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Allelopathic effects of root exudates of some weeds on germinability and growth of radish (*Raphanus sativus* L.) and cucumber (*Cucumis sativus* L.)

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ABSTRACT
The Allelopathy phenomenon is an important component in a natural and an agro-ecosystems causing a stimulatory or inhibitory effect in crop production through the release of allelochemicals to the environment. In this study, the allelopathic effect on seed seed germination, seedling growth, fresh weight, dry weight, vigor index, total chlorophyll and carotenoid contents of radish (*Raphanus sativus* L.) and cucumber (*Cucumis sativus* L.) to the root exudates of *Ageratum conyzoides* L. (T1), *Leucas aspera* (Willd.) Link. (T2), *Scoparia dulcis* L. (T3), *Spilanthes acmella* L. (T4) and *Vernonia patula* (Dryand.) Merr. (T5) were studied in the laboratory. Results showed that the concentrated aqueous root exudates of T5 exerted highly reduced the effect of all the parameters as compared to control (distilled water) followed by T4. However, T2, T3, and T1 showed the least inhibitory effect on both the test crops. In comparison to cucumber, radish was more sensitive to inhibitory effects of aqueous root exudates of these five weeds. The result suggested that the root exudates may affect radish and cucumber seeds due to the inhibitory effect of allelochemicals which were present in the tested weeds.

Key words: Allelopathy, Pigments, Root exudate, Seed geminability, Weed plants.

INTRODUCTION

Weeds are highly successful undesirable plants in nature which compete for the utilization of land, moisture, nutrients with the neighboring crop plants for their adaptation and dissemination (Blanco *et al.*, 2017) although its beneficial effect as bio-utilization has been found in the literature (Raj and Syriac, 2016). However, the presence of weed species in a crop field causes a substantial yield loss (Channabasavanna *et al.;* 2017, Jat *et al.*, 2011) by releasing a wide array of allelochemicals (Salgude *et al.*, 2015). These chemicals can be synthesized in different plant organs including plant tissues, leaves, flowers, fruits, stems, roots, rhizomes and seeds under favorable conditions (Haig, 2008). Generally, the allelochemicals are secreted by various processes: volatilization, leaching, decomposition of plant parts and root exudation (Cimmino *et al.*, 2014).

Root exudation is one form of the natural process of releasing allelochemicals composed primarily of carboxylic acids, amino acids, lipids, alcohols, amines, sugars, phenols, flavonoids, as well as an array of secondary metabolites termed as root exudates, which is a part of rhizodeposition and thus clearly represent a significant carbon cost to the plant (van Dam and Bouwmeester, 2016). The exudation from roots may change at different growth and developmental stages, when confronted with diverse biotic and abiotic factors (Vranova *et al.*, 2013). For the last decade, literature exists about the importance of root exudates in mediating complex interactions among organisms in the rhizosphere environment (Badri and Vivanco, 2009). Root exudates have significant implications on soil-root contact, affecting the physical and chemical properties of the soil and shaping a specific rhizosphere bacterial communities (Hu *et al.*, 2018) in the root zone, and facilitate plant establishment, growth and development. Recently, a growing body emphasizing on the effect of root exudate compounds, such as enhancing or reducing germination rates of seeds, increasing root peroxidase and superoxide dismutase activities, as well as enhancing membrane peroxidation (Gfeller *et al.*, 2018). Various studies have reported that allelochemicals are responsible for collective interference on seedling or plant growth (An *et al.*, 2013). During physiological activity in case of growth and development of plants, some known allelopathic compounds showed the inhibitory effect on seed germination (Zhang *et al.*, 2010). Batish *et al.* (2008) analysis the rhizospheric soil and found more phenolics which effect on rhizosphere environment.

The allelopathic effects of root exudate from weed species depend on target host plant. Thus, it is crucial to evaluate the interaction of root exudates of weed species with various crop plants to understand more deeply about the allelopathic interaction between weed and crop plants. The purpose of this study was to evaluate the allelopathic effect of root exudates from five weed species, namely a) *Ageratum conyzoides* L. b) *Leucas aspera* (Wild) Link. c) *Scoparia dulcis* L. d) *Spilanthes acmella* L. e) *Vernonia*

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patula (Dryand.) Merr. on two agricultural crops i.e. radish (*Raphanus sativus* L.) and cucumber (*Cucumis sativus* L.) under laboratory conditions.

MATERIALS AND METHODS

This experiment was undertaken in the laboratory of Department of Botany, University of Chittagong. Five common weeds such as *Ageratum conyzoides* L, *Leucas aspera* (Willd.), *Scoparia dulcis* L. Link., *Spilanthes acmella* Murr., *Vernonia patula* (Dryand.) Merr. were collected from around the crop fields of Chittagong University campus in September 2017 and termed as treatments: T1, T2, T3, T4 and T5 respectively. The collected weeds identified by the botanist and comparing with the authentic certified specimens at the Botany department herbarium, Universty of Chittagong. The seeds of radish (*Raphanus sativus* L.) and cucumber (*Cucumis sativus* L.) were collected from Hathazari Local Bazar, Hathazari, Chittagong.

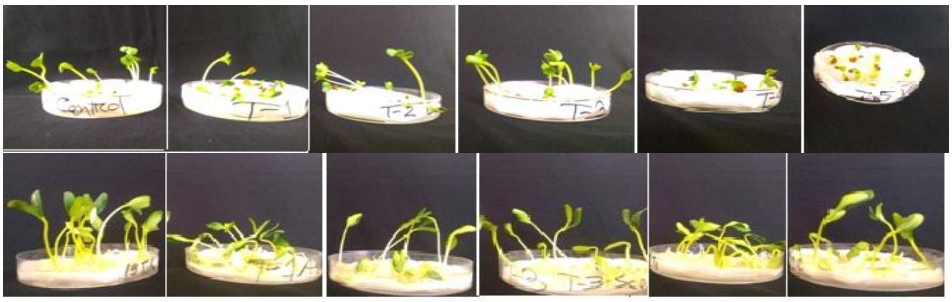
About 40 plants of each weed species were uprooted from the soil of the research area (Chittagong University Campus).The roots were washed with running tap water, then rinsed with distilled water. The plant roots were immediately transferred to the conical flask containing 300 ml of distilled water and kept for 5 h under sunlight for root exudation. The water extract of root exudates were collected by filtering through filter paper (whatman No. 1). The collected exudates were then concentrated upto one-fourth of the original solution using water bath at 60 °C and preserve in refrigerator for further experiment.

Uniform size, shape, a color of the seeds of radish and cucumber were selected and surface sterilized with 70% ethanol for 1-2 minutes, subsequently rinsed with sterile distilled water for 5 times to remove chemicals. Fifteen seeds of tested crops were spread on 9 cm glass petri dishes containing a two-fold filter paper moistened with 5 ml of root exudates (Treatments T1-T5). Distilled water was used as control (T0). The plates with the seeds were kept at room temperature allowing to grow for 7 days. Throughout the experiment period, care was taken to add an equal volume of root exudates in each petri dish periodically. The treated seeds were observed every day. The seeds were considered germinated when radicle length was over 2 mm. After 7 days, seed germinability, radicle and hypocotyl length, seedling vigour index, contents of chlorophyll and carotenoid of the tested crops were recorded. The germination percentage and vigour index (VI) were calculated according to method defined by Iman (2006) and Abdul-Baki and Anderson (1973) respectively. The data obtained expressed as a mean ± standard error of the mean (SEM) of three replicates.

To determine the plant pigments, primary leaves of one week of seedlings were used. One gram (approx.) of fresh leaves were extracted by 2 mL of 80% (v/v) acetone filtered through a filter paper (Whatman No. 1). After filtration, 10 mL of 80% (v/v) acetone were added and the volume of the filtrate was made up to 10 mL. The mixture was then thoroughly mixed with a vortex mixer and the absorbance of the samples is determined spectrophotometrically at 646 nm, 663 nm (chlorophyll) and 470 nm (carotenoid). The concentrations of the different pigments were estimated using Lichtenthaler and Wellburn (1983) formula.

RESULTS AND DISCUSSION

The response of cucumber and radish germination to root exudates: Seed germination is a key stage for plant productivity but an infestation of weeds in the crop fields play a negative role by secreting host specific metabolites (Ebrahimi and Hassannejad, 2015). In this study, root exudate of five weed species showed an inhibitory effect on the germination and seedling emergence of cucumber and radish seeds. All aqueous root exudate extracts reduced the seed germination compared with that of control (T0) (Fig.2). In our findings, we found that root exudate of T5 revealed the lowest seed germination (below 50%), while T1, T2, and T4 (above 70%) showed the least reduced incidence on both the tested crops. On the other hand, the germination percentage at T3 (Above 80%) found to be closest to control indicating the less inhibitory effect on both tested species (Fig.1 and Fig. 2). The present results are in conformity to



T0 T1 T2 T3 T4 T5

Treatments

Fig 1: Shows the seedling emergence of radish (above) and cucumber (below) after 7 days incubation treated with aqueous root exudates of five weeds.

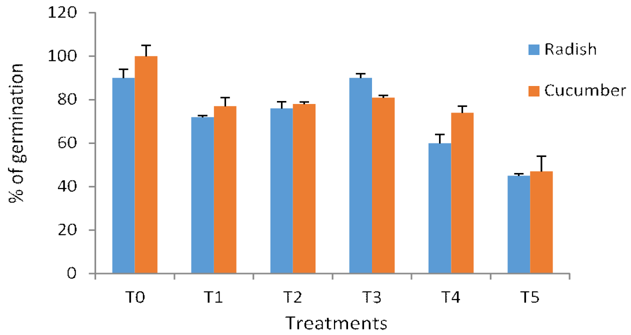


Fig 2: Effect of aqueous extracts of root exudates of five weeds on the percentage of seed germination of radish and cucumber after 7 days incubation.

Xuan *et al.* (2005) who have concluded the root exudates of barnyardgrass suppressed the growth of rice, lettuce (*Lactuca sativa* L.) and monochoria (*Monochoria vaginalis*) during the early growth stages. Similarly, Otusanya *et al.* (2008) found the inhibitory effect of the water-soluble root exudate (WRE) of *Tithonia diversifolia* on the germination, growth of pepper (*Capsicum annum* L.) and tomato (*Lycopersicon esculentum* Mill.). In addition, Aquoeous extract of *Ageratum conyzoides*, *Lantana camera* and *Cyperus rotundus* caused significant reduction in groundnut germination (Ghosh *et al.*, 2000). However, in our result, radish exhibited a lower germination percentage compared to cucumber (Fig. 2) and the rate of germination varied with the different weeds and tested crops. The result was agreed to Meissner *et al.* (1982), who found water extracts of *Cyperus rotundus* decreased the seed germination and seedling growth of *Cucumis sativus* L. Similar inhibitory effect was also observed on *Zea mays*, *Phaseolus vulgaris*, *Cucurbita pepo* and *Lycopersicon esculentum* by the application of Mexican desert plants (Romero-Romero *et al.*, 2002). The inhibitory effect on seed germination and seedling growth can be induced by synthesizing inhibitors of phenols, terpenoids, p-coumaric acid, gallic acid, ferulic acid, p-hydroxybenzoic acid, and anisic acid during physiological activity of seed germination (Zhang *et al.*, 2010; Batish *et al.* 2008). In addition, some regulatory polyphenols bind with other hormones and caused reduction of seedling growth (Ali *et al.* 2012). For example, ferulic acid, t-cinnamic acid, chlorogenic acid, p-coumaric acid, coumarin interact with ABA and showed additive inhibitory effects, both on seed germination and seedling in mung bean (Batish *et al.*, 2008), which cause the reduction of crop productivity (Channabasavanna *et al.*, 2017).

Growth performance of seedlings: A noticeable effect of root exudate extracts of 5 weeds on radicle and hypocotyl length of the radish and cucumber are presented in Table 1. In cucumber, the average length of radicle (7.26 ± 0.02 cm g/plant) and hypocotyl (12.91 ± 0.03 cm g/plant) was found to be the highest in T1 compared to radish (Table 1, Fig. 1). This indicates that the inhibitory effect of root exudate of T1 was minimum in cucumber than radish. A mild reduced length of radicle and hypocotyl growth was appeared in T3 in both tested crops while the medium effect was estimated for radish (hypocotyl 5.48±0.02 cm g/plant and radicle 2.91 ± 0.04 cm g/plant) and cucumber (hypocotyl 9.42±0.01cm g/plant and radicle 4.91±0.01cm g/plant) by T4 root exudate (Table 1). Interestingly, the lowest average radicle and hypocotyl length as well as the inhibition effect of T5 was maximum amongst the five weeds for both the tested crops (Table 1). Same findings were observed when root exudate from wild rice applied to *Sisybrium officinale* (Mahmoodzadeh *et al.*, 2011). Also, the retardation of radicle length in *L. esculentum* by water-soluble root exudate of *Tithonia diversifolia* was reported by Otusanya *et al.* (2008). Further, the inhibition of seed germination, the plumule and radicle length were reduced as influenced by aqueous extract of *Galinsoga paviflora* and *Bidens pilosa* (Singh *et al.*, 1996). The effect of allelochemicals present in root exudates may cause deep oxidative stresses in target tissues degrading antioxidant mechanisms or probable the accumulation of chemical compounds that delay or hinder the arrangement of microtubule during cell division resulting the reduction of hormonal activity, less ion uptake, inhibition of protein and enzyme activation and reduction of cell permeability (Sitthinoi *et al*,. 2017) These actions may inhibit the radicle and hypocotyl length of crops (Aenavoli *et al.*, 2006). Baleroni *et al.* (2000) demonstrated that another phenolic compound, p-coumaric acid at 1mM severely affects the root growth and fresh weight of canola (*Brassica napus*).

The seedling fresh and dry weight are important traits and accumulation of dry matter in plants present a possible way for plant health. In this study, the fresh and dry weight of seedlings found to be consistency with the height of radicle and hypocotyl growth. The seedling vigor index was higher in cucumber than radish which showed an adverse effect of root exudates on radish (Table 1). A similar result was also reported by Leela (2014) during seed germination studies in wheat (*Triticum durum*). Lettuce (*Lactuca sativa* L.) seedling growth was also reduced by the application of increased concentration of root exudate of switchgrass (*Panicum virgatum* L.) studied by An *et al.* (2013) whose report was in accordance with our result.

A reduction of the seedling emergence, vigor index, fresh and dry weight of test crops indicates that the infested weeds in the crop fields may contain phytotoxins, which affected the target crops (Tanvir *et al.*, 2010). Kaur *et al.* (2005) reported that benzoic acid, a potential allelochemical suppressed mustard growth by producing of abnormal and disorganized tissues interfering mitotic cell division with increasing concentrations. In a study, conducted by Travols *et al.* (2008) demonstrated that the extract of root exudates from Bermuda buttercup inhibit biomass production of tomato, oat and lettuce plants showing its allelopathic

Table 1: The effect of aqueous extracts of root exudates of five weeds on the seedling growth of radish and cucumber after 7 days incubation.

Test crops	Treatments	Hypocotyl Length(cm) (g/plant)	Radicle Length(cm) (g/plant)	Seedling Fresh Weight (g/plant)	Seedling dry weight (g/plant)	Seedling vigour index
	T0	11.88±0.01	5.30±0.01	0.89±0.01	0.12±0.01	1718.00±0.48
	T1	10.12±0.02	4.32±0.02	0.65±0.03	0.08±0.02	1301.40±0.36
Radish	T2	8.58±0.03	4.26±0.02	0.58±0.01	0.07±0.01	869.60±0.33
	T3	10.55±0.01	4.34±0.02	0.67±0.01	0.08±0.02	1027.20±0.58
	T4	5.48±0.02	2.91±0.04	0.14±0.01	0.02±0.01	587.30±0.33
	T5	3.78±0.01	2.52±0.01	0.12±0.01	0.02±0.01	378.00±0.67
	T0	14.32±0.02	8.32±0.01	1.30±0.03	0.17±0.02	2037.60±0.61
	T1	12.91±0.03	7.26±0.02	1.10±0.03	0.14±0.01	1613.60±0.42
Cucumber	T2	10.86±0.04	5.51±0.03	0.95±0.02	0.12±0.03	1145.90±0.55
	T3	11.83±0.03	6.21±0.01	0.80±0.02	0.11±0.01	1490.40±0.29
	T4	9.42±0.01	4.91±0.01	0.68±0.01	0.09±0.01	0.09±0.01
	T5	7.51±0.01	3.42±0.02	0.55±0.01	0.07±0.01	546.50±0.28

Table 2: Effects of aqueous extracts of root exudates of five weeds on the chlorophyll and carotenoid contents of the 7– day - old radish and cucumber seedlings.

Test crops	Treatments	Chlorophyll a (mg/f.wt.)	Chlorophyll. b (mg/f.wt.)	Total chlorophyll (mg/f.wt.)	Carotenoids (mg/f.wt.)
	T0	0.782±0.017	0.415±0.001	1.197±0.012	0.461±0.001
	T1	0.572±0.002	0.221±0.001	0.793±0.005	0.231±0.000
Radish	T2	0.473±0.001	0.184±0.001	0.657±0.005	0.228±0.000
	T3	0.663±0.002	0.218±0.001	0.881±0.007	0.376±0.000
	T4	0.351±0.000	0.173±0.000	0.526±0.004	0.133±0.000
	T5	0.234±0.000	0.122±0.000	0.356±0.001	0.125±0.000
	T0	1.474±0.019	1.122±0.012	2.596±0.017	0.661±0.002
	T1	1.221±0.013	0.724±0.001	1.945±0.020	0.602±0.002
Cucumber	T2	0.955±0.012	0.671±0.001	1.626±0.013	0.593±0.001
	T3	0.895±0.014	0.724±0.002	1.619±0.014	0.610±0.002
	T4	0.903±0.012	0.553±0.001	1.456±0.013	0.573±0.001
	T5	0.767±0.002	0.425±0.000	1.192±0.011	0.468±0.000

potential to respective crops. In one experiment it was found that catechol, gallic acid or pyrogallic acid significantly decreased the germination percentage, growth of radicle and hypocotyls and the fresh and dry weight of cucumber seedlings. Among them, pyrogallic acid was the inhibitor (Ali *et al.*, 2012). However, some studies showed that the weed aqueous extract did not suppress the crop plant rather it exhibited biostimulant in the crop field (Raj and Syriac, 2016). Those findings are not consistent with our present result.

Chlorophylls and carotenoid contents: Amongst other parameters, chlorophyll contents, are greatly affected by root exudates. In the experiment, reduction of total chlorophyll and carotenoid was observed on both test crops (Table 2). Relative to control plants, the decreased levels of total chlorophyll contents in T1, T2, T3, T4 and T5 was 0.793±0.005, 0.657±0.005, 0.881±0.007, 0.526±0.004, 0.356±0.001 mg/f.wt in radish whereby the higher amount was noticed in cucumber (Table 2). The result showed that there were high chlorophyll (2.596±0.017 mg/f.wt) and carotenoid (0.661±0.002 mg/f.wt) accumulation in cucumber control plant followed by radish (Chlorophyll 1.197±0.012 mg/f.wt and carotenoid 0.461±0.001 mg/f.wt) where the most pronounced reduction was found in T5 (Table 2). Reduction of chlorophyll a, chlorophyll b, and total chlorophyll and carotenoids contents in many plants have been studied in past. For example, changes in chlorophyll contents could be because of some secondary metabolites such as 3-3´-5-Trihidroxi-4´-7-dimethoxyflavone and 3-3´-5-Trihidroxi-4´-7-dimethoxyflavone-3-O-sulphate, might be playing a major role to change the chlorophyll and carotenoid contents in cucumber (Devi *et al.*, 2014). Variations in chlorophyll contents were also reported by Abu-Rommann *et al.* (2010) in *Triticum durum.* Einhellig *et al.* (1993) examined that root exudate of sorgoleone (SGL), a p-benzoquinone from *Sorghum bicolor* act as a powerful inhibitor of a chlorophyll synthesis which indicate an effective blocked of photosynthesis in soybean and pea resulting an ultimate reduction of the growth of plants. Elisante *et al.* (2013) revealed that the allelochemicals present in *Datura stramonium* inhibited to chlorophyll synthesis resulting in the reduction of total chlorophyll contents which corroborates with our study. However, it is likely that the allelochemicals in the root exudates may induce deleterious effects on protective enzyme systems results in the reduction of chlorophyll synthesis (Ding *et al*., 2016).

CONCLUSION

This study showed that the root exudates of five weeds have strong allelopathic potential activity on seed germination and seedling emergence, the fresh and dry weight of radish and cucumber. Moreover, the exudates reduced the chlorophyll and carotenoid contents in the test species. However, with the positive outcomes from the current study, further efforts should be focused to screen the groups of allelochemicals present in the root exudates of weeds and investigated their role in the crop fields to boost up the production in sustainable agriculture.

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