

Determination of allelopathic properties of *Acacia catechu* (L.f.) Willd.

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Abstract

Plants possessing allelopathic potential could be used as a source of bio-herbicide to help decrease the use of synthetic herbicides. *Acacia catechu* (L.f.) Willd., a deciduous tree from the Mimosaceae family, has been reported to have medicinal properties. However, there have been no reports on the allelopathy of this tree. Therefore, the leaf extracts of *A. catechu* were examined for allelopathic potential using six concentrations: 0.001, 0.003, 0.01, 0.03, 0.1, and 0.3 g dry weight equivalent extract mL⁻¹. The aqueous methanol extracts of *A. catechu* significantly suppressed the seedling growth of six test plants such as alfalfa, cress, lettuce, barnyard grass, Italian ryegrass, and timothy. The extracts inhibited the six test plants in a concentration-dependent manner. The shoot and root growth of all the test plant species were completely inhibited from the concentration of 0.3 g of dry weight equivalent extract mL⁻¹, except the shoot growth of barnyard grass. Concentrations needed for 50% growth inhibition (I_{50} values) ranged from 0.004 to 0.043 g dry weight equivalent extract mL⁻¹ for shoot growth, and 0.003 to 0.019 g dry weight equivalent extract mL⁻¹ for root growth. Moreover, the I_{50} values indicated that the root growth of all the test plants was more susceptible to the *A. catechu* extracts than the shoot growth. The inhibitory effects of the extracts of *A. catechu* suggest that the extracts may contain allelopathic potential and, therefore, may be a potential candidate for the isolation and characterization of allelochemicals to develop an ecofriendly bio-herbicide.

Keywords: *Acacia catechu*; allelopathy; bio-herbicides; weed control

Introduction

Weeds are plants that negatively affect farming and forestry industries, such as growing of crops, grazing of animals, and planting of forest trees (Popay, 2008). Most species of weed spread very fast and compete with crops for water, soil, nutrients, light, and space, resulting in crop yield reduction in agricultural ecosystems (McErlich and Boydston, 2013). It has been reported that weed infestation causes about 34% loss of major crop yields throughout the world (Jabran *et al.*, 2015). Synthetic herbicides are commonly applied to control weeds. Indiscriminate use of herbicides results in the development of herbicide resistance in weeds, a serious risk to humans and animals (Mazumder, 2011; Daniel *et al.*, 2013; Heap, 2014). Currently 514 unique cases (species x site of action) of weed have developed resistance to many herbicides (Heap, 2019). Therefore, it is crucial to find alternative ways to manage weeds that is sustainable and eco-friendly. The biological approach is considered a natural method of weed management in farming systems (Hunt *et al.*, 2017; Xiao *et al.*, 2017;

Ojija *et al.*, 2019). Biological approach of weed control is a method which integrates the use of various natural organisms and approaches like allelopathy, competition of crop, and different agronomic practices (Sodaiezhadeh and Hosseini, 2012). Among them allelopathy is considered as one of the most important biological approach. The releasing of bioactive derivative metabolites by different organisms such as plants, insects, microbes, algae, and fungi, and the successive interaction with other organisms, inducing positive or negative effects, is termed allelopathy (Fujii, 2003; Weston and Duke, 2003; Zeng *et al.*, 2008). The application of allelopathic plant species and allelopathic substances for sustainable weed control or management in agriculture has been investigated in various studies (Islam *et al.*, 2019; Rob *et al.*, 2020). Allelochemicals are released into the environment through leaching, root exudation, volatilization, or decomposition of crop residues (Fujii, 2003). Searching for allelopathic potential of various plants is an ongoing process and is the most important first step towards developing bio-herbicides in order to reduce dependence on synthetic herbicides.

Acacia catechu is a deciduous plant with a light feathery crown that reaches heights of 9-15 m. The plant grows well in Bangladesh, India, Pakistan, Sri Lanka, Bhutan, Indonesia, Mauritius, Myanmar, Nepal, Taiwan, Thailand, USA, Vietnam, and China in both natural and managed plantations (Chakrabarty and Gangopadhyay, 1996), and is an important species for bioresources and multipurpose use. *Acacia catechu* is the leading source of tannin, which is one of the important forest products currently traded globally (Singh and Lal, 2006). This plant is commonly known as khair in Bangladesh. Katha and cutch are obtained from extracts of the heartwood of *A. catechu*. Katha is used in betel chewing together with the leaf of *Piper betle* L. Cutch is a substance marketed as a solid extract and used as dye, polishes for furniture, and paints for various uses (Singh, 2000), and for tanning leather. *Acacia catechu* also has many medicinal properties. The extract of *A. catechu* has been recorded to have different pharmacological effects such as immunomodulatory (Ismail and Asad, 2009), antioxidative (Li *et al.*, 2010; Lakshmi *et al.*, 2011), antibacterial (Lakshmi and Arvind, 2011), antipyretic, hypoglycaemic (Singh *et al.*, 1976), antidiarrhoeal (Ray *et al.*, 2006), and hepatoprotective (Ray *et al.*, 2006). It is beneficial for the treatment of colds and coughs (Anonymous, 2002; Wallis, 2005), ulcers, bleeding piles, boils and other skin eruptions, atonic dyspepsia, uterine haemorrhage, and chronic bronchitis. The medicinal properties of *A. catechu* are well known. The medicinal plants have relatively potent allelopathic potential (Fujii *et al.*, 2003; Morikawa *et al.*, 2012; Appiah *et al.*, 2015).

Recently, different potent allelochemicals have been isolated from medicinal plants (Kato-Noguchi *et al.*, 2013; Piyatida *et al.*, 2013; Bich and Kato-Noguchi, 2014; Kato-Noguchi *et al.*, 2014; Suwitchayanon *et al.*, 2015; Appiah *et al.*, 2017; Raihan *et al.*, 2019). While the medicinal and other uses of *A. catechu* are well known, there is only one report in the literature about the allelopathic potential of this plant (Singh *et al.*, 2006). Singh *et al.* (2006) did their study in field condition and they used only crop species for their study but in this study, we used crop and weed species. Therefore, this study was undertaken to evaluate and confirm the allelopathic activity of *A. catechu*.

Materials and Methods

Plant materials

Medium age and size plants were selected during warm and moist weather condition. These types of plants contain all of the typical characteristics of trees. Mature and healthy leaves (leaf size and number: 1-2 cm, approximately 10000 leaves) from middle part of *Acacia catechu* plant were collected from the Noakhali Science and Technology University (22° 47' 31" N and 91° 06' 07" E), Noakhali, Bangladesh, in August 2019. Professor Sarwar AKM Golam (Department of Crop Botany, Bangladesh Agricultural University) identified the species as *Acacia catechu*. The authors preserved a voucher and the voucher number is AGNSTU 19MP-0001 deposited in the Medicinal Plant Herbarium, Department of Agriculture, Noakhali Science and Technology University (NSTU), Bangladesh. The collected leaves were washed in running water to clear

debris. The leaves were then dried in the shade until constant weight was reached. Finally, the dried leaves were ground into powder, which was kept in a polyethylene bag, and stored in a refrigerator at 2 °C until use.

Test plant species

Six test plant species were selected for this experiment: alfalfa (*Medicago sativa* L.), cress (*Lepidium sativum* L.), lettuce (*Lactuca sativa* L.), Italian ryegrass (*Lolium multiflorum* Lam.), barnyard grass (*Echinochloa crus-galli* (L.) P. Beauv), and timothy (*Phleum pratense* L.). The first three plants are dicotyledonous and the latter three are monocotyledonous. Alfalfa, lettuce, cress, and timothy were chosen for their well-known growth characteristics, while Italian ryegrass and barnyard grass were chosen for their wide distribution, mostly in crop lands (Rob and Kato-Noguchi, 2019).

Extraction of Acacia catechu leaves

The leaf powder (50 g) of *A. catechu* was extracted with 450 mL of 70% (v/v) aqueous methanol for 48 h and filtered with one layer of filter paper (No. 2, 125 mm; Advantec Toyo Roshi Kaisha, Ltd., Tokyo, Japan) through a vacuum pump. The residue was re-extracted again with an equal amount of 100% (v/v) methanol for 24 h and filtered. Both filtrates were then mixed and evaporated until complete dryness through a rotary evaporator at 40°C.

Growth bioassays

The crude extract of *A. catechu* was dissolved in 150 mL of methanol to make six bioassay concentrations (0.001, 0.003, 0.01, 0.03, 0.1, and 0.3 g dry weight equivalent extract mL⁻¹). To produce those concentrations, aliquots of the methanol extract of *A. catechu* (3, 9, 30, 90, 300, and 900 µL, respectively) were put onto sheets of filter paper (No. 2, 28 mm; Advantec) in Petri dishes (28 mm). The filter papers were then desiccated in a draft chamber and wetted with 0.6 mL of 0.05% (v/v) aqueous solution of polyoxyethylene sorbitan monolaurate (Tween20; Nacalai Tesque, Inc., Kyoto, Japan). Tween 20 was used as a surfactant and had no noxious effects on the growth of the seedlings (Islam and Kato-Noguchi, 2016). Next, 10 seeds of cress, alfalfa, and lettuce, and 10 sprouted seeds of barnyard grass, Italian ryegrass, and timothy (seeds were moistened for 24 h in distilled water and then permitted to germinate at 25 °C in darkness for 72, 60, and 48 h, respectively) were placed on the filter papers (No. 2, 28 mm; Advantec) in Petri dishes (28 mm), and the seeds or sprouted seeds were also placed on a filter paper soaked with 0.6 mL of 0.05% (v/v) aqueous solution of Tween 20 as a control and kept in the growth chamber. After 48 h of incubation in the dark at 25 °C the growth seedling of the tested plants was estimated. The percentage of the seedling length growth was measured by referring to the control seedling lengths.

Statistical analysis

All the bioassay experiments were undertaken in a completely randomized design (CRD) with three replications and repeated twice using 10 seedlings. The resulting data were analyzed using SPSS software version 16.0 (IBM Corp., 2007). The data obtained from each experiment were then subjected to analysis of variance (ANOVA), and the significant differences between the mean of treatments and control were calculated using Tukey's HSD test at the 0.05 probability level. The I_{50} values (Concentrations needed for 50% growth inhibition) were determined for each test plant species using a regression equation. The correlation of coefficient (R) between the extract concentrations and seedling growth was analyzed using a two-tailed Pearson correlation test (SPSS version 16.0) of the test plant species.

Results

Effect of aqueous methanol extracts of Acacia catechu on the shoot growth of the test plants

The aqueous methanol extracts of *A. catechu* significantly inhibited the shoot growth of the tested plants (Figures 1 and 2). At a concentration of 0.03 g dry weight equivalent extract mL⁻¹, the shoot growth of alfalfa, cress, and lettuce was less than 20% of control shoot growth, while Italian rye grass, barnyard grass, and timothy were 31.75, 64.57, and 32.62% of control shoot growth, respectively. Likewise, at a concentration of 0.1 g dry weight equivalent extract mL⁻¹, the shoot growth of the tested plants was inhibited more than 90% by the extracts, except barnyard grass. In contrast, the shoot growth of cress and lettuce was inhibited more than 60%, while alfalfa, barnyard grass, Italian ryegrass, and timothy were inhibited to 54.21, 26.47, 29.94, and 32.76% of control, respectively, at the concentration of 0.01 g dry weight equivalent extract mL⁻¹ (Figure 2). With exposure to the concentration of 0.3 g dry weight equivalent extract mL⁻¹, the shoot growth of the tested plants was completely inhibited, except barnyard grass. The correlation coefficient (R) measures the direction and strength of a linear relationship. It always has a value between 1 and -1. Strong positive linear relationships have values R of closer to 1. Strong negative linear relationships have values of R closer to -1. The correlation coefficient (R) between shoot growth and the concentration of the extracts of *A. catechu* ranged from -0.781 to -0.908 (Table 1). The concentration required for 50% growth inhibition (I_{50}) by the extracts of *A. catechu* on the shoot growth of the tested plants ranged from 0.004 to 0.043 g dry weight equivalent extract mL⁻¹ (Table 2). Further, the I_{50} values indicated that the lettuce shoots were the most sensitive to the extracts, while the barnyard grass shoots were the least sensitive.

Table 1. Correlation coefficient between the seedling growth of the tested plants and the concentration of the extracts of *A. catechu*

Test plant species	Correlation coefficient (R)	
	Shoot	Root
Alfalfa	-0.864**	-0.849**
Cress	-0.908**	-0.931**
Lettuce	-0.884**	-0.909**
Italian ryegrass	-0.781**	-0.881**
Barnyard grass	-0.798**	-0.854**
Timothy	-0.830**	-0.870**

** Indicates significance of correlation at $p < 0.01$.

Table 2. The concentration needed for 50% growth inhibition (I_{50} values) of the shoot and root growth of the tested plants by the extracts of *Acacia catechu*

Test plant species	I_{50} (g dry weight equivalent extract mL ⁻¹)	
	Shoot	Root
Alfalfa	0.008	0.006
Cress	0.005	0.004
Lettuce	0.004	0.003
Italian ryegrass	0.018	0.010
Barnyard grass	0.043	0.019
Timothy	0.017	0.006

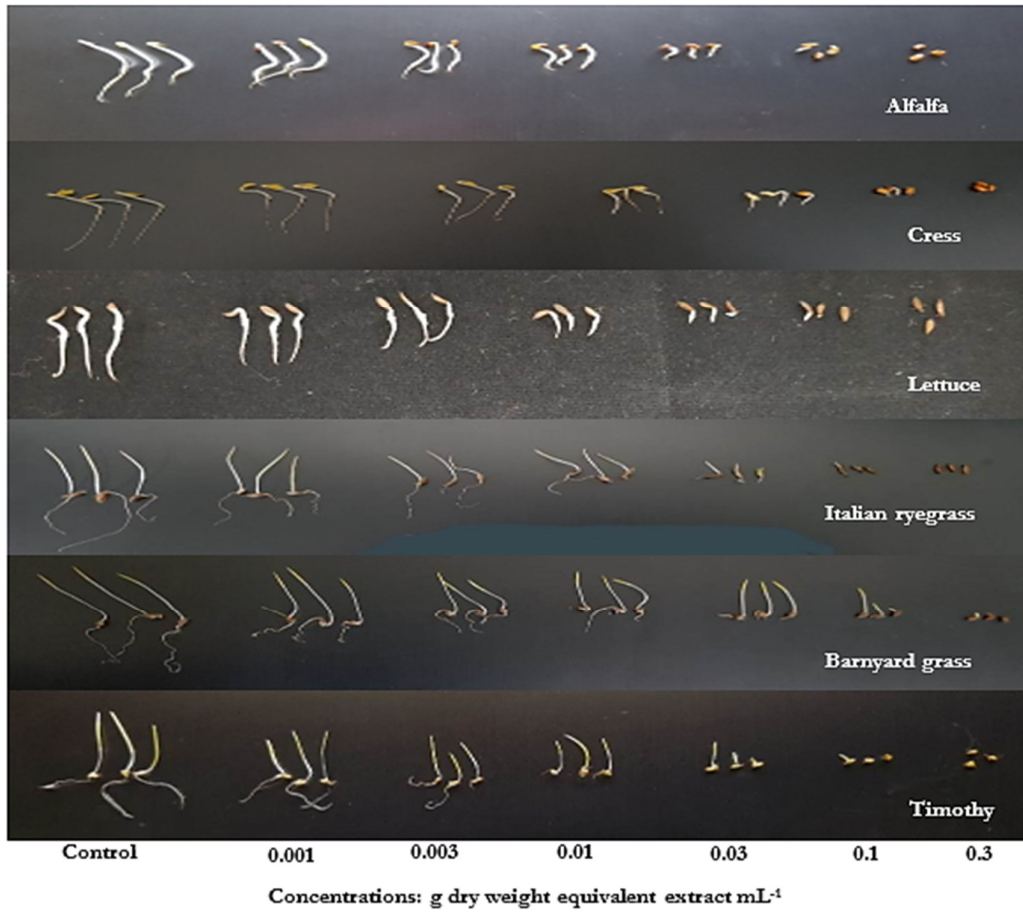


Figure 1. Effect of *Acacia catechu* extracts on the seedling growth of alfalfa, cress, lettuce, Italian ryegrass, barnyard grass, and timothy

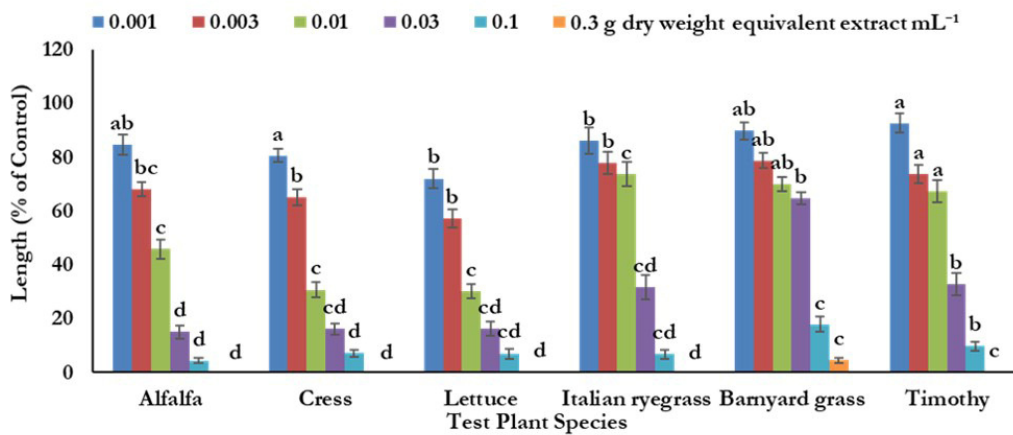


Figure 2. Effect of *A. catechu* extracts on the shoot growth of the six tested plants. The tested plants were exposed to concentrations of 0.001, 0.003, 0.01, 0.03, 0.1, and 0.3 g dry weight equivalent extract of *A. catechu* mL⁻¹

Mean ± SE from 2 independent experiments with 3 replications for each treatment are presented (number of seedlings per treatment=10, n=60). Standard error of the mean is depicted by a vertical bar. The different letters in the same panel indicate significant difference according to Tukey's HSD test at the 0.05 probability level.

Effect of aqueous methanol extracts of Acacia catechu on the root growth of the test plants

The aqueous methanol extracts of *A. catechu* significantly inhibited the root growth of the tested plants (Figures 1 and 3) in a concentration-dependent manner. At a concentration of 0.3 g dry weight equivalent extract mL⁻¹, the root growth of the tested plants was completely inhibited (100%) by the extracts (Figure 3). The root length of alfalfa, cress, lettuce, and timothy was less than 45% compared with control, while the root length of Italian ryegrass and barnyard grass was restricted to 52.23 and 59.47% of control, respectively, by 0.01 g dry weight equivalent extract mL⁻¹. When the tested plants were exposed to a concentration of 0.1 g dry weight equivalent extract mL⁻¹, the root growth of the tested plants was inhibited to below 5% of control, except cress root length (Figure 3). In addition, the root lengths of the tested plants were markedly decreased by the concentration of 0.03 g dry weight equivalent extract mL⁻¹, with alfalfa exhibiting the strongest inhibition (92.74%), followed by Italian ryegrass (89.4%), timothy (87.7%), cress (84.9%), lettuce (84.62%), and barnyard grass (64.58%). The correlation coefficient between the extract of *A. catechu* and root length varied from -0.849 to -0.931 (Table 1). The *I*₅₀ values for the root growth of the tested plants varied from 0.003 and 0.019 g dry weight equivalent extract mL⁻¹ (Table 2), with lettuce being the most susceptible to the extracts, while barnyard grass was least susceptible. In addition, the decrease in root growth of the tested plants was greater than that of the shoots.

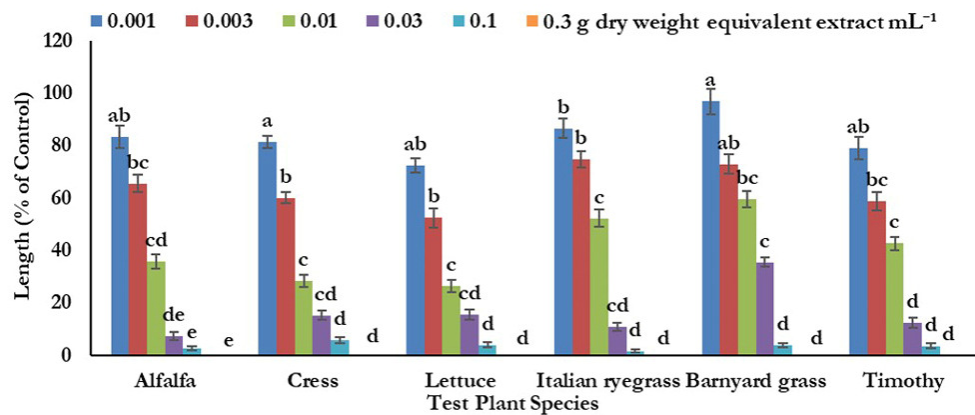


Figure 3. Effect of *A. catechu* extracts on the root growth of the six tested plants. The tested plants were exposed to concentrations of 0.001, 0.003, 0.01, 0.03, 0.1, and 0.3 g dry weight equivalent extract of *A. catechu* mL⁻¹

Mean \pm SE from 2 independent experiments with 3 replications for each treatment are presented (number of seedlings per treatment=10, n=60). Standard error of the mean is depicted by a vertical bar. The different letters in the same panel indicate significant difference according to Tukey's HSD test at the 0.05 probability level.

Discussion

The extracts of *A. catechu* significantly inhibited the growth of alfalfa, cress, and lettuce (dicotyledonous plants) and Italian ryegrass, barnyard grass, and timothy (monocotyledonous plants). Stronger inhibitory effects were found at higher extract concentrations, indicating that inhibition was concentration dependent. This type of concentration-dependent inhibition has been reported by Sinha and Samar (2004), Swain *et al.* (2005), Ishak and Sahid (2014), Gulzar *et al.* (2016), Al-Harbi (2018), Mushtaq *et al.* (2018), Zaman *et al.* (2020), and Hossen *et al.* (2020). The extracts of *A. catechu* had different inhibitory activities on different tested plants, showing that the growth inhibitory effects of this plant's extracts depended on the target plant species. Rob and Kato-Noguchi (2019), and Islam and Kato-Noguchi (2016) also reported different inhibition of target plants by growth inhibitory substances, and some species are more susceptible compared with other species. The results of this study showed that the shoot growth of the tested plants was less sensitive to the

extracts than the root growth. The root system plays a vital role in plant adaptation to edaphic limitations, and biotic and abiotic stimuli (Yan *et al.*, 1995). The measurement of shoot and root elongation is commonly used to determine allelopathic activity (Wu *et al.*, 1999). However, many researchers also note that the inhibitory activity of plant extracts is more effective against root growth than shoot growth (Pukclai *et al.*, 2010; Netsere and Mendesil, 2012; Sbai *et al.*, 2016; Liu *et al.*, 2018; Islam *et al.*, 2017). Exposed roots are more sensitive to extracts because of direct contact with allelochemicals (Islam and Kato-Noguchi, 2016) as well as root tissue being more permeable to allelochemicals than shoot tissue (Nishida *et al.*, 2005; Yoshimura *et al.*, 2011). On the other hand, root growth is based on cell proliferation, which is severely affected by phytochemicals, leading to arrested root growth (Yoshimura *et al.*, 2011; Tanveer *et al.*, 2012). Moreover, based on the I_{50} values of the tested plants, the results of this study revealed a significant difference in sensitivity of the tested plants to the extracts of *A. catechu*. These results suggest that different allelochemicals have species-specific inhibition against test plant species (Mushtaq *et al.*, 2018).

Conclusions

The aqueous methanol extracts of *Acacia catechu* inhibited the shoot and root growth of the tested dicotyledonous and monocotyledonous plants in a concentration- and species-dependent manner. These results indicate that the plant possesses strong allelopathic potential and may contain allelochemicals. Thus, the leaf extracts of *A. catechu* could be considered a viable candidate for the isolation and characterization of allelopathic substances

Authors' Contributions

KH conducted the whole experiment, recorded and analyzed the data and wrote the manuscript. HKN designed and supervised the experiment. He also helps to improve the quality of manuscript by editing and giving proper guideline.

The authors read and approved the final manuscript.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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