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Allelopathic influence of leaf and leaf litter of white cedar (*Melia azedarach* L.) on eggplant and okra

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ABSTRACT

In laboratory bioassay and pot culture, we studied the allelopathic effects of white cedar (*Melia azedarach* L.) leaves on germination and seedling growth of eggplant (*Solanum melongena* L) and okra (*Abelmoschus esculentus* L.). In lab bioassay, we determined the effects of leaf aqueous extracts (control, 25, 50, 75 and 100 %) on test crops. The allelopathic effects of leaf litter (control, 12.5, 25.00, 37.50 and 50 g) were determined in pot culture on later stages of growth, biomass and fruit yield of test crops. GC-MS analysis revealed many compounds [phenolic acids and their derivatives, alkaloids, methyl ketones (volatile allele-chemical), unsaturated fatty acids, omega-3 fatty acid, benzofuran, propargyl acid, benzoxepine, fluorobenzoic acid, silicyclobutane, palmitic acid, etc.] in leaf litter of *Melia azedarach.* The leaf aqueous extract and leaf litter inhibited the germination and seedling growth of eggplant and okra both in laboratory bioassay and pot culture. In pot culture (done till maturity of test crops) leaf litter did not show significant allelopathic effects on growth, biomass and fruit yield, thus, the allelopathic influence of leaf litter is transient in nature. Hence, these crops may be cultivated as intercrop in the *Melia azedarach* stands.

KEYWORDS: *Abelmoschus esculentus*, allelopathy, eggplant, GC-MS analysis, germination, laboratory bioassay, *Melia azedarach*, okra, pot culture, *Solanum melongena* L., white cedar.

INTRODUCTION

In agroforestry, there may be positive and/or negative effects on both plant components in above and belowground growth and biomass. Some antipathies of one component may be attributed to allelochemicals, which are released through leaf or stem leaching, volatilization, root exudates and decomposition of plant residues (13). New tree-crop combinations may lead to inhibitory or stimulatory effects on under storey crops (2,9). Although research on allelopathy in agroforestry has been done but very little on Allelopathic interactions between the component crops. Therefore, new agroforestry technologies, involving woody and non-woody components, need to be investigated for alleged deleterious allelopathic effects. White cedar (*Melia azedarach* L.) is perennial, multipurpose fast growing, deciduous tree native to Asian countries and introduced to Australia, Brazil, Ethiopia, France, Greece, Iran, Iraq, Italy, Kenya, Korea, Mexico, Mozambique, Namibia, Philippines, Portugal, South Africa, Spain, Swaziland, Turkey, Uganda, United Kingdom, United States of America, Zanzibar (17). Hence, it is planted by

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farmers in agroforestry systems with different intercrops, as shade tree in coffee and abaca (*Musa textilis*) plantations, sugarcane, vegetables, pulses and grain crops (15,17,18). Studies on its allelopathic potential are mostly restricted to laboratory bioassays (3,20,27,31) and further evidences of such effects in soil or pot culture up to maturity of test crops are needed. We selected eggplant and okra as test crops because in India, eggplant contributes 8.1% of total vegetable production, and India ranks first in okra production in world (73%) and contribute 3.9% of total vegetable production (32). We determined the effects of leaf extract or leaf residue of *M. azedarach* on germination, growth and yield of eggplant and Okra.

MATERIALS AND METHODS

This investigation was done in our University laboratory and green house at 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.

Phytochemical analysis of leaf litter: The allelopathic compounds in leaf litter of *M. azedarach* were determined by Gas Chromatography-Mass Spectrometery (GC-MS) analysis as per Murugesan *et al*. (14).

Preparation of aqueous extracts

The leaves (mixture of young and mature leaves) of *M. azedarach* were collected during October 2014. These were air dried at room temperature and later at 65°C in hot air oven until constant dry weight was reached (19). The dried leaf litter was stored at room temperature and was used for both petridish bioassay and pot experiments. Aqueous extracts were prepared by soaking 200 g grounded dried leaf litter in 1 L distilled water (24). The solution was stirred and kept at room temperature (20-25°C) for 24 h. The filtrate was centrifuged, supernatant was decanted and filtrate was considered as 100 % extract (21). From this, 25, 50, 75 and 100% concentrations were prepared and distilled water was used as control (0 %). The treatments were replicated five times in completely randomized design (CRD).

Petridish bioassay: The seeds of eggplant and okra were pre-treated with Thiram [(Ectoparasiticide fungicide) at 2g/kg]. We used 5- treatments: 0, 25, 50, 75, 100% of aqueous extracts of *M. azedarach* in 9 cm dia petridish replicated five times in complete randomized design. Fifty seeds each test crop were placed on filter paper in sterilized petridishes. Five ml aqueous extracts were applied per petridish on first day and later, 2 ml on alternate days to keep the filter paper moist till the end of experiment (6). Seeds were considered germinated, when radicle emerge (>1 cm length) from seeds. Seed germination was recorded daily till 12 (okra) and 13 (eggplant) days after the start of experiment. Twelve days after sowing, the seedling shoot and root length and biomass were recorded from 10-randomly selected seedlings of each test crop per petridish. Germination (%) and Germination Rate Index (GRI) were calculated using formulae as suggested by Association of Official Seed Analysts. Root and shoot portion were separated and dried in hot air oven at 60^0 C for 48 h and then dry weight was recorded.

Pot experiment: Pot experiments were done in green house to find the effects of leaf litter of *M. azedarach* on germination, GRI, initial growth and biomass of both test crops. Fifty seeds were sown in the plastic pots [28 cm dia x 26 cm height (16007 cc) containing 10.00

kg field soil (N, P and K contents were 84.82, 17.85 and 80.35 ppm, respectively]. Course grounded leaf litter was applied at 0,12.5, 25, 37.5, 50 g per pot and mixed in the upper soil layer (30). For control pots, leaf litter was not added. The replications and statistical design was same as used in petridish bioassay. The litter dose was calculated on the basis of annual average litter fall (8), the leaf litter fall of 3 months was recorded by placing 1 m^2 traps under 5 to 6-years old plantation of *M. azedarach*. The mean litter fall of 3-months was 446.43 g/m^2, which comes approximately 27.68 g per pot (used in experiments). The mulch treatments were calculated based on the range of litter fall as mentioned above.

Pots were irrigated at 2 L / pot daily prior to seed sowing and later with 1 L water was added, as and when required to keep the soil moist. The seedling emergence and seedling growth were recorded at 14 day from start of experiment. The germination (%) and GRI was calculated as per petridish experiment.

To evaluate the plant growth, biomass and yield of each test crop, another pot experiment was done in green house. The treatments were replicated five times (3-plants per replication). In each pot, 5-seeds were sown and one healthy seedling was retained 2-weeks after sowing. At maturity (90 days after sowing), fresh and dry biomass of plant and fruit were recorded. Fruits were harvested from the plants, when they attained harvestable size (vegetable purpose).

Statistical analysis

The experimental data recorded for all parameters in these experiments were statistically analysed following completely randomized design (CRD) and F-test was done and ANOVA was constructed following Sheron et al. (29). Treatment means were compared at $P<0.05$.The percentage data were arcsine transformed before statistical analysis.

RESULTS AND DISCUSSION

Leaf litter allelochemicals

Using the gas chromatography mass-spectrometry (GCMS), 18-types of compounds were detected in leaf litter samples used for bioassay and pot culture experiments (Table 1). The chromatograms showing the relative abundance, retention time and area under curve of chemical compounds detected are depicted in Figure 1. The GC-MS studies of *M. azedarach* leaf extracts was done as per Sharma and Paul (28), they also reported similar chemical compounds. Out of detected compounds the benzoic acids, benzofuran and benzopyran, cyclohexanone, octanoate, dicarboxylic acid, icosapentaenoic acid, 5-methyl (5-8 dihydro 1-4 Naphthoquinone), Cyclohexanone, 3-hydroxy-3-phenyl-, 1-Pentanone, 1-(4-methoxyphenyl)-Oxim Or 1-(4-Methoxyphenyl)-1-pentanone oxime etc. are inhibitory to germination and growth of test crops and many plant species either as extract or leaf litter (12,22,23,33).

Petridish bioassays

The aqueous leaf extract of *M. azedarach* significantly inhibited the germination and germination rate index of eggplant and okra (Table 2). The inhibition (%) in germination and GRI over control, increased with increase in extract concentration (Figure 2 and 3). The suppression effect increased with increase in the extract concentration or leaf

litter quantity i.e. the inhibitory effect was concentration dependent. The extracts were inhibitory to seedling growth over the control. The effects increased with increase in extract concentration (Table 2). The reduction in growth attributes was proportional to extract concentration (Figure 2 and 3). The inhibition (%) was more pronounced in root than on shoot growth, in both test crops.

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

Sr. No.	Compound name	Retention time	Peak Area
1	1-benzofuran-2,3-dione	5.49	192729
2	4 methylbenzoic acid, Propargyl acid	9.71	90293
3	2,3-Benzofurandione,2-oxime	10.34	143614
4	2,3,4,5-tetrahydro-1-benzoxepine	10.57	815388
5	3-Fluorobenzoic acid, 4-nitrophenyl ester	11.04	117505
6	1-cyclohexyloxy-1-methyl-1-silicyclobutane	11.30	-
7	4H-Pyrazino[2,3-b]indole, 6,7,8,9-tetrahydro-	11.41	237741
8	Cyclohexanone, 3-hydroxy-3-phenyl-	12.14	138784
9	1,4-Dithiepan-2-one, 3-phenyl	12.93	74168
10	1,4,7,10,13,16-Hexaoxacyclooctadecane-2,5,9-trione,3-(phenylmethyl)-	14.24	-
11	1-Decen-3-yne	14.33	-
12	2-Methyl-3,5-dodecadiyne	15.14	3884632
13	Methyl 5,7 hexadeadiynoate (Palmitic acid methyl ester; Hexadecanoic acid, methyl ester; Palmitic acid)	16.23	191202
14	Methyl 8-(5-octyl-1,2,4-trioxolan-3-yl) octanoate	16.79	115882
15	Methyl (4E,7E,10E)-Hexadeca-4,7,10-Trienoate	21.83	1157941
16	1,3-Dioxolane-4-methanol, 2-pentadecyl-, acetate, cis-	24.02	162408
17	Spiro [adamantine-2,2-(1,3) dithiolane]-1,5-dicarboxylic acid, 6 oxo		
18	1-Pentanone, 1-(4-methoxyphenyl)-Oxim Or 1-(4-Methoxyphenyl)-1-pentanone oxime Or p-Methoxyvalerophenone oxime	27.20	1370885

Pot culture

Leaf litter inhibited the germination and GRI of eggplant and okra (Table 3) and the effect was proportional to litter quantity. The suppression enhanced with the increase in litter quantity. The maximum inhibition ($p < 0.05$) was recorded at 20 g litter dose. The reduction (%) over control increased with increase in litter amount (Figure 2). Leaf litter of *M. azedarach* was inhibitory to the seedling growth of eggplant and okra (Table 3). The inhibitory effects increased with increase in litter quantity and it was maximum at 20 g litter dose. The % reduction gradually increased with increase in litter quantity over the control (Figure 2). The reduction percentage was more marked in root length as compared to shoot height, indicating organ specific sensitivity to allelochemicals. The leaf litter inhibited the biomass (Table 2) of germinated seedlings over the control (Table 3). The inhibitory effects gradually increased with increase in leaf litter quantity. The magnitude of % reduction in biomass over control (no litter application) was proportional to litter application on both test crops (Figure 2).

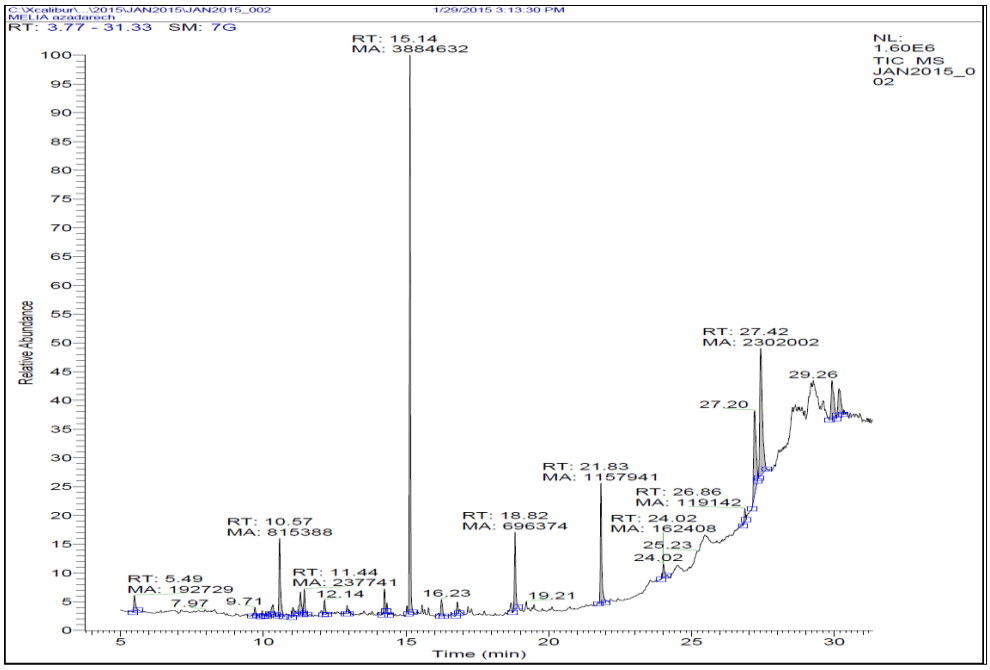

Figure 1. GC-MS chromatogram showing retention time and peaks of different chemical compounds in *M. azedarach* leaf litter

Table 2. Allelopathic effect of aqueous leaf extracts of *M. azedarach* on germination (%), GRI, initial growth and biomass of eggplant and okra in laboratory bioassay.

Extract concentration (%)	Germination (%)	GRI	Growth		Biomass (DM mg/plant)		
			Shoot length (cm)	Root length (cm)	Shoot	Root	Total
Eggplant							
0% control	78.80 (62.63)	8.72	5.62	4.84	21.56	11.8	33.36
25%	76.40 (60.95)	7.58	4.61	4.22	20.99	10.52	31.51
50%	62.40 (52.18)	5.43	4.12	3.66	17.52	9.22	26.74
75%	60.00 (50.76)	5.41	3.53	3.10	14.41	7.48	21.89
100%	52.80 (46.65)	4.29	3.32	2.11	12.94	6.35	19.29
SE	1.63	0.26	0.08	0.12	0.69	0.85	1.19
CD at 5%	4.83	0.77	0.25	0.36	2.04	2.50	3.50
Okra							
0% control	87.60 (69.50)	18.27	5.71	3.96	51.79	28.58	80.37
25%	74.00 (59.51)	11.94	5.22	3.03	50.81	23.73	74.54
50%	59.20 (50.38)	7.38	4.25	2.46	41.80	21.98	63.78
75%	46.80 (43.11)	5.35	3.62	2.10	40.62	17.58	58.20
100%	43.20 (41.00)	4.36	3.06	2.02	28.98	17.14	46.11
SE	2.64	0.62	0.06	0.04	5.32	1.16	5.34
CD at 5%	7.85	1.84	0.17	0.12	15.81	3.43	15.85

DM=Dry Matter; SE=standard error; Values in parenthesis are arcsine transformation; Treatment means were compared at P<0.05

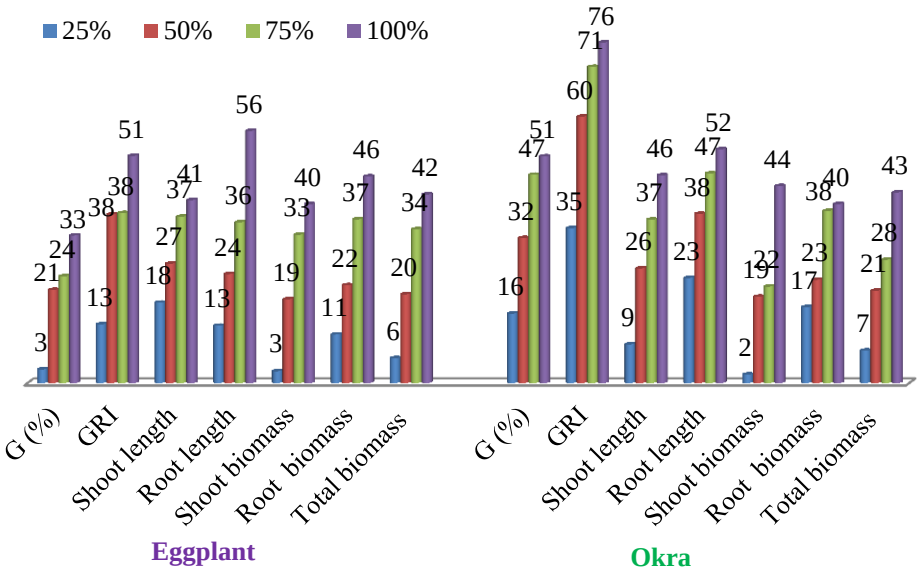

Figure 2. Per cent inhibitory effects (over control) of aqueous leaf extract concentrations of *M. azedarach* on germination, GRI, growth and biomass of eggplant and okra

Table 3. Allelopathic effect of leaf litter of *M. azedarach* on germination (%), GRI, growth and biomass of eggplant and okra in pot culture.

Leaf litter (g/pot)	Germination (%)	GRI	Growth		Biomass (DM mg/plant)		
			Shoot length (cm)	Root length (cm)	Shoot	Root	Total
Eggplant							
No litter	90.80 (72.69)	8.73	8.22	6.40	48.92	25.93	74.85
12.5 g	89.20 (71.01)	7.80	7.33	5.48	40.35	22.68	63.03
25 g	74.80 (59.91)	5.89	6.35	4.03	29.85	18.68	48.53
37.5 g	68.00 (55.66)	5.57	5.60	2.60	20.57	15.16	35.73
50 g	61.20 (51.45)	4.81	4.83	2.49	10.33	11.27	21.60
SE	1.57	0.24	0.13	0.20	2.27	0.86	3.13
CD at 5%	4.65	0.72	0.37	0.60	6.74	2.56	9.30
Okra							
No litter	89.20 (70.86)	12.64	12.52	6.48	96.81	39.76	136.57
12.5 g	88.00 (70.64)	10.83	11.61	5.17	81.81	34.89	116.69
25 g	75.20 (60.51)	7.98	10.73	3.78	71.79	31.63	103.42
37.5 g	66.40 (54.58)	6.63	10.00	2.86	65.42	29.57	94.99
50 g	61.60 (51.70)	5.94	9.46	2.68	61.13	28.18	89.31
SE	2.08	0.40	0.12	0.27	0.70	0.21	0.90
CD at 5%	6.18	1.19	0.34	0.80	2.08	0.63	2.65

DM=Dry Matter; SE=standard error; Values in parenthesis are arcsine transformation; Treatment means were compared at P<0.05

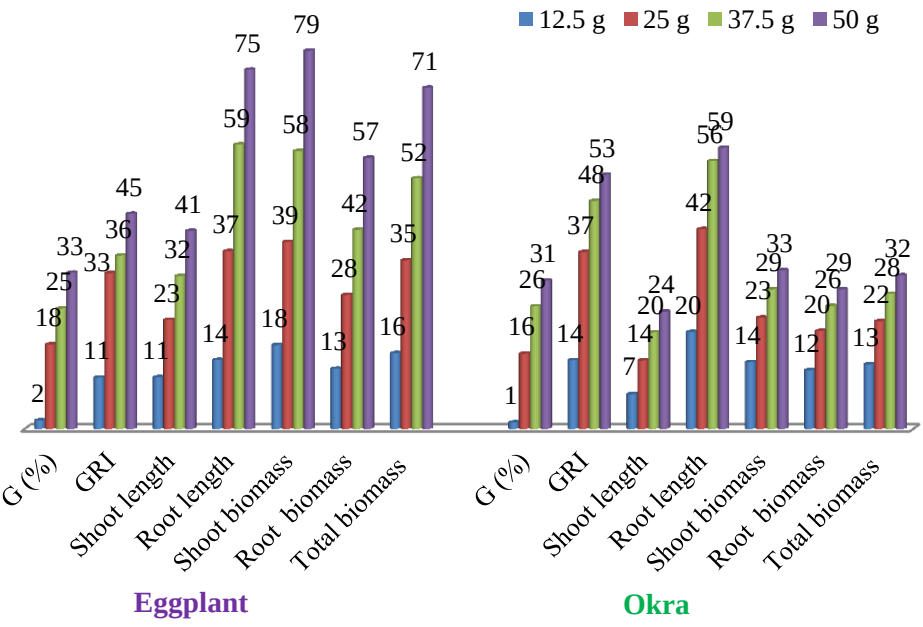

Figure 3. Per cent inhibitory effects (over control) of leaf litter treatments of *M. azedarach* on germination, GRI, growth and biomass of eggplant and okra

The inhibitory effects are due to the water soluble allelochemicals in extracts (25) and leaf litter. The leaf extracts of *M. azedarach* inhibits the germination and seedling growth in various crops (3,20,27,31). In our study the inhibitory effects on test vegetable crops was due to the presence of allelochemicals in the leaf litter of donor species as per results of GC-MS analysis. Our laboratory and pot culture experiments evinced that the magnitude of inhibition on germination traits, initial growth and biomass of eggplant and okra increased with incremental extract concentration. These inferences are in conformity with Akacha *et al*. (3), Phuwiwat *et al*. (20) and Tur *et al*. (31).

In our study, the inhibitory effects of leaf aqueous extract and leaf litter, relative to control, was significantly higher ($P < 0.05$) on root growth than shoot growth in laboratory and pot experiments. Similar organ specific effects of leachates on pulse and vegetable crops against aqueous leaf leachates of *Azadirachta indica* has been reported on radish, barnyard grass, pea and *Lactuca sativa* (29,30). Because the roots first come in contact with allelochemicals and absorb them from the environment in which they are growing (25). The cell death and tissue browning occur in the root apical zone, an area with active cell division, when roots are exposed to allelopathic agents (7). Several pot culture studies reports similar organ specific effects (25,26,30). In bioassay and pot experiments, the leaf extract and leaf litter of white cedar, inhibited the germination, initial growth and biomass of both the test crops. The aqueous leaf extracts of plant species may hamper the

physiological processes of germinating seeds and growing seedlings. Akacha *et al*. (3) reported that *M. azedarach* allelochemicals produced an imbalance in the oxidative status of cells and they observed 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 (3). They observed changes in membrane lipid peroxidation and electrolytes leakage in radish seedlings against *M. azedarach*. Phuwiwat *et al.* (20) observed that water uptake and α-amylase activity of *Echinochloa crusgalli* was inhibited by aqueous extracts (12.5 to 100 mg/mL) of young leaves of *M. azedarach* and concluded that water soluble allelochemicals caused inhibition of both water uptake and α-amylase activity during germination process compared to control (20). The germination inhibition is the result of induction of oxidative stress (11). Allelochemicals decreases the stomatal conductance by inducing ABA production, which impact on the rates of photosynthesis and transpiration of germinated seedlings (3). Phytotoxic substances also decrease the respiration and uncoupling oxidative phosphorylation (1). Multiple physiological effects [reduction in plant growth, absorption of water and mineral nutrients, ion uptake, leaf water potential, shoot turgor pressure, and osmotic potential (25)] have been observed from treatments with many phenolics. The growth inhibition occurs because the allelochemicals cause physiological drought in plants in laboratory and pot culture bioassays (5).

Table 4. Allelopathic effects of leaf litter of *M. azedarach* on growth, biomass (DM g/ plant) and fruit yield (at 90 days old) of eggplant and okra in pot culture.

Leaf litter (g/pot)	Plant Height (cm)	Root length (cm)	No. of fruits/ plant	Fruit yield (FW g/plant)	Biomass (DM g/ plant)
Eggplant					
No litter	53.36	28.74	3.96	147.57	105.51
12.5 g	54.65	28.58	4.43	164.96	107.07
25 g	57.88	27.87	4.21	156.87	109.60
37.5 g	55.00	29.68	4.84	180.46	111.43
50 g	51.24	28.65	4.96	184.91	116.53
SE	2.75	0.92	0.64	23.82	10.22
CD at 5%	N.S.	N.S.	N.S.	N.S.	N.S.
Okra					
No litter	31.17	12.55	4.70	26.64	31.38
12.5 g	32.57	13.50	4.62	26.16	32.27
25 g	31.71	12.44	5.73	32.48	29.26
37.5 g	31.44	13.71	4.90	27.80	31.35
50 g	32.91	14.14	5.50	31.19	31.46
SE	2.58	1.09	0.38	2.14	3.30
CD at 5%	N.S.	N.S.	N.S.	N.S.	N.S.

MAS: Months after sowing; DM: Dry Matter; FW: Fresh Weight; SE: Standard error; Treatment means were compared at $P < 0.05$

Growth and fruit yield in pot culture

There was no significant effect of mulch treatments of 12.5, 25, 37.5 and 50 g per pot on growth, biomass and fruit yield of both the test crops (Table 4).

Shapla *et al*. (27) reported that *M. azedarach* mulch (20 g/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 several tree species have been reported on various test crops (26,30), which differ from to the present findings. These studies have reported the inhibitory effect of mulch only up to one month. However, our study reports the results of growth, yield and biomass till crops maturity. This may be attributed to faster mulch decomposition, leaching out of allelochemicals due to frequent irrigation done to maintain the moisture in the pots, ephemeral nature of allelochemicals present in leaf mulch especially phenolics. Hossain and his co-worker have reported faster decomposition of leaf litter of *M. azedarach* than other tree species (10) and the phytotoxicity due to crop residue disappears quickly after the decomposition.

The highest concentrations of allelochemicals are near the soil surface and are more rapidly lost due to volatilization, moreover allelopathic compounds in plant residues are mainly phenolic (4). The addition of plant residues into the growth environment of another plant can inhibit the germination and growth and may deplete the nitrogen. Addition of readily decomposable organic matter of wide C : N ratio to soil, enhances the microbial activity leading to nitrogen immobilization, thereby depress the plant growth, however, watering and addition of nitrogen, overcome such growth reduction (16). Management practices like frequent watering may have resulted in faster decomposition of leaf litter of *M. azedarach* in pots, hence did not pose any significant inhibitory effects on growth, yield and dry matter production of test crops in our study.

CONCLUSIONS

Our laboratory studies found that, the leaves of *M. azedarach* contained different phytotoxic chemicals as detected by GC-MS analysis and these were inhibitory to the seed germination, seedlings growth of eggplant and okra. However, long term pot culture studies with leaf litter, showed that litter did not show any significant allelopathic effects on final growth, biomass and fruit yield of both test crops, perhaps because the phytotoxic compounds in leaves of *M. azedarach* are transient in nature and their effect got alleviated over a period of time. The study showed that allelopathic effects occur only on germination seedlings growth, without any deleterious effects on final growth and yield of crops; hence, these vegetable crops can be grown under *M. azedarach* plantations.

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