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Effect of leaf aqueous extract and leaf litter of chinaberry tree as transient allelopathic influence on growth and yield of chilli and tomato

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ABSTRACT

Through gas chromatography mass-spectrometry (GC-MS), we detected many compounds in leaf litter of *Melia azedarach* which are phenolic acids and their derivatives, alkaloids, methyl ketones (volatile allelochemical), unsaturated fatty acids, omega-3 fatty acid, benzofuran, propargyl acid, benzoxepine, Fluorobenzoic acid, silicyclobutane, palmitic acid. The leaf aqueous extract and leaf litter inhibited the germination, germination rate index (GRI), initial growth, and biomass of Chilli and Tomato. However, the results of pot experiment carried out till maturity of test crops showed that there was no significant allelopathic effects of leaf litter on growth, biomass and fruit yield of both the test crops. The allelochemicals inhibited the germination, initial growth and biomass of chilli and tomato in petridish bioassay and pot experiments. However, in pot experiments conducted up to maturity of test crops, the effect of allelochemicals might have diluted through cultural practices like regular watering. The detected allelochemicals are ephemeral in nature hence; there was no allelopathic effect of leaf litter of *M. azedarach* on growth, biomass and fruit yield of chilli and tomato. It is evident from these results that chilli and tomato can be successfully cultivated under canopy of chinaberry without any deleterious effect.

Key words: *Melia azedarach*, *Capsicum annuum, Solanum lycoperisicon,* germination traits, laboratory bioassay.

INTRODUCTION

Melia azedarach L. is perennial, deciduous woody species which is native in Bangladesh, India, Indonesia, Laos, Malaysia, Myanmar, Nepal, Pakistan, Papua New Guinea, Sri Lanka, Thailand, Vietnam and exotic in Australia, Brazil, China, 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. It is a commercial multipurpose fast growing tree, commonly known by many names like chinaberry tree, white cedar, bead-tree, Cape lilac, Syringa berry tree, Persian lilac, Indian lilac *etc*. It is planted by farmers in agroforestry systems either in block or as boundary plantations, preferred in alley cropping system of agroforestry and ornamental purpose (Nandal and Kumar, 15). The industrial and ecological importance of the species has encouraged the farmers to take large scale plantations with different intercrops, as shade tree in coffee and abaca (*Musa textilis*) plantations, sugarcane, vegetables, pulse and grain crops (Nandal and Kumar, 15 and Patil *et al*., 17). Positive and or negative effects may arise both above and below ground in land use systems involving woody and non woody components co-existing on same piece of land.

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This antagonism of one component on another is also attributed to allelochemicals, released in the process of leaf or stem leaching, decomposition of plant residue (Mensah *et al*., 13).

Tree-crop combinations may lead to inhibitory or stimulatory effects on under storey crops (Abugre *et al*., 2, Kumar *et al*., 8). Research on allelopathy in agroforestry has been carried out to a large extant nevertheless, agroforestry systems with new tree-crop combinations are evolving which are being adopted and practiced by the farmers. Therefore, such new agroforestry technologies, involving woody and non woody components, need to be investigated for alleged positive or negative effects. Studies on its allelopathic nature of chinaberry tree are only limited to laboratory bioassays and evidences of such effects in soil or pot culture up to maturity of test crops are needed. In this perspective, the present study was intended to illustrate the effects of leaf aqueous extract or leaf litter of *M. azedarach* on germination, growth, biomass and yield of chilli and tomato.

MATERIALS AND METHODS

This study was accomplished in the laboratory and green house complex of College of Forestry, ACHF, Navsari Agricultural University, Navsari, Gujarat (20.95° N latitude, 75.90° E longitude with an altitude of 10 m above MSL) in 2014 and 2015.

Phytochemical analysis of leaf litter of donor species

To determine the allelopathic compounds in leaf litter samples of *M. azedarach* used in this study, Gas Chromatography-Mass Spectrometery (GC-MS) analysis was done following Murugesan *et al*. (14) and chemical compounds were identified.

Donor plant material and preparation of aqueous extracts

The leaves (mixture of young and mature leaves showing signs of senescence) of *Melia azedarach* were collected during October 2014. The leaves were dried at room temperature and later at 65° C in hot air oven until constant dry weight was reached (Perez-Corona *et al*., 18). The dried leaf litter was stored at room temperature and was used for both peteriplate bioassay and pot experiments. Aqueous extracts were prepared by soaking 200 g of grounded dried leaf litter in 1L distilled water (Reigosa *et al*., 21). The solution was stirred and kept at room temperature (20-25°C) for 24 h. The filtrate was centrifuged and supernatant was decanted and the filtrate was considered as 100 per cent extract (Prasad *et al*., 20). From this 25, 50, 75, 100 per cent concentrations were prepared and distilled water was used as control T_1 (0 %). The treatments were replicated five times in completely randomized design (CRD).

Petridish bioassay: The pre-treated seeds (treated with Thirum @ 2g/kg) of chilli and tomato were used as test crops. In the laboratory experiment, 5-different treatments *viz*., 0, 25%, 50%, 75% and 100% of aqueous extracts of *M. azedarach* were used and replicated five times. Each petridish of size 90 mm diameter is considered as replication. Total 50 seeds of each test crop were placed on filter paper in sterilized petridishes. Five ml aqueous extracts were applied on first day and afterwards, 2 ml on alternate days to keep the filter paper moist till the completion of experiment (Bhat *et al*., 6). Seeds were considered germinated upon radicle emergence (>1 cm length) from seeds. Germination was counted daily till 13 (chilli) and 12 (tomato) days after the start of experiment. The seedling shoot and root length and biomass were recorded from 10-randomly selected seedlings of each test crop per petridish. Germination (%0 and Germination Rate Index (GRI) were calculated as per formulae given by Association of Seed Analysts. Root and shoot portion were separated and dried in hot air oven at 60^0 C for 48 h and then sample were weighed. The germination and GRI were calculated as per standard procedures.

Pot experiment: Pot experiments were done in greenhouse to find the effects of leaf litter of *M. azedarach* on germination, GRI, initial growth and biomass of both the test crops. Fifty seeds were sown in the plastic pots [28 cm diameter x 26 cm height (16007 cc) containing approximately 10 kg normal soil (N, P and K content and they 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. 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 annual average litter fall (Gonzalez-Munoz *et al*., 10). We recorded the leaf litter fall of 3-months by placing the 1 m^2 traps under 5 to 6 years old plantation of *M. azedarach*. The average litter fall was 446.43 g/m^2, which comes approximately 27.68 g per pot (used in the experiments). Hence, the mulch treatments were calculated based on the range of litter fall as mentioned above. Pots were irrigated (2 L/ pot) with water (pH 7.71, electrical conductivity, 1.752dS/m) a day prior to seed sowing and approx. 1 L on subsequent days as and when required to keep the soil moist. The seed germination and seedling growth (at 14 day from start of experiment) were recorded as done in Petri Plate bioassay on 14 day, except that here seedling emergence from the soil was recorded. The germination (%) and GRI was worked out as per standard procedure followed in the petridish bioassay experiment.

To evaluate the plant growth, biomass and yield of each test crop, a separate pot experiment was done in greenhouse. Each litter treatment was replicated five times (3 plants per replication). In each pot, only 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 separated from the plants as and when they attained harvestable size (vegetable purpose).

Statistical analysis: The experimental data recorded for all the parameters in different experiments were the statistically analysed following completely randomized design (CRD) and F-test was done and ANOVA was constructed following Sheron *et al*. (25). Treatment means were compared at P≤0.05.

RESULTS AND DISCUSSION

In gas chromatography mass-spectrometry, 18 different types of compounds were detected in leaf litter samples of *M. azedarach*, which were used for bioassay and pot culture experiments (Table 1). The detected compounds are phenolic acids and their derivatives, omega-3 fatty acid, alkaloids, methyl ketones (volatile allelochemical), unsaturated fatty acids, aromatic ketone, phenolic acids and their

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	Area under Curve
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-Methoxy valerophenone oxime	27.20	1370885

derivatives, alkaloids, methyl ketones (volatile allelochemical), unsaturated fatty acids, omega-3 fatty acid, benzofuran, propargyl acid, benzoxepine, Fluorobenzoic acid, silicyclobutane, palmitic acid. Chromatograms showing the relative abundance, retention time and area under curve of chemical compounds detected Fig. 1.

The GC-MS studies of *M. azedarach* leaf extracts carried out by Sharma and Paul (24) also reported the similar chemicals. The phytochemicals like 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., detected in leaf litter of *M. azedarach* in our study, were reported with inhibitory effect on germination and growth of test crops either in extract form or as leaf litter of many plant species (Walsh *et al*., 27).

The aqueous leaf extract and leaf litter of *M. azedarach* significantly (P≤0.05) suppressed the germination and germination rate index of chilli and tomato (Table 2 and 3, Fig. 2).

The suppression effect increased with increase in the extract concentration or leaf litter quantity. The inhibitory effect was concentration or litter amount dependent. The inhibition (%) in germination and GRI (over control), increased with increase in extract concentration or litter quantity (Fig. 3 and 4).

Significant inhibitory effects against aqueous extract and leaf litter application over the control on shoot and root length, shoot and root biomass and total biomass of germinated seedlings (Table 2 and 3, Fig. 2). The effects increased with increase in extract strength or litter quantity. The percentages of reduction, over control, in growth parameters were relative to extract concentration and litter amount (Fig. 3 and 4). The per cent inhibition was more prominent on root growth as compared to shoot growth, in both the test crops, in petri dish bioassay and pot culture, except in case of chilli in petri dish culture.

The leaf extracts (bioassay culture studies) of *M. azedarach* is reported to inhibit the germination and initial growth and biomass of germinated seedlings of various test crops (Tur *et al*., 26; Akacha *et al*., 3, Phuwiwat *et al*., 19; Shapla *et al*., 23). The inhibitory

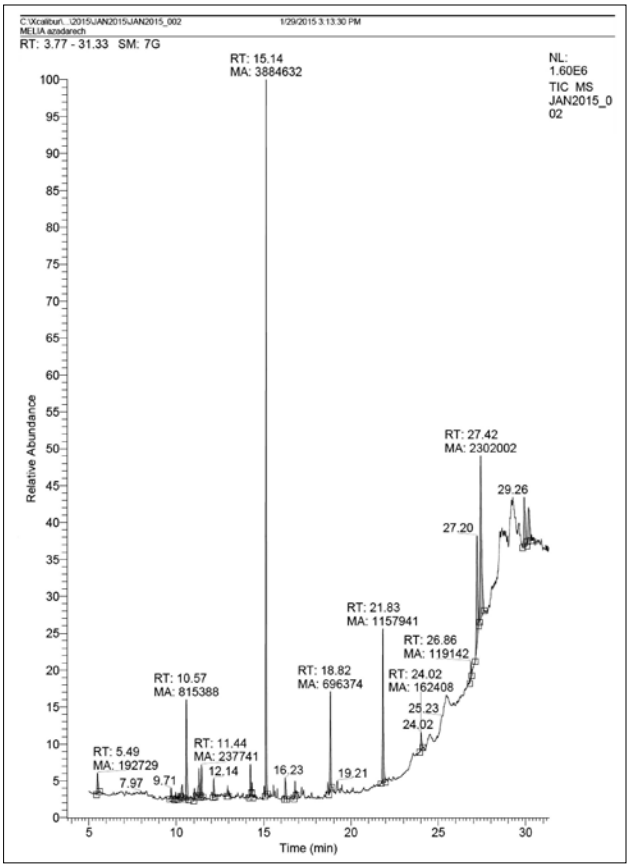

Fig. 1. GC-MS Chromatogram showing retention time and peaks of different chemical compounds in *Melia azedarach* leaf litter.

effect on vegetable crops in our study is due to the presence allelochemicals in the leaf litter of donor species, which was underpinned by GC-MS analysis. The compounds are mainly phenolic acids. The addition or incorporation of plant residues into the growth environment of another plant can result in germination and growth inhibition and addition of leachates and plant debris to the growing medium may deplete nitrogen. Our laboratory and pot culture experiments evinced that the magnitude of inhibition on germination traits, initial growth and biomass of chilli and tomato increased with incremental extract intensity. These inferences are also in conformity with those of Tur *et al*. (26), Akacha *et al*. (3) and Phuwiwat *et al*. (19).

The percent inhibitory effects of leaf aqueous extract and leaf litter, relative to control, was more on root growth as compared to shoot growth in laboratory and pot culture experiments. Similar organ specific effect of leachates on pulse and vegetable crops against aqueous leaf leachates of *Azarachta indica* on radish, barnyardgrass, pea, and *Lactuca sativa* as

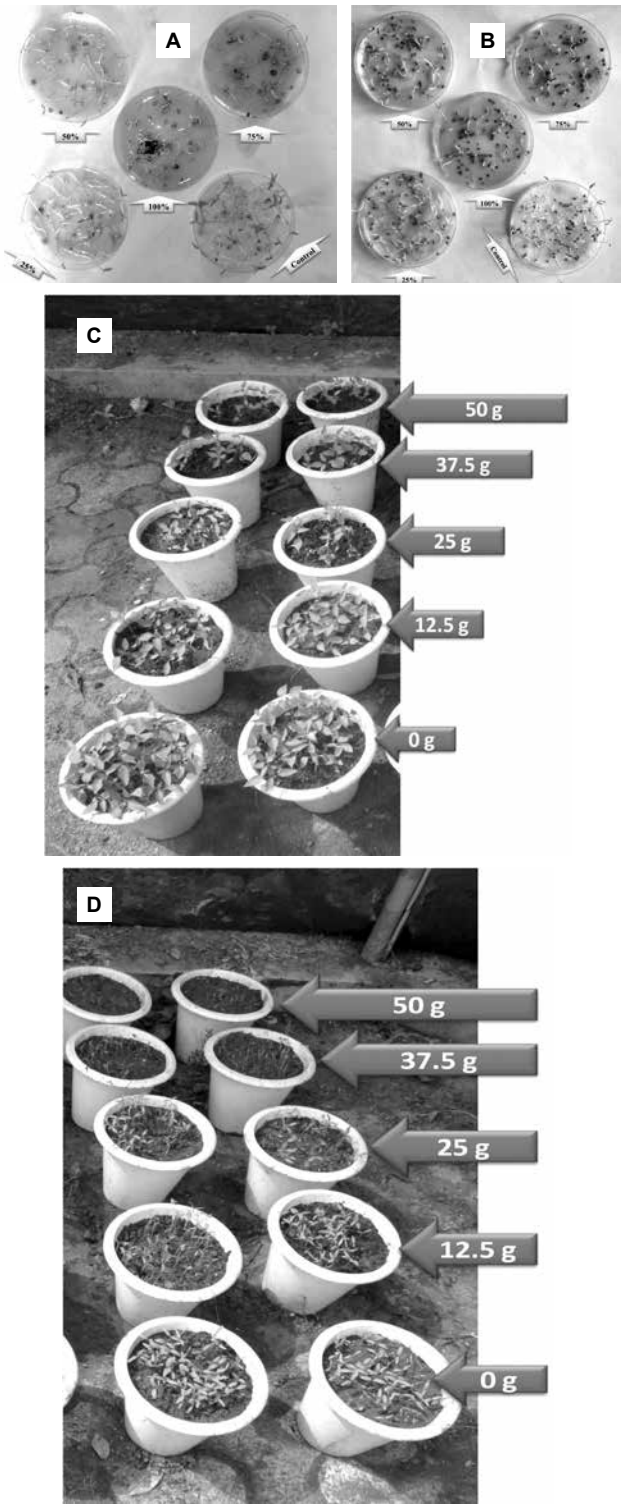

Fig. 2. Showing the allelopathic influence of aqueous leaf extracts [0 (distilled water), 25, 50, 75 and 100%] and leaf litter [0 (no leaf litter), 12.5, 25, 37.5 and 50 g/pot] of *M. azedarach* on germination and initial growth of chilli and tomato in laboratory (a and b) and pot culture bioassays (c and d).

Table 2. Allelopathic effect of aqueous leaf extracts of *M. azedarach* on germination, GRI, initial growth and biomass of chilli and tomato in laboratory bioassay.

Extract concentration (%)	Germination (%)	GRI	Growth		Biomass (DM mg/plant)		
			Shoot length (cm)	Root length (cm)	Shoot	Root	Total
			Chilli				
0% control	81.20 (64.37)	4.60	5.75	4.36	23.32	9.62	32.94
25%	63.20 (52.64)	3.31	4.87	4.28	20.49	8.75	29.24
50%	55.20 (47.97)	2.72	4.26	3.64	18.59	7.06	25.65
75%	51.60 (45.42)	2.35	3.9	3.24	14.51	6.03	20.55
100%	40.40 (39.43)	1.94	3.71	2.88	12.18	5.38	17.56
CD at 5%	4.519	0.36	0.12	0.16	4.65	0.87	4.81
SEm±	1.521	0.12	0.04	0.05	1.57	0.29	1.62
			Tomato				
0% control	81.60 (64.71)	5.24	6.90	4.16	18.97	12.25	31.21
25%	63.20 (52.64)	3.72	6.44	3.23	16.48	9.97	26.30
50%	53.60 (47.05)	2.95	5.41	2.66	13.16	9.82	23.13
75%	47.20 (43.38)	2.41	4.79	2.30	13.15	6.47	18.38
100%	42.00 (40.33)	2.24	4.22	2.09	11.09	5.23	17.57
CD at 5%	5.27	0.43	0.18	0.12	4.21	3.82	5.67
SEm±	1.78	0.15	0.06	0.04	1.42	1.29	1.91

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

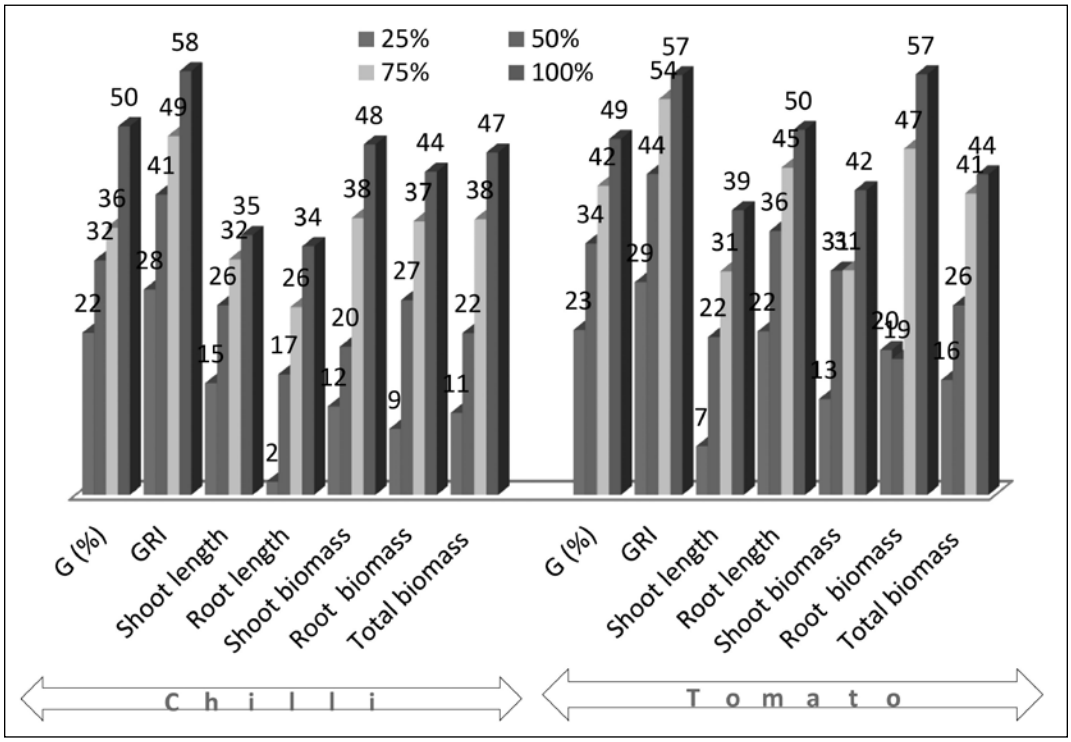

Fig. 3. Inhibitory effects (per cent over control) of aqueous leaf extract concentrations (%) of *M. azedarach* on germination, GRI growth and biomass of chilli and tomato.

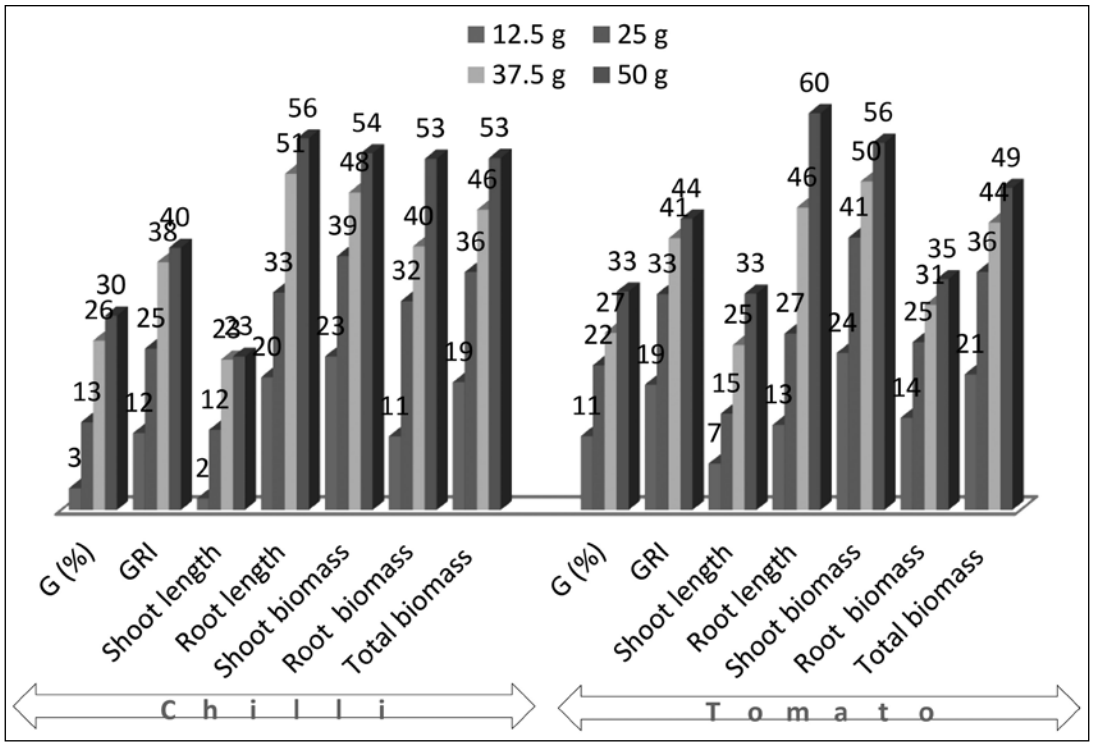

Fig. 4. Inhibitory effects (per cent over control) of leaf litter treatments (g/pot) of *M. azedarach* on germination, GRI growth and biomass of chilli and tomato.

Table 3. Allelopathic effect of leaf litter of *M. azedarach* on germination, GRI, growth and biomass of chilli and tomato in pot culture.

Leaf litter (g/pot)	Germination (%)	GRI	Growth		Biomass (DM mg/plant)		
			Shoot length (cm)	Root length (cm)	Shoot	Root	Total
			Chilli				
No litter	84.00 (66.54)	5.53	8.54	5.21	64.72	21.09	85.80
12.5 g	81.20 (64.33)	4.88	8.39	4.16	50.41	18.73	69.14
25 g	72.80 (58.56)	4.17	7.50	3.49	40.39	14.40	54.80
37.5 g	62.40 (52.19)	3.45	6.59	2.55	34.03	12.65	46.69
50 g	59.20 (50.29)	3.33	6.55	2.27	30.11	9.84	39.95
CD at 5%	3.21	0.44	1.07	0.54	1.90	1.57	2.89
SEm±	1.08	0.15	0.36	0.18	0.64	0.53	0.97
			Tomato				
No litter	89.20 (71.81)	6.67	10.12	5.52	66.44	32.93	99.37
12.5 g	79.20 (62.88)	5.40	9.41	4.81	50.59	28.32	78.91
25 g	69.60 (56.54)	4.48	8.64	4.04	38.94	24.54	63.48
37.5 g	65.20 (53.88)	3.92	7.58	2.98	33.30	22.68	55.98
50 g	59.60 (50.54)	3.72	6.79	2.19	29.39	21.39	50.77
CD at 5%	5.52	0.50	0.36	0.36	6.24	2.01	8.09
SEm±	1.86	0.17	0.12	0.12	2.10	0.68	2.73

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

reported by Phuwiwat *et al*. (19) and Akacha *et al*. (3). Kumar *et al*. (8) reported similar allelopathic effects on pulse crops against aqueous extracts and leaf litter of *Melia composita*. The roots first come in contact with allelochemicals and absorb them from the environment in which they are growing. The cell death and tissue browning occur in the root apical zone, an area with active cell division, when roots exposed to allelopathic agents (Ding *et al*., 9). Several pot culture studies reports similar organ specific effect (Sale and Oyun, 22). 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 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. They observed changes in membrane lipid peroxidation and electrolytes leakage in Radish seedlings against *M. azedarach*. Phuwiwat *et al*. (19) 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 concluded that water soluble allelochemicals caused inhibition of both water uptake and α-amylase activity during germination process compared to control. The germination inhibition is the result of induction of oxidative stress (Javed, 12).

Allelochemicals decrease stomatal conductance by inducing ABA production, which impact on the rates of photosynthesis and transpiration of germinated seedlings (Akacha *et al*., 3). Phytotoxic substances also decrease respiration and uncoupling oxidative phosphorylation (Abrahim *et al*., 1). Multiple physiological effects have commonly been observed from treatments with many phenolics. These effects include reduction in plant growth, absorption of water and mineral nutrients, ion uptake, leaf water potential, shoot turgor pressure, and osmotic potential. The growth inhibition is explained by the fact that allelochemicals cause physiological drought in plants (Barkosky and Einhellig, 5) in laboratory and pot culture bioassays.

There was no significant effect of litter treatments of 12.5, 25, 37.5 and 50 g per pot on growth, biomass and fruit of both the test crops (Table 4), despite the fact that we detected alleged allelochemicals in leaf litter of *M. azedarach*.

It has been reported that *M. azedarach* 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 (Shapla *et al*., 23). Similar adverse effect of leaf mulch of several tree species have been reported on various test crops (Sale and Oyun, 22) which are also divergent to the present findings. These studies have reported inhibitory effect of mulch only up to a month or so. However, our study analyzed the results of growth, yield and biomass till maturity crops. Similar to our findings, Kumar *et al*. (8) have also reported no allelopathic effects of *Melia dubia* leaf litter on growth, biomass and grain yield of black gram. 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 *et al*. (11) have reported faster decomposition rate of leaf litter of *M. azedarach* compared to other tree species and it has been observed that phytotoxicity due to crop residue disappears quickly upon decomposition.

Further, it is observed that highest concentrations of allelochemicals are near the soil surfaces and are more rapidly lost volatilization (Chen *et al*., 7).

Table 4. Allelopathic effects of leaf litter of *M. azedarach* on growth, biomass (DM g/ plant) and fruit yield (at 90 days old) of chilli and tomato 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)
			Chilli		
No litter	80.64	34.67	1.90	7.98	73.61
12.5 g	76.79	34.27	1.66	7.00	51.22
25 g	74.02	35.62	1.62	6.80	57.80
37.5 g	78.57	30.16	2.07	8.68	64.00
50 g	64.07	30.89	2.24	9.41	66.12
CD at 5%	N.S.	N.S.	N.S.	N.S.	N.S.
SEm±	2.54	1.49	0.34	1.45	9.64
			Tomato		
No litter	95.02	32.01	6.84	96.00	113.38
12.5 g	90.63	34.64	7.29	102.31	85.76
25 g	87.68	32.63	7.05	98.99	95.18
37.5 g	91.00	31.64	7.17	100.73	111.39
50 g	81.98	30.98	7.82	109.83	113.24
CD at 5%	N.S.	N.S.	N.S.	N.S.	N.S.
SEm±	3.68	1.32	0.64	8.97	6.36

MAS=Months after sowing; DM=Dry Matter; FW=Fresh Weight; Treatment means were compared at P<0.05

Allelopathic or phytotoxic compounds are known to be mainly phenolic acids and these phenolic compounds degrade with decomposition of plants residues, resulting in the alleviation of phytotoxicity of the decomposing plant residues (Ampofo, 4). It is advocated that addition of readily decomposable organic matter of wide C: N ratio to soil, enhances the microbial activity leading to nitrogen immobilization, thereby depressing the plant growth, however, watering and addition of nitrogen, overcome such growth decreases (Narwal *et al*., 16). Management practices like frequent watering may have resulted in faster decomposition of leaf mulch of *M. azedarach,* hence their mulch did not exhibited any significant inhibitory effect on growth, yield and dry matter production of test crops in our study.

Laboratory and pot culture studies revealed that the leaf litter of *M. azedarch* possess different phytotoxic chemicals, detected through GC-MS analysis, with alleged inhibitive potential on seed germination, initial growth and biomass of chilli and tomato. However, pot culture studies, intended to examine the effect of leaf leachates through litter, diulged that, different litter treatments did not show any significant negative effect on final growth, biomass and fruit yield. The second investigation brought out that the phytotoxic compounds in *M. azedarach* leaf litter are of transient in nature and their effect got alleviated with the passage of time. Hence, these vegetable crops can be grown under canopy of *M. azedarach.*

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