly with distilled water and air-dried at room temperature for 96 hours. After air drying, the leaves were chopped and kept in the oven at 28 ^{0}C for 72 hours. The dried leaves were ground with the help of electronic grinder, to make fine powder. The aqueous leaf extracts of 1, 2, 3, 4, 5 and 6% concentrations were prepared by

taking 10, 20, 30, 40, 50 and 60 g of leaf powder and each was added into 1 L distilled water (*w*/*v*) separately, macerated manually and shook for 15 minutes on electric shaker. After shaking, extracts were placed at room temperature for 24 hours. These extracts were then filtered with double muslin cloth, followed by Whatman filter paper No. 1. Apart from these six concentrations of aqueous leaf extracts, control (0% i.e. distilled water) was also included in the study.

Bio-assay experiment: The seeds of maize were surface-sterilized with 0.1% mercuric chloride for 5 minutes, and then washed four to five times with distilled water and dried on an absorbent to eliminate fungal attack. Ten sterilized seeds of maize were evenly placed on two layers of filter paper in sterile petri dishes (9 cm) and lid was placed back on, to prevent evaporation. Thereafter, 5 ml of different concentration of the aqueous leaf extracts (1, 2, 3, 4, 5 and 6%) and distilled water (control) were added to petri dishes containing seeds. Afterwards, 2 ml of different concentrations were applied on alternate days to keep the filter paper moist till the completion of experiment. All petri dishes were placed in laboratory at room temperature. Treatments were arranged in completely randomized block design and all the treatments were replicated five times. The number of seeds germinated were recorded every day and germination percentage (%) was calculated. At the end of the experiment (after 7 days), shoot length (cm), root length (cm) and collar diameter (mm) of the plants were measured with the help of a ruler. Thereafter, five plants were randomly selected from each petri dish and analyzed for fresh and dry weight (mg). The dry weight was determined by drying the plant materials in an oven at 60 °C for 24 hours and expressed in mg/5 plants. The generated data were used to calculate the seedling vigour index. The vigour index was evaluated as the combination of early seedling growth and germination traits, and calculated with equation (P + R) × G, where P and R are the shoot (plumule) length and root (radical) length, respectively at the end of the experiment for seedlings and G is the final germination percentage.

Statistical analysis: All the data were subjected to one-way analysis of variance (ANOVA). Least Significant Difference (LSD) was used to compare treatment differences ($P<0.05$). The statistical analysis was performed by using the statistical package SYSTAT version 12.

RESULTS AND DISCUSSION

Effect on germination of *Z. mays*

Per cent seed germination was recorded maximum in control (100%) which was at par with the germination recorded in 1 and 2% aqueous leaf extract concentrations (Figure 1). Further increase in the concentrations (3 and 4%) reduced the germination percentage up to 96%. Minimum germination per cent was recorded in 5 and 6% concentrations (86%).

Effect on growth of *Z. mays*

Among various tested concentrations, maximum shoot and root length were recorded at 1% concentration which were significantly higher than control (Table 1). Minimum values of these parameters were recorded at 6% concentration. More or less similar results were recorded in terms of collar diameter. It was also recorded maximum at 1% but the value was comparable with the value recorded in the control. The minimum collar diameter was recorded at 5% concentration which was at par with the value recorded at 6% concentration.

Effect on fresh weight of *Z. mays*

Maximum shoot fresh weight was recorded at 1% concentration which was comparable with control. Its minimum value was recorded at 6% which was at par with 4 and 5%. Significantly higher fresh root weight was recorded in control and its least value was recorded in 6% concentration which was comparable with 4 and 5%. Total fresh weight of *Z. mays* was also recorded maximum in control which was comparable with the total fresh weight recorded in 1% concentration. The minimum weight was recorded at 6% and it was found comparable with 4 and 5% concentrations (Table 2).

Effect on dry weight of *Z. mays*

Maximum shoot dry weight was recorded in 1% concentration which was comparable with control. Its minimum value was recorded in 6% concentration. Significantly higher root dry weight was recorded in control. Its least value was recorded in 5% concentration which was comparable with 4 and 6% concentrations. Total dry weight was also recorded maximum in control which was comparable with the dry weight recorded in 1% concentration. The minimum dry weight was recorded at 6% concentration (Table 3).

Vigour index

Maximum value of vigour index was recorded in 1% concentration of aqueous leaf extract of *M. dubia*, followed by control (0%), 2%, 3%, 4%, 5% and 6% concentrations. All these values were significantly different from each other (Figure 2).

Thus, the results of this study showed that aqueous leaf extracts of *M. dubia*at low concentrations (up to 2%) did not have deleterious effect on seed germination. Moreover, 1% concentration of the leaf extract exhibited stimulatory effect in terms of enhanced shoot length, root length, shoot fresh and dry weight over control. Such stimulatory effects of lower concentration of extracts have been reported by various researchers in different crops [14, 15]. Phiri [16] reported stimulatory effect of *Moringa oleifera* leaf extracts. The lower concentration of *M. oliefera* enhanced germination of sorghum by 29%, hypocotyls length of wheat by 14.9% and radical length of maize by 77.8%. Chon *et al*. [17, 18] reported 13-33% increase in root length of alfalfa upon application of low concentration of aqueous extracts of some plants from Asteraceae family. El-Rokiek *et al*. [19] also recorded enhanced shoot length and shoot biomass at low and moderate concentrations of rice straw aqueous extract. Application of rice hull extract showed stimulatory effect on stem length of *Silybum marianum* and also enhanced the dry weight of *Echinochloa crusgalli* [20].

Table 1. Effect of aqueous leaf extract of *Meliadubia* on shoot length, root length and collar diameter of *Zea mays*.

Concentration of extracts (%)	Shoot length (cm)	Root length (cm)	Collar diameter (mm)
0	5.13±0.66	5.54±0.51	2.33±0.19
1	6.38±1.31	6.77±0.14	2.39±0.20
2	4.32±0.07	4.64±0.66	2.02±0.06
3	3.39±0.51	3.17±0.47	1.62±0.02
4	2.52±0.30	2.07±0.25	1.46±0.07
5	1.74±0.12	1.55±0.14	1.08±0.11
6	1.50±0.33	1.18±0.14	1.18±0.18
F-ratio	25.953	93.196	46.713
P-value	<0.001	<0.001	<0.001
LSD0.05	1.08	0.67	0.24

Table 2. Effect of aqueous leaf extract of *M. dubia* on shoot, root and total fresh weight of *Zea mays*.

Concentration of extracts (%)	Shoot fresh weight (mg)/5 plants	Root fresh weight (mg)/5 plants	Total fresh weight (mg)/5 plants
0	918.67±231.16	1021.00±270.75	1939.67±467.94
1	1105.33±249.03	735.33±174.15	1840.67±320.01
2	751.67±88.49	631.00±180.34	1383.00±166.59
3	554.0±116.63	552.67±35.02	1106.67±133.82
4	340.67±18.04	330.33±27.54	671.00±43.09
5	215.33±21.22	215.67±15.57	431.00±28.36
6	197.67±29.74	207.00±24.52	404.67±52.29
F-ratio	19.128	13.523	23.006
P-value	<0.001	<0.001	<0.001
LSD0.05	246.35	246.74	403.91

Table 3. Effect of aqueous leaf extract of *M. dubia* on shoot, root and total dryweight of *Zea mays*.

Concentration of extracts (%)	Shoot dry weight (mg)/5 plants	Root dry weight (mg)/5 plants	Total dry weight (mg)/5 plants
0	170.667±55.717	190.333±44.433	361.000±95.828
1	201.667±12.22	136.667±1.528	338.333±10.693
2	108.000±28.213	126.667±15.044	234.667±19.502
3	105.333±32.624	123.33±10.214	228.667±27.154
4	74.667±6.506	100.667±2.309	175.333±6.807
5	58.000±3.000	73.667±4.933	131.667±7.572
6	53.667±3.786	75.667±5.033	129.333±3.512
F-ratio	12.997	14.434	17.181
P-value	<0.001	<0.001	<0.001
LSD0.05	47.634	32.169	67.921

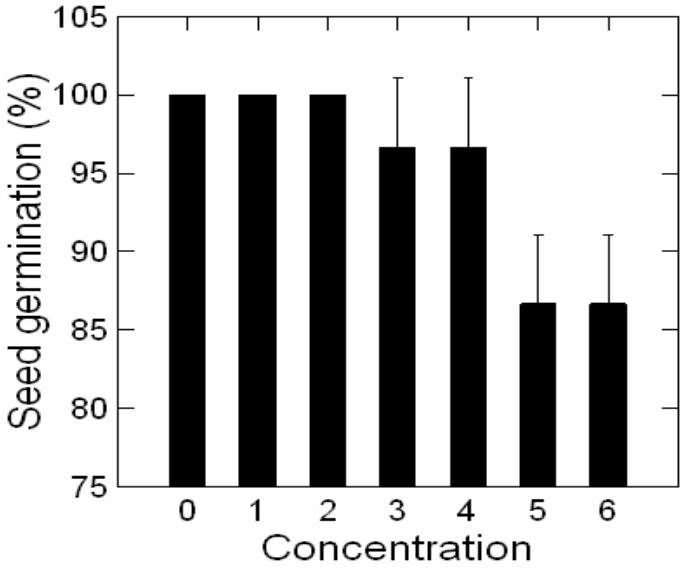

Figure 1. Effect of aqueous leaf extract of *Melia dubia* on seed germination (%) of *Zea mays*.

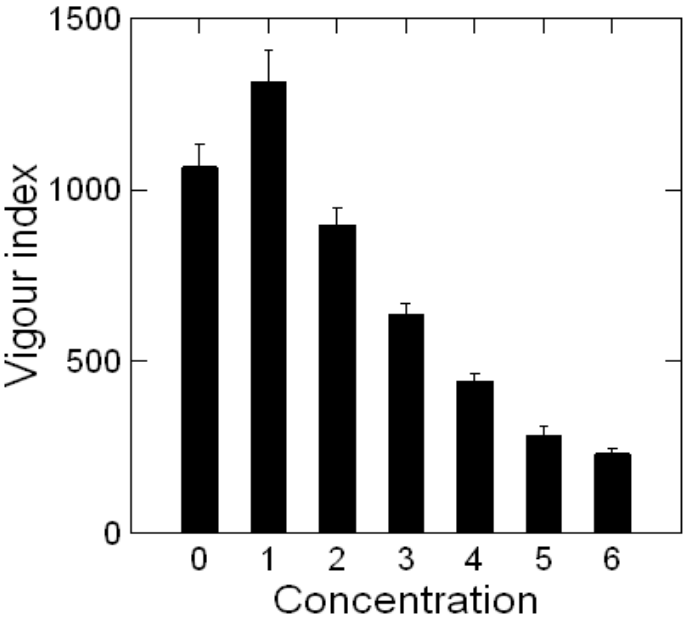

Figure 2. Effect of aqueous leaf extract of *Melia dubia* on vigour index of *Zea mays*.

Results also showed that >2% concentration of aqueous leaf extract of *M. dubia* inhibited the germination of the studied crop. The reduction in the seed germination could be due to less water uptake and alteration in the activity of gibberellic acid [21] which is known to regulate *de novo* production of α-amylase during germination [22]. Thakur *et al*. (2017) analyzed the aqueous leaf extract of *M. dubia* through Gas Chromatography Mass-Spectrometry (GC-MS) and recorded 18 different types of phyto-chemicals which consisted of phenolic acids and their derivatives, omega-3 fatty acid, alkaloids, methyl ketones (volatile allelochemical), unsaturated fatty acids, aromatic ketone and chromene. The alkaloids [23] and phenolics [8] are known to have inhibitory effects. Further, results showed that >1% concentration of the extract significantly affected the studied growth parameters of *Z. mays*, and the degree of the inhibition increased with increase in concentration of the extracts. Different concentrations of the extracts showed different levels of negative effect on total fresh and dry weight. The per cent reduction in total fresh weight and total dry weight of treated plants over control ranged from 5.10-79.14% and 6.28-64.1%, respectively. Our results are in good accord with recent studies [24, 25]. Thakur *et al*. [24] also reported inhibitory effect of different concentrations of *M. dubia* leaf extracts on germination, growth (shoot length, root length and vigour index) and initial biomass (shoot, root and

total biomass) of green-gram and chickpea. Later, Parmar *et al*. [25] assessed the allelopathic effect of aqueous leaf extract of *M. dubia* on chilli and eggplant, and reported similar results. Decreased growth of test crop in present study could be due to inhibition in cell division, as allelo-chemicals reported to inhibit the functions of gibberellin and indole acetic acid that cause reduction in plumule length [26]. Anaya [27] stated that a decrease in plumule and radicle length might be due to decrease in cell division, which ultimately inhibited the fresh and dry weight of the test crops. Baziramakenga *et al.* [28] postulated that development of root is highly susceptible to the presence of allelo-chemicals in the rhizosphere because root tissues are more permeable to these chemicals than shoot tissues. Further, results showed that reduced plumule and radical length leads to reduced shoot and root weight, and thus total fresh as well as dry weight of test crop. Similar results have also been reported by various researchers [29-30]. According to Khan *et al*. [31], presence of proteases in aqueous leaf extracts could be the reason of reduction in total dry weight.

From the present study, it may be concluded that the aqueous leaf extract of *M. dubia*at low concentrations did not have deleterious effect on seed germination. Moreover, 1% concentration of the leaf extract exhibited stimulatory effect in terms of enhanced shoot length, root length, shoot weight and vigour index.The higher concentrations of aqueous leaf extract resulted deleterious effect on growth parameters, but detailed study in field conditions is required to draw the conclusion.

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