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RESEARCH ARTICLE

ALLELOPATHIC EFFECTS, YIELDS AND QUALITATIVE PHYTOCHEMICAL SCREENING OF ROOT EXUDATES OF FIVE WEED SPECIES

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ABSTRACT

This research investigated the allelopathic effects, the yields and qualitative phytochemical screening of the water extract of root exudates of five weed species i.e. *Cyperus rotundus* L. (T1), *Marselia quadrifolia* L. (T2), *Ludwigia hyssopifolia* (G. Don) Exell, (T3) *Pistia stratiotes* L. (T4) and *Colocasia esculenta* L. (T5). The allelopathic tests of root exudates on five weed species showed that all the extracts had the pronounced inhibitory effect on cowpea and mungbean (tested crops). The yields of root exudates of the selected weed species varied. Root exudate of T3 showed the highest yield whereby T1 contained the lowest one. A preliminary phytochemical test showed the positive result of alkaloids, flavonoids, phenols and carbohydrates whereas proteins, amino acids, tannins, saponins, have been found to be absent in the root exudates of tested weeds. The results evidenced that these mentioned weeds contain compounds in their root exudates which may cause allelopathic effects on both tested crops.

KEYWORDS

Allelopathy, Colocassia esculenta. Cyperus rotundus, Ludwigia hyssopifolia, Marselia quadrifolia, Pistia stratiotes and Root exudates

1. INTRODUCTION

Allelopathy, a term was first coined by a researcher that concerned a biological and chemical interaction within the communities by the addition of certain compounds to the rhizosphere environment and thus influence the agricultural systems [1]. In cultivated crops, weeds are highly successful organisms in nature and established as an integral part of our agroecosystem [2]. Weeds always compete for the light, moisture, macro, and micronutrients with the neighboring crop plants for their growth and development and influence to the crop productivity by liberating a number of chemicals in the soil through roots [3]. The biologically active chemicals that released to the soil are so-called allelochemicals [4]. They are present in varieties of plant tissues including leaves, flowers, fruits, stems, roots, rhizomes and seeds [5]. Allelochemicals are released by means of volatilization, decomposition of plant residues and triggered either synergistic or antagonistic phenomena or displays allelopathic stress [6].

Root exudation is another source of allelochemicals released by plant roots, most of which are organic and normal plant components are formed during photosynthesis and accompanying plant process [7]. Thus, it signifies the carbon cost to the plant during their lifetime altogether up to17 % of the photosynthetically fixed carbon [8,9]. The reported amount, however, can vary depending on the author; maximum values range around 30 %. The deposition of root exudates in the rhizosphere is determined by the various biotic and abiotic factors [10]. High root densities, the cultivars, plant age and the degree of environmental stress greatly influence the amount of root exudation [11]. In addition, the transportation mechanism of metabolites is also one of the major causes in releasing of exudate compounds [12]. Based on the number of literatures, it is conceived that the exude compounds are separated by low- and high- molecular- weight compounds that are present in the intercellular space of root tip tissues and root hairs. They may leak either from root cells or be transported via the phloem from other tissues [13,14]. The low-molecular-weight compounds belong to primary and secondary metabolites of which carbohydrates, amino acids, phenolic with a variety of other metabolites are allegedly thought to be the mainstream components of root exudates [15]. Compounds such as Isoflavone, phytoanticipin-antimicrobal components, allelopathic chemicals are associated with various functions that nourish the rhizosphere environment [16].

Several investigators have been reported the implications of root exudates in interactions among plants and soil microorganisms and plant-plant interaction and their crucial role in nutrient mobilization through direct coordination complex formation of micronutrients [17]. Studies showed that it acts as an inhibitor to plant growth. For example, sorgoleone a hydrophobic allelopathic compound was identified as root exudates from the roots of *Sorghum bicolor* L. Moench) and showed a potent inhibitory effect on the growth of several broadleaf plants [18]. However, literature regarding the allelopathic effect of root exudate on seed germination and seedling growth under laboratory conditions is insufficient [19]. Hence, the objective of these study is to evaluate the yields, phytochemical analysis and allopathic effects of water extracts of root exudates of *Cyperus rotundus* L., *Marselia quadrifolia* L., *Ludwigia hyssopifolia* (G. Don) Exell, *Pistia stratiotes* L. and *Colocasia esculenta* L. on seed germination and seedling growth of mungbean and cowpea under laboratory conditions.

2. MATERIALS AND METHODS

2.1 Plant materials

Five common weed species namely *Cyperus rotundus* L. (T1), *Marselia quadrifolia* L. (T2), *Ludwigia hyssopifolia* (G. Don) Exell. (T3), *Pistia stratiotes* L. (T4) and *Colocasia esculenta* L. (T5) were selected on the campus of Chittagong University (Hathazari. Chittagong, Bangladesh) in

September 2017 and identified by a botanist at Chittagong university. The seeds of mungbean (*Vigna radiata* L. R.Wilczek), and cowpea (*Vigna unguiculata* L. Walp.) were collected from Hathazari Local bazar, Hathazari, Chittagong.

2.2 Preparation of Root Exudates

Approximately 50 plants of each weed species were uprooted around the crop fields without hampering its roots and washed with running tap water (three times) followed by 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 collecting root exudation. The water extract of root exudates was collected by filtering through Whatman filter paper (No. 1). The exudates were dried using the water bath at 60 °C and measured. About half of the dried exudates were redissolved in 50 ml sterile distilled water used for allelopathy experiment on mungbean and cowpea. The rest of the dried material was preserved at 4 °C refrigerator for phytochemical analysis.

2.3 Bioassay technique

Seeds of mungbean and cowpea were surface sterilized with 70% ethanol for 3 minutes, subsequently, they were washed gently with sterile distilled water up to 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. Seeds were soaked in sterile distilled water used as control (T0). All the treatments including control group were kept in the dark chamber for 2 days. The plates were then transferred at room temperature for the next 8 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 10 days, seed germinability, radicle and hypocotyl length, fresh and dry weight of seedlings of the tested and control crops were recorded.

2.4 Phytochemical analysis of root exudates

The dry root exudate sample had suspended with 10 ml of distilled water and incubated for 72 hours at room temperature. The supernatant was used to detect the various bioactive compounds according to the method described by a researcher [20].

2.5 Statistical analysis

The experiment was conducted for thrice and the data were expressed as mean ± standard deviation (SD) using Microsoft excel 2010.

3. RESULTS AND DISCUSSION

3.1 Effect of root exudate on seed germination of mungbean and cowpea

The data presented in Figure 1 revealed that the root exudate of five weed species had an inhibitory effect on seed germination of mungbean and cowpea and the degree of the inhibition varied depending on the species tested. In case of mungbean and cowpea, the germination was ranged 60% to 80% and 40% to 80% irrespective of all the treatments respectively, while control on both crops set 90% of germinated seeds. This occurrence representing as an assurance of seed quality under laboratory conditions before field trial. The inhibitory effect was found to be in the proportion of the germination percentage where the maximum germination having recorded in T1 and T3 followed in mungbean. In contrary, the highest and the lowest seed germination were recorded in T5 and T4 respectively in cowpea. Our results indicate that root exudate from weed species affects greatly on mungbean than cowpea in terms of germination ability.

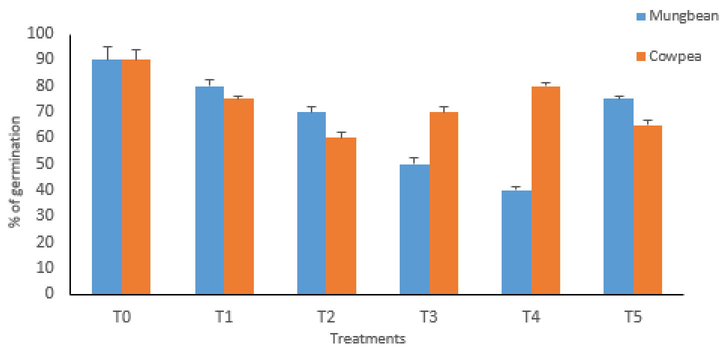

Figure 1: Effect of water extracts of root exudate of five weeds on the percentage of seed germination of mungbean and cowpea after 10 days incubation

3.2 Effect of root exudate on radicle and hypocotyl growth of mungbean and cowpea

Among two crops on-test, the length of the radicle and hypocotyl in control plants was found to be maximum whereas root exudate of weed species clearly showed inhibition of both tested crops (Figure 2 and 3). In mungbean, the length of the radicle ranged from 0.82 ± 0.25 to 2.95 ± 0.57 cm and the hypocotyl length varied from 2.18 ± 0.58 to 5.50 ± 1.28 cm treated with T1 toT5 root exudates (Table 1). However, it was not the same in cowpea. Cowpea, comparatively showed a higher range of the radicle (3.70 ± 1.89 cm. to 5.40 ± 1.75 cm.) and hypocotyl length (3.84 ± 0.78 cm. to 4.95 ± 1.45 cm.) compared to control (Table 1). In case of hypocotyl length, Root exudate of T3 (71.6 %) and T4 (67.3 %) showed higher inhibition effect followed by T2 (31.5%), T5 (37.3 %) and T1 (18.2 %) in mungbean while T4 (9.3%) was found to be the lowest inhibitory effect followed by T2 (22.2 %) in cowpea (Figure 2 and 3). It was noticed that the hypocotyl length was increased twice than radicle length in mungbean. The maximum inhibitory effect on radicle growth was found in T4 (79.4 %) followed by T3 (71.3%) in mungbean (Figure 3). Both radicle and hypocotyl affected by root exudates of the tested weeds in mungbean in comparison to cowpea. Consequently, the ratio of radicle and hypocotyl was found to be lower in mungbean (0.33 to 0.54) than cowpea (0.76 to 1.28) for all the treatments (Table 1). The control plants revealed good health of radicle and hypocotyl in both the tested crops.

Table 1: Effects of root exudates of five weeds on the length (cm) and the ratio of radicle and hypocotyl of mungbean and cowpea (mean ± SD)

Treatments	Test crops					
	Mungbean			Cowpea		
	RL (cm)	HL (cm)	R : H	RL (cm)	HL (cm)	R : H
T0	4.64 ± 1.14	7.68 ± 1.30	0.61	6.97 ± 1.15	5.46 ± 1.25	1.28
T1	2.95 ± 0.57	5.50 ± 1.28	0.54	3.70 ± 1.89	3.84 ± 0.78	0.96
T2	2.56 ± 0.50	5.26 ± 1.49	0.49	5.40 ± 1.75	4.25 ± 1.23	1.27
T3	1.16 ± 0.37	2.18 ± 0.58	0.53	4.58 ± 1.03	3.85 ± 1.20	1.19
T4	0.82 ± 0.25	2.51 ± 1.20	0.33	3.75 ± 1.20	4.95 ± 1.45	0.76
T5	1.86 ± 0.68	4.82 ± 1.47	0.39	5.00 ± 1.24	3.95 ± 1.15	1.27

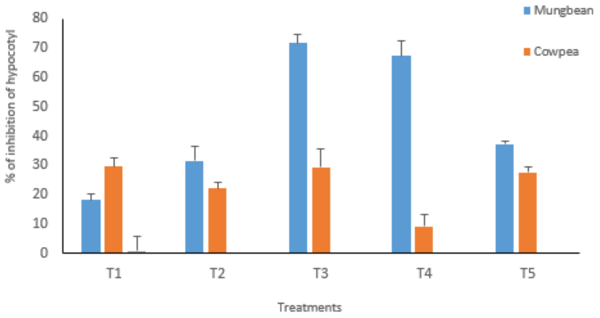

Figure 2: Inhibition of hypocotyl elongation of mungbean and cowpea as affected by root exudates of selected weeds, after 10 days incubation.

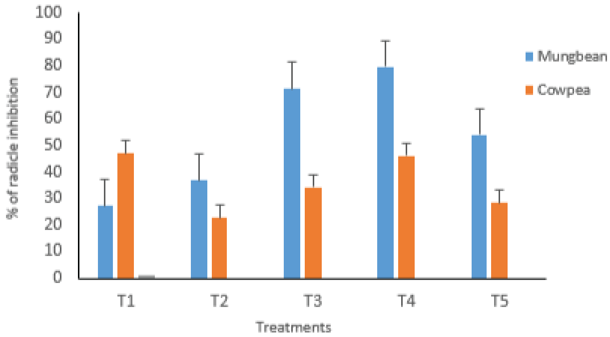

Figure 3: Inhibition of radicle elongation of mungbean and cowpea as affected by root exudates of selected weeds, after 10 days incubation

During physiological activity in case of growth and development of plants, some known allelopathic compounds are synthesized and showed the inhibitory effect on seed germination [21]. For example, Inhibitors such as phenols and terpenoids found to be involved in the allelopathic activity on crop plants [22]. Besides, the presence of p-coumaric acid, gallic acid, ferulic acid, p-hydroxybenzoic acid, and anisic acid can be secreted as a potent exudates and induced as an inhibitors on seed germination [23]. In addition, some regulatory polyphenols bind with other hormones and caused reduction of seedling growth. 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. A researcher 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 [24]. Similarly, the water-soluble root exudate (WRE) of Tithonia diversifoliaon effect on the the germination, growth of pepper (*Capsicum annum* L.) and tomato (*Lycopersicon esculentum* Mill.) were recorded [25]. However, in our result, radish exhibited a lower germination percentage compared to cucumber (Figure 1).

From the present work, it was observed that the metabolites present in root exudate effect seedling growth (radicle and hypocotyl length) corroborated with a researcher who observed that water-soluble root exudate of *Tithonia diversifolia s*uppressed the seedling growth of pepper and tomato. The Similar results were also found on lettuce growth [26]. Further, root exudates of *Burmuda buttermut* showed 34-42 % inhibition of the tested crops which agree with our findings [27]. The inhibitory effect was also observed by a researcher by aqueous extracts of four native Mexcan desert plants in *Zea mays*, *Phaseolus vulgaris*, *Cucurbita pepo* and *Lycopersicon esculentum* [28]. Another researcher reported that p-hydroxyl madelic acid as an root exudate substance which cause inhibition of root elongation in rice released by the young barnyard grass [29]. As phenolic compounds released from the weed roots or residues to the soil and effect on the seed germination and seedling growth. The action of such allelopathic compounds influences on specific plant processes such as- cell division and elongation, the action of inherent growth regulators, mineral uptake, photosynthesis, respiration, stomata opening, protein synthesis, membrane permeability and specific action [30].

3.3 Root exudate yields

The extraction yield was expressed as gm from 50 plants from five weed species and illustrated in Figure 4. The yields ranged from 1.34 to 2.50 gm among the weed species. *Ludwigia hyssopifolia* (T3) and *Colocasia esculenta* L. (T5), comparatively showed the better yield (2.50 gm. And 2 gm.) than in *Cyperus rotunus L.* (T1)), *Marselia quadrifolia* (T2) and *Pistia stratiotes* L. (T4) which produced 1.34 gm., 1.65 gm., 1.63 gm. respectively. Nearly 30-40% carbon-based compound released to the soil as root exudate but it depends on the plant species, maturity and environmental condition and the sampling method for root exudate collection [31]. The site of the plant is also important. a previous researcher demonstrated that the root apex is the predominant site of exudation in healthy young plants which is clearly separated from older tissues and concluded the main site of exudation belonging to the immediately behind the root tips named as longitudinal cell junctions [32]. Considering the fact we selected the mentioned weed species randomly from different crop fields at a young stage and immediately collected root exudates by distilled water to give a natural environment for root exudate collection and measured. Although the exudate yields varied to different solvent extraction methods, however, water is rather more suitable for exudate collection and gives more yields than that of other solvents [33].

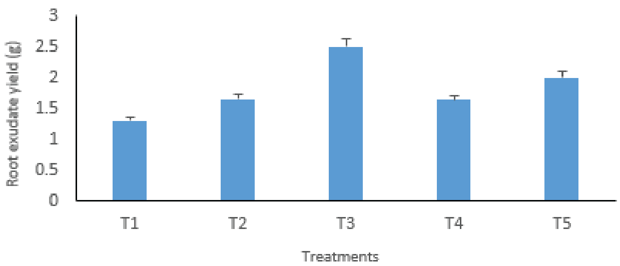

Figure 4: Yields of root exudate (water extract) of five weed species

3.4 Phytochemical analysis

Phytochemical analysis plays a major source of information on the analytical and instrumental methodology in plant sciences. As shown in Table 2, it is clear that all the root exudates in different reagent showed a positive response for alkaloids. Root exudates of T1 to T5 showed '+++' (high concentration) in Dragendroff's reagent while Hager's reagent, Mayer's reagent, and wagner's reagent gave '++' (low concentration). In the tannic acid test, T3, T4, and T5 exhibited '++' while very low concentration, '+' was found in T1 and T2 root exudates as $FeCl_3$ test. In addition to alkaloids, qualitative assessment for four other secondary metabolites, viz. carbohydrate, flavonoids, phenols, amino acid, and protein, tannins, and saponins test were also done. Carbohydrate, flavonoid and phenols were present in all the exudates. Tannins, saponins, amino acids, and proteins were not found to be present. Reported from the literature, the above-mentioned allelochemicals were present in leaves, flowers, stem, and roots in the weed species [34]. The concentration was varied with the extraction procedures [35]. Since the root exudate yields lower than other plant parts, thus it is difficult to detect all of the secondary metabolites investigated in the present study by the traditional method. Therefore, high-throughput screening method would the best choice to detect the metabolites in the root exudates.

Table 2: Qualitative phytochemical screening test of root exudates of five weed species

		Treatments				
Name of the test	Type of test	T1	T2	T3	T4	T5
Alkaloids	Dragendroff	+++	+++	+++	+++	+++
	Wagner	++	++	++	++	++
	Mayer	++	++	++	++	++
	Hager	++	++	++	++	++
	Tannic	+	+	++	++	++
	$FeCl_3$	+	+	+	+	+
Carbohydrates		+	+	+	+	+
Flavonoids		+	+	+	+	+
Phenols		+	+	+	+	+
Tannins		-	-	-	-	-
Saponins		-	-	-	-	-
Proteins		-	-	-	-	-
Amino acid		-	-	-	-	-

* (-: Not detected, +: Low concentration, ++: Moderate concentration. +++: High concentration

4. CONCLUSION

On the basis of observation and experimental output, the study indicates that the root exudates of five weeds species have the negative effect on the germination of seeds as well as the growth of seedlings of tested crops (mungbean and cowpea). This interrelationship between weed and crops proved that the resulted effect was due to the presence of allelochemicals in the root exudates of the weeds. In the ecological niche, the crops and the weeds compete for the nutrition and in some cases has an antagonistic effect. So, the presence of these weeds beside the crop field should be checked and uprooted in the early stage of seedlings before cultivation. And in the meantime, the field should be checked frequently after sowing the seed to evade the computational effect of the weeds. Furthermore, the allelochemicals present in the root exudates of these weed species should be identified by high throughput technique for the deeper understanding of weed and crop interactions.

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