



## Allelopathic Potential of Aqueous Leaf Extract and Leaf Litter of *Melia dubia* Cav. on Tomato and Okra

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### ABSTRACT

#### Key Words:

Allelopathy, Gaschromatography, germination, *Melia dubia*, okra, tomato

The leaf aqueous extract and leaf litter of *M. dubia* inhibited the germination traits (germination per cent, mean daily germination, peak value, germination value and germination rate index), initial growth and biomass of both the test species. The allelochemicals present in leaf litter might have inhibited the germination, initial growth and biomass of test crops in laboratory and pot culture bioassays. However, the results of the pot experiment carried out till crop maturity, showed that final growth, biomass and fruit yield was not affected due to leaf litter. The allelochemicals might have diluted through cultural practices or ephemeral nature of allelochemicals and hence, there was no allelopathic effect of leaf litter of *M. dubia* on later growth, biomass and yield of tomato and okra.

### INTRODUCTION

Allelopathy may occur through release of allelochemicals from plant parts through leaching, root exudation, volatilization, residue decomposition and other processes both in natural and agricultural systems (Gupta et al. 2007; Narwal et al. 2011; Gunarathne and Perera 2016). Research on allelopathy has been carried out to a great extent, however new tree-crop models are evolving with time and are adopted farmers. *Melia dubia* is an industrially and economically important fast growing short rotation tree species. Outside India, it is found in Australia, Sri

Lanka, Malaysia, Java and China. It is indigenous to the Western Ghats of Southern India and is common in moist deciduous forests of Kerala (Saravanan et al. 2013). It is valued for its high-quality termite and fungus resistant timber for furniture, agricultural implements and house construction, as alternative pulp wood species, fuel wood and fodder and has medicinal properties as well (Vijayan et al. 2004; Parthiban et al. 2009; Sinha et al. 2019; Singh et al. 2019, Dhaka et al. 2020). 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). This species is need to be tested for its allelopathic effects, if any, on crops to be integrated in agroforestry models. Hence, keeping in view the increasing popularity of *M. dubia* the present study was undertaken to investigate the alleged allelochemicals in leaf litter of *M. dubia* and to bring out the beneficial or antagonistic effect on germination, growth, biomass and yield test crops through laboratory bioassays and pot cultures.

## **MATERIALS AND METHODS**

This study was accomplished in the laboratory and green house complex of College of Forestry, 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.

### ***Donor plant material and preparation of aqueous extracts***

The leaves (mixture of young and mature leaves showing signs of senescence) of *Melia dubia* were collected from 3-year old plantations in October 2014. The leaves were initially dried at room temperature and later at 65° C in hot air oven until constant dry weight was reached. The dried leaf litter was stored at room temperature and was used for both petriplate bioassay and pot experiments. Aqueous extracts were prepared by soaking 200 g grounded dried leaf litter in 1L distilled water. 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 % extract. From this 25, 50, 75, 100 % concentrations were prepared and distilled water was used as control 0 (%) (Kumar et al. 2017a). The treatments were replicated five times in completely randomized design (CRD).

### ***Petridish bioassay***

The pre-treated seeds (treated with Thirum @ 2g/kg) of tomato (*Lycopersicon esculentum* Mill.) and

okra [*Abelmoschus esculentus* (L.) Moench] were procured from Agricultural Technology Information Centre (ATIC), Navsari Agricultural University, Navsari, Gujarat, India. In laboratory experiment, 5-treatments [T<sub>1</sub> (0), T<sub>2</sub> (25%), T<sub>3</sub> (50%), T<sub>4</sub> (75%) and T<sub>5</sub> (100%)] of aqueous extracts of *M. dubia* were used and replicated five times. Each petridish (90 mm dia) was considered as replication. Fifty 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 (Parmar et al. 2019a). Seeds were considered germinated when > 1 cm radicle emerges from the seed. Germination was counted daily till 15 and 13 days, for tomato and okra, respectively after the start of experiment. The seedlings growth shoot and root length and biomass were recorded from 10-randomly selected seedlings per petridish on 15<sup>th</sup> and 12<sup>th</sup> day for tomato and okra, respectively after sowing. Germination (%) and Germination Rate Index (GRI) were calculated following AOSA (1983). Root and shoot portion were separated and dried in hot air oven at 60° C for 48 h and then sample were weighed.

### ***Pot experiments***

Pot experiments were done in green house to find the effects of leaf litter of *M. dubia* on germination, GRI, initial growth and biomass of test crops. Fifty seeds were sown in plastic pots [28 cm dia x 12 cm height (16007 cc)] containing 10 kg soil (N, P and K content were 84.82, 17.85 and 80.35 ppm). Course grounded leaf litter was applied at 0, 12.5, 25, 37.5, 50 g per pot and mixed in upper soil layer (Thakur 2014) and a control (T<sub>1</sub>) without leaf litter was used. The replications and statistical design was same as used in petridish bioassay. The litter dose was calculated on the annual average litter fall (Thakur et al. 2017d). We recorded the leaf litter fall of 3-months by placing the 1 m<sup>2</sup> traps under 3 years old plantation of *M. dubia*. It was 216.45 g/m<sup>2</sup> (2.16 t/ha), which comes to 13.42 g/ pot (used in present study).

Hence, the mulch treatments fixed fall within the range of natural litter fall.

Pots were irrigated (2L/ pot) with water (pH 7.71, electrical conductivity 1.752dS/m) a day prior to seed sowing. Approx. 1 L on subsequent days or as and when required to keep the soil moist. Daily germination count was made up to the last seed to germinate *i.e.*, 14 days for both the test species. The growth and biomass of test crops were recorded on the day when last germination count was made. The seed germination and seedlings growth and biomass were recorded and calculated as done in laboratory bioassay, except that here seedling emergence from the soil was recorded.

To evaluate the plant growth, biomass and yield, till crop maturity, a separate pot experiment was done in green house (50 per cent relative shading). 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 yield were recorded.

### **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. (1998). Treatment means were compared at  $P < 0.05$

## **RESULTS AND DISCUSSIONS**

### **Allelopathic effects on germination attributes of test crops**

Petridish and pot culture bioassays revealed that, aqueous leaf extract concentrations (0, 25, 50, 75, 100%) and leaf litter quantities (0, 12.5, 25, 37.5 and 50 g/pot) of *M. dubia* significantly ( $P < 0.05$ ) suppressed the germination (%) and germination rate index (GRI) of tomato and okra (Table 1 and 2), relative to control. The inhibitory effect gradually increased with increase in extract concentrations (Fig.

1a and b) or litter leaf litter amount (Fig. 1c and d), over the control in each test crop. The per cent inhibition, over control, in all the germination traits of tomato and okra (Fig. 2 and 3) increased with increase in extract concentrations and leaf litter quantities as well, and was greatest at maximum extract concentration (100%) or litter amount (50 g/pot). The per cent inhibition on the germinations attributes was higher in laboratory bioassays as compared to pot culture in both the test crops.

### **Initial growth and biomass of test crops**

The leaf aqueous extracts, in laboratory and leaf litter in pot culture, exhibited significant ( $P < 0.05$ ) inhibitory effect on shoot and root length of germinated seedlings of tomato and okra (Table 1 and 2). The growth parameters had gradual inhibitory effect as the extract concentration or litter quantity increased ( $T_2$  to  $T_5$ ) compared to control ( $T_1$ ). The magnitude of per cent inhibition, over control, gradually increased with increase in aqueous extract concentration or leaf litter quantity, showing maximum reduction at 100% extract or 50 g leaf litter/pot (Fig. 2 to 3). The per cent diminution was higher on shoot growth of tomato and okra against leaf litter and extract application, respectively. Whereas, it was more marked on root growth of tomato and okra against aqueous extract in laboratory and against leaf litter in pot culture bioassays, respectively.

The aqueous leaf extracts and leaf litter exhibited significant inhibitory effect on shoot, root and total dry biomass of germinated seedlings of both the test crops (Table 1 and 2). The magnitude of diminution progressed gradually with increase in extract concentration or litter amount, with maximum at 100% extract concentration and 50 g leaf litter/pot in tomato as well as okra.

The intensity of per cent suppression in growth and biomass parameters increased with incremental extract concentration or litter quantity (Fig. 3 to 4). The percent

diminution was pronounced on root biomass in laboratory bioassays as compared to shoot and it was reverse in pot culture in both the test crops. The growth attributes experienced greater magnitude of reduction in laboratory bioassays, as compared litter application, over control treatments. The magnitude of inhibition on germination traits and initial seedlings growth and biomass increased with incremental extract concentration or leaf litter quantity in present study.

**Table 1.** Allelopathic influence of leaf aqueous extract of *M. dubia* on germination traits, growth and biomass of tomato and okra in laboratory bioassay.

Extract concentrations (%)	Germination traits		Growth (cm)		Biomass (DM mg/plant)		
	G (%)	GRI	Shoot length	Root length	Shoot	Root	Total
Tomato							
T <sub>1</sub> (0% control)	82.80 (65.57)	5.40	6.40	3.86	20.01	9.34	29.27
T <sub>2</sub> (25%)	66.00 (54.33)	3.91	5.94	2.93	18.13	9.22	27.35
T <sub>3</sub> (50%)	59.60 (50.54)	3.25	4.91	2.36	15.75	8.04	24.03
T <sub>4</sub> (75%)	57.20 (49.16)	2.83	4.29	2.00	13.03	6.31	19.34
T <sub>5</sub> (100%)	54.80 (47.74)	2.81	3.72	1.79	11.37	4.78	16.15
CD at 5%	5.19	0.39	0.18	0.12	5.52	1.42	5.84
SE(m)	1.75	0.13	0.06	0.04	1.86	0.48	1.97
Okra							
T1 (0% control)	83.60 (66.16)	15.98	6.21	3.86	50.23	18.89	69.12
T2 (25%)	80.80 (64.03)	14.59	5.05	3.70	45.51	16.12	61.63
T3 (50%)	62.00 (51.94)	9.23	4.65	3.18	41.32	12.35	53.67
T4 (75%)	52.80 (46.60)	6.72	3.61	2.77	35.88	9.88	45.77
T5 (100%)	46.00 (42.69)	4.66	2.62	2.48	28.60	7.95	36.55
CD at 5%	3.42	1.97	0.21	0.66	12.64	5.55	16.76
SE(m)	1.15	0.66	0.07	0.22	4.26	1.87	5.64

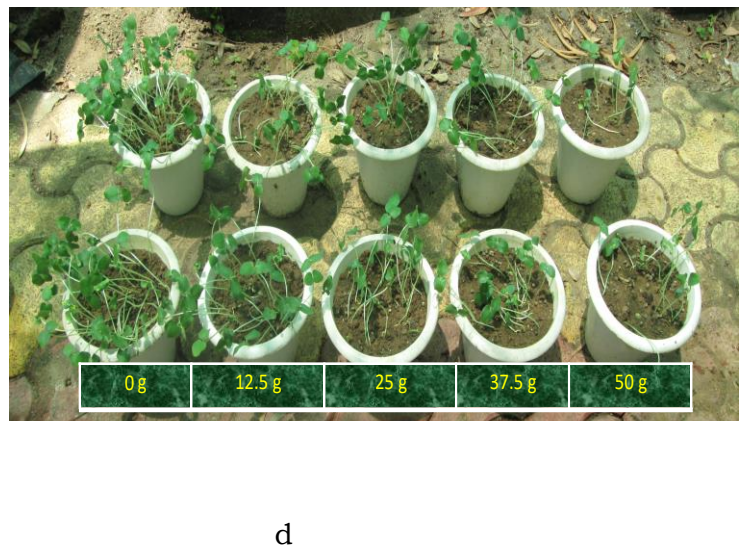
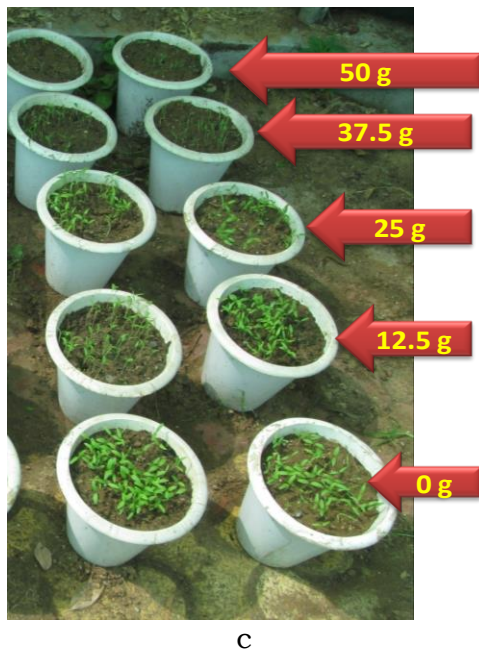
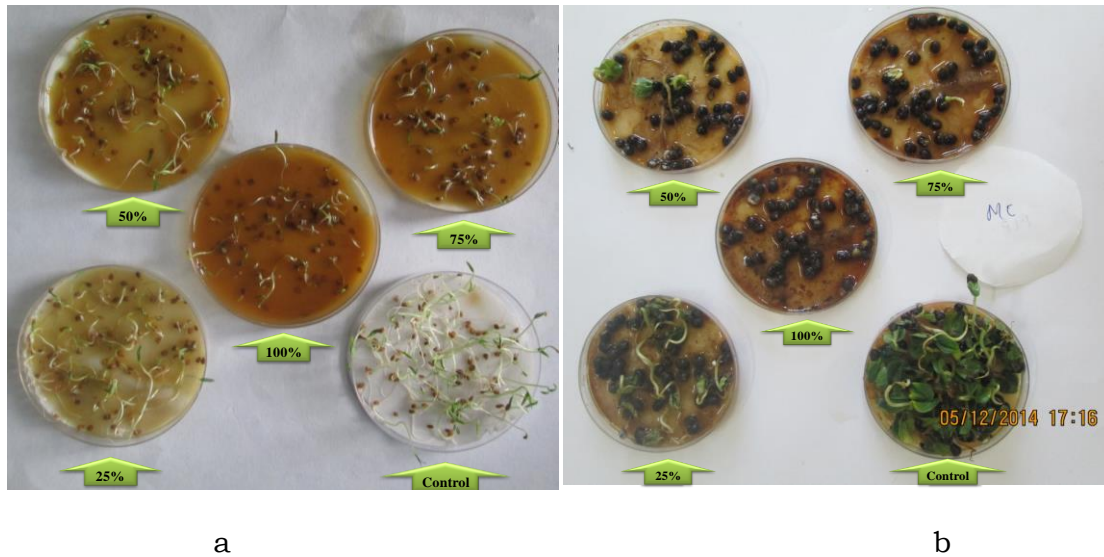
\*Transformed values; **G**=Germination; **GRI**=Germination Rate Index **DM**=Dry Matter

**Table 2.** Allelopathic influence of leaf litter of *M. dubia* on germination traits, initial growth and biomass of tomato and okra in pot culture.

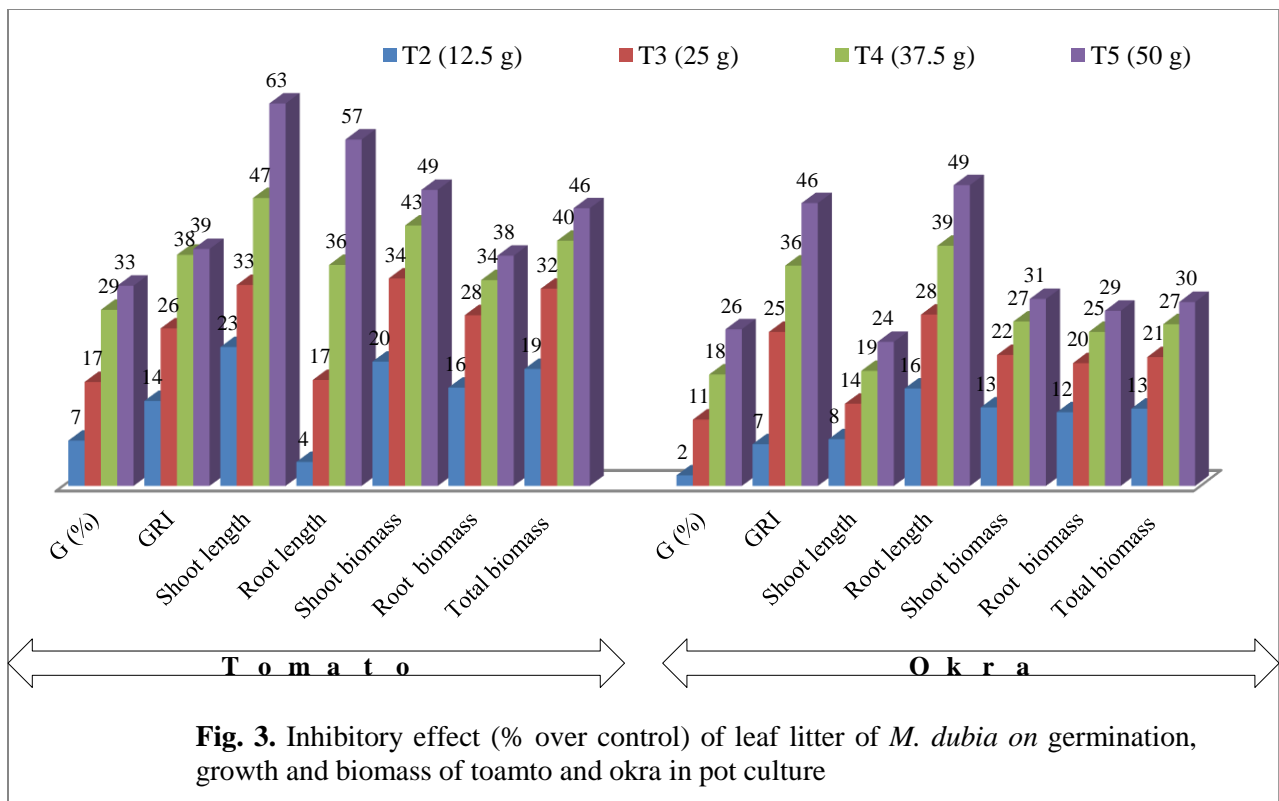
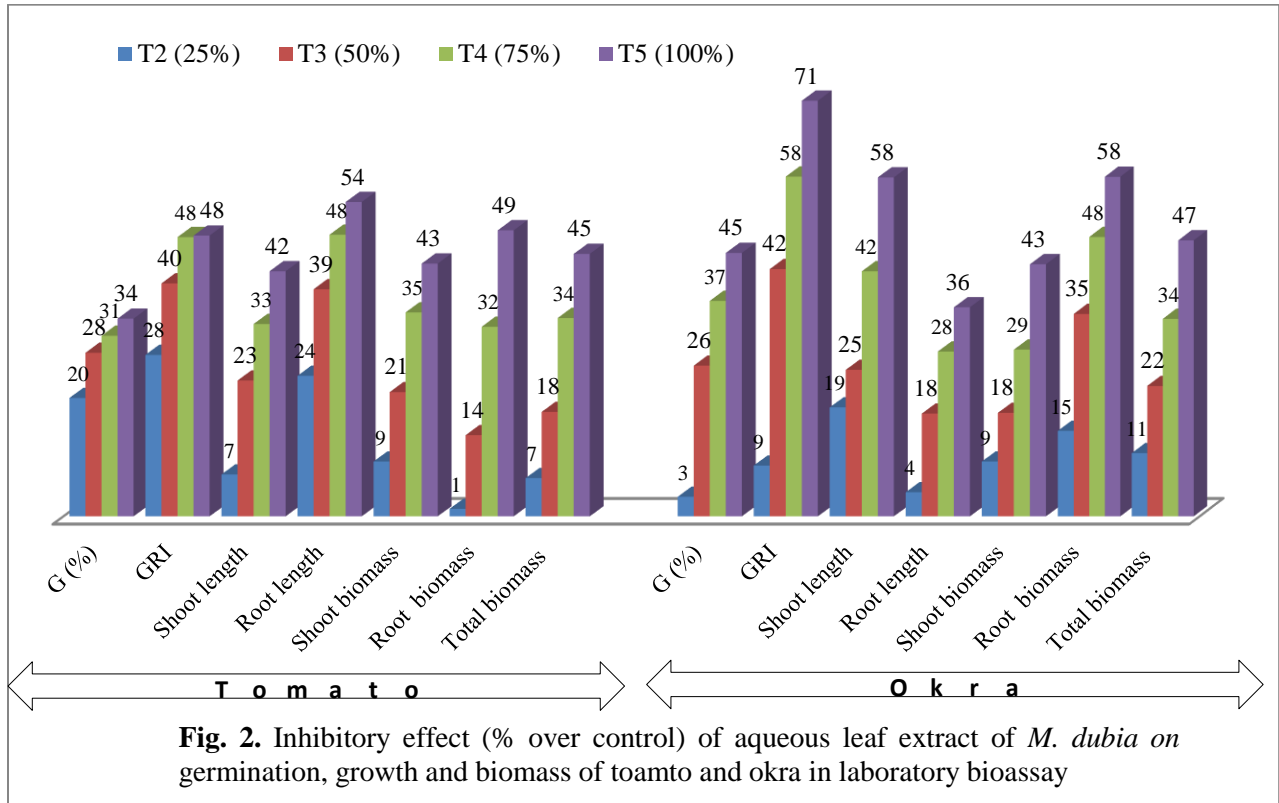
Leaf litter (g/pot)	Germination traits		Growth (cm)		Biomass (DM mg/plant)		
	G (%)	GRI	Shoot length	Root length	Shoot	Root	Total
Tomato							
T <sub>1</sub> (No litter)	91.20 (73.09)	6.30	6.39	4.82	82.00	31.70	113.70
T <sub>2</sub> (12.5 g)	84.40 (66.80)	5.40	4.93	4.63	65.20	26.60	91.80
T <sub>3</sub> (25 g)	75.60 (60.42)	4.60	4.28	3.98	54.00	22.80	76.80
T <sub>4</sub> (37.5 g)	64.80 (53.61)	3.90	3.37	3.07	46.90	21.00	67.90
T <sub>5</sub> (50 g)	61.20 (51.47)	3.80	2.38	2.08	42.10	19.70	61.90
CD at 5%	3.76	0.40	0.7	0.69	2.30	2.00	3.50
SE(m)	1.27	0.10	0.2	0.23	0.80	0.70	1.20

	Okra						
T1 (No litter)	91.60 (73.19)	13.87	13.46	6.46	116.15	40.00	156.14
T2 (12.5 g)	90.00 (71.84)	12.92	12.42	5.42	101.14	35.15	136.29
T3 (25 g)	81.60 (64.63)	10.36	11.64	4.64	91.12	31.92	123.04
T4 (37.5 g)	74.80 (60.10)	8.85	10.91	3.91	84.75	29.87	114.62
T5 (50 g)	68.00 (55.55)	7.43	10.27	3.27	80.47	28.48	108.95
CD at 5%	4.05	0.89	0.43	0.43	2.09	0.68	2.77
SE(m)	1.36	0.30	0.14	0.14	0.71	0.23	0.93

\*\*Transformed values; **G**=Germination; **GRI**=Germination Rate Index **DM**=Dry Matter



**Fig. 1** 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. dubia* on germination and initial growth of tomato and okra in laboratory (a and b) and pot culture bioassays (c and d), respectively.





### Effects of leaf litter on growth, biomass and fruit yield

There was no significant effect of different litter treatments (12.5, 25, 37.5 and 50 g/pot) and that of no litter treatment on growth, biomass and fruit yield of tomato (Table 3) and okra (Table 3). Despite validation of allelochemicals in *M. dubia* through GC-MS analysis (Kumar et al. 2017b; Parmar et al. 2019b), the leaf litter did not exhibit inhibitory or stimulatory effect on final growth and yield of tomato and okra.

Through gas Chromatography Mass-Spectrometry (GCMS) analysis 18 different chemical compounds have detected in leaf litter of *M. dubia*, which are phenolic acids and their derivatives, alkaloids, methyl ketones (volatile allelochemical), omega-3 fatty acid, unsaturated fatty acids, aromatic ketone and chromene (Kumar et al. 2017; Parmar et al. 2019b).

We found that the magnitude of inhibition on germination and initial seedlings growth and biomass increased with incremental extract concentration or leaf litter quantity in present study. Similar concentration dependent inhibitory trend has been reported earlier on various test crops against *M. azedarach* (Akacha et al. 2013; Phuwiwat et al. 2012, Thakur et al. 2017d), another species of same family, and other donor species (Zhang et al. 2015). This is the result of water soluble allelochemicals in the extracts or leaf litter and their allelopathic effect on the measured parameters of test crops (Rezaeinodehi et al. 2006).

Petridish and pot culture experiments revealed that the per cent inhibitory effect was more pronounced on root growth of tomato and okra. However, shoot growth of tomato, in pot culture, and okra, in petridish bioassays, experienced more suppression as compared to root growth.

**Table 3.** Allelopathic influence of leaf litter of *M. dubia* on growth, biomass (DM g/plant) and yield of tomato and okra in pot culture.

Leaf litter (g/pot)	Plant height (cm)	Collar diameter (mm)	Root length (cm)	No. of fruits/plant	Fruit yield (FW g/plant)	Biomass (DM g/plant)
Tomato						
T <sub>1</sub> (No mulch)	89.83	7.13	38.04	7.19	112.62	94.68
T <sub>2</sub> (12.5 g)	85.71	6.41	38.58	8.41	131.74	102.67
T <sub>3</sub> (25 g)	82.85	7.24	38.19	8.17	128.04	107.35
T <sub>4</sub> (37.5 g)	86.79	7.16	37.98	8.29	129.98	128.90
T <sub>5</sub> (50 g)	75.03	7.15	37.29	8.94	140.13	111.93
CD at 5%	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
SE(m)	2.84	0.39	0.92	0.78	12.16	11.94
Okra						
T <sub>1</sub> (No mulch)	28.87	3.29	13.55	5.36	28.85	37.63
T <sub>2</sub> (12.5 g)	31.03	3.74	14.16	5.53	29.76	45.44
T <sub>3</sub> (25 g)	28.63	3.35	13.79	5.70	30.68	36.93
T <sub>4</sub> (37.5 g)	31.52	2.99	13.67	4.95	26.61	43.19
T <sub>5</sub> (50 g)	32.52	3.84	14.31	5.52	29.68	45.34
CD at 5%	N.S.	0.61	N.S.	N.S.	N.S.	N.S.
SE(m)	2.507	0.20	1.12	0.42	2.27	3.09

MAS= Months after sowing; FW= Fresh Weight; DM=Dry Matter

Similar organ specific effects of *M. azedarach* leaf aqueous extracts have been reported earlier (Phuwiwat et al. 2012; Akacha et al. 2013, Thakur et al. 2017d). This may be attributed to the fact that, roots first come in contact with allelochemicals and absorb them from the environment in which they are growing. The young seedlings, especially the roots, are more sensitive to allelopathic agents than adult plants or other plant organs (Zhang et al. 2015).

The aqueous leaf extracts of plant species hamper physiological processes of germinating seeds and growing seedlings (Kaushal et al. 2011). Phuwiwat et al. (2012) observed that water uptake and  $\alpha$ -amylase activity of test species was inhibited by aqueous extracts of young leaves of *M. azedarach*. Studies show that germination inhibition is 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 tomato and okra in the present study.

Addition of leachates or incorporation of plant residues into the growth environment of another plant 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). Akacha et al. (2013) reported that allelo-chemicals produce an imbalance in the oxidative status of cells and they observed changes in activity of catalase, ascorbate peroxidase, guaiacol peroxidase as well as in the levels of  $H_2O_2$  and assimilatory pigments. Allelochemicals decrease the stomatal conductance by inducing ABA production, which indirectly impacts on the rates of photosynthesis, transpiration, respiration and uncoupling oxidative phosphorylation. Multiple physiological effects, which include reduction in plant growth, absorption of water and mineral nutrients, ion uptake, leaf water potential, shoot turgor pressure, physiological drought and osmotic potential (Rezaeinodehi et al. 2006; Bagavathy and Xavier 2007) are attributed to reduction in

germination, growth and biomass seedlings both in laboratory bioassay and pot experiments. Similar effects might have resulted in reduced germination, growth and biomass of test crops in present study against leaf litter of *M. dubia* as compared to control.

Unlike allelochemicals obtained directly by extraction from living organs or tree litter, allelochemicals released from decomposed leaf litter are influenced by soil; thus their concentration, composition, structure, and activity might be extremely different. The important circumstances in which allelopathic effects appear occur when allelochemicals reach the recipient plant in their active structure and at their effective concentration, thus extracts of litter and decomposed litter (or the decomposed medium) often show different allelopathic effects (Zhang et al. 2015).

Most of the allelopathic studies allege the donor species as potential allelopathic based on the laboratory bioassays. Our study, confirm the allelopathic nature till maturity of the test crops. Despite validation of allelochemicals in *M. dubia* through GC-MS analysis in our study, and their corroboration for allelopathic potential from other plants with earlier studies, the leaf litter did not exhibit inhibitory or stimulatory effect on final growth and yield of tomato and okra. Similar to present study no significant allelopathic effect of *M. azedarach* leaf litter was reported on eggplant and okra (Thakur et al. 2017d). In contrary, Shapla et al. (2011) reported that *M. azedarach* mulch application inhibited the growth and biomass of test crops. Similar adverse effects of leaf mulch, in pot culture studies, of donor species on other test crops have also been reported earlier (Sale and Oyun 2013; Thakur 2014). These workers have reported inhibitory effect of leaf litter only up to a month or so. However, our study, results of growth, biomass and yield of the test crops are recorded till maturity. 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



allele-chemicals, loss from soil through volatilization, especially phenolics (Ampofo 2009; Narwal et al. 2011, Thakur et al, 2017d). These evidences may be attributed to non-significant effect of litter treatments of *M. dubia* on growth, biomass and fruit yield of tomato and okra in the present study. Similar results have been reported earlier on pulse and other vegetable crops using same donor species (Kumar et al. 2017b, Thakur et al. 2017a,b; Parmar et al. 2019b). This expresses that, over the longer period, the effect of leaf litter allelochemicals of *M. dubia* got alleviated.

### CONCLUSIONS

The study revealed that, the leaf litter of *M. dubia* contain different types of phytotoxic chemicals with putative inhibitive potential on seed germination, initial growth and biomass of tomato and okra. However, pot culture studies carried out till maturity of test crops, revealed that, there was no inhibitory effect of leaf litter treatments on later growth, biomass and fruit yield. Hence, allelopathic potential of *M. dubia* leaf litter are transient in nature and their effect alleviated over of time.

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