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Allelopathic impact of *Anthocephalus cadamba* (Roxb.) Miq. and *Melia dubia* Cav. on *Triticum aestivum* L.: Bioassay study

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Abstract

Anthocephalus cadamba and *Melia dubia* are being promoted under agroforestry in various parts of the country due to their fast growth and multifarious uses. Therefore, it is necessary to study the allelopathic impact of these tree species on understory crops. Hence, present study was conducted to assess the allelopathic effect of aqueous leaf extracts of *A. cadamba* and *M. dubia* on *Triticum aestivum* (wheat) under laboratory conditions. Study consisted of seven concentrations of aqueous leaf extracts of *A. cadamba* and *M. dubia* (0.5, 1, 2, 3, 4, 5 and 6%) along with a control (0%). The results showed that lower concentrations (0.5, 1 and 2%) of aqueous leaf extract of *A. cadamba* had no allelopathic impact, however higher concentration (6%) showed maximum inhibitory allelopathic impact on seed germination (90%). Higher concentrations of *M. dubia* leaf extract also exhibited allelopathic effect on seed germination and resulted in minimum (63.3%) germination at 6% concentration. Similarly, higher concentrations of leaf extracts of both the species resulted in more pronounced allelopathic effect on growth and shoot and root weight of wheat.

Keywords: Allelopathic effect, *Melia dubia*, *Anthocephalus cadamba*, wheat, shoot and root weight

Introduction

Anthocephalus cadamba (Roxb.) Miq. (Rubiaceae) is an important fast growing agroforestry tree species of India. It is distributed naturally in Sub-Himalayan tract, Bihar, Chhattisgarh, Madhya Pradesh, Andhra Pradesh and evergreen forest of Karnataka to Kerala [1-3]. Its wood is utilized for making building, furniture, pulp, plywood, toys and packing cases etc. [4]. *Melia dubia* Cav. (Meliaceae) is also an important fast growing agroforestry tree species of India, which is distributed naturally in tropical and sub-tropical regions [5]. Its wood is utilized for making, furniture, pulp, plywood, packing cases, agricultural implements, pencils, splints etc. and as a source of bio-energy [6].

Nowadays, fast growing tree species are being promoted under agroforestry to sustain food security, livelihood security, environment security vis-a-vis developing a highly productive land use system to reduce the impact of climate change. Thus, compatibility of all the components specially tree and crop is crucial for successful establishment of any agroforestry system. Allelopathy study is one of important aspects of determining compatibility of trees and crops, therefore, it is imperative that tree species having no or positive allelopathic impact on understory crops should be selected for agroforestry system [7]. Inhibited or slow seed germination rate, reduced radicle and plumule growth, necrosis of root tips, the root axis curling and discoloration of tissues are indicator of Allelopathy [8]. Tree species posing negative allelopathic impact on understory crops has been reported by many researchers [4, 9-12]. It has been reported that leachates from leaf possess metabolites that have toxic effects and these metabolic toxins are species or genus specific [13, 14].

Materials and Methods

Fresh, fully matured, disease free leaves of *A. cadamba* and *M. dubia* were collected from established agroforestry systems at central research farm of ICAR-Central Agroforestry Research Institute, Jhansi (24° 11' N latitude and 78° 17' E longitude). Leaves were washed with distilled water and then air-dried at room temperature till constant weight, and later these were oven dried at 68 ^{0}C for 72 hours. The dried samples were made into fine powder with the help of electronic grinder.

Preparation of aqueous leaf extracts
Leaf powder (150 g) was added to 1 L distilled water (*w*/*v*), mixed manually with the help of glass rod and then kept on electric shaker for 15 minute for achieving uniform mixing. Thereafter, shaking mixture of leaf power and distilled water was kept in at room temperature for 24 hours for extraction of water soluble petro-chemicals. Then this extract was filtered by using muslin cloth, followed by Whatman filter paper No. 1. This extract was used as a stock solution (15%) and then various other concentrations viz. 0.5, 1, 2, 3, 4, 5 and 6 were made separately in flasks, labeled and stored in a refrigerator, when not used. One more treatment, control (0% i.e. distilled water or no leaf extract) was also included in the experiment.

Bio-assay experiment
The seeds of *Triticum aestivum* L. (wheat) were surface-sterilized with 0.1% mercuric chloride for 5 minutes, and then washed properly five to six times to remove traces of mercuric chloride. After washing, seeds were dried on an absorbent paper. Ten sterilized seeds of wheat were evenly placed on filter paper in sterile petri dishes (4 replications each treatment) at room temperature in laboratory. 2 ml of various concentrations of the aqueous leaf extracts (0.5, 1, 2, 3, 4, 5 and 6%) and distilled water (control) were added to Petri dishes containing seeds.

Observation
Seed germination percentage (radicle protuberance of at least 1 mm), shoot length (cm) and root length (cm) of the crop was recorded. At the end of the experiment i.e. after 15 days, shoot length and root length of the crop were measured with the help of a ruler. Thereafter, five plants were randomly selected from each replication and analyzed for fresh and dry weight. 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.

Statistical analysis
All the data were subjected to two-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
Seed germination
Higher concentrations of aqueous leaf extract of *A. cadamba* and *M. dubia* affected the seed germination of wheat. However, leaf extract of *M. dubia* showed higher inhibitory effect and only 63.3% germination was resulted in comparison to *A. cadamba* (90%) at 6% concentration. In *A. cadamba,* up to 2% concentration the germination was not affected and 100% germination was recorded. The impact of interaction of leaf extract concentrations and tree species was observed to be non-significant (Table 1).

Growth parameters
Data presented in Table 2 revealed that with the increase in concentration of leaf extract the shoot and root length of wheat decreased. The lowest values of shoot length were recorded at 6% concentration of *A. cadamba* (9.7 cm) and *M. dubia* (9.5 cm). Similarly, the lowest root length was also recorded at 6% concentration of *A. cadamba* (8.7 cm) and *M. dubia* (5.6 cm). These results showed that inhibitory effect was relatively higher in *A. cadamba* than *M. dubia.* The observations on wheat biomass showed that both fresh as well as dry shoot and root weight was recorded maximum in control. It decreased with the increase in concentration of aqueous leaf extracts of *A. cadamba* and *M. dubia*. The lowest fresh and dry shoot and root weight of wheat was observed at 6% concentration of leaf extract of *A. cadamba* and *M. dubia*. The impact of interaction of leaf extract concentration and tree species on fresh and dry shoot as well as root biomass was observed to be non-significant (Table 3 and 4). Overall impact of aqueous leaf extract of *A. cadamba* and *M. dubia* on total fresh and dry biomass in wheat crop has been presented in Figure 1 and revealed that aqueous leaf extract of *A. cadamba* showed higher inhibitory impact than aqueous leaf extract of *M. dubia*.

Thus, results showed that higher concentration **of** aqueous leaf extract of *M. dubia* and *A. cadamba* had inhibitory impact on seed germination of test crop. Perusal of various allelopathic studies revealed that inhibition of seed germination is generally attributed to the change in pH [15, 16], presence of phenolic content in plant extracts [17] and due to alterations in the enzymatic activity of seeds. Similarly, in our study reduction in germination percentage with increase in aqueous leaf extract concentration may be attributed to the increase in acidic nature of aqueous extracts, alteration of enzymatic activities and presence of allelochemicals [18]. Phytotoxic chemicals impact gibberellic acid activities in seed which is important for production of α-amylase responsible for degradation of reserved carbohydrate to soluble sugars, therefore stored compounds mobility is impacted leading to inhibited or reduced seed germination [18]. Leaf litter of *M. dubia* contains phenolic acids derivatives, unsaturated fatty acid, alkaloids, methyl ketones etc. which inhibits the seed germination and with increase in leaf extract concentration, the germination inhibition also increased in *Vigna radiata* L. and *Cicer arietinum* L. [19]. Almost similar results have been observed in our study.

Concentration dependent effects on germination percentage, shoot length, root length, shoot biomass, root biomass and total biomass were observed which are in confirmation with the studies carried out on *M. dubia* leaf extract [19] and *M. azedarach* leaf extract impact on pulse crops [20, 21]. *A. cadamba* leaf also possesses alkaloids [22] and its leaf extract has also been reported to inhibit crop growth as compared to control [23] which is also in confirmation with our study. The results showed that aqueous leaf extracts of *A. cadamba* and *M. dubia* inhibited the shoot and root length of wheat crop and degree of inhibition increased with increase in concentration of leaf extracts. Enzymatic activities of plants receiving allelopathic compounds are impacted via increased production of reactive oxygen species that leads to oxidative stress [24-26]. Allelo-chemicals present in extract inhibit cell division and impact functions of gibberellin and indole acetic acid which reduces the plumule length [27]. Further physiological changes occur in plants exposed to allelo-chemicals which impacts seedling growth [28]. Root tissues are more permeable to allelopathic compounds [29] which inhibit cell division in meristematic region of root tip [30-32]. Decrease in cell division leads to decrease in plumule and radicle length that ultimately inhibits the fresh and dry weight of crops. Allelo-chemicals present in the aqueous extracts of the *A. cadamba* and *M. dubia* have exerted inhibitory effects on shoot and root growth, dry shoot and root weight as well as total dry weight of wheat crop. Similar results have also been reported by various researchers [33-35].

Table 1: Effect of different concentrations of aqueous leaf extracts of *A. cadamba* and *M. dubia* on seed germination (%) of wheat

Concentration (%)	**Tree species**		**Mean**
	A. cadamba	***M. dubia***	
0	100.0±0.0	100.0±0.0	100.00
0.5	100.0±0.0	96.7±5.8	98.3
1.0	100.0±0.0	93.3±11.5	96.7
2.0	100.0±0.0	93.3±5.8	96.7
3.0	93.3±5.8	90.0±10.0	91.7
4.0	93.3±11.5	89.7±0.6	91.5
5.0	93.3±11.5	86.7±5.8	90.0
6.0	90.0±0.0	63.3±15.3	76.7
Mean	96.3	91.50	
	Tree species	Concentration	Interaction
F-ratio	6.064	11.242	1.874
P-value	<0.001	0.002	0.107
$LSD_{0.05}$	8.7	4.3	NS

Table 2: Effect of different concentrations of aqueous leaf extracts of *A. cadamba* and *M. dubia* on shoot and root length of wheat

Concentration (%)	**Tree species**		**Mean**
	A. cadamba	***M. dubia***	
Shoot length (cm)			
0	11.6±0.7	12.5±1.0	12.1
0.5	11.0±0.3	12.1±0.3	11.6
1.0	10.5±0.9	12.1±0.2	11.3
2.0	10.4±0.5	11.9±0.2	11.2
3.0	10.3±0.3	11.8±0.9	11.1
4.0	9.8±0.5	11.3±0.5	10.6
5.0	9.6±0.9	10.6±0.7	10.1
6.0	9.5±0.1	9.7±1.2	9.6
Mean	10.4	11.5	
Root length (cm)			
0	19.5±1.0	19.8±1.4	19.7
0.5	16.7±0.3	18.2±0.9	17.5
1.0	15.7±0.6	16.8±0.8	16.3
2.0	9.1±1.0	15.4±0.5	12.3
3.0	7.4±0.5	14.3±1.2	10.9
4.0	6.3±0.3	12.1±0.2	9.2
5.0	5.9±0.3	11.2±0.9	8.6
6.0	5.6±0.7	8.7±0.4	7.1
Mean	10.8	14.6	

	Shoot length			**Root length**		
	***F*-ratio**	***P*-value**	**$LSD_{0.05}$**	**F-ratio**	**P-value**	**$LSD_{0.05}$**
Tree species	35.591	<0.001	0.4	280.296	<0.001	0.5
Concentration	8.946	<0.001	0.8	205.275	<0.001	0.9
Interaction	0.727	0.650	NS	16.302	<0.001	1.3

Table 3: Effect of different concentrations of aqueous leaf extracts of *A. cadamba* and *M. dubia* on fresh weight (mg)/5 seedlings of wheat

Concentration (%)	**Tree species**		**Mean**
	A. cadamba	***M. dubia***	
Fresh shoot weight (mg)/5 seedlings			
0	457.67±24.71	563.00±64.63	510.33
0.5	407.33±55.63	526.67±45.62	467.00
1.0	384.33±7.43	507.33±39.53	445.83
2.0	336.33±10.50	502.67±79.26	438.17
3.0	373.67±40.50	485.33±16.62	419.67
4.0	354.00±50.90	467.67±4.93	408.00
5.0	348.33±16.50	450.33±39.53	393.33
6.0	336.33±35.08	361.33±35.23	346.33
Mean	374.13	483.04	
Fresh root weight (mg)/5 seedlings			
0	661.67±38.50	613.33±134.45	637.50
0.5	636.00±46.29	606.67±67.21	621.33
1.0	513.67±76.35	564.33±24.01	539.00
2.0	362.67±16.07	537.00±147.00	449.83
3.0	362.67±16.07	460.33±15.95	411.50
4.0	282.00±16.01	382.67±121.74	332.33
5.0	236.33±17.21	345.33±46.06	290.83

6.0	225.00±30.81		304.00±32.19			264.50
Mean	410.00		476.71			
	Fresh shoot weight			**Fresh root weight**		
	***F*-ratio**	***P*-value**	**$LSD_{0.05}$**	***F*-ratio**	***P*-value**	**$LSD_{0.05}$**
Tree species	85.249	<0.001	24.09	11.470	0.002	40.22
Concentration	8.757	<0.001	48.18	27.093	<0.001	80.44
Interaction	0.974	0.467	NS	1.765	0.129	NS

Table 4: Effect of different concentrations of aqueous leaf extracts of *A. cadamba* and *M. dubia* on dry weight (mg)/5 seedlings of wheat

Concentration (%)	**Tree species**					**Mean**
	A. cadamba		***M. dubia***			
Dry shoot weight (mg)/5 seedlings						
0	48.60±4.00		63.00±4.28			55.58
0.5	47.97±3.81		61.60±8.18			54.78
1.0	47.60±8.00		60.93±5.30			54.27
2.0	46.50±7.37		58.00±6.00			52.25
3.0	44.50±2.71		57.57±1.39			51.03
4.0	43.53±0.32		56.77±2.65			50.15
5.0	43.03±2.38		42.47±3.80			42.75
6.0	42.93±4.74		40.93±23.90			41.93
Mean	45.58		55.16			
Dry root weight (mg)/5 seedlings						
0	63.07±7.70		58.73±8.19			60.90
0.5	55.07±8.27		55.33±5.77			55.20
1.0	52.33±4.58		50.37±7.21			51.35
2.0	42.80±2.63		54.27±2.00			48.53
3.0	42.17±2.25		46.50±14.27			44.33
4.0	40.77±1.08		51.63±4.70			46.20
5.0	38.13±4.15		42.13±2.67			40.13
6.0	36.83±6.32		38.40±2.25			37.62
Mean	46.40		49.67			
	Dry shoot weight			**Dry root weight**		
	***F*-ratio**	***P*-value**	**$LSD_{0.05}$**	***F*-ratio**	***P*-value**	**$LSD_{0.05}$**
Tree species	18.966	<0.001	4.49	3.358	0.076	NS
Concentration	2.917	0.018	8.98	9.305	<0.001	7.30
Interaction	1.182	0.341	NS	1.251	0.305	NS

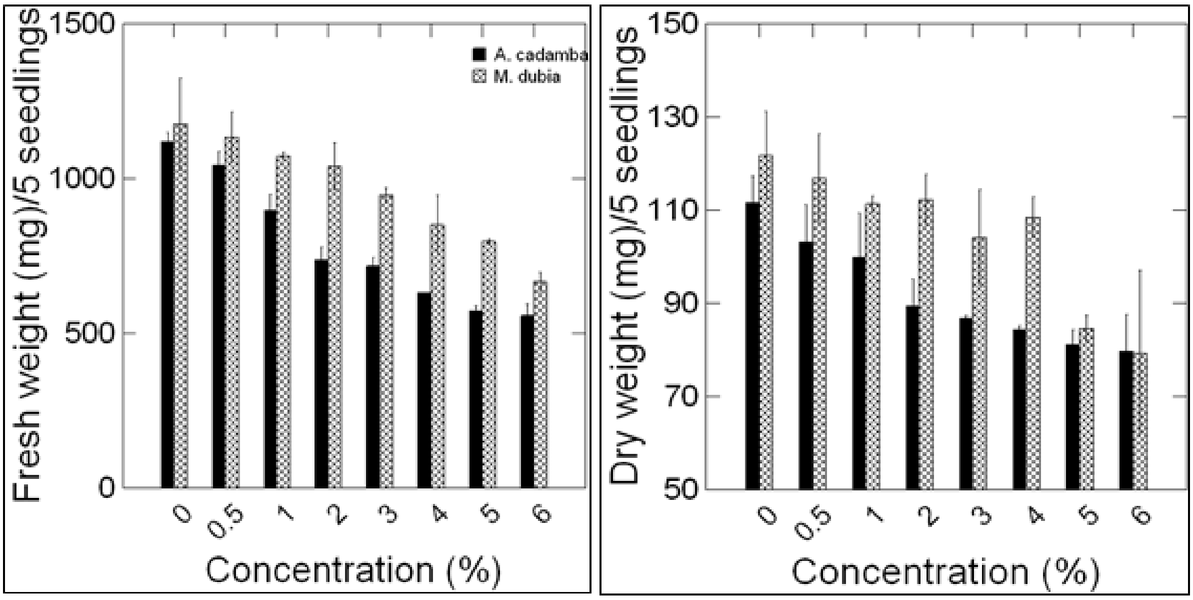

Fig 1: Effect of different concentrations of aqueous leaf extracts of *A. cadamba* and *M. dubia* on fresh and dry weight of wheat

Conclusion

From the present study, it may be concluded that the aqueous leaf extract of *A. cadamba* up to 2% concentration had no allelpathic effect on seed germination, but at higher concentrations allelopathic effect was observed. Whereas, in *M. dubia* the germination was affected even at lower concentrations but the effect was more at higher concentration. Similarly, growth attributes of wheat crop was adversely affected at higher concentrations of leaf extracts of both the species, but detailed study in field conditions is required to draw the conclusion.

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