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Allelopathic effect of aqueous leaf extracts of selected trees on germination and seedling growth of rice

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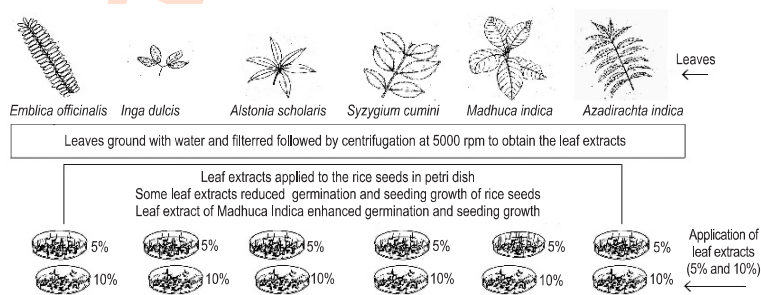
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Abstract

Aim : The present investigation aimed to study the allelopathic effects of aqueous leaf extracts of *Emblica officinalis*, *Inga dulcis*, *Alstonia scholaris*, *Syzygiumcuminis*, *Madhuca indica* and *Azadirachta indica* trees on germination and seedling growth of rice.

Methodology : The studies on germination and seedling growth was conducted in a BOD incubator. The number of seeds germinated daily was counted to study speed of germination, germination energy and number of days to 50% germination. DMSO was used to extract the chlorophyll and carotenoid pigments of leaves. The chlorophyll and carotenoid contents were estimated by measuring the optical density through UV-VIS spectrophotometer. Relative watercontent of leaves was determined by soaking of leaves in water followed by oven drying.



Results : The effect of leaf extracts (5 and 10%) of *Emblica officinalis*, *Inga dulcis*, *Alstonia scholaris*, *Syzygium cuminis*, *Madhuca indica* and *Azadirachta indica* was studied on two rice cultivars viz. NK-5251 (Hybrid) and Gontra Bidhan-2 (high yielding variety). The experimental findings revealed that leaf extracts of different trees had significant effect on the germination and seedling growth of rice. The leaf extract of most of the trees, except *Madhuca indica*, reduced germination, chlorophyll content and RWC at higher concentration. The aqueous extracts of *Madhuca indica* significantly increased germination, seedling vigour, seedling dry weight, germination energy, speed of germination, chlorophyll content and RWC as compared to control. However, 5% extract of *Madhuca indica* recorded the highest germination percentage, seedling vigour, speed of germination, germination energy, seedling dry weight, chlorophyll content and RWC among all the treatments.

Interpretation : The leaf extract of *Emblica officinalis*, *Inga dulcis*, *Alstonia scholaris*, *Syzygium cuminis* and *Azadirachta indica* plants at higher concentration reduced germination and early seedling growth of rice. The leaf extract of *Madhuca indica* plant, however, enhanced the performance. The positive allelopathic effect of *Madhuca indica* leaf extract may be attributed to the presence of phytohormones and biostimulants.

Key words: Allelopathy, Chlorophyll, Germination rate, Leaf extracts, Rice, *Syzygium cuminis*

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Introduction

Allelopathy includes all of the effects that directly and indirectly result from biochemical substances transferred from one plant to another (Molisch, 1937). It is also defined as any direct or indirect harmful or beneficial effect by one plant (including microorganisms) on another through production of chemical compounds that escape into the environment (Rice, 1984). It is also defined as any process involving secondary metabolites produced by plants, algae, bacteria and fungi that influences the growth and development of agriculture and biological systems" (International Allelopathy Society, 1996). According to Ferguson and Rathinasabapathi (2009), allelopathy includes beneficial as well as harmful effects of one plant on another plant, both crop and weed species, by releasing biochemicals known as allelochemicals, from plant parts by leaching, root exudation, volatilization, residue decomposition, and other processes in both natural and agricultural systems. The allelochemicals are non-nutritive substances, which are mainly produced as plant secondary metabolites or decomposition products of microbes (Cheng and Cheng, 2015). Allelochemicals are classified into 14 categories based on chemical similarity and consist of various chemical families (International Allelopathy Society, 1996).

These are water-soluble organic acids, straight-chain alcohols, aliphatic aldehydes, and ketones; simple unsaturated lactones; long-chain fatty acids and polyacetylenes; benzoquinone, anthraquinone and complex quinones; simple phenols, benzoic acid and its derivatives; cinnamic acid and its derivatives; coumarin; flavonoids; tannins; terpenoids and steroids; amino acids and peptides; alkaloids and cyanohydrins; sulfide and glucosinolates; and purines and nucleosides. The plant growth regulators such as gibberellic acid, salicylic acid, ethylene, etc. are also considered as allelochemicals (Cheng and Cheng, 2015). Allelopathic chemicals are found in different plant parts such as leaves, flowers, roots, fruits, or stems. They are not only found in the plant body but also in the rhizosphere. The leaf extracts of different allelopathic trees have been found to inhibit germination of different crop species. Soaking of seeds of wheat in the aqueous leaf extracts of *Alstonia scholaris* and *Syzygium cumini* (L.) for short duration (10 min) has been found to enhance germination of wheat (*Triticum aestivum* L.) and reduce the occurrence of seed-borne mycoflora (Shafique et al., 2007). Leaf extracts of many trees such as *Juglans regia*, *Quercus cerris*, *Populus tremula*, *Pinus nigra*, *Platanus orientalis*, *Salix alba* and crops such as sunflower (*Helianthus annuus* L.), sorghum (*Sorghum bicolor* L.) and sesame (*Sesamum indicum* L.) and weeds such as *Echinochloa colona* and *Cyperus iria* L. were also found to have inhibitory effect on seed germination and seedling growth of different plants (Terzi et al., 2013; Javaid et al., 2006; Duary, 2002; Chopra et al., 2017; Swain et al., 2012).

Though several reports on negative allelopathic effect of plant extracts has been reported, the positive allelopathic effect has rarely been studied by researchers. The extracts of

Chenopodium album and *Reseda lutea* was found to promote the germination of *Abutilon theophrastii* Medik (Kadioglu and Yanar, 2004). The inhibitory effect of leaf extracts on germination and growth of crops is influenced by the concentration of aqueous extract. While higher concentration of leaf extracts was found to have negative effect on germination and growth of many crops, lower concentration had stimulatory effect (Kumar and Gautam, 2008; Majeed et al., 2017; Wang et al., 2018). In view of the above, the present investigation was under taken to study the allelopathic effects of aqueous leaf extracts of some trees on germination and its attributes, seedling growth and some physiological and biochemical parameters of rice seedlings.

Materials and Methods

The allelopathic effect of aqueous leaf extracts of *Embllica officinalis*, *Inga dulcis*, *Alstonia scholaris*, *Syzygium cuminis*, *Madhuca indica* and *Azadirachta indica* on germination and seedling growth of rice was studied on two cultivars of rice NK-5251 (Hybrid) and Gontra Bidhan-2 (High yielding variety). The seeds were sterilized in 1% HgCl₂ solution for 3 min before sowing, rinsed with sterilized water and air dried. The experiment was laid out in a complete randomized design (CRD) with twenty-six treatment combinations, each replicated thrice. Leaf extracts were prepared by grinding of 100 g leaves in mixer grinder followed by filtration in muslin cloth. The filtrate was centrifuged at 5000 rpm at 25°C for 10 min to obtain the desired leaf extracts suitable for application to seeds. The supernatant liquid was used to prepare different concentration of leaf extract (5 and 10%). Hundred seeds of each variety in three replications were placed in Petri dishes. Two layers of filter papers were laid in each petridish. The filter papers were moistened with 10 ml of tree leaf extracts to facilitate seed germination.

The petri dishes were placed in a BOD incubator at 28 ± 2°C with 12 hr photoperiod for 10 days. Seeds of two cultivars were subjected to two levels (5 and 10%) each of extracts prepared from six plants viz. *Embllica officinalis*, *Inga dulcis*, *Alstonia scholaris*, *Syzygium cuminis*, *Madhuca indica* and *Azadirachta indica* and a control. In this experiment, two different concentrations of leaf extracts viz. 5% and 10% were prepared to study the response of rice seeds to low and high concentration of allelochemicals present in the leaf leachates. The extracts of tree leaves were applied to the seeds in the petri dishes at a regular interval of 2 days to maintain adequate moisture for germination and growth of rice seedlings. The numbers of seedlings emerging daily were counted from the beginning of the experiment till germination was completed and was expressed as germination percentage in each treatment. For counting germination percentage, radicle protrusion of 2 mm or more was scored as germinated. The seedlings were evaluated as described in Seedling Evaluation Handbook (Association of Official Seed Analysis, 1991). The seedlings were harvested after ten days and used for various physiological and biochemical analyses. Germination speed was calculated by using the following formula; $GS = n / d$, where, n is the number of seedlings emerging on day

'd' and d is the day after sowing (ISTA, 2005). The data for shoot and root length (mm), fresh weight (mg) of plumule and radicle and dry weight (mg) of plumule and radicle were measured at 10th day after germination (International Allelopathy Society, 1996). Dry weights were measured after drying the seedlings at 70°C for 48 hr in an oven. Vigor index of the seedlings was estimated by the method of Abdul-Baki and Anderson (1973). Mean germination time (MGT) was calculated by the equation given by Ellis and Roberts (1981). The germination index (GI) was calculated by the formula of AOSA (1983). The formula of Coolbear *et al.* (1984) modified by Farooq *et al.* (2005) was used to calculate the time to 50% germination (T₅₀). The method of Ruan *et al.* (2002) was used to determine germination energy (GE). It was expressed as the percentage of germinating seeds five days after planting relative to the total number of seeds tested.

Chlorophyll content of leaves was estimated by following the method of Hiscox and Israelstam (1979), using dimethyl sulfoxide (DMSO). Leaf material of 50 mg was taken from fully emerged leaf and placed in a test tube, and 10 ml of DMSO was added and is kept in an oven at 65°C for about 4 hours. After 4 hours the chlorophyll was extracted in the liquid form without any grinding. The extract was taken in a measuring cylinder and final volume was made up to 10 ml by using DMSO. The absorbance of the solution was read at 663 nm, 645 nm and 470 nm using

spectrophotometer against the DMSO blank. The chlorophyll and carotenoids content was determined by using the formula given by Arnon (1949) and expressed as mg/g of fresh leaf.

Leaf relative water content (RWC) was determined by using the method of Weatherley (1950). The leaf samples weighing 0.5 g was taken and incubated in 100 ml of distilled water for 4 hrs. The weight of the turgid leaf was taken and packed in butter paper bags and dried at 75°C for 48 hr till constant weights were obtained. The relative leaf water content was estimated by dividing the difference of fresh weight and dry weight with the difference of turgid weight and dry weight and was expressed in percent. The analysis of variance of data and comparison of the means on the basis of least significant difference (LSD) were carried out using MS-EXCEL.

Results and Discussion

The leaf extracts at different concentrations had significant effect on the germination (Table 1) of both the cultivars of rice viz. NK-5251 (Hybrid) and Gontra Bidhan-2 (high yielding variety). Highest germination percent (96.7%) was recorded from 5% *Madhuca indica* extracts followed by 10% *Madhuca indica* extracts and 5% *Emblica officinalis* extract, whereas *Azadirachta indica* (10%) extract recorded the lowest germination (83.8%).

Table 1: Allelopathic effect of tree leaf extracts on germination and germination attributes of rice

| Treatments | Germination (%) | Vigour Index | Speed of germination | Germination energy | Time taken to 50% germination (days) | Mean germination time (days) |
|---------------------|-----------------|--------------|----------------------|--------------------|--------------------------------------|------------------------------|
| Cultivars | | | | | | |
| V1 | 92.1 | 160.3 | 19.77 | 63.94 | 2.64 | 3.44 |
| V2 | 86.8 | 148.0 | 20.02 | 59.35 | 2.73 | 3.56 |
| Sem (±) | 0.60 | 1.2 | 0.20 | 0.50 | 0.03 | 0.04 |
| CD (0.05) | 1.7 | 3.5 | NS | 1.42 | 0.09 | 0.13 |
| Leaf extract | | | | | | |
| Control | 90.8 | 149.8 | 23.58 | 71.55 | 2.27 | 3.00 |
| LE1 | 92.7 | 158.3 | 22.17 | 69.40 | 2.48 | 3.17 |
| LE2 | 87.8 | 138.9 | 20.92 | 64.53 | 2.73 | 3.43 |
| LE3 | 91.3 | 156.3 | 19.90 | 63.62 | 2.55 | 3.58 |
| LE4 | 87.5 | 143.0 | 18.33 | 56.28 | 2.78 | 3.63 |
| LE5 | 88.5 | 150.7 | 18.67 | 65.57 | 2.58 | 3.75 |
| LE6 | 86.3 | 140.2 | 16.18 | 56.00 | 2.95 | 3.98 |
| LE7 | 89.8 | 156.1 | 20.15 | 61.07 | 2.92 | 3.72 |
| LE8 | 85.7 | 139.2 | 17.57 | 50.45 | 3.22 | 3.97 |
| LE9 | 96.7 | 195.3 | 23.57 | 74.58 | 2.20 | 2.80 |
| LE10 | 94.2 | 175.8 | 21.95 | 71.68 | 2.37 | 3.18 |
| LE11 | 87.3 | 158.7 | 19.12 | 54.52 | 2.67 | 3.37 |
| LE12 | 83.8 | 141.3 | 16.55 | 42.13 | 3.20 | 3.93 |
| Sem (±) | 1.54 | 3.2 | 0.51 | 1.27 | 0.08 | 0.11 |
| CD (0.05) | 4.4 | 9.0 | 1.45 | 3.61 | 0.22 | 0.32 |

V₁=NK-5251 (Hybrid); V₂=Gontra Bidhan; LE₁=*Emblica officinalis* (5%); LE₂=*Emblica officinalis* (10%); LE₃=*Inga dulcis* (5%); LE₄=*Inga dulcis* (10%); LE₅=*Alstonia scholaris* (5%); LE₆=*Alstonia scholaris* (10%); LE₇=*Syzigium cuminis* (5%); LE₈=*Syzigium cuminis* (10%); LE₉=*Madhuca indica* (5%); LE₁₀=*Madhuca indica* (10%); LE₁₁=*Azadirachta indica* (5%) and LE₁₂=*Azadirachta indica* (10%). SEM=Standard Error of Mean; CD=Critical Difference

Table 2: Allelopathic effect of tree leaf extracts on seedling growth of rice

| Treatments | Seedling dry wt. (mg) | Shoot length (cm) | Root length (cm) |
|---------------------|-----------------------|-------------------|------------------|
| Cultivars | | | |
| V1 | 19.50 | 9.3 | 8.1 |
| V2 | 18.25 | 9.0 | 8.0 |
| Sem (\pm) | 0.22 | 0.1 | 0.1 |
| CD (0.05) | 0.63 | 0.2 | 0.2 |
| Leaf extract | | | |
| Control | 17.77 | 8.8 | 7.7 |
| LE1 | 20.55 | 9.1 | 8.0 |
| LE2 | 19.55 | 8.4 | 7.4 |
| LE3 | 18.72 | 9.1 | 8.1 |
| LE4 | 17.20 | 8.8 | 7.6 |
| LE5 | 16.87 | 9.1 | 7.9 |
| LE6 | 17.70 | 8.7 | 7.6 |
| LE7 | 17.08 | 9.2 | 8.2 |
| LE8 | 16.92 | 8.6 | 7.6 |
| LE9 | 22.57 | 10.8 | 9.4 |
| LE10 | 21.48 | 9.8 | 8.9 |
| LE11 | 20.18 | 9.8 | 8.4 |
| LE12 | 18.82 | 9.1 | 7.7 |
| Sem (\pm) | 0.56 | 0.2 | 0.2 |
| CD (0.05) | 1.59 | 0.5 | 0.5 |

V₁=NK-5251 (Hybrid); V₂=Gontra Bidhan; LE₁=*Emblca officinalis* (5%); LE₂=*Emblca officinalis* (10%); LE₃=*Inga dulcis* (5%); LE₄=*Inga dulcis* (10%); LE₅=*Alstonia scholaris* (5%); LE₆=*Alstonia scholaris* (10%); LE₇=*Syzygium cuminis* (5%); LE₈=*Syzygium cuminis* (10%); LE₉=*Madhuca indica* (5%); LE₁₀=*Madhuca indica* (10%); LE₁₁=*Azadirachta indica* (5%) and LE₁₂=*Azadirachta indica* (10%). SEM=Standard Error of Mean; CD=Critical Difference

The leaf extracts also had significant effect on the vigour index of both the cultivars of rice. Highest vigour index of rice seedlings was recorded from *Madhuca indica* extracts followed by *Emblca officinalis* extract whereas lowest vigour index recorded was obtained from the application of *Azadirachta indica* (10%) extract. The application of aqueous extract of neem (*Azadirachta indica* A. Juss) was also found to reduce germination of wheat and Jowar (Kasarkar and Barge, 2016).

The lower concentration of leaf extracts (5%) of *Emblca officinalis*, *Inga dulcis*, *Alstonia scholaris* and *Syzygium cuminis* was found to have positive effect on the germination of rice whereas higher concentration (10%) had negative effect on germination. The stimulatory effect of lower concentration of aqueous leaf extracts on the germination of seeds was also reported by Ahmed et al. (2008) in cowpea, arhar and chick pea, Majeed et al. (2017) in wheat and Kumar et al. (2018) in maize. The speed of germination as well as germination energy was found to be significantly affected by the application of different concentrations of leaf extracts (Table 1). The time taken to 50% germination, mean germination time of rice was found to be delayed by seed soaking in different leaf extracts, except 5%

extract of *Madhuca indica*. However, 10% extract of *Emblca officinalis*, *Inga dulcis*, *Alstonia scholaris*, *Syzygium cuminis* and *Azadirachta indica* (10%) extract significantly increased the above parameters delaying the time required for germination. The time taken to 50% germination and mean germination time was found to be lowest from *Madhuca indica* extracts (5%) treatment indicating faster germination response. The leaf extracts of *Moringa oleifera* was also found to have positive effect on the germination of cowpea (Phiri and Mbewe, 2010). Leaf extracts of *Azadirachta indica* (10%), *Syzygium cuminis* (10%) and *Alstonia scholaris* (10%) significantly reduced the germination and vigour index of rice seeds as compared to control (Table 1). Among the cultivars, NK-5251 (Hybrid) performed better in terms of germination and vigour index. However, the genotype Gontra Bidhan-2 (HYV) recorded higher speed of germination, germination energy, time taken to 50% germination, mean germination time than NK-5251. Interaction effect of cultivars and leaf extracts on germination and vigour index was significant.

Aqueous leaf extracts contain different growth regulators, inhibitors and nutrients which influence germination and seedling growth of crops when applied exogenously. The results are in conformity with the findings of Kawaguchi et al. (1997). While *Madhuca indica* extracts (both 5% and 10% concentration) enhanced the germination and vigour of rice, higher concentrations (10%) of *Azadirachta indica*, *Syzygium cuminis* and *Alstonia scholaris* significantly reduced these parameters of rice. The inhibitory effect of higher concentration of plant extracts on germination was also reported by Kumar and Gautam (2008), Swain et al. (2012) and Sahoo et al. (2015).

The aqueous leaf extracts had significant effect on the seedling dry weight, shoot length and root length of rice (Table 2). NK-5251 recorded highest seedling dry weight, shoot length and root length. Seed soaking in *Madhuca indica* extracts (5 and 10%) significantly increased the above seedling growth parameters with maximum (22.57 mg, 10.8 cm and 9.4 cm, respectively) in 5% followed by 10% concentration among different treatments. The lower concentration of *Inga dulcis* (5%) leaf extract was found to increase the seedling dry weight whereas higher concentration (10%) had negative effect. Lower concentration (5%) of *Alstonia scholaris* and *Syzygium cuminis* leaf extracts was found to increase the root and shoot length whereas higher concentration (10%) decreased these seedling parameters of rice.

The positive effect of lower concentration of leaf extracts on seedling growth was also reported by Kumar and Gautam (2008) in sunflower. The lowest seedling dry weight (16.87mg) was recorded from *Alstonia scholaris* (5%) whereas lowest shoot and root length (8.4cm and 7.4cm) was obtained from *Emblca officinalis* (10%). Seed soaking with aqueous leaf extracts had significant effect on the chlorophyll and carotenoids content of rice seedlings (Table 3). NK-5251 recorded highest chlorophyll a, chlorophyll b total chlorophyll and carotenoids contents. The highest values of these pigment components (1.697, 0.577, 2.274 and 0.316 mg g⁻¹ of chlorophyll a, b, total chlorophyll and

Table 3: Allelopathic effect of tree leaf extracts on chlorophyll, carotenoid content and relative water content of rice seedling

| Treatments | Chlorophyll a (mg g ⁻¹) | Chlorophyll b (mg g ⁻¹) | Total chlorophyll (mg g ⁻¹) | Carotenoids (mg g ⁻¹) | RWC (%) |
|---------------------|--|--|--|--------------------------------------|---------|
| Cultivars | | | | | |
| V1 | 1.458 | 0.525 | 1.982 | 0.277 | 83.0 |
| V2 | 1.403 | 0.490 | 1.893 | 0.258 | 82.3 |
| SEm (±) | 0.013 | 0.010 | 0.018 | 0.005 | 0.3 |
| CD (0.05) | 0.038 | 0.028 | 0.051 | 0.014 | NS |
| Leaf extract | | | | | |
| Control | 1.491 | 0.562 | 2.052 | 0.275 | 84.7 |
| LE1 | 1.526 | 0.569 | 2.095 | 0.295 | 82.8 |
| LE2 | 1.473 | 0.488 | 1.961 | 0.275 | 80.0 |
| LE3 | 1.432 | 0.455 | 1.886 | 0.251 | 84.0 |
| LE4 | 1.392 | 0.522 | 1.914 | 0.258 | 80.1 |
| LE5 | 1.393 | 0.507 | 1.899 | 0.270 | 82.2 |
| LE6 | 1.281 | 0.459 | 1.740 | 0.243 | 80.2 |
| LE7 | 1.283 | 0.504 | 1.786 | 0.243 | 82.6 |
| LE8 | 1.274 | 0.463 | 1.737 | 0.248 | 80.1 |
| LE9 | 1.697 | 0.577 | 2.274 | 0.316 | 89.8 |
| LE10 | 1.606 | 0.498 | 2.104 | 0.302 | 88.0 |
| LE11 | 1.395 | 0.511 | 1.906 | 0.253 | 79.6 |
| LE12 | 1.354 | 0.479 | 1.832 | 0.250 | 76.1 |
| SEm (±) | 0.034 | 0.025 | 0.045 | 0.012 | 0.7 |
| CD (0.05) | 0.096 | 0.072 | 0.129 | 0.035 | 2.1 |

V₁=NK-5251 (Hybrid); V₂=Gontra Bidhan, LE₁=*Emblca officinalis* (5%); LE₂=*Emblca officinalis* (10%); LE₃=*Inga dulcis* (5%); LE₄=*Inga dulcis* (10%); LE₅=*Alstonia scholaris* (5%); LE₆=*Alstonia scholaris* (10%); LE₇=*Syzygium cuminis* (5%); LE₈=*Syzygium cuminis* (10%); LE₉=*Madhuca indica* (5%); LE₁₀=*Madhuca indica* (10%); LE₁₁=*Azadirachta indica* (5%) and LE₁₂=*Azadirachta indica* (10%), SEm=Standard Error of Mean; CD=Critical Difference

carotenoids) were recorded from *Madhuca indica* extracts (5%) followed by *Madhuca indica* extracts (10%) whereas the lowest values (1.274, 0.463, 1.737 and 0.248 mg g⁻¹, respectively) were obtained from *Syzygium cuminis* (10%) followed by *Syzygium cuminis* (5%). This implies *Syzygium cuminis* extracts significantly reduced the pigment content whereas *Madhuca indica* extracts significantly increased the pigment contents of rice seedlings. Inhibitory effect of leaf extracts on chlorophyll content of leaves was also reported by Wang *et al.* (2018). Soaking of seeds in aqueous leaf extracts had significant effect on the plant water status expressed as relative leaf water content (RWC).

Among different leaf extracts highest RWC (89.8%) was obtained from seed soaking with 5% *Madhuca indica* extracts closely followed by 10% *Madhuca indica* extracts whereas the lowest RWC (76.1%) was obtained from *Azadirachta indica* (10%). The positive allelopathic effect of *Madhuca indica* leaf extracts can be attributed to the presence of some phytohormones and biostimulators in the aqueous extracts. However, no study has been carried out to identify and characterize the allelochemicals present in *Madhuca indica* leaves. Besides, higher concentration (10%) of *Emblca officinalis*, *Inga dulcis*, *Alstonia scholaris*, *Syzygium cuminis* and *Azadirachta indica* significantly reduced the RWC of rice seedlings. The negative effect of leaf extracts on the relative water

content was also reported by Shankar *et al.* (2014) in red gram. The study has concluded that lower concentration of leaf extracts of most of the plants enhanced germination and seedling growth whereas higher concentration had inhibitory effect. Among different tree species studied, *Madhuca indica* was found to have positive allelopathic effect both at higher and lower concentration.

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