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Bioassay screening of tropical tree sawdust for allelopathic properties and their field performance against paddy weeds

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ABSTRACT

The present study investigated the allelopathic potential of sawdust obtained from eleven tropical tree species available in Bangladesh *viz.*, *Azadirachta indica*, *Swietenia macrophylla*, *Acacia auriculiformis*, *Tamarindus indica*, *Eucalyptus camaldulensis*, *Syzygium cumini*, *Mangifera indica*, *Albizia saman*, *Artocarpus heterophyllus*, *Diospyros discolor* and *Tectona grandis*. Four concentrations of aqueous sawdust (1:5, 1:10, 1:15, 1:20 (w/v)) were tested for their potentiality in inhibiting seedling growth of allelopathic sensitive plant *Raphanus sativus* under laboratory condition. A control (distilled water without extract) was also maintained in each cases. The results of this experiment showed that *S. macrophylla*, *E. camaldulensis*, *M. indica* and *A. saman* inhibited more than 90% shoot and root growth of *R. sativus*. The sawdust of these four plant species were selected to evaluate their potentiality in controlling paddy field weeds under filed condition. A total of 16 weed control treatments were considered in the field experiment *viz.*, sawdust of selected four tree plant species at three application rates (1, 2 and 3 t ha^{-1}), manual weeding (three times), chemical control (pre- + post- emergence herbicides), chemical + manual control and season long weedy (control). The results showed that the effect of different sawdust on the weed control varied significantly. Weed growth suppression by the sawdust was increased with the increase in application rate. The results revealed that manual, chemical weed control and application of *E. camaldulensis* saw dust @ 3 t ha^{-1} reduced the weed density by 79, 77 and 72%, respectively, and weed biomass by 86, 84 and 79%, respectively. On the other hand, manual weed control offered 100% rice yield increase while chemical control and *E. camaldulensis* saw dust @ 3 t ha^{-1} both resulted in 92% rice yield increase over control. Although manual and chemical weed control offered efficient weed control and resulted in higher rice yield, from environmental viewpoint application of *E. camaldulensis* sawdust @ 3 t ha^{-1} may be considered for sustainable weed management in rice.

Keywords: Allelopathy, sawdust, tropical tree species, bio-herbicide, weed management

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1 Introduction

Weed management strategies can differ between countries but mainly rely on chemical herbicides (Thill et al., 1991). The labor-force in several developing countries including Bangladesh has shifted to industries with higher wages than agriculture, increasing the need to use herbicides. In Bangladesh the herbicide use has increased 37-fold in the last three decades (Islam et al., 2018). Intensive use of chemical herbicides has considerably increased crop productivity but sacrificing environmental and ecological issues (Aktar et al., 2009; Dass et al., 2017). Moreover, 500 known cases of herbicide-resistant weedshave been detected globally with most of these cases in developed countries (Heap, 2019), and weed management approaches should be changed to face this issue (Colbach et al., 2017).

The allelopathic plants have been proposed as an important option for alternative weed management in sustainable agriculture (Macías et al., 2007; Islam et al., 2018a). These plants could be exploited in weed management through a number of processes. For example, use them as cover/smother crops (Milchunas et al., 2011), rotational/companion crops (Putnam and Duke, 1978), application of their extracts (Jabran et al., 2010), incorporation of their residues (Cheema et al., 2000), allelopathic substances as natural herbicides (Lovett, 1990), allelopathic plant extracts with lower herbicide doses (Razzaq et al., 2010) or allelopathic crop cultivars developed by breeding program (Wu et al., 2011). In addition to weed control by incorporating allelopathic plants or their residues, some plant nutrients become available in the soil after their decomposition, which ultimately reduced the number of fertilizer requirements (Xuan et al., 2005).

The sawmill industry of different tropical countries generates a lot of sawdust. Some part of it is used as raw materials to feed the boilers for kilns, manufacturing plywood, particle board, and charcoal (Ng'andwe et al., 2015). A little amount is used for preparing compost or applies as mulch. However, a large amount of the produced sawdust is left to waste. If the farmers can introduce the sawdust of allelopathic tree species in their crop field either directly as mulch or their extract to control weeds, the environmental pollution caused by chemical herbicide can be minimized. Beside this, it will increase the organic matter status of the soil. However, a very few research works has been conducted on the use of sawdust allelopathy as a weed control agents (Gruber et al., 2008; Abouziena and Radwan, 2015). In this viewpoint, this study was designed to evaluate the allelopathic properties of 11 tropical trees sawdust of Bangladesh. Further, we investigated the herbicidal potential of selected plant sawdust in suppressing paddy field weeds under field condition.

2 Materials and Methods

2.1 Laboratory experiment

2.1.1 Experimental site and plant materials

The experiment was conducted at the Agro Innovation Laboratory, Department of Agronomy of Bangladesh Agricultural University (BAU), Mymensingh, Bangladesh. Sawdust of 11 locally available tropical tree species used in the experiment was collected from different locations of Mymensingh regions of Bangladesh. The scientific and family names of those trees are listed in Table 1.

Table 1. Plant materials used in the experiment with their local, scientific, and family name

Scientific name	Family
Azadirachta indica A. Juss	Meliaceae
Swietenia macrophylla King	Meliaceae
Acacia auriculiformis A. Cunn. ex Benth.	Fabaceae
Tamarindus indica L.	Fabaceae
Eucalyptus camaldulensis Dehnh.	Myrtaceae
Syzygium cumini (L.) Skeels	Myrtaceae
Mangifera indica L.	Anacardiaceae
Albizia saman F. Muell.	Fabaceae
Artocarpus heterophyllus Lam.	Moraceae
Diospyros discolor Willd.	Ebenaceae
Tectona grandis L.f.	Lamiaceae

2.1.2 Extraction and bioassay procedure

The fresh sawdust of those selected tree plant species was collected from different sawmills of Mymensingh region. Hundred gram sawdust of each plant species was then crushed into a paste by a grinder and soaked with 400 mL distilled water and homogenized in a warring blender for 5 min at 25°C. The extract was then filtered through one layer of filter paper (No. 2; Double Rings ® Hangzhou Xinhla Paper Industry Co. Ltd., China). The filtrate was then put into 500 mL volumetric flask and filled with distilled water up to the mark, and homogenized by manual shaking. The prepared concentration was considered as full strength concentration, *i.e.*, 1:5 (w/v) and was then stored at 4°C in a refrigerator until used. The extraction of each tree species was done separately.

The prepared aqueous extracts were then diluted into another three concentrations, *viz.*, 1:10, 1:15, and 1:20 (w/v), and control (distilled water without extract) was also maintained. A small amount (2.0 mL) of sawdust extract concentration was added to a sheet of filter paper (No. 2) in a 28 mm Petri dish. Twenty seeds of radish (*R. sativus*) were then arranged on the filter paper in each Petri dish. The experiment was conducted following a completely randomized block design with three replications. The bioassay experiment for plant sawdusts was done separately. *Raphanus sativus* was used as an indicator plant because it is very sensitive to allelopathic substances at low concentration (Tsuzuki et al., 1995). After 48 h of incubation at room temperature (25°C±2°C), the shoot and root growth of *R. sativus* were measured. The percentage of inhibition was then calculated according to the equation described by (Islam et al., 2018b) as stated below:

$$I = 1 - \left(\frac{L_E}{L_C} \times 100 \right) \quad (1)$$

where, I = inhibition (%), L_E = seedling length with aqueous extract (mm), and L_C = seedling length in control treatment (mm).

2.2 Field experiment

2.2.1 Experimental site

This experiment was conducted at the Agronomy Field Laboratory (AFL) of Bangladesh Agricultural University during the period from December 2016 to May 2017. The site is located at 24°43′8.3″N, 90°25′41.2″E. The experimental field was medium high land belonging to Old Brahmaputra Flood plain Agro-ecological zone (AEZ-9) of Bangladesh.

The soil of the experimental plot was more or less neutral in reaction with pH value 6.8, low in organic matter content (1.96%). The soil was silty-loam in texture and contained 0.1% total nitrogen, 50.4 ppm available phosphorus, 7.4 ppm available sulfur and 0.2% exchangeable potassium. During the growing season, the monthly average temperature ranged from 17.4 to 33.6°C and relative humidity was about 80%. On the other hand, monthly total rainfall and sunshine hours were 0.3–13.0 mm and 140–171 h, respectively.

2.2.2 Treatments and design

The experiment was conducted with 16 weed management practices *viz.*, (i-xii) sawdust of *Swietenia macrophylla*, *Mangifera indica*, *Albizia saman* and *Eucalyptus camaldulensis* at three application rates (1, 2 and 3 t ha^{-1}), (xiii) manual weed control [three hand weeding at 15 d interval starting from 25 d after transplanting (DAT)], (xiv) chemical weed control [pretilachlor followed bypenoxsolum (pre + post-emergence) herbicide at 2 and 21 DAT], (xv) chemical [pretilachlor at 2 DAT] + manual [one hand weeding at 35 DAT] weed control, and (xvi) season long weedy or control [no sawdust, no weed control measure]. Sawdust of *S. macrophylla*, *M. indica*, *A. saman*, and *E. camaldulensis* was used because of their higher inhibitory activity (more than 80%) in a laboratory experiment. The control treatment was also maintained where no weeding was performed or sawdust was applied. The experiment was laid out in a randomized complete block design (RCBD) with three replicates. The total number of the plot was 48. Each plot size was 2 m^2 (2 m × 1 m). The distance maintained between the individual unit plots was 0.5 m and the distance between blocks was 1.0 m.

2.2.3 Crop husbandry

BRRI dhan28, a high yielding variety of rice developed and released by Bangladesh Rice Research Institute (BRRI) was used in this experiment.The experimental plot was fertilized with urea, triple superphosphate, muriate of potash, gypsum, and zinc sulphate at the rate of 22, 12, 7.5, 6, and 1 g m^{-2}, respectively. Except for urea, the whole amount of other fertilizers was applied during final land preparation. Urea was top dressed in three equal splits at 15, 30, and 45 DAT. The sawdust of the selected timber plant species was also applied at the time of final land preparation as per the treatments mentioned above. Sawdust was then mixed well with the soil by using a spade. Seedlings (30 days old) were transplanted in the well-puddled plot at 3 seedlings hill^{-1} maintaining the spacing of 25 cm × 15 cm on January 15, 2017.

2.2.4 Data collection

Data on weed population were collected from each plot by using 25 cm^2 quadrate based on the method described by Cruz et al. (1986). The weeds within the quadrate were counted and converted to number per square meter. After counting the weed density, the weeds inside each quadrate were uprooted, cleaned, separated species-wise, and dried in an electric oven at a temperature of 60±2°C until constant weight. The dry weight of each species was taken by an electric balance and expressed in g m^{-2}.

2.2.5 Sampling, harvesting, and processing

The crops were harvested at full maturity, *i.e.*,when 90% of the grains became golden yellow in color. The harvested crops of each plot were bundled separately, properly tagged, and brought to the threshing floor. The crops were then threshed, sun-dried, cleaned, and grain and straw weight was recorded. The final grain weight was adjusted to moisture content of 14% and converted to t ha^{-1}.

2.3 Statistical analyses

The recorded data were compiled and tabulated for statistical analyses. Analysis of variance was done following completely randomized design (CRD) for laboratory experiment and randomized complete block design (RCBD) for field experiment. The statistical analyses were performed with the statistical program 'XLStat'. The mean differences among the treatments

Table 2. Dominant weed species found in the control plots with their scientific name, family name, and type

Sl. no.	Scientific name	Family	Weed type
1	*Panicum disticum* Lam.	Gramineae	Grass
2	*Echinochloa crusgalli* (L.) P. Beauv	Gramineae	Grass
3	*Cynodon dactylon* (L.) Pers.	Gramineae	Grass
4	*Digitaria sanguinalis* (L.) Scop.	Gramineae	Grass
5	*Echinochloa colonum* (L.) Link.	Gramineae	Grass
6	*Physalis minima* L.	Solanaceae	Broadleaf
7	*Mimosa pudica* L.	Leguminosae	Broadleaf
8	*Polygonum orientale* L.	Polygonaceae	Broadleaf
9	*Polygonum hydropiper* (L.) Delabre	Polygonaceae	Broadleaf
10	*Eclipta alba* L.	Compositae	Broadleaf
11	*Commelina bengalensis* L.	Commelinaceae	Broadleaf
12	*Cyperus rotundus*	Cyperaceae	Sedge
13	*Cyperus nemoralis*	Cyperaceae	Sedge

were adjudged following Duncan's Multiple Range Test.

3 Results

3.1 Bioassay

3.1.1 Effect on shoot growth

The aqueous sawdust extract of all the tree species, except *S. cumini* and *D. discolor*, significantly inhibited the shoot length of *R. sativus* at concentrations more than 1:20 (w/v) (Figure 1a). The inhibitory activity was concentration dependent. At 1:5 (w/v) concentration, the highest shoot growth inhibition (95%) was observed by *M. indica* sawdust extract followed by *A. saman* (94%), *S. macrophylla* (84%), and *E. camaldulensis* (81%) (Figure 1a). The lowest inhibition was observed in *D. discolor* (37%) followed by *A. heterophyllus* (47%). On the other hand, other plant species inhibited the shoot growth of *R. sativus* from 50 to 70% (Fig. 1a). At lower concentration, *A. indica*, *A. auriculiformis*, *T. indica*, *S. cumini*, and *D. discolor* plant extracts stimulated the shoot growth of *R. sativus*, but stimulation decreased with increasing concentration.

3.1.2 Effect on root growth

The aqueous sawdust extract of all the tree species, except *S. cumini* and *D. discolor*, significantly inhibited the root length of *R. sativus* at concentrations more than 1:20 (w/v) (Fig. 1b). Here, the inhibitory activity was also concentration dependent. At 1:5 (w/v) concentration, the highest root growth inhibition (97%) was found in *E. camaldulensis* followed by *S. macrophylla* (95%), *M. indica* (94%), and *A. saman* (93%) (Fig. 1b).The lowest inhibition was observed in *D. discolor* (63%) followed by *A. heterophyllus* (64%). Other plant species showed 73% root growth inhibition on an average and their inhibition values ranged from 68% to 77% (Fig. 1b). Similar to shoot growth, the root growth of *R. sativus* was also stimulated by sawdust extract of *A. indica*, *A. auriculiformis*, *T. indica*, *S. cumini*, and *D. discolor* at a lower concentration.

3.2 Field experiment

3.2.1 Effect on weed density and biomass

Thirteen weed species of seven different families were observed in control plots (season long weedy), among which six were broadleaf, five grasses, and two sedges (Table 2). Weed density and biomass were significantly influenced by the different treatments used in the experiment (Table 2). The control plot (season long weedy) gave the highest weed density (187) and biomass (1475 g m^{-2}) followed by *A. saman* sawdust at 3 t ha^{-1} and *S. macrophylla* sawdust at 1 t ha^{-1} in case of weed density (152) and biomass (1228 g m^{-2}), respectively (Fig. 2). Weed density and biomass were decreased with the increase in the rate of sawdust application. The lowest weed density (35) was observed when *M. indica* sawdust was applied at 3 t ha^{-1} followed by three times manual weeding (40), chemical control (43), chemical + manual (50), and *S. macrophylla* sawdust @ 3 t ha^{-1} (50) (Fig. 2). On the other hand, three times manual weeding gave the lowest weed biomass (208 g m^{-2}) followed by chemical control (pre + post-emergence) (235 g m^{-2}), *E. camaldulensis* sawdust at 3 t ha^{-1} (314 g m^{-2}) and *S. macrophylla* sawdust @ 3 t ha^{-1} (315 g m^{-2}) (Fig. 2).

3.2.2 Effect on rice yield

The results showed that application of sawdust and weed control methods significantly influenced the grain and straw yield of rice. The lowest grain yield (2.6 t ha^{-1}) was found in season-long weedy treatment followed by *M. indica* sawdust applied at 1 t ha^{-1} (Fig. 3a). The highest grain yield (5.2 t ha^{-1}) was

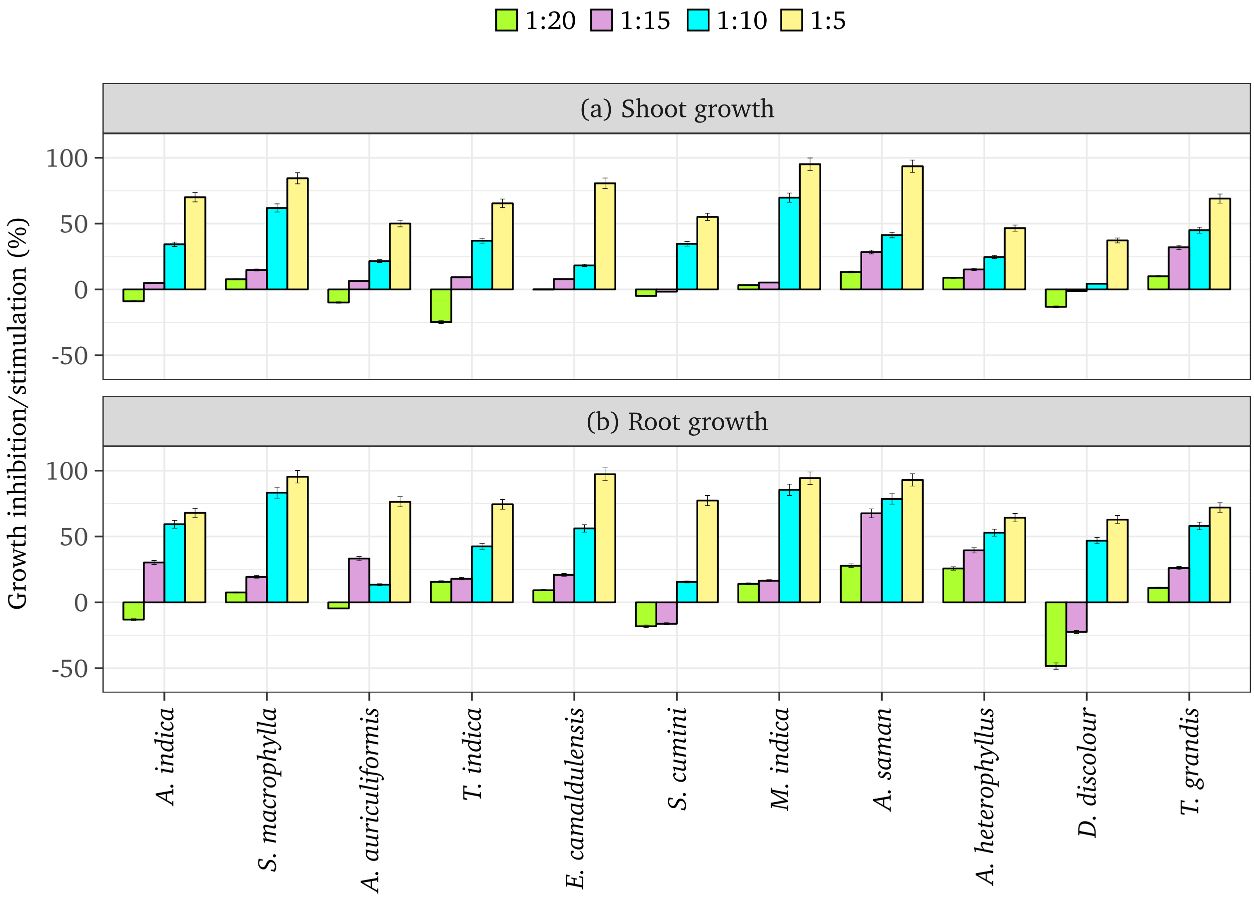

Figure 1. Effect of aqueous extracts of timber plant species on growth inhibition/stimulation of (a) shoot, and (b) root of *R. sativus*. The (+) values in Y-axis shows inhibition and (−) values indicate stimulation

obtained from three times manual weeding followed by *E. camaldulensis* saw dust at 3 t ha^{-1}(5.0 t ha^{-1}) and chemical control of weeds (pre + post-emergence) (5.0 t ha^{-1}) (Fig. 3a). The lowest straw yield (3.7 t ha^{-1}) was observed in season-long weedy treatment followed by *M. indica* sawdust applied at 2 t ha^{-1} (Fig. 3b). The highest straw yield (6.5 t ha^{-1}) was observed in three times manual weeding followed by chemical control (pre + post-emergence) (6.4 t ha^{-1}) and *A. saman* sawdust applied at 3 t ha^{-1} (Fig. 3b).

3.2.3 Weed infestation and rice yield

A negative relation of rice grain yield with weed density ($r = -0.587$, $p = 0.016$) and weed biomass ($r = -0.808$, $p < 0.001$) was observed (Fig. 4). Results showed that weed biomass (80%) could explain rice grain yield more than weed density (58%).

4 Discussion

Inhibitory activities of the aqueous extracts of sawdust of eleven tree species against *R. sativus* seedling growth increased with extract concentration. In some cases, stimulatory activity was also found at lower concentrations. However, the degree of inhibition of *R. sativus* by the sawdust extract of different tree species was variable. This type of growth inhibition by allelopathic plants extracts was also reported by other workers elsewhere (Batlang and Shushu, 2007; Pukclai and Kato-Noguchi, 2011). In comparison to the shoot growth of *R. sativus*, its root growth was more sensitive to the aqueous sawdust extracts of 11 timber plant species. These results have been supported by earlier findings of Levizou et al. (2002); Islam and Kato-Noguchi (2012, 2013) and Islam et al. (2018b) who reported that the extracts of allelopathic plants had higher root growth inhibition than the shoot. These results confirm that the inhibitory potential of the plant species is due to their allelopathic properties.

Although a plant shows strong allelopathic activity on target plant in laboratory experiments, the magnitude of its activity might differ in the field conditions due to the influence of environmental conditions (Qasem, 2010). That's why the field trial was

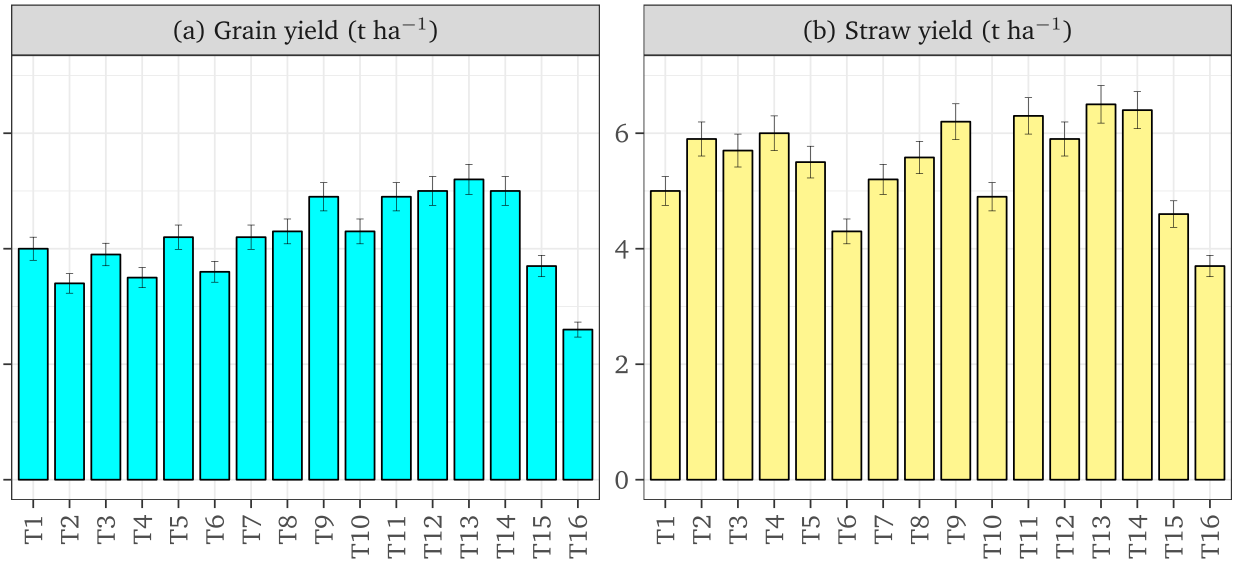

Figure 2. Effects of different plant species sawdusts on (a) weed density, and (b) biomass. Here, T1 = *S. macrophylla* sawdust at 1 t ha^{-1}, T2 = *M. indica* sawdust at 1 t ha^{-1}, T3 = *A. saman* sawdust at 1 t ha^{-1}, T4 = *E. camaldulensis* sawdust at 1 t ha^{-1}, T5 = *S. macrophylla* sawdust at 2 t ha^{-1}, T6 = *M. indica* sawdust at 2 t ha^{-1}, T7 = *A. saman* sawdust at 2 t ha^{-1}, T8 = *E. camaldulensis* sawdust at 2 t ha^{-1}, T9= *S. macrophylla* sawdust at 3 t ha^{-1}, T10 = *M. indica* sawdust at 3 t ha^{-1}, T11 = *A. saman* sawdust at 3 t ha^{-1}, T12 = *E. camaldulensis* sawdust at 3 t ha^{-1}, T13 = manual weed control, T14 = chemical weed control, T15 = chemical + manual weed control, and T16 = season long weedy (control)

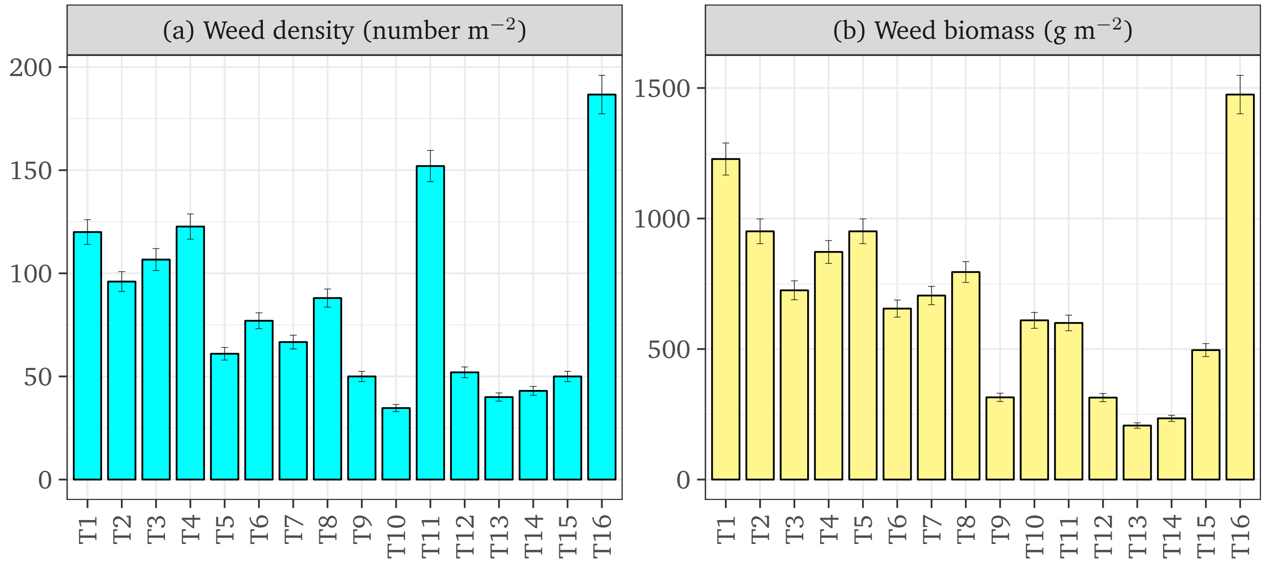

Figure 3. Effects of different plant species sawdusts on (a) weed density, and (b) biomass. Here, T1 = *S. macrophylla* sawdust at 1 t ha^{-1}, T2 = *M. indica* sawdust at 1 t ha^{-1}, T3 = *A. saman* sawdust at 1 t ha^{-1}, T4 = *E. camaldulensis* sawdust at 1 t ha^{-1}, T5 = *S. macrophylla* sawdust at 2 t ha^{-1}, T6 = *M. indica* sawdust at 2 t ha^{-1}, T7 = *A. saman* sawdust at 2 t ha^{-1}, T8 = *E. camaldulensis* sawdust at 2 t ha^{-1}, T9= *S. macrophylla* sawdust at 3 t ha^{-1}, T10 = *M. indica* sawdust at 3 t ha^{-1}, T11 = *A. saman* sawdust at 3 t ha^{-1}, T12 = *E. camaldulensis* sawdust at 3 t ha^{-1}, T13 = manual weed control, T14 = chemical weed control, T15 = chemical + manual weed control, and T16 = season long weedy (control)

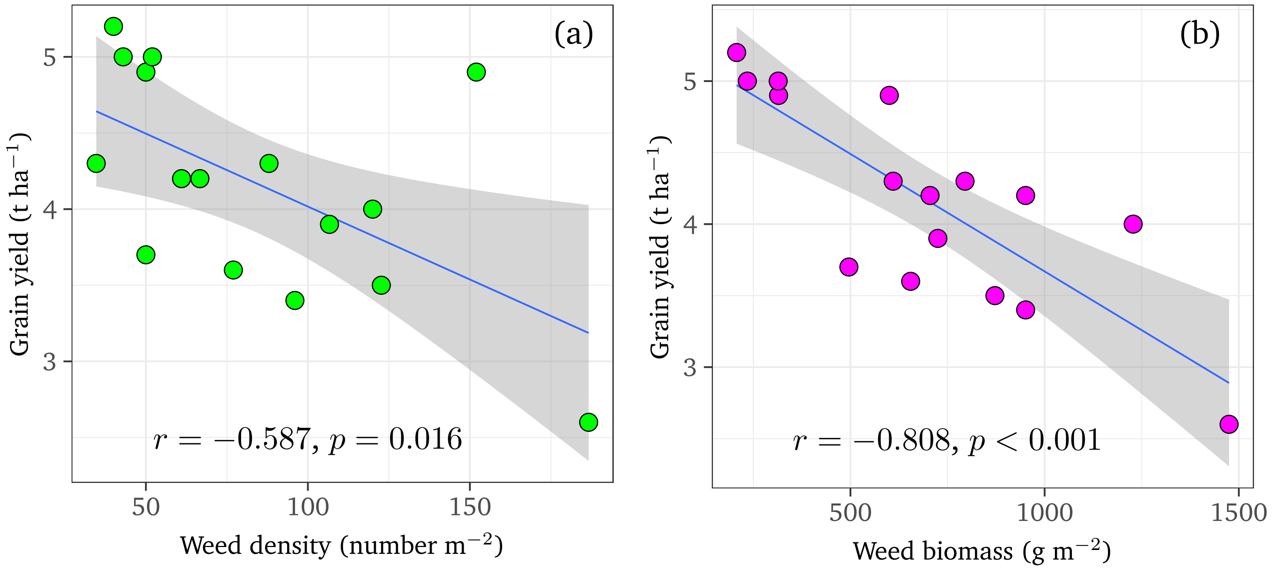

Figure 4. Relationship of weed density and weed biomass with rice grain yield. The shade denotes the region of standard error.

conducted to evaluate the activity of selected sawdust under field condition. As *E. camaldulensis*, *S. macrophylla*, *M. indica*, and *A. saman* had shown more than 80% shoot-root growth inhibition under laboratory conditions, these four plants were used for the field experiment. Here, the activity of this plant sawdust was compared with other conventional weed management options. The results revealed that manual weed control (farmers' practice) reduced 79% weed density and 86% weed biomass, whereas chemical weed control (application of pre- + post-emergence herbicide) reduced 77% and 84% weed density and biomass, respectively. On the other hand, 72% weed density and 79% weed biomass reduction were obtained by *E. camaldulensis* sawdust at 3 t ha^{-1}. Although a huge number of reports have been published about the allelopathic potential of different plant parts, surrounding soil extract or litter fall of *E. camaldulensis*, its field performance is very scant (May and Ash, 1990; Espinosa-Garcia et al., 2008; Khan et al., 2008; Ahmed et al., 2008; Samedani et al., 2013; Chu et al., 2014; Abdullahi et al., 2017). The current research explores the field performance of *E. camaldulensisin* suppressing weeds.

On the other hand, manual weed control offered 100% yield increase while chemical control and *E. camaldulensis* saw dust at 3 t ha^{-1} both gave 92% yield increase over control. Even though the lowest weed density and biomass and higher grain yield were observed in manual weed control (three times manual weeding), *E. camaldulensis* sawdust at 3 t ha^{-1} was the best from a sustainability point of view. In that case, only 3.8% yield the farmers have to sacrifice, which is negligible when environmental pollution is of huge concern nowadays. Some other researchers have also conducted similar types of research. For example, alfalfa pellets at 1-2 t ha^{-1} completely inhibited the germination of *Dopatrium junceum*, *Lindernia pyxidaria*, and *Elatine triandra*, and also reduced the density and biomass of *Echinochloa oryzicola* (Xuan et al., 2001). Hong et al. (2004) worked with ten allelopathic higher plants, and all the species at 2 t ha^{-1} significantly reduced rice weed growth and promoted the rice growth and yield. Xuan et al. (2001) observed Japanese alfalfa variety (Rasen) inhibited 80% total weed biomass and promoted 81% rice yield when compared with the control (without any weed and fertilizer management). They also observed that herbicide treatment suppressed 75% paddy weeds and increased rice yield by only 10%, whereas those of hand weeding were about 70% and 25%, respectively.

In addition, an indiscriminate use of chemical herbicides increases the herbicide resistance in weeds and ultimately developed herbicide-resistant weed biotypes (Juraimi et al., 2013; Anwar et al., 2014). Currently, there are 500 unique cases (species × site of action) of herbicide resistant weeds have been discovered globally, with 256 species (149 dicots and 107 monocots) (Heap, 2019). Therefore, it is much difficult to control them through chemical herbicides. Allelopathic plants showed promising results in controlling those weeds. For example, (Xuan et al., 2005) reported that application of alfalfa and rice byproducts at the rate of 1–2 t ha^{-1} could completely inhibit the emergence of *Rotala indica*, an herbicide resistant noxious weed of Japan.

5 Conclusions

All the studied tree species except *D. discolor* and *A. heterophyllus* showed allelopathic potential. In addition, *E. camaldulensis*, *S. macrophylla*, *M. indica*, and *A. saman* had shown strong shoot and root growth inhibition of *R. sativus* under laboratory condition and performed accordingly in suppressing weed growth under field condition. Weed growth suppression by the sawdust increased with the increase of application rate. Although lowest weed density and biomass and higher grain yield were observed in three times manual weeding or weed control through pre- + post-emergence herbicide application, sawdust of *E. camaldulensis* at 3 t ha^{-1} also gave very close result in terms weed density and biomass reduction, and rice yield performance. Based on these results, we may conclude that the tropical tree species *E. camaldulensis* has very strong allelopathic potential and thus sawdust of this species could be used for the development of eco-friendly bio-herbicide. As this is the first report on the application of sawdust of those plant species, multi-location trials at different agro-climatic conditions considering more tropical tree speciesare strongly recommended for identifying the most allelopathic potential tree species toward developing bioherbicides for sustainable weed management in rice. In addition, residues of *E. camaldulensis* may also affect crops in rotation unless the land is permanently devoted to rice cultivation and hence, this issue also has to evaluate before recommending it for weed control.

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Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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