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ALLELOPATHIC PROPENSITY OF THE AQUEOUS LEAF EXTRACT AND LEAF LITTER OF *Melia dubia* CAV. ON PULSE CROPS

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Pot culture

ABSTRACT

Gas Chromatography Mass-Spectrometry (GC-MS) analysis revealed that leaf litter of *Melia dubia* contain phenolic acids and its derivatives, unsaturated fatty acid, alkaloids, methyl ketones (volatile allelochemical), aromatic ketone, chromene *etc.* Further it was reported that the aqueous leaf extracts (0, 25, 50, 75 and 100% concentration) and leaf litter (0, 5, 10, 15 and 20 g/pot) inhibited the germination, growth (shoot length, shoot length and vigour index) and initial biomass (shoot, root and total biomass) of green gram and black chickpea. Percentage of inhibition in germination and initial growth parameters increased with the increasing the concentration of aqueous extract or litter amount of *M. dubia*. However, pot experiments, carried out till crop maturity, revealed that there was no significant allelopathic effect on growth, biomass and grain yield of the test crops. This indicates that the allelochemicals present in *M. dubia* leaf litter are volatile in nature and their effect is transient in nature.

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1 Introduction

Melia dubia Cav. (Syn. *Melia composita* Willd.) is being planted in agroforestry system either in block plantation or along the farm boundary. Commonly known as Malabar neem/ Burma neem, is an industrially and economically important fast growing tree species, which can be harvested on a short rotation (Chauhan & Ritu 2005; Chavan et al., 2015). It is indigenous to the Western Ghats of Southern India and is common in moist deciduous forests of Kerala and outside India, it is found in Sri Lanka, Malaysia, Java, China and Australia (Saravanan et al., 2013). It is valued for its high-quality termite and fungus resistant timber for furniture, agricultural implements and house construction (Suprapti et al., 2004), as alternative pulp wood species, fuel wood and leaf used as a fodder (Parthiban et al., 2009) and has medicinal properties as well (Vijayan et al., 2004). The industrial and ecological importance of *M. dubia* has encouraged the farmers to take large scale plantations with different intercrops (Parthiban et al., 2009; Nuthan et al., 2009).

In some of the woody species, in agroforestry systems, allelopathic effects on under storey crops have been reported (Gupta et al., 2007; Narwal et al., 2011; Gunarathne & Perera, 2016). Hence to answer such queries of farmers who are interested to integrate this valuable species allelopathic investigations are to be done.

Therefore, keeping in view the importance and increasing popularity of this species, the present investigation was undertaken to investigate the allelochemicals in leaf litter of *M. dubia* to divulge the beneficial or antagonistic effect of aqueous leaf extract and leaf litter on germination, growth, biomass and yield of pulse crops in laboratory bioassay and pot culture.

2 Materials and methods

The present investigations were accomplished in the agroforestry laboratory as well as in the green house complex of College of Forestry, ACHF, Navsari Agricultural University, Navsari, Gujarat, India (20.95° N latitude, 75.90° E longitude with an altitude of 10 m above MSL) during November 2014 to April 2015.

2.1 Leaf litter chemical analysis

The alleged allelopathic compounds in leaf litter samples of *M. dubia*, used in the present study, were detected through Gas Chromatography-Mass Spectrometry (GC-MS) as described by Murugesan et al. (2013).

2.2 Allelopathic studies

2.2.1 Plant material and preparation of aqueous extracts

The leaf litter (mixture of young and mature leaves showing signs of senescence) of *M. dubia* were collected from 3 year old plantations during October-November 2014. Leaf litter was initially dried at room temperature and later at 65° C in hot air oven until constant dry weight was reached (Perez-Corona et al., 2013). The dried leaf litter was stored at room temperature and was used for both petriplate and pot experiments bioassay. Aqueous extracts were prepared by soaking 200g of grounded dried leaf litter in 1L distilled water. The solution was stirred and kept at room temperature (20-25°C) for 24 hours. The filtrate was centrifuged and supernatant was decanted (Prasad et al., 2011). The filtrate was defined as 100 per cent extract and was further diluted with distilled water at 25, 50, 75, 100 per cent concentrations (Nikneshan et al., 2011) while distilled water was used as control T₁ (0 %) (Lawan et al., 2011).

2.3 Petridish bioassay experiment

The seeds (treated with Thirum @ 2g/kg) of green gram [*Vigna radiata* (L.)] and black chickpea (*Cicer arietinum* L.) were procured from Pulses and Castor Research Unit, Navsari Agricultural University, Navsari, Gujarat, India. In the laboratory experiment, five treatments of leaf aqueous extracts (0 to 100% concentration) of donor species were used with five replications for each. Each petridish of size 90 mm diameter was considered as replication. Total 50 seeds of both the test crops were placed on filter paper in sterilized petridishes and 5 ml of aqueous extract was applied on first day and after that, 2 ml was applied at alternate day to keep the filter paper moist till the completion of experiment (Bhat et al., 2011). Seeds were considered germinated upon radicle emergence. Daily germination count was made up to 9th day from day after extract treatment (DAET). Growth and biomass attributes were recorded by randomly selecting 10 seedlings from each replication on 11th DAET. Germination percentage and Germination Rate Index (GRI) were calculated following standard procedure (Anonymous 1983; Anonymous 1985). The shoot, root length and biomass were estimated randomly selecting ten seedlings from each replication on the 11th DAET. Root and shoot portion was dried separately in hot air oven at 60° C for 48 h and then samples were weighed using sensitive balance to estimate biomass.

2.4 Pot experiment

Pot experiments were conducted to investigate the effect of leaf litter of *M. dubia* on germination indices, initial growth and biomass of both the test crops. Leaf litter (course grounded mixture of young and mature leaves showing signs of senescence)

was used in five concentrations viz., T₁ (Control), T₂ (5 g), T₃ (10 g), T₄ (15 g) and T₅ (20 g), these concentration were mixed in the upper soil layer of the pots of concern treatment (Thakur 2014). Soil without leaf litter was used as a control treatment. Each treatment was replicated five times. The litter treatments imposed were according to annual average litter fall (Li et al., 2013), where leaf litter fall of three months data was recorded by placing the 1 m² traps under 3 years old plantation of *M. dubia* and average leaf litter fall of three months (216.45 g/m²) was considered and extrapolated for pot with top area of 0.026 m² i.e. 5.63 g/pot. Hence, the leaf litter treatments were fixed within the range of average litter fall. Total 50 seeds of each crop were sown in the plastic pots [18 cm diameter x 16 cm height (4070 cc)] containing approximately 2.5 kg soil having N, P and K content of 84.82, 17.85 and 80.35 ppm, respectively. Pots were irrigated with tap water (pH 7.71 and electrical conductivity 1.752dS/m) and seeds were sown after 24 hours. Daily germination count was made up to the last seed to germinate i.e., 8 days. Shoot and root length, and biomass were recorded on the 11th day randomly selecting 10 seedlings from each replication. Shoot and root samples were dried in hot air oven at 60^o C for 48 h and then dry biomass was recorded. Germination percentage and Germination Rate Index (GRI) were calculated as per standard procedure followed in the laboratory bioassay.

2.5 Pot experiments (up to crop maturity)

In order to evaluate the allelopathic effect of leaf litter till crop maturity a separate experiment was laid out by following same

procedure as adopted in pot experiment to study the germination, initial growth and biomass. Each litter treatment was replicated five times (three plants per replication). However, in each pot, only five seeds were sown and one seedling was retained after two weeks of sowing for further observations. The pots were kept in green house with 50 per cent relative shading. Growth parameters such as plant height, root length, no. of leaves, no. of branches, no. of flower, no. of pods per plant and average leaf area per plant was recorded. Furthermore, fresh and dry biomass of plant and grain yield was also recorded at crop maturity, when 80 percent of pods matured (3 months after sowing). Pods were separated from the plants and threshed to record the grains.

2.4 Statistical analysis

The experimental data of all the parameters studied in different experiments were subjected to the statistical analysis following completely randomized design (CRD) and F-test was done and ANOVA was constructed following Sheron et al. (1998). Treatment means were compared at P<0.05. Further, Duncan's multiple range test (MRT) was used to compare the sets of means of each treatment.

3 Results and discussion

3.1 Leaf litter Phytochemicals

Gas Chromatography Mass-Spectrometry (GC-MS) screening of *M. dubia* leaf litter revealed the presence of 18 different types of phytochemicals (Table 1). Among the detected compounds most

Table 1 Chemical compounds, their retention times and area under curve detected in (GC-MS) analysis of *M. dubia* leaves

Sr. No.	Compound name	Retention time	Area under Curve
1	Ethanone 1 (2-aminophenyl), Acetophenone	5.47	131541
2	2 (3-hydroxy-4-methoxyphenyl) 3,5,7 Trimethoxy 4-H-chroman-4-one	6.79	114900
3	Papaveroline 6-O-methyl Or 1,2-Benzenediol,4-[(7-hydroxy-6-methoxy-1- isoquinolinyl) methyl]	9.66	408430
4	5-methyl (5-8 dihydro 1-4 Naphthoquinone) or 1,4-aphthalenedione, 5,8-dihydro-5-methyl-	9.70	203565
5	2-propanone, 1-1-diethoxy- or 1,1-diethoxypropan-2-one	9.77	95117
6	2,3,4,5-tetrahydro-1-benzoxepine	10.57	415469
7	1,5 Anhydro-3-6 di-O-acetyl (2,4 di-O-methyl D-glucitol or D-Glucitol, 1,5-anhydro-2,4-di-O-methyl-, diacetate	11.30	696171
8	4-Piperidinol, 1-(2-phenoxyethyl)-4-phenyl-	12.99	55688
9	2,4-Dimethyl-5,6-dithia-2,7-nonadienal, 5-oxide	14.79	348465
10	2-Methyl-3,5-dodecadiyne	15.14	608896
11	1,4 dithiepan-2-one-3-phenyl	15.39	417663
12	1,3-Dioxolane-4-methanol, 2-pentadecyl-, acetate, trans-	15.56	23571
13	2, methyl, 2 phenyl-5 (1-4, dihydropyridine- 4-yidene)-1,3-dioxan-4-6 dione	15.64	52239
14	Methyl 4-6 tetradecadiynoate	16.24	115370
15	1H-Purine-2,6-dione, 8-(1,2-dibromo-2-phenylethyl)-3,7-dihydro-1,3,7-trimethyl	16.95	347653
16	3,9 Epoxypregnane-11,14,18 triol-20-one 16 cyano-3methoxy, 11 acetate	20.74	280670
17	Eicosapentaenoic Acid or Icosapent	21.83	98308
18	Oxazole, 5-ethyl-2-methyl-4-benzoyl-	26.87	352502

common are phenolic acids and their derivatives, omega-3 fatty acid, alkaloids, methyl ketones (volatile allelochemical), unsaturated fatty acids, aromatic ketone and chromene. Chromatograms showing the relative abundance, retention time and area under curve of chemical compounds detected and are presented in figure 1. The phytochemicals detected in this study

through GC-MS have also been reported by Valentina et al. (2013) and Murugesan et al. (2013) in *M. dubia* extract. Further, the phytochemicals like 3, 4-Dihydroxyacetophenone, 4-hydroxybenzoic acid, Piperidinol, Tetradecanoic acid, 2,4-Dimethyl-5,6-dithia-2,7-nonadienal, 5-oxide, Icosapentaenoic acid, Naphthoquinone, Purine, D-Glucitol etc., detected in leaf

Figure 1 GC-MS chromatogram showing retention time and peaks of different chemical compounds in *Melia dubia* leaf litter

litter of *M. dubia* in this study, have been reported in other woody and non woody species and are alleged for their inhibitory allelopathic effect (extract or leaf litter) on germination and growth of various test crops (Suzuki et al., 1996; Duke et al., 2000; Kim & Kil 2001; Rezaeinodehi et al., 2006; Peneva 2007; Hongying & Hong 2008; Kato-Naguchi 2008; Koder 2011; Ruan et al., 2011; Jones et al., 2012; Aslani et al., 2014). However, this study has first report of allelopathic nature of leaf litter of *M. dubia*.

3.2 Laboratory and pot culture bioassays: Germination and its attributes

Laboratory and pot culture bioassays revealed that, aqueous leaf extract and leaf litter of *M. dubia* significantly ($P < 0.05$) inhibited the germination (%) and germination rate index (GRI) of green gram and black chickpea (Table 2) relative to control (distilled water or no litter). The inhibitory effect gradually increased with incremental extract concentrations or leaf litter amount, over the control (Figure 2 A to D).

Figure 2 Showing the allelopathic influence of aqueous leaf extracts [0, (distilled water), 25, 50, 75 and 100%] and leaf litter [0 (no leaf litter), 5, 10, 15 and 20 g/pot] of *M. dubia* on germination and initial growth of green gram and black chickpea in laboratory (a and b) and pot culture bioassays (c and d), respectively.

The magnitude of per cent inhibition, over control, in all the germination parameters of green gram and black chickpea (Figure 3 to 6) increased with increase in extract concentration and leaf litter quantities with greatest at maximum concentration (100%) or litter amount (20 g/pot). The per cent reduction on germinations attributes was higher in laboratory bioassays as compared to pot culture.

3.3 Initial growth and biomass

The leaf aqueous extract under laboratory and leaf litter in pot culture, exhibited significant ($P < 0.05$) inhibitory effect on growth parameters viz., shoot and root length of germinated seedling of green gram and black chickpea (Table 2 and 3). The data indicates that, growth parameters had gradual inhibitory effect as the extract concentration or litter quantity increased when compared with the control treatment. The magnitude of per cent inhibition in growth parameters of green gram (Figure 3 & 5) and black chickpea (Figure 4 & 6) over control, against aqueous extract and leaf litter, gradually increased with increase in extract concentration or litter amount with maximum at 100% extract concentration or maximum litter application i.e. 20 g litter/pot. The per cent reduction was more marked in root growth as compared to shoot except in black chickpea, where shoot length experienced little higher percent reduction, in laboratory bioassay. The reduction percentage was more in laboratory bioassay as compared to pot experiments.

The leaf extracts as well as leaf litter exhibited significant inhibitory effect on shoot, root and total dry biomass of germinated seedlings (Table 2 and 3). The magnitude of diminution progressed gradually with increase in leachate concentration or litter application over the control with maximum at 100% extract concentration and 20 g leaf litter/pot.

Intensity of per cent inhibition, over control, in growth traits of green gram and black chickpea (Figure 3 to 6), increased with increase in extract concentration or leaf litter quantities of *M. dubia*. All the biomass attributes experienced greater magnitude of reduction due to aqueous extracts compared to litter application in pots, over control treatments.

The magnitude of inhibition on germination indices, initial growth and biomass of seedlings increased with incremental aqueous extract concentration and leaf litter quantity. This showed concentration dependent effect of

Figure 3 Per cent inhibition (over control) in germination, growth and biomass of green gram against aqueous leaf extract of *M. dubia*

Figure 4 Per cent inhibition (over control) in germination, growth and biomass of black chickpea against aqueous leaf extract of *M. dubia*

Figure 5 Per cent inhibition (over control) in germination, growth and biomass of green gram against leaf litter of *M. dubia*

aqueous extract and leaf litter. The findings are in congruence with earlier laboratory bioassays of *M. azedarach* on pulse crops (Phuwiwat et al., 2012; Akacha et al., 2013). This may be attributed to water soluble nature allelochemicals (Table 4) in leaf litter (Rezaeinodehi et al., 2006). Petridish bioassay and pot culture experiments revealed that per cent depression effect was more pronounced on root growth in laboratory bioassay as well as in pot experiments. Similar organ specific effects of *M. azedarach* leaf aqueous extracts have been reported earlier (Lungu et al., 2011; Phuwiwat et al., 2012; Akacha et al., 2013). This may be attributed to the fact that roots first come in contact with allelochemicals and are the first to absorb them from the environment in which they are growing and cell death and tissue browning frequently occur in the root apical zone, an area with active cell division (Rezaeinodehi et al., 2006; Ding et al., 2007).

Similar to present findings, it has been reported that young seedlings are more sensitive to allelopathicals compared to adult plants or other plant organs, especially the roots (Wu et al., 2007; Zhang et al., 2010).

Table 2 Effect of aqueous leaf extract of *M. dubia* on germination traits, initial growth and biomass green gram and black chickpea in bioassay culture

Extract concentration (%)	Germination traits		Growth (cm)		Biomass (DM mg/plant)		
	G (%)	GRI	Shoot length	Root length	Shoot	Root	Total
Green gram							
Control (0%)	97.60 (77.17) ^a	91.36 ^a	7.31 ^a	2.90 ^a	13.82 ^a	8.83 ^a	22.65 ^a
25%	87.60 (69.29) ^b	63.16 ^b	6.15 ^b	2.89 ^a	12.27 ^b	7.58 ^b	19.85 ^b
50%	82.00 (65.04) ^c	51.86 ^c	5.75 ^c	2.11 ^{ab}	10.11 ^c	6.52 ^c	16.63 ^c
75%	77.20 (60.95) ^c	47.14 ^c	4.71 ^d	1.81 ^b	8.53 ^d	5.44 ^d	13.97 ^d
100%	69.20 (55.19) ^d	42.19 ^c	3.72 ^e	1.61 ^b	7.47 ^e	4.56 ^e	12.04 ^e
CD (P ≤ 0.05)	2.99	2.45	0.20	0.22	0.79	0.33	1.05
SEm (±)	1.01	0.83	0.07	0.08	0.27	0.11	0.35
Black chickpea							
Control (0%)	97.20 (80.47) ^a	37.95 ^a	3.40 ^a	4.00 ^a	23.33 ^a	21.25 ^a	44.80 ^a
25%	80.80 (64.02) ^b	30.91 ^{ab}	2.50 ^b	3.10 ^b	21.07 ^b	19.25 ^b	40.32 ^b
50%	63.60 (52.89) ^c	25.01 ^{bc}	2.10 ^c	2.50 ^c	18.60 ^c	17.00 ^c	35.60 ^c
75%	60.00 (50.82) ^c	19.50 ^c	1.70 ^d	2.10 ^d	16.01 ^d	14.63 ^d	30.63 ^d
100%	48.00 (43.83) ^d	14.96 ^c	1.40 ^e	1.90 ^e	13.08 ^e	11.95 ^e	25.03 ^e
CD (P ≤ 0.05)	4.10	1.49	0.12	0.12	1.29	1.05	2.48
SEm (±)	1.38	0.50	0.04	0.04	0.44	0.35	0.84

*Figures in parenthesis are the transformed values; G=Germination; GRI=Germination Rate Index; MDG=Mean Daily Germination; DM=Dry Matter; CD= Critical difference; SEM= Standard error of mean, Letter different in same vertical column are significantly different according to Duncan's multiple range test (P ≤ 0.05).

Table 3 Effect of leaf litter of *M. dubia* on germination traits, initial growth and biomass of green gram and black chickpea in pot culture

Leaf litter (g/pot)	Germination traits		Growth (cm)		Biomass (DM mg/plant)		
	G (%)	GRI	Shoot length	Root length	Shoot	Root	Total
Green Gram							
No litter	94.80 (77.07) ^a	24.24 ^a	15.50 ^a	12.20 ^a	47.52 ^a	26.61 ^a	74.12 ^a
5 g	86.20 (68.18) ^b	23.44 ^{ab}	14.40 ^b	10.90 ^b	40.19 ^b	22.51 ^b	62.70 ^b
10 g	78.20 (62.15) ^c	20.82 ^b	13.60 ^c	9.60 ^c	34.80 ^c	19.49 ^c	54.30 ^c
15 g	70.40 (57.03) ^d	18.05 ^c	12.90 ^d	8.30 ^d	32.20 ^{cd}	18.03 ^{cd}	50.23 ^{cd}
20 g	62.20 (52.05) ^e	16.37 ^c	12.30 ^e	7.30 ^e	30.39 ^d	17.02 ^d	47.41 ^d
CD (P ≤0.05)	2.61	0.71	0.43	0.94	2.34	0.89	4.50
SEm (±)	0.88	0.24	0.14	0.32	0.79	0.30	1.51
Black chickpea							
No litter	94.40 (76.75) ^a	12.19 ^a	17.20 ^a	18.60 ^a	59.27 ^a	32.04 ^a	91.31 ^a
5 g	87.20 (69.04) ^b	10.70 ^a	16.60 ^{ab}	17.90 ^{ab}	52.78 ^b	28.53 ^b	81.30 ^b
10 g	80.00(63.44) ^c	10.74 ^a	15.80 ^{bc}	17.00 ^{bc}	48.44 ^c	26.18 ^c	74.62 ^c
15 g	74.80 (59.87) ^d	8.36 ^b	14.70 ^c	16.10 ^c	45.68 ^d	24.70 ^d	70.38 ^d
20 g	68.00 (55.53) ^e	8.33 ^b	13.90 ^c	15.30 ^c	43.83 ^e	23.69 ^e	67.52 ^e
CD (P ≤0.05)	3.09	0.63	0.41	0.68	0.91	0.49	1.40
SEm (±)	1.04	0.21	0.14	0.23	0.31	0.17	0.47

*Figures in parenthesis are the transformed values; G=Germination; GRI=Germination Rate Index; MDG=Mean Daily Germination; DM=Dry Matter; CD= Critical difference; SEM= Standard error of mean, Letter different in same vertical column are significantly different according to Duncan's multiple range test ($P \leq 0.05$).

This study is the first to report the allelopathic nature of *M. dubia* leaf extract and leaf litter. Similar compounds have been detected in *M. azedarach*, member of same family, alleged for allelopathic influence on germination and initial growth and biomass of various crops (Mulatu et al., 2011; Shapla et al., 2011). The aqueous leaf extracts of plant species may hamper physiological processes of germinating seeds and growing seedlings. Such physiological hindrances might have resulted due to allelochemicals of *M. dubia* in present investigations. Phuwiwat et al. (2012) observed that water uptake and α -amylase activity of *Echinochloa crusgalli* was inhibited by aqueous extracts of young leaves (12.5 to 100 mg/mL) of *M. azedarach* and water soluble allelochemicals caused inhibition of both water uptake and α -amylase activity during germination process as compared to control. Metabolism activation within seed occurs in adequate moisture (Chong et al., 2002), which may be restricted in germinating seeds due to inhibition of specific enzymes. Germination inhibition could be the result of induction of oxidative stress (Javed, 2011).

All these findings may be ascribed to the inhibitory effect of *M. dubia* aqueous extracts on seed germination of green gram and black chickpea in the present study. Earlier studies showed that addition of leachates or incorporation of plant residues into the growth environment of another plant can result in inhibition effect on germination and growth due to depletion of the nitrogen content and impeding of the physiological processes of the seedlings growing in such environment (Al-Khatib et al., 1997). Similar effects might have resulted in reduced germination, growth and biomass of black gram against leaf mulch of *M. dubia* as compared to control in the present study. Akacha et al. (2013) reported that *M. azedarach* allelochemicals produced an imbalance in the oxidative status of cells and these allelochemicals made changes in activity of catalase (CAT), ascorbate peroxidase (APX), guaiacol peroxidase (GPX) as well as in the levels of H_2O_2 and assimilatory pigments.

Allelochemicals have been alleged to decrease the stomatal conductance due to induced ABA production, which indirectly impact the photosynthesis, transpiration, respiration rates and

Table 4 Effect of leaf litter of *M. dubia* on growth, biomass and grain yield (3 MAS) of green gram and black chickpea in pot culture

Leaf litter (g/pot)	Plant Height (cm)	Collar diameter (mm)	Root length (cm)	Grain yield (g/ plant)	Biomass (DM g/ plant)
Green Gram					
No litter	54.43	2.62	21.40	2.20	7.69
5 g	48.94	2.71	20.90	2.23	7.11
10 g	49.93	2.70	22.93	2.18	7.76
15 g	50.94	2.38	21.91	2.50	7.70
20 g	49.52	2.52	20.35	2.01	6.30
CD (P ≤0.05)	N.S.	N.S.	N.S.	N.S.	N.S.
SEm (±)	2.50	0.19	2.64	0.20	1.08
Black chickpea					
No litter	35.25	2.83	6.78	*	4.72
5 g	33.43	2.80	7.02	*	4.33
10 g	34.33	3.23	7.19	*	4.68
15 g	32.01	2.78	6.03	*	4.36
20 g	34.74	2.82	7.03	*	4.61
CD (P ≤0.05)	N.S.	N.S.	N.S.	*	N.S.
SEm (±)	1.98	0.29	0.42	*	0.24

MAS= Months after sowing; DM=Dry Matter; *Grain formation did not occur; CD= Critical difference; SEM= Standard error of mean

uncoupling oxidative phosphorylation (Yu et al., 2003; Bagavathy & Xavier, 2007). Multiple physiological effects, such as reduction in plant growth, absorption of water and mineral nutrients, ion uptake, leaf water potential, shoot turgor pressure, physiological drought and osmotic potential have been attributed to reduction in germination, growth and biomass seedlings both in laboratory bioassay and pot experiments (Barkosky & Einhellig 2003; Rezaeinodehi et al., 2006).

3.4 Allelopathic effect of leaf litter on growth, biomass and grain yield at harvesting

The data on growth, biomass and grain yield (3 months after sowing) attained by green gram and black chickpea (Table 4) expressed that there was no significant effect of leaf litter of *M. dubia* applied @ 0, 5, 10, 15 and 20 g/pot, on growth, biomass and grain yield both the test crops.

Despite validation of allelochemicals in *M. dubia* through GC-MS analysis, the leaf mulch treatments did not exhibit inhibitory or stimulatory effect on later stage of growth of green gram and black chickpea in the present study. In contrary to present findings, Shapla et al. (2011) reported that *M. azedarach*, sister species of *M. dubia*, mulch application @ 20 gm/pot inhibited the growth (shoot and root length, number of leaves) and biomass (shoot, root and total fresh and dry) of mung bean and soybean. Similar adverse effects of leaf mulch of fruit and timber tree

species on other pulse and cereal crops have also been reported (Sale & Oyun 2013; Thakur, 2014). Studies on pot culture carried out by Divya et al. (2004) and Hossain et al. (2002) are also divergent to the present findings. These studies have reported inhibitory effect of leaf litter application only up to a month or so. However, in this study, results of growth, biomass and yield are reported till maturity of the test crops.

This may be attributed to faster mulch decomposition, leaching out of allelo-chemicals due to frequent irrigation done to maintain the moisture in the pots, ephemeral nature of allelo-chemicals, loss from soil through volatilization, especially phenolics (Ampofo 2009; Narwal et al. 2011). Management practices like frequent watering may have resulted in faster decomposition of leaf mulch of *M. dubia*, hence did not exhibited any significant inhibitory effect on growth, yield and dry matter production of pulse crops in present study. The mulch used in the present study was crushed and reduced in size before application, which might have resulted in quick decomposition, thus, alleviating the allelochemicals. These evidences may be attributed to non-significant effect of mulch treatments of *M. dubia* on growth, biomass and grain yield of test crops in the present study.

Laboratory bioassay and pot culture studies divulged that, the leaf litter of *M. dubia* contain different types of phytotoxic chemicals, as evident from the GC-MS analysis, with putative inhibitive potential on seed germination, initial growth and biomass of green

gram and black chickpea. However, pot culture studies, revealed that there was no significant allelopathic effect on later growth, biomass and grain yield of both the test crops. The second investigation brought out that allelochemicals in leaf litter of *M. dubia* are of ephemeral nature and their effect got alleviate over of time.

Conflict of Interest

Authors would hereby like to declare that there is no conflict of interests that could possibly arise.

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