

Allelopathic propensity of *Melia dubia* Cav. leaf litter on the growth performance of leguminous crops

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Abstract

Allelopathy is a biological occurrence in which an organism exudate one or more biochemicals that alters the survival, growth or replication of other adjacent organisms. These biochemicals are known as allelochemicals which have either beneficial (positive allelopathy) or detrimental (negative allelopathy) effects on the target organisms. The present study has been carried out using the leaf litter collected from commercial varieties of *Melia dubia* Cav. Varsha (FRI/MD/232) and Shashi (FRI/MD/349) to analyse its allelopathic potency on the growth and enhancement of leguminous crops. The presence of different phytoconstituents in the leaf litter was analysed through Gas Chromatography Mass Spectrometry analysis (GC-MS) and their allelopathic influence on leguminous plants was examined through germination tray experiments using various concentrations of leaf litter (1g, 2.5g and 5g/seedling pit). The per cent germination, initial growth and biomass of leguminous plants such as Black gram (*Vigna mungo* L.) and Black chickpea (*Cicer arietinum* L.) was observed. The amendment of leaf litter was found to inhibit the root length, shoot length and biomass of both black gram and black chick pea at an earlier stage. But, on later stages, it had no such inhibitory effect on their growth and development. Thus, the allelopathic effect of analysed phytochemicals were ephemeral or transitory in nature and it may have lessened with the passage way of time in soil which may be due to the process of volatilization. *Melia dubia* leaf litter contain allelochemicals that inhibit the growth of weeds nearby agricultural crops and thereby increasing the yield which resulting in better yields.

Keywords: *Melia dubia*, allelopathy, leaf litter, black gram, black chickpea

Introduction

Allelopathy is defined as the direct release of various biochemicals from one plant to an adjacent plant which either enhances or suppresses the growth and development of the neighbouring plant. The release of these chemicals is due to the decomposition of plant residues in the soil. Allelochemicals are a subcategory of secondary metabolites that are not involved in metabolism (i.e) growth, development and reproduction of the allelopathic organism. The term 'Allelopathy' or 'Teletoxy' is derived from the Greek language and was introduced by Molisch (1937) [1]. 'Allelo' means 'each other' and 'patho', means an 'expressing the concurrence of disease'. Allelopathic potential is notably seen in plants, algae and microbes and their interactions play an important role in determining species distribution and abundance in plants community (Cheng & Cheng, 2015) [2].

These allelochemicals may increase or decrease the germination per cent and other growth parameters of an adjacent plants. In plantation, allelopathic effect of one plant may be either advantageous or detrimental to adjacent plant due to the process of liberation of various allelochemicals. It can be released from any part of the plant parts by exudation, leaching, putrefaction of residue and various other processes in nature (Gupta *et al.*, 2007; Narwal *et al.*, 2011) [3, 4]. The non-woody and woody tree species are mandatory to check for its allelopathic consequence by screening the presence of various phytochemicals present in it to design as an agroforestry systematic tree species (Orwa *et al.*, 2009) [5].

Melia dubia Cav. belongs to the family Meliaceae is a fast-growing short rotation tree species with significant industrial importance for its valuable, durable and termite-resistant timber (Chauhan & Ritu, 2005; Chauhan *et al.*, 2015) [6, 7]. It is an indigenous tree species to the Western Ghats of South India and most common in moist deciduous forests of Kerala (Saravanan *et al.*, 2014) [8]. The wood is utilized for various industrial purposes which includes paper wood, pencil, pulpwood, packing cases and splints. (Parthiban *et al.*, 2009) [9]. In addition, the wood is resistant to termites and fungus and thereby considered as a suitable wood for making furniture, agricultural implements and house construction works (Thakur *et al.*, 2019) [10]. Owing to its multipurpose uses, it is recommended and promoted as an agroforestry tree species in various parts of the country. This tree species is also under study in agroforestry plantation system at experimental farm land of ICAR-Central Agroforestry Research Institute (CAFRI), Jhansi (Srivastav *et al.*, 2024) [11].

Melia dubia Cav. has great environmental importance as a soil fertilizer, forestry tree species and its fruits as a food for livestock (Melak *et al.*, 2023). It has been reported as a suitable tree species for timber-based cultivation with high nutrient yields, without exhibiting significant allelopathic effects on the yields. The trees generally bear fruits in 10-12 years. It has been found that *M. dubia* based farming system is more profitable than mono-grafting system (Mohanty *et al.*, 2019) [10].

Considering the allelopathic potential of exotic species, there is a need to manage degraded areas, the potential for persistent vegetation establishment and reforestation. This study was conducted to evaluate the allelopathic effects of Varsha (FRI/MD/232) and Shashi (FRI/MD/349) using aqueous leaf extract of *M. dubia* on seed germination and growth of leguminous plants namely black chickpea (*Cicer arietinum*) and black gram (*Vigna mungo*).

Black chickpea is a healthy legume that many people eat for their health benefits. They are an excellent source of plant-based protein, which supports muscle growth and muscle repair. They are high in fiber, which supports the digestive system and helps to prevent constipation. Their high iron content helps to lower blood pressure and increase hemoglobin levels. Black gram also contains essential minerals such as calcium, magnesium and potassium, which support bones and cardiovascular health. They are rich in antioxidants such as phenolic compounds and flavonoids, which help to reduce stress and inflammation. In addition, legumes have a low glycemic index, making them beneficial for diabetics in controlling blood sugar levels. Due to their high vitamin B content, regular consumption helps to restore the immunity. They are also known for their skin and hair conditioning benefits due to their nutritional benefits. Overall, legumes are an important food source that promotes health and helps in disease prevention (USD, 2023; FAO, 2021) [38].

Materials and Methods

1. Sample collection and preparation of leaf litter amended soil

The fallen leaves collected from northern commercial varieties of *Melia dubia* Cav. namely Varsha (FRI/MD/232) and Shashi (FRI/MD/349) were washed with double-distilled water and shade-dried at room temperature followed by shade-drying for 72 hours. The dried leaf litter samples were further made into fine powder with the help of mortar and pestle. The powdered leaf litter was stored in an air-tight container at room temperature and used for an allelopathic experimental study (Perez-Corona *et al.*, 2013) [14].

2. Phytochemical analysis of leaf litter of *Melia dubia* Cav.

Melia dubia leaf extract was prepared by cold percolation technique using (1:5) (plant sample: solvent) ratio and was incubated for 48 hours and then subjected to Gas Chromatography Mass Spectrometry (GC-MS) at Sophisticated Instrumentation Facility (SIF), Chemistry Division, School of Advanced science, VIT University, Vellore to identify the bioactive compounds analysis. The bioactive phytocompounds present in the leaf litter of two commercial varieties of *M. dubia* Varsha

(FRI/MD/232) and Shashi (FRI/MD/349) was analysed using Gas Chromatography-Mass Spectrometry (GC-MS) (Murugesan *et al.*, 2013) [15].

Allelopathic experiment was studied using the collected leaf litter of two commercial varieties of *Melia dubia* Varsha and Shashi leaf litter were prepared in different concentrations of leaf litters were employed (T1 (soil + no leaf litter) (control), T2 (soil + 1.25g of leaf litter) (25%), T3 (soil + 2.5g of leaf litter) (50%) and T4 (soil + 5g of leaf litter) (100%)) at each pit of germination tray. Leaf litter of two commercial varieties of *Melia dubia* were used (Nikneshan *et al.*, 2011; Lawan *et al.*, 2011) [16, 17]. The germination trays amended with leaf litter were moistened at regular intervals for 7 days to allow proper decomposition of leaf litter which may release the leaf leachates on future stages.

The allelopathic effect of *Melia dubia* leaf litter of two commercial varieties was investigated using the seeds of leguminous crops namely *Vigna mungo* (black gram) and *Cicer arietinum* (black chickpea). The seeds of *Vigna mungo* (Black gram) (3 seeds) and *Cicer arietinum* (3 seeds) were used for the germination study. Five sets of triplicates with fifteen seeds per concentration were sown and watered at regular intervals. Per cent germination was recorded from day 1 followed by the measurement of root length, shoot length and biomass after 60th day of sowing was calculated (Kader, 2005; Scott *et al.*, 1984; Majeed *et al.*, 2017) [18, 19, 20].

$$\text{Per cent Germination (\%)} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds}} \times 100$$

$$\text{Biomass} = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Dry weight} \times 100}$$

Results and Discussion

The allelopathic propensity of two commercial varieties of leaf litter of *Melia dubia* Cav. Varsha (FRI/MD/232) and Shashi (FRI/MD/349) were collected and tested on the growth and development of black chickpea and black gram and their per cent germination and other growth parameters such as per cent germination, root length, shoot length and biomass were observed.

1. Gas Chromatography Mass Spectroscopy (GC-MS)

Gas Chromatography Mass Spectroscopy (GC-MS) analysis of leaf extract Varsha (FRI/MD/232) and Shashi (FRI/MD/349) had revealed the presence of various phytochemical compounds such as Humulene, Germacrene-D, Hexadecenoic acid Caryophyllene, Octadecanoic acid and Dodecanoic acid, which represents the presence of various classes of phytoconstituents in the leaf litter of commercial varieties of *M. dubia*.

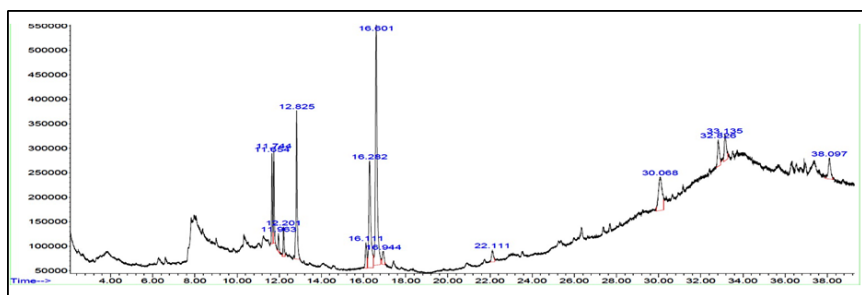


Fig 1: Gas Chromatography Mass Spectroscopy (GC-MS) analysis

2. Per cent germination

Per cent germination had revealed significant variation for all the leaf amended soil in each test crop with respect to its control (soil + no leaf litter amendment). Maximum per cent of germination (100%) was found in all the concentrations except 5 g/pit in both commercial varieties of *Melia dubia* Cav. against *Vigna mungo* and *Cicer arietinum* with respect to the control and minimum (88%) was recorded in 5 g/pit at Varsha against *Cicer arietinum* (Table 1-4). At lower concentrations (0.5–2%), *Melia dubia* leaf extract did not show a significant effect on wheat (*Triticum aestivum*) germination. This suggests that the minor allele does not cause seedling-induced defects and can promote seed germination. In a study conducted by Naresh Kumar *et al.* (2019) [32], leaf litter concentrations ranging from 1% to 6% were tested; the control group (0%) showed 100% production. Similarly, lower concentrations (0.5–2%) maintained growth rates similar to those of the control strain, indicating no chemotaxis at this concentration. However, as the seed germination rate increased, there was a tendency toward inhibition; the higher growth rate (6%) resulted in a decrease in growth of 63.3%. This reduction suggests a concentration-dependent chemotaxis, as the concentration of phytotoxic compounds affects plant growth processes such as water content, enzyme activity, and cellular respiration. Overall, the results of this study showed that low and moderate doses of *Melia dubia* leaf extract did not inhibit plant growth, while higher doses inhibited seed germination, which decreased from 100% to 63.3% (Naresh Kumar *et al.*, 2019) [32].

3. Shoot length

No significant variation was observed in the shoot length of *Vigna mungo* and *Cicer arietinum* at all the concentrations of leaf litter of *Melia dubia*. Maximum (15.3 cm) shoot length was recorded at 100% of concentration of Shashi against *Cicer arietinum* and minimum (4.1 cm) in control (soil + no leaf litter amendment) of Varsha against both *Vigna mungo* and *Cicer arietinum*. (Table 1-4). *Melia dubia* leaf extract shows a growth inhibitory effect that is consistent with the dose-response curve. This suggests its role in the regulation of plant growth. At low concentrations, the extracts promote seed germination, root development, and plant height, resulting in a beneficial effect, representing the inhibition of seedling growth. For example, high concentrations reduce plant growth,

suppress germination signals, and show the effects of plant growth inhibition. These results suggest that *Melia dubia* allelochemicals can act as promoters or inhibitors of germination, depending on their concentrations. Studies such as (Rajendra *et al.*, 2023) as well as by demonstrated that incorporation of these extracts at appropriate concentrations increased the biomass of aerial and root parts and prolonged the survival of aloe vera plants, indicating that it possessed good growth inhibitory activity. However, in general, leaf removal can reduce shoot growth, inhibit root and shoot growth, and inhibit plant growth, highlighting the importance of careful consideration in assessing the nature and magnitude of allelopathic effects (Parameswari *et al.*, 2018) [34].

4. Root length

No substantial variation was noted in shoot length of *Vigna mungo* and *Cicer arietinum* at all concentrations of leaf litter of *Melia dubia*. The maximum root length (9.6 cm) was recorded at 100% concentration of Shashi against *Cicer arietinum* and minimum (1 cm) was recorded at 25% of concentration of Varsha against *Vigna mungo* (Table 1-4). The aqueous extract of *Melia dubia* shows allergic and toxic effects on cultivated plants, depending on the dose used. These factors affect plant growth, such as growth rate, plant height, plant height, and vigor. At low concentrations, the extracts support metabolism by increasing enzymatic activity and nutrient uptake, thereby increasing seed germination rate, increasing root and shoot length and increasing plant vigor. This beneficial effect may be due to the presence of bioactive compounds that act as growth promoters. However, when applied in large quantities, these substances can become toxic, which can reduce crop germination, inhibit root and shoot growth, and reduce plant vigor. These inhibitory effects are associated with disruption of cellular function, membrane permeability, and hormonal balance. As noted by Edwina & Leila (2021), low doses of organic matter can enhance growth under controlled conditions. This highlights the anti-*Melia dubia* chemical activity and their use in agriculture when used in appropriate concentrations.

5. Fresh weight

No significant variation was observed in shoot length of *Vigna mungo* and *Cicer arietinum* at all concentrations of leaf litter of *Melia dubia* (Table 1-4). *Melia dubia* leaf litter extracts at concentrations ranging from 0 to 20 g/pot were tested on green gram (*Vigna radiata*) and black chickpea (*Cicer arietinum*) to assess their effects

on plant growth under controlled conditions. The fresh biomass of fruits and seeds was recorded at maturity (90 days after sowing). The results showed that although some inhibition was observed at the early stages of plant growth, there was no significant increase or decrease in fresh and dry weight at the early stages of plant growth. This implies that the seedlings overcame the initial stress, showed a high rate of compensation, and ultimately accumulated biomass efficiently at maturity. This response indicates that the effects of herbicides are transient and not specific to the growing season. These results indicate that foliar herbicides have a moderate, non-destructive effect on crop germination, which can be considered as a beneficial effect in agricultural systems. Therefore, incorporation of these fertilizers into soil does not affect crop yield and is compatible with sustainable agricultural practices (Thakur *et al.*, 2017) [35].

6. Dry weight

The dry weight of *Vigna mungo* and *Cicer arietinum* had revealed no significant difference among the treatments with leaf litter amendment (Table 1-4). In the present study, per cent germination and growth parameters such as root length, shoot length and biomass of the test crops were observed. The allelopathic effects were observed on germination and growth only at an initial stage and no negative significant effects were recorded on the allelopathic proclivity of leaf litter of *Melia dubia* on black gram found resemblance with the test results of Kumar *et al.*, (2017) [21]. This may be associated to the rapid decomposition or leachates of leaf litter out of allelochemicals due to regular irrigation done to uphold the moistness in the pots Hossain *et al.*, (2011) [22]. Parmar, Thakur and Gunaka (2018) investigated the effects of *Melia dubia* leaves in agricultural areas and reported that these effects were temporary.

In their study of important crops, such as pepper (*Capsicum frutescens*) and eggplant (*Solanum melongena*), they found that the presence of *Melia dubia* leaves, especially in the early stages of flowering, significantly reduced symptoms of senescence, including both fresh and dry leaves. This initial inhibition may be due to the release of allyl compounds that can diffuse into water as plant fibers decompose. However, as the harvest time approached, the inhibitory effect decreased significantly, and there was no significant difference in biomass compared to the control sample. These studies show that these contaminants tend to diffuse, translocate, or decompose in soil, reducing their long-term phytotoxicity resistance. An important finding of this study is that although *Melia dubia* initially shows growth-inhibiting effects, these effects are transient. The seeds can germinate and grow more efficiently, therefore, these results support the safety and efficacy of using *Melia dubia* with other plants in agriculture, as it has no adverse effects on plant growth (Parmer *et al.*, 2018). Due to the presence of phytotoxins, crop remnants tend to decompose rapidly (Burnsid *et al.*, 1985) [23]. The phytotoxic effect due to the addition of leaf amendments is comparatively minimum under field conditions as the phytotoxins degrade faster in the field conditions than in the laboratory (Tian, 1992) [24].

In spite of authentication of superficial allelochemicals present in *M. dubia* leaf litter, the allelopathic studies have revealed that there are no significant adverse effects using different concentrations of leaf litter applications. The percent germination and growth parameters including root length, shoot length and biomass of both the test crops has revealed that there is no reduction among all concentrations used in the germination tray experiment instead enhanced the root, shoot and biomass of both black gram and black chickpea (Table 1-4). However, (Shapla *et al.*, 2011) [25] reported that *M. azedarach* leaf mulch application at 20 g/pot had inhibited the biomass and growth of soybean and mung bean at an initial stage. Similar adverse effects of leaf mulch were recorded for the numerous tree species (Sale & Oyun, 2013; Thakur, 2014) [26, 27]. However, these studies had reported an inhibitory effect of leaf litter on test crops only up to 10-15 days.

The present study on leguminous plants had revealed that *Melia dubia* leaves have certain allelopathic effect at an initial stage since the presence of allelochemicals in the leaf litter is ephemeral in nature it got evaporated over the period of time due to regular irrigation. It enhanced the growth and development of the leguminous crops as it had enhanced the root length, shoot length and biomass of test crops where per cent germination of black gram and black chickpea were found to be significant.

Further, it is concluded that maximum concentration of allelochemicals near the surface of the soils are more quickly vanished in the soil due to the process of volatilization (Chen *et al.*, 1991) [28]. Phytotoxic or allelopathic compounds are from phenolic acids (Glass, 1976) [29] and these phenolic group of compounds are degraded automatically with respect to the decomposition of plants remnants, resulting in the mitigation of phytotoxins (Ampofo, 2009) [30]. The accumulation of readily degradable organic natural matter of wide carbon: nitrogen ratio into the soil improves the microbial action resulting in nitrogen immobilization which affects the plant development (Harper, 1977) [31]. Watering frequently and addition of nitrogen rich compounds, can overcome such growth reductions (Narwal *et al.*, 2011) [4]. Management practices like watering at regular intervals leads to a faster degradation or break down of leaf litters of *M. dubia*. Hence, it does not cause any inhibitory effect on yield, growth and biomass of leguminous crops. The leaf litters used in the present study was shade-dried, powdered, crushed with mortar and pestle, which might have helpful in earlier break down and thereby it lessens the effect of allelochemicals. Similar results have been drawn in an earlier study by Ampofo, (2009) [30]. These evidences may be accredited to a non-significant effect of leaf litters treatments of *M. dubia* on growth, root, shoot length and biomass of tested leguminous crops.

The GC-MS analysis had revealed that leaf litter of *M. dubia* contains phenolic acids and their derivatives which had been revealed an inhibitory effect on root, shoot length and growth of test crops at an initial stage. Laboratory bioassay and pot culture studies had revealed that detected allelochemicals have presumed inhibitory potential on seed per cent germination, initial growth and biomass of black chickpea and black gram at an initial stage. However, once after the seed was

germinated it has observed that no significant deleterious allelopathic effects on per cent inhibition, root, shoot growth and bio mass of test leguminous crops were observed. Thus, the phytotoxins or

allelochemicals present in the leaf litters of *M. azedarach* are of ephemeral or short-lived in nature and their effect got alleviated or lessened over of the period of time (Kumar *et al.*, 2017) [21].

Table 1: Allelopathic effect of leaf powder amendment of *Melia dubia* Cav.- Varsha (FRI/MD/232) on percent germination, growth and biomass of *Vigna mungo*

Concentration (g)	Percent germination	Root Length (cm)	Shoot length (cm)	Fresh weight (g)	Dry weight (g)
C	100	1.0	4.1	3.3	0.48
1 g	100	2.0	5.3	4.25	0.54
2.5 g	100	3.3	7.2	4.50	0.59
5 g	92	4.2	9.1	5.25	0.66

Table 2: Allelopathic effect of leaf powder amendment of *Melia dubia* Cav.- Varsha (FRI/MD/232) on percent germination, growth and biomass of *Cicer arietinum*

Concentration (g)	Percent germination	Root Length (cm)	Shoot length (cm)	Fresh weight (g)	Dry weight (g)
C	100	4.2	4.1	12	1.09
1 g	100	5.3	8.0	16.5	1.35
2.5 g	100	7.4	12.4	17	1.52
5 g	88	9.2	14.3	20.5	1.86

Table 3: Allelopathic effect of leaf powder amendment of *Melia dubia* Cav.- Shashi (FRI/MD/349) on percent germination, growth and biomass of *Vigna mungo*

Concentration (g)	Percent germination	Root Length (cm)	Shoot length (cm)	Fresh weight (g)	Dry weight (g)
C	100	1.5	4.7	4.12	0.65
1 g	100	2.6	5.4	5.25	0.73
2.5 g	100	3.7	8.1	5.50	0.74
5 g	90	4.4	12.2	6.32	0.77

Table 4: Allelopathic effect of leaf powder amendment of *Melia dubia* Cav.- Shashi (FRI/MD/349) on percent germination, growth and biomass of *Cicer arietinum*

Concentration (g)	Percent germination	Root Length (cm)	Shoot length (cm)	Fresh weight (g)	Dry weight (g)
C	100	3.1	4.5	14.12	1.18
1 g	100	4.1	8.3	18.24	1.58
2.5 g	100	7.4	13.3	19.45	1.84
5 g	89	9.6	15.3	21.23	1.97



Fig 2: Allelopathic experiment of *Melia dubia*- Varsha (FRI/MD/232)



Fig 3: Allelopathic experiment of *Melia dubia*- Shashi (FRI/MD/349)

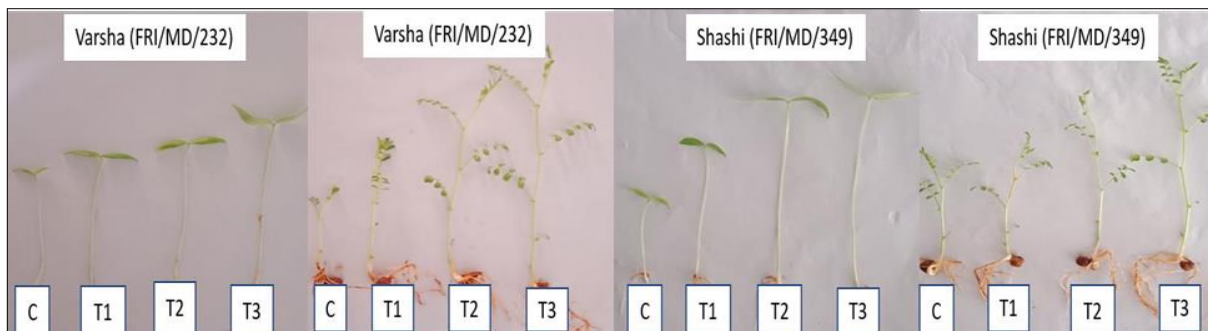


Fig 4: Allelopathic experiment of *Melia dubia* Cav.- Varsha (FRI/MD/232) and Shashi (FRI/MD/349)

Conclusion

The present study is the strong evidence that the two commercial varieties of *Melia dubia* does not cause any inhibitory or deleterious effects on the growth and development of black gram and black chickpea at its later stage. Hence, *Melia dubia* has the tendency to serves itself as an agroforestry tree species which also promotes the beneficiary effects by enhancing the growth. Since the allelochemicals found in *Melia* species are transient in nature, thereby it can be recommended as an agroforestry model. The allelopathic effect of leaf litter amendment of *Melia dubia* varieties with different concentrations had revealed no adverse effects on per cent germination and growth parameters such as per cent germination, root length, shoot length, fresh weight and dry weight. *Melia dubia* has been emerging as a multipurpose tree species but gained only limited research attention pertaining to phytoremediation and as an agroforestry tree species in wasteland management or agroforestry model for sustainable utilization. The phytotoxins present in the leaf litters of *Melia dubia* Cav. are short-lived in nature since it undergoes the process of volatilization over the period of time. Hence, indigenous knowledge pertaining to its beneficiary allelopathic effects of *Melia dubia* has been recognized and need to be documented scientifically through large scale field studies by introducing *M. dubia* as an agroforestry tree species has to be done. Further, conservation of germplasm from superior commercial varieties of *Melia dubia* Cav. as well as its wild native species are essential as they represent the valuable resource for the sustainable utilization in future.

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