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Evaluation of Allelopathic Activity of Three Mango (*Mangifera indica*) Cultivars

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Abstract: The present study was undertaken to investigate the allelopathic potential of three mango (*Mangifera indica*) cultivars: Khirshapat, Himsagor and Sinduri. The aqueous methanol extracts of leaves at four different concentrations were examined against germination and seedling growth of cress (*Lepidum sativum*), lettuce (*Lactuca sativa*), alfalfa (*Medicago sativa*), Italian rye grass (*Lolium multiflorum*), barnyard grass (*Echinochloa crus-galli*) and timothy (*Phleum pratense*). The germination and seedling growth of all test plant species were inhibited significantly by selected mango leaf extracts at concentrations greater than 0.01 g dry weight (DW) equivalent extract mL^{-1}. The inhibitory activities of the extracts were proportional to the extract concentrations. At 0.1 g DW equivalent extract mL^{-1}, a significant delay or complete inhibition of germination were observed on all test plant species except for Sinduri extracts on barnyard grass. Alternatively, all extracts showed more than 70% hypocotyl/coleoptile and root growth inhibition of all test plant species except barnyard grass at the same concentration. The concentration required for 50% growth inhibition (I_{50}) on the hypocotyls/coleoptiles and roots of the test plants ranged from 0.003-0.103 g DW equivalent extract mL^{-1}. These results suggest that all three mango cultivars have allelopathic properties and thus allelopathic substances. As no prominent differences in the inhibitory activity were found among the three mango cultivars, all of them might be useful candidates for isolation and identification of allelopathic substances which may lead the basis for new natural herbicides development.

Key words: Allelopathy, growth inhibitor, mango cultivars, germination, seedling growth

INTRODUCTION

Over reliance on synthetic herbicides may often create overwhelming environmental side effects due to their persistence behavior with lower biodegradability (Macias *et al*., 1999). The herbicides are also responsible for evolution of herbicide-resistant weed biotypes (De Prado *et al*., 1997). To invert these virulent trends of heavy reliance on synthetic herbicides, researchers are now interested in eco-friendly alternatives and sustainable biological solutions to reduce environmental impacts produced by synthetic herbicide (Xuan *et al*., 2005; Petroski and Stanley, 2009). In this context, allelopathic plants may play an important role because of their allelopathic metabolites that have potential to suppress the growth and establishment of susceptible neighboring plant species (Bais *et al*., 2006). These substances are usually produced and accumulated in different plant organs like leaves, stems, roots, flowers, pollens, fruits or seeds of allelopathic plants (Weir *et al*., 2004), but their quantities may vary among the organs (Grisi *et al*., 2012). According to Dorning and Cipollini (2006) and Tanveer *et al*. (2010) leaves are the most consistent source of allelopathic substances and produced the greatest allelopathic effects on target species. Isolation and identification of these allelopathic substances from different organs of allelopathic plants, may served the basis for biodegradable natural product based herbicides development.

Mango (*Mangifera indica*) belonging to Anacardiaceae family is one of the major tropical evergreen economic fruit plants. It is also well known for many medicinal properties and is used for the remedy of diabetes, asthma, diphtheria, diarrhea, tetanus, gastrointestinal disorders, anemia, bronchitis, rheumatism, piles, miscarriage and many other diseases (Shah *et al*., 2010). In addition, mango have antioxidant (Ajila *et al*., 2007), anti-inflammatory (Garrido *et al*., 2004), anti-allergic, anthelmintic (Garcia *et al*., 2003), antiviral (Makare *et al*., 2001), antifungal and antibacterial properties (Kanwal *et al*., 2010).

Beside their pharmacological properties, few reports are available in the literature about the allelopathic potential of mango on different weed or crop species

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(Sahoo *et al*., 2010; El-Rokiek *et al*., 2010; Ashafa *et al*., 2012; Ferguson *et al*., 2003; Yan *et al*., 2006). However, to the best of our knowledge, not a single study has been done to check whether there have any differences in their allelopathic potentiality at the cultivar level. Although it is reported by some researchers that allelopathic potentiality varied among the cultivars of the same species (Putnam and Duke, 1974; Xuan and Tsuzuki, 2002; Kabir *et al*., 2010). Furthermore, all the works with mango allelopathy has been done with their aqueous extracts rather than organic solvent extracts which cannot dissolved most of the non-polar bioactive substances under room temperature.

Based on the above facts, the present study was designed to:

- Evaluate the varietal differences in allelopathic potential of three mango cultivars using aqueous methanol extracts of their leaves
- Identify the cultivars with strongest potential for isolation and identification of allelopathic substances which may lead the new natural herbicides development

MATERIALS AND METHODS

Plant materials: Mature leaves of three mango (*Mangifera indica* L.) cultivars (Khirshapat, Himsagor and Sinduri) were collected from Bangladesh Agricultural Research Institute, Gazipur-1701, Bangladesh. Leaves were washed with tap water, sun dried and kept under refrigeration at 4°C until extraction.

Test plants: Seeds of six test species, viz., cress (*Lepidium sativum* L.), lettuce (*Lactuca sativa* L.), alfalfa (*Medicago sativa* L.), Italian ryegrass (*Lolium multiflorum* Lam.), barnyard grass (*Echinochloa crus-galli* (L.) Beauv.) and timothy (*Phleum pratense* L.) with uniform shape and size were selected for the current research. Among these, first three are dicotyledonous and the rest of them are monocotyledonous. The seeds of cress and lettuce were obtained from Nakahara Seed Product Co. Ltd. (Fukuoka, Japan) and Tohoku Seed Co. Ltd. (Utsunomiya, Japan), respectively. The seeds of alfalfa, Italian rye grass and timothy were obtained from Takii Co. Ltd. (Kyoto, Japan) and barnyard grass seeds were from the farmer's fields (Kagawa, Japan).

Extraction: Leaves of mango (50 g dry weight) were cut into small pieces and extracted with 400 mL of 80% (v/v) aqueous methanol for 48 h. The extract was filtered through one layer of filter paper (No. 2; Toyo Ltd., Japan), using a vacuum pump. The residue was re-extracted with equal volume of methanol for 24 h and filtered. The two filtrates were combined and evaporated to dryness using a rotary evaporator at 40°C. All the activities (washing, drying, storing, extraction) were done separately for each mango cultivars during the whole research work.

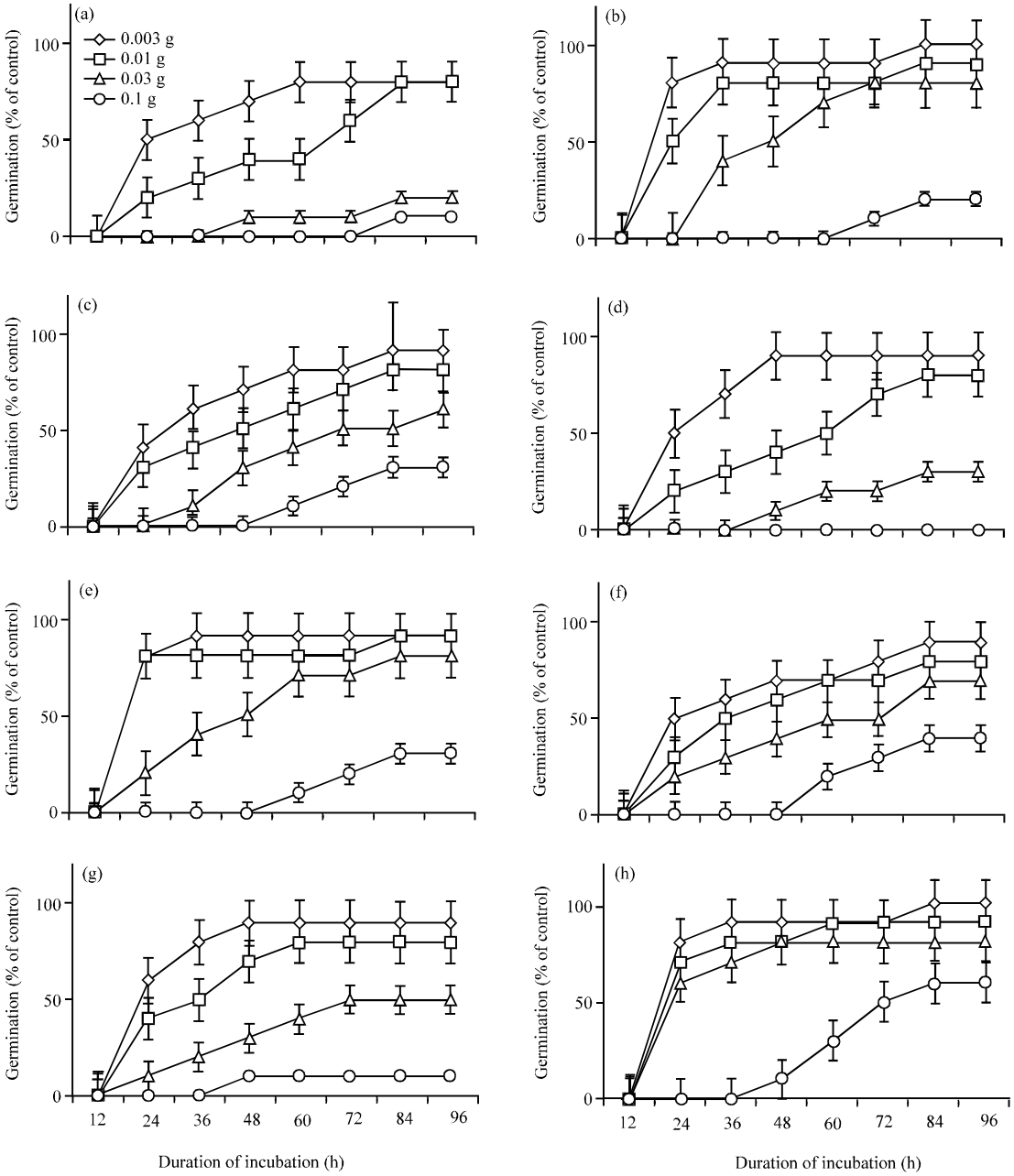
Germination bioassay: Required volume of the extract was dissolved in methanol to get four different extract concentrations of 0.003, 0.01, 0.03 and 0.1 g dry weight (DW) equivalent extract mL^{-1}. The extract was then added to a sheet of filter paper (No. 2) in 2.8 cm Petri dishes. The methanol was evaporated in a draft chamber and then the filter paper was moistened with 0.6 mL of 0.05% (v/v) aqueous solution of Tween 20 (polyoxyethylene sorbitan monolaurate; Nacalai, Kyoto, Japan): a surfactant that is non-toxic to seeds. A Petri dish without leaf extract served as control containing only Tween 20 on filter paper. The bioassay was carried out with three replications and repeated twice. Before starting the germination bioassay, seeds of timothy, Italian ryegrass and barnyard grass were soaked in distilled water for 24 h to imbibe the seeds. Ten seeds of cress, lettuce, alfalfa, Italian rye grass, barnyard grass or timothy were placed on filter paper in Petri dishes, covered with aluminum foil and kept in a germination chamber at 25°C in dark. Germination was measured by counting the number of germinated seeds at every 12 h of interval up to 96 h according to the procedure stated by Islam and Kato-Noguchi (2013a).

Growth bioassay: The extract preparation and placing in Petri dishes were done according to the same procedure as described in previous section. Then 10 seeds of cress, lettuce or alfalfa, or 10 germinated seeds of Italian ryegrass, barnyard grass or timothy (germinated in the darkness at 25°C for 24-72 h) were arranged on the filter paper. All of the Petri dishes were kept in a growth chamber for 48 h at 25°C in dark. The shoot and root lengths of the emerged seedlings were measured and inhibition percentage was determined using the equation as prescribed by Islam and Kato-Noguchi (2012).

Statistical analysis: Each experiment was carried out using a Completely Randomized Design (CRD) with three replications and the experiments were repeated twice. All the data were analyzed by MSTAT-C (Russell, 1984) and are subjected to three-way ANOVA. The concentration at which the growth of test plants was reduced by 50% (I_{50}) was performed on the basis of curve fitting to a logistic equation, using Graph Pad Prism 6 (Graph Pad, Inc., San Diego, CA, USA).

RESULTS AND DISCUSSION

Effect of leaf extracts of three mango cultivars on the germination: Effect of three mango leaf extracts on the germination of six test species are shown in Fig. 1 and 2. The three-way ANOVA showed that the effects of test species, concentrations, incubation time and their interaction on seed germination were significant at $p<0.001$ (Table 1). The germination of all test species was inhibited by selected mango leaf extracts at concentrations greater than 0.003 g DW equivalent extract mL^{-1} (Fig. 1 and 2). A complete or delayed germination was observed on all test species at concentration 0.1 g DW equivalent extract mL^{-1} except Sinduri extract on barnyard grass (Fig. 1 and 2). The inhibitory activity of the extracts was increased with the



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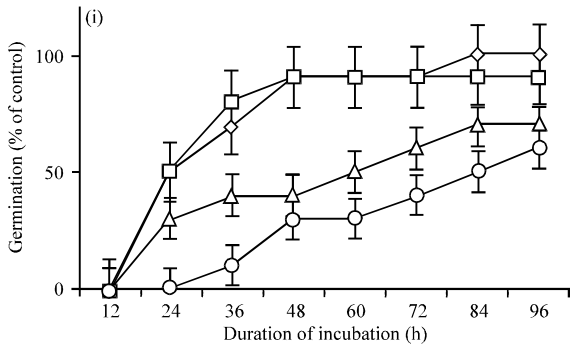
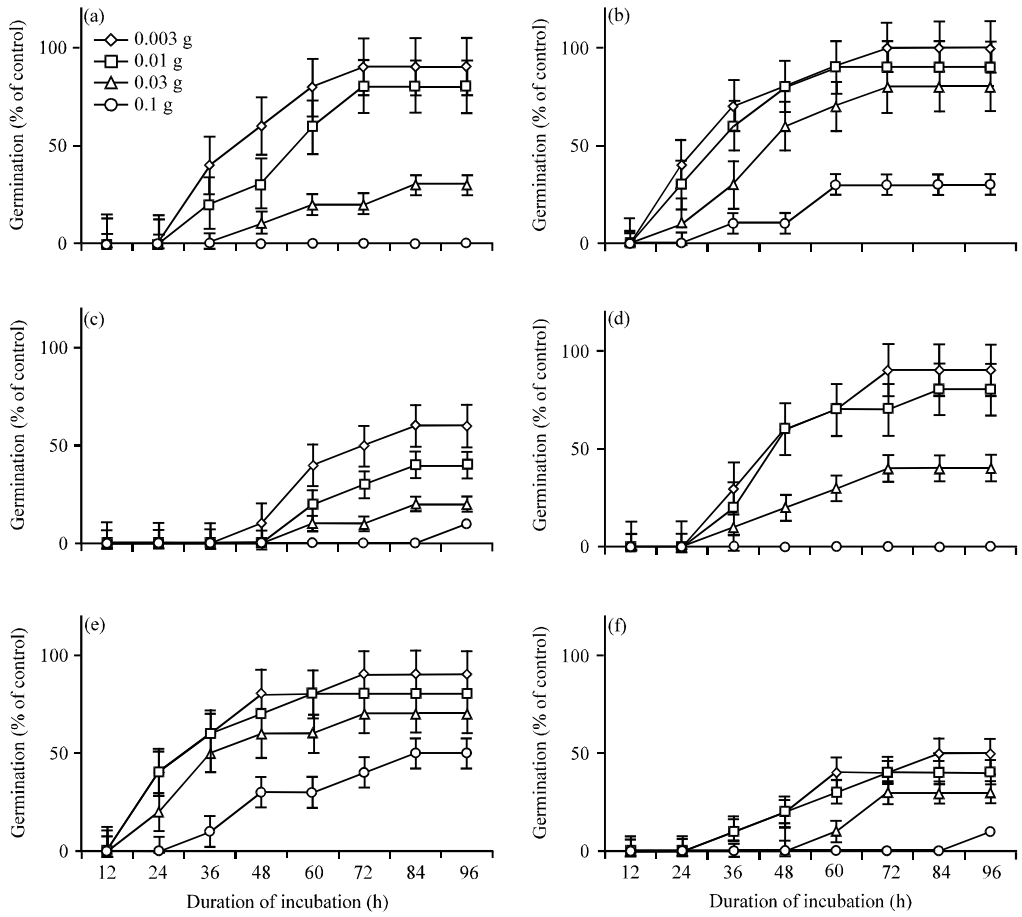


Fig. 1(a-i): Effect of aqueous methanol extracts of the leaves of three mango cultivars on the germination of cress, lettuce and alfalfa at different incubation periods. Concentrations of tested samples corresponded to the extract obtained from 0.003, 0.01, 0.03 and 0.1 g dry weight of each mango leaves extract. Vertical bars represent standard error deviations. Means±SE from three independent experiments with 10 seeds for each determination are shown. All the values are statistically significant at $p<0.001$, (a) Khirshapat-Cress, (b) Khirshapat-Lettuce (c) Khirshapat-Alfalfa, (d) Himsagor-Cress, (e) Himsagor-Lettuce, (f) Himsagor-Alfalfa, (g) Sinduri-Cress, (h) Sinduri-Lettuce and (i) Sinduri-Alfalfa



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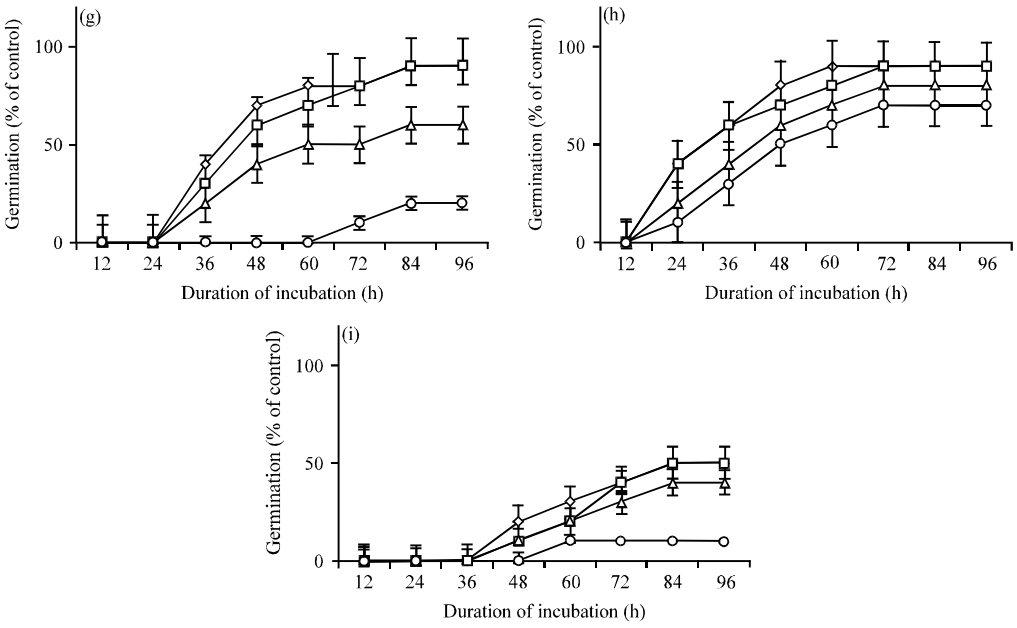

Fig. 2(a-i): Effect of aqueous methanol extracts of the leaves of three mango cultivars on the germination of Italian rye grass, barnyard grass and timothy at different incubation periods. Other details are same as Fig. 1 (a) Khrishapat-Italian ryegress, (b) Khrishapat-Barnyard grass, (c) Khrishapat-Timothy, (d) Himsagor-Italian rye grass, (e) Himsagor-Barnyard grass, (f) Himsagor-Timothy, (g) Sinduri-Italian ryegrass, (h) Sinduri-Barnyard grass and (i) Sinduri-Barnyard grass

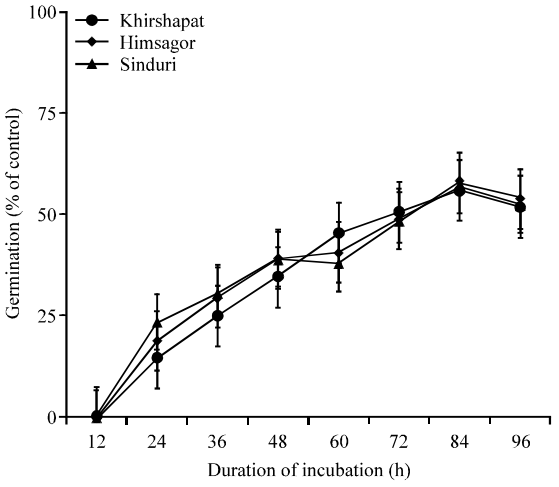

Fig. 3: Overall effect of the leaf extracts of three mango cultivars on the germination of all test plant species at different incubation periods. Vertical bars represent error bars with standard deviations

increase of concentration. However, no significant differences among the three mango extracts were observed for their inhibitory activity on germination of the test species (Fig. 3).

Table 1: Analysis of variance (ANOVA) for germination of six test plant species treated with the leaf extracts of three mango cultivars at four different concentrations

		Germination (%)		
Source of variation	df	Khirshapat	Himsagor	Sinduri
Test species (A)	5	5128.8***	3907.5***	3306.2***
Concentration (B)	4	12196.2***	10995.6***	11006.1***
A×B	20	2712.5***	2453.8***	2192.7***
Incubation time (C)	7	27106.6***	25775.2***	25577.3***
A×C	35	4551.1***	4169.0***	4099.7***
B× C	28	4966.1***	4596.4***	4501.6***
A×B×C	140	1235.7***	1087.5***	995.3***
Error	480	236.8	279.2	314.4

***p<0.001, df: degrees of freedom

Effects of leaf extracts of three mango cultivars on the seedling growth: The three way ANOVA showed that three mango leaf extracts, test plant species, extract concentrations and their interaction possessed significant effects on the hypocotyl/coleoptile and root growth of all test species at $p<0.01$ (Table 2). The aqueous methanol extract of three mango leaves inhibited significantly the hypocotyl/coleoptile and root growth of six test species at concentrations greater than 0.01 and 0.003 g DW equivalent extract mL^{-1}, respectively (Fig. 4 and 5). Similar to germination bioassay, the inhibitory effects of the extracts were concentration dependent. At 0.1 g DW equivalent extract mL^{-1}, the average inhibition on the

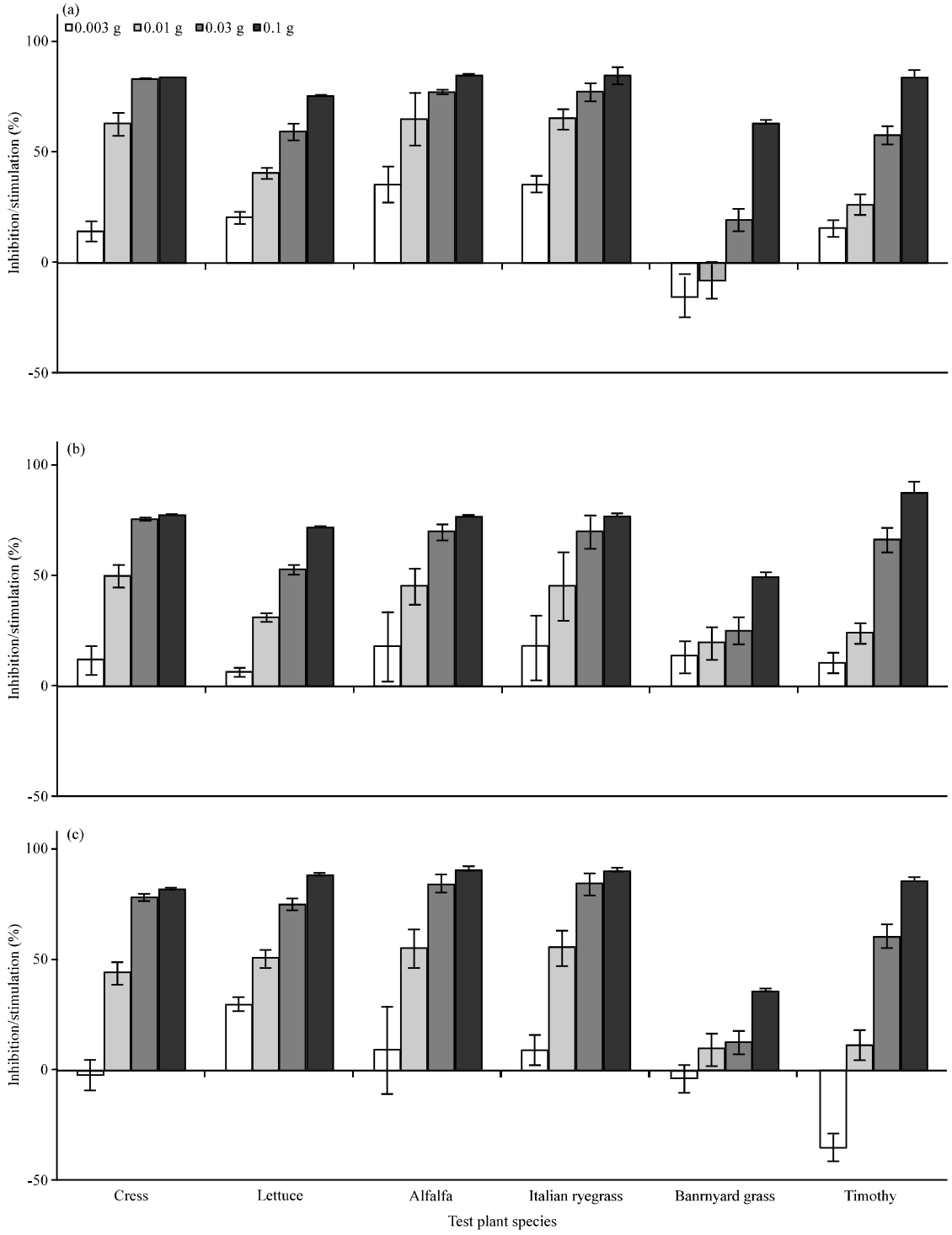

Fig. 4(a-c): Effect of aqueous methanol extracts of Khirshapat, Himsagor and Sinduri on hypocotyl/coleoptile growth of six test plant species. Concentrations of tested samples corresponded to the extract obtained from 0.003, 0.01, 0.03 and 0.1 g dry weight of Khirshapat, Himsagor and Sinduri leaves extract. Vertical bars represent standard error deviations. Means±SE from three independent experiments with 10 seedlings for each determination are shown. The positive (+) value indicates inhibition and negative (–) value indicates stimulation of the hypocotyl/coleoptile growth

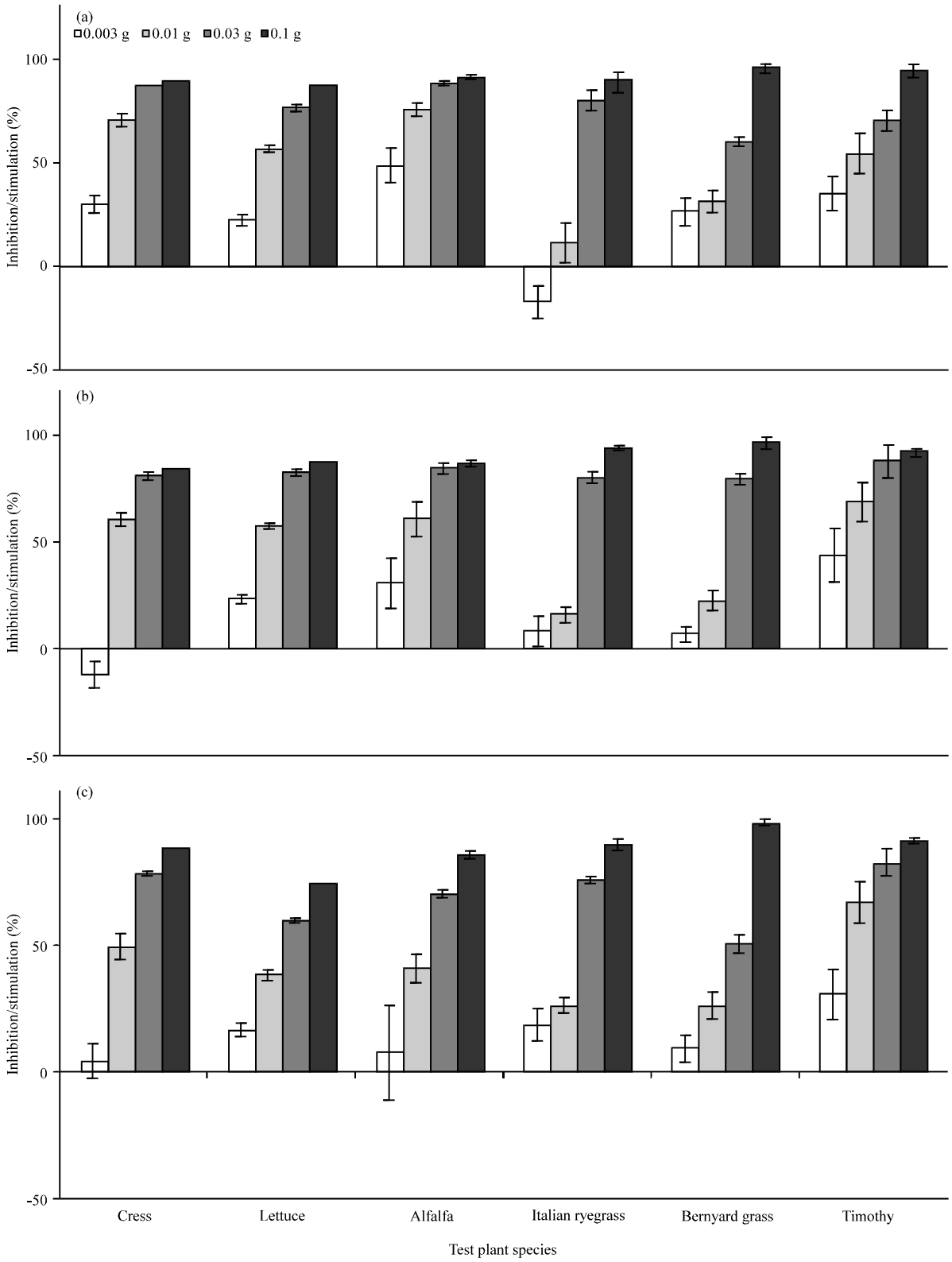

Fig. 5(a-c): Effects of aqueous methanol extract of Khirshapat, Himsagor and Sinduri on root growth of six test plant species. Other details are similar to Fig. 4

Table 2: Analysis of variance (ANOVA) for hypocotyl/coleoptile and root growth of six test plant species treated with the leaf extracts of three mango cultivars at four different concentrations

		Inhibition (%)	
Source of variation	df	Hypocotyl/coleoptile growth	Root growth
Cultivar (A)	2	1298.5***	287.3***
Test species (B)	5	11942.3***	6610.1***
A×B	10	556.0***	1502.8***
Concentration (C)	3	51256.6***	57870.7***
A×C	6	114.4***	132.2***
B×C	15	1253.1***	1725.0***
A×B×C	30	229.6**	381.1***
Error	144	35.7	18.6

**p<0.01 and ***p<0.001, df: degrees of freedom

Table 3: I_{50} values of the aqueous methanol extracts of the leaves of three mango cultivars on hypocotyl/coleoptile and root growth of six test plant species

		I_{50} value (g DW equivalent extract mL^{-1})	
Extracts	Test species	Hypocotyl/coleoptile	Root
Khirshapat	Cress	0.008	0.005
	Lettuce	0.018	0.008
	Alfalfa	0.005	0.003
	Italian ryegrass	0.017	0.019
	Barnyard grass	0.099	0.033
	Timothy	0.018	0.003
Himsagor	Cress	0.012	0.009
	Lettuce	0.028	0.008
	Alfalfa	0.016	0.006
	Italian ryegrass	0.014	0.012
	Barnyard grass	0.099	0.024
	Timothy	0.022	0.003
Sinduri	Cress	0.013	0.011
	Lettuce	0.020	0.008
	Alfalfa	0.013	0.009
	Italian ryegrass	0.020	0.016
	Barnyard grass	0.103	0.026
	Timothy	0.027	0.007

Values were determined by a logistic regression analysis after bioassays

hypocotyl/coleoptile growth of the test species were 80, 74 and 79% by Khirshapat, Himsagor and Sinduri leaf extracts, respectively. In contrast, at the same concentration the average inhibition on the root growth of the test species were 91, 90 and 88% by Khirshapat, Himsagor and Sinduri, respectively.

The concentration required for 50% hypocotyl/coleoptile growth inhibition (I_{50}) of the test species were ranged 0.005-0.099, 0.012-0.099, 0.013-0.103 g DW equivalent extract mL^{-1} for Khirshapat, Himsagor and Sinduri leaf extracts, respectively (Table 3). On the other hand, the I_{50} values of root growth were ranged from 0.003 to 0.033, 0.003-0.024 and 0.007-0.026 g DW equivalent extract mL^{-1} for those leaf extracts, respectively (Table 3). Compare to the hypocotyl/coleoptile growth, root growth of the test species was more sensitive to the leaf extracts. Similar to the germination bioassay, there was no prominent differences observed for the inhibitory activity of three mango cultivars on the seedling growth of the test species (Fig. 6).

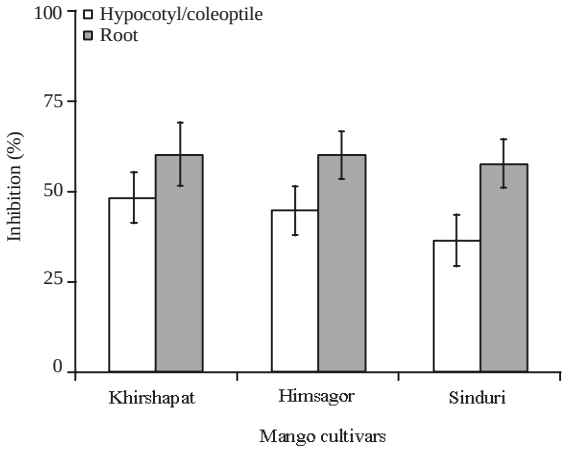

Fig. 6: Overall effect of the leaf extracts of three mango cultivars on the hypocotyl/coleoptile and root growth of all test plant species. Vertical bars represent error bars with standard deviations

The aqueous methanol extract of the leaves of three mango cultivars significantly inhibited both seed germination and seedling growth of cress, lettuce, alfalfa, Italian ryegrass and barnyard grass. In addition, the inhibitory activity of the extracts was much more prominent at higher concentrations, while the lowest concentration stimulates the germination or growth in some cases. Lovett *et al.* (1989) and Liu *et al.* (2011) reported that allelopathic substances can stimulate the seedlings growth at lower doses but inhibited the growth at higher doses. On the other hand, the higher sensitivity of root growth to the extracts compare to their hypocotyls/coleoptiles observed in our research might be due to the more intensive contact of roots to the extracts than their hypocotyls/coleoptiles (Qasem, 1995; Islam and Kato-Noguchi, 2013b). Based on the results and discussion we may summarize that all three mango cultivars possess allelopathic properties and therefore contains allelopathic substances. Similar trend on inhibitory activity of mango leaf water extracts were reported by Sahoo *et al.* (2010) and Ashafa *et al.* (2012).

CONCLUSION

The aqueous methanol extract of three mango cultivars (Khirshapat, Himsagor and Sinduri) have allelopathic properties and therefore, contain allelopathic substances. As their inhibitory activity not varied among the cultivars, all of them may be potential candidate for isolation and identification of allelopathic substances. These substances may finally serve the chemical basis for new natural bio-degradable herbicide development for sustainable and eco-friendly weed management.

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