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RESEARCH ARTICLE

ALLELOPATHIC RESPONSE OF ROOT EXUDATES OF FIVE COMMON WEEDS IN YARD LONG BEAN (*VIGNA UNGUICULATA* SUBSP. *SESQUIPEDALIS* L. VERD] AND MAIZE (*ZEA MAYS* L.)

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ABSTRACT

The allelopathic effect on seed germination, seedling growth, fresh weight, dry weight, total chlorophyll and cell metabolic activity of yard long bean (*Vigna unguiculata* subsp. *sesquipedalis* L.verd) and maize (*Zea maize* L.) to the water extract of root exudates of *Polygonum hydropiper* L. (T1), *Amaranthus spinosus* L. (T2), *Eclipta alba* L. (T3), *Mimosa pudica* L. (T4) and *Cynodon dactylon* (G. Don) Exell (T5) were investigated in this study. Allelopathic testing of root exudates on five weed species revealed that all extracts had a noticeable inhibitory effect on tested crops. The results showed that the concentrated aqueous root exudates of T1, T4 and T5 reduced all parameters on both test crops. Yard long bean was more sensitive to the inhibitory effects than maize. The findings suggested that root exudates may affect yard long bean and maize seeds due to the inhibitory effect of allelochemicals present in the tested weeds.

KEYWORDS

Allelopathy, Polygonum hydropiper L. *Amaranthus spinosus* L., *Eclipta alba* L., *Mimosa pudica* L. *Cynodon dactylon and Root exudates.*

1. INTRODUCTION

Weed infestation is a severe constraint to the farm and biodiversity due to population pressure and more intensive use of cultivated land (Soltani et al., 2016). Due to shorter life span and faster growth rate under the adverse condition than most crops grown in association, they compete consistently for the light, moisture, nutrients, and space and thus adversely affect commercial agricultural production (Sharma et al., 2021). In the early stages of crop growth, losses due to weeds are estimated up to 90% in worst cases, costing more than $40 billion per annum in global economic loss (Fahad et al., 2015). It varies with the type of associated crops and weeds, density, agricultural operation, competition interval, and weather condition. Despite the detrimental effects, weeds also contribute to agriculture positively (Raj and Syriac, 2016). Therefore, crop weed interaction in agriculture has been considered a new strategy or tool for sustainable agriculture (Akter et al., 2019; Cheng et al., 2015). In agro-ecosystems, these positive or negative interactions of different plants and weeds occurred due to liberating several allelochemicals (Salgude et al., 2015). These metabolites are leached from various plant parts through volatilization, leaching, decomposition, and root exudation (Cimmino et al., 2014). Like all other compounds, these organically active compounds are categorized into primary and secondary. The first one, known as primary metabolites, includes carbohydrates, amino acids, proteins, and lipids that assist in the growth of plants (Canarini et al., 2019). The latter consists of secondary metabolites; the principal function is to safeguard the plant from unfavorable situations (Strehmel et al., 2014). They are called root exudates because they are both primary and secondary metabolites. They are constantly released into the rhizosphere through rhizodeposition (Semchenko et al., 2021). The amount of root exudates and their nature is determined by various factors (Wang et al., 2021). Previous studies showed that these compounds mediate complex interactions with plant roots underneath the soil (Tian et al., 2021). These interactions profoundly affect soil structure physically and chemically affect plant growth and development. Most literature regarding root exudates has discussed collection procedures, nutrient solubilization, and microbial allelopathy (Tian et al., 2021; Sasse et al., 2018). Besides, extensive research on allelopathy of leaves, stems, roots, rhizome, flowers, fruits, and seeds extract is vast in laboratory and field conditions. But least in root exudates. Thus, root exudate with allelopathic properties has focused on many kinds of research in recent years (Akter et al., 2019; Bicharanloo et al., 2020; Mitić et al., 2018). Literature is insufficient on root exudate and its Allelopathic activities on the plant. So, this study aims to see how water extracts of root exudates from five weeds affect seed germination and seedling growth in *Vigna unguiculata* subsp. *Sesquipedalis* L.verd] and *Zea mays* L.

2. MATERIALS AND METHODS

2.1 Plant materials

Five common weed species, *Polygonum hydropiper* L. (T1), *Amaranthus spinosus* L. (T2), *Eclipta alba* L. (T3), *Mimosa pudica* L. (T4) and *Cynodon dactylon* (G. Don) Exell (T5) were collected from the Chittagong University campus (Hathazari, Chittagong, Bangladesh). In addition, yard long bean (*Vigna unguiculata* subsp. *Sesquipedalis* L.verd] and maize (*Zea mays* L.) seeds were collected in Hathazari Local Bazar, Chittagong.

2.2 Collection of root exudates

Approximately 50 plants of each weed species were pulled without

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harming their roots and cleaned three times with running tap water before being cleansed with distilled water. The plant roots were immediately transferred to a conical flask holding 300 mL of distilled water and maintained in direct sunlight for 5 hours to collect root exudate. Filtering the water extract of root exudates with Whatman filter paper (No. 1). was used to collect the water extract. After that, the exudates were concentrated to 50 ml in a water bath at 60°C for the allelopathy experiment.

2.3 Seed germination assay

Yard long bean and maize seeds were surfaces sterilized for 3 minutes with 70 percent ethanol, and then gently washed with sterile distilled water up to 5 times to eliminate chemicals. On 9 cm glass Petri dishes with a two-fold filter paper soaked with 5 ml of root exudates, fifteen seeds of the tested crops were distributed. The seeds were immersed in sterile distilled water (T0) as a control. For two days, all treatments, including the control group, were housed in a dark environment. After that, the plates were kept at room temperature for the next eight days. An equal volume of root exudates was added to each Petri dish regularly throughout the experiment. Every day, the treated seeds were observed. When the radicle length exceeded 2 mm, the seeds were declared germinated. Seed germinability, radicle and hypocotyl/plumule length, and fresh and dry weight of seedlings of the tested and control crops were measured after ten days.

The following calculation was considered for the data collection.

$$\text{Germination percentage}(\%) = \frac{\text{No. of germinated seeds}}{\text{Total no. of seeds}} \times 100 \quad \text{(Iman, 2006)}$$

$$\text{Inhibition percentage}(\%) = \frac{\text{Control (C0) - Treatment (Tn)}}{\text{Control (C0)}} \times 100 \quad \text{(Chung } et\ al.\text{, 2003)}$$

Here, n= T1, T2……………T5.

2.4 Cell metabolic activity

Fresh plant material (100 mg of radicle) of germinated seeds or seedlings of yard long bean and Maize from all the treatments were washed and dried quickly using blotting paper, then incubated in 5 mL of TTC (0.2 percent, pH= 7) at 37 °C for four hours in the dark. The reaction was stopped by adding 0.5 mL of sulfuric acid (1 M). Following that, the plant material was removed, washed with distilled water, quickly dried between filter paper, and ground in a mortar placed in ice containing 3.5 mL of ethyl acetate. The homogenate was filtered through a paper Whatman No. 1 filter, and the volume was adjusted to 7 mL with ethyl acetate. The absorbance was measured at 485 nm, and the amount of formazan was calculated using formula (Sampietro et al., 2006).

2.5 Chlorophyll content

Primary leaves from one week old seedlings were used to determine plant pigments. Two mL of (80 %) (v/v) acetone, filtered through filter paper, extracted one gram (approx.) of fresh leaves (Whatman No. 1). Following filtration, 10 mL of 80 percent (v/v) acetone was added to bring the filtrate volume to 10 mL. The samples' absorbance was measured spectrophotometrically at 646 nm, 663 nm (chlorophyll), and 470 nm after the mixture was thoroughly mixed with a vortex mixer (carotenoid). The concentrations of the various pigments were calculated using the formula developed by formula (Lichtenthaler and Wellburn, 1983).

2.6 Statistical analysis

The experiment was repeated three times, with the results expressed as mean, standard deviation (SD) in Microsoft Excel 2010.

3. RESULTS AND DISCUSSION

3.1 Yard long bean and maize germination in reaction to root exudates:

Although seed germination is essential for plant productivity, weed infestations in crop fields can negatively impact by secreting host-specific chemicals (Ebrahimi and Hassannejad, 2015). In this study, the germination and seedling emergence of yard long bean and maize seeds were inhibited by root exudate from five weed species. Compared to the control (T0), all aqueous root exudate extracts reduced seed germination (Figure 1 and 2). Our findings discovered that T5 root exudate had the lowest seed germination (below 50%) in yard long bean, whereas T1, T2, T3 and T4 showed 55%, 65%, 50% and 60% respectively. On the other hand, the germination percentage at T3 (85%) in maize was found to be closest to control, indicating that *Eclipta alba* could not affect much in case maize seed germination (Figure 2). Extracts from *Polygonum hydropiper* L., and *Amaranthus spinosus* L. correspomding to T1 and T2 showed lower seed germination followed by T4 (*Mimosa pudica* L.).

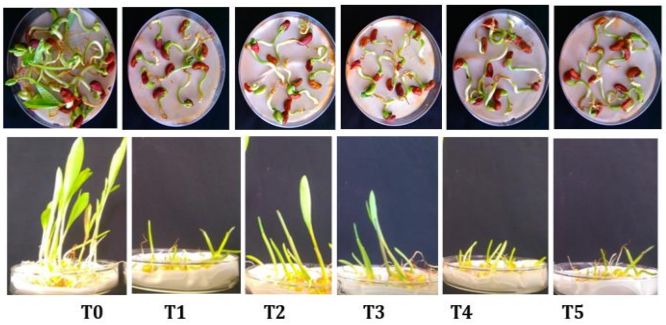

Figure 1: Shows the seedling emergence of yard long bean (above) and maize (below) after 10 days incubation treated with aqueous root exudates of five weeds

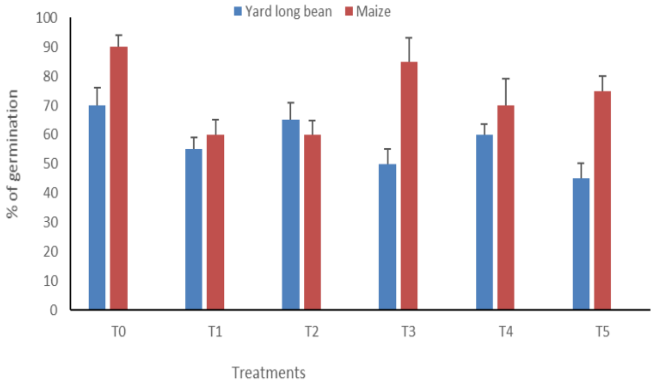

Figure 2: Effect of water extracts of root exudate of five weeds on the percentage of seed germination of yard long bean and maize after 10 days incubation.

The current findings are consistent with previous research that found barnyard grass root exudates reduced the growth of rice, lettuce *(Lactuca sativa* L.), and monochoria (*Monochoria vaginalis*) during the early stages of growth (Xuan et al., 2005). Similarly, the water soluble root exudate (WRE) of *Tithonia diversifolia* suppressed pepper (*Capsicum annum* L.) and tomato (*Lycopersicon esculentum* Mill.) germination and growth (Otusanya et al., 2008). However, when compared to maize, yard long bean had a lower germination percentage (Figure 2), and the rate of germination varied depending on the weeds and crops studied. Water extracts of *Cyperus rotundus* were reported to reduce seed germination and seedling growth of *Cucumis sativus* L., according (Meissner et al., 1982). The application of Mexican desert plants had a similar inhibitory impact on *Zea mays*, *Phaseolus vulgaris, Cucurbita pepo,* and *Lycopersicon esculentum* (Romero-Romero et al., 2002).

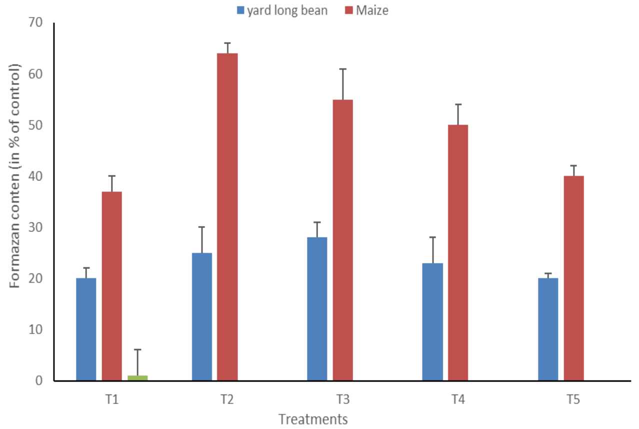

Figure 3: Effect of water extracts of root exudate of five weeds on formazan content of yard long bean and maize after 10 days incubation.

Synthesizing inhibitors of phenols, terpenoids, p-coumaric acid, gallic acid, ferulic acid, p-hydroxybenzoic acid, and anisic acid during the physiological activity of seed germination can have an inhibitory effect on seed germination and seedling growth (Zhang et al., 2010; Batish et al., 2008). Furthermore, some regulatory polyphenols bind to other hormones, causing seedling growth to be reduced. For example, ferulic acid, t-cinnamic acid, chlorogenic acid, p-coumaric acid, and coumarin, for example, interact with ABA and have additive adverse effects on seed germination and seedling growth in mung bean resulting in lower crop productivity (Batish et al., 2008). The average length of the radicle and hypocotyl in control plants was maximum, whereas root exudate from weed species inhibited both crops (Table 1).

Table 1: Effect of water extracts of root exudate of five weeds on seedling growth of yard long bean after 10 days incubation (mean ± SD).

Treatments	Radicle length (cm)	Hypocotyl length (cm)	Radicle: Hypocotyl	Fresh weight (g.Seedling^{-}1)	Fresh weight (g.Seedling^{-}1)
T0	3.39 ± 0.52	3.65 ± 0.61	0.93 ± 0.08	6.56 ± 1.42	1.38 ± 0.30
T1	1.31 ± 0.31	2.12 ± 0.54	0.61 ± 0.06	3.36 ± 1.03	0.87 ± 0.06
T2	1.17 ± 0.29	2.6 ± 0.46	0.45 ± 0.03	3.88 ± 1.12	1.09 ± 0.12
T3	1.12 ± 0.32	2.5 ± 0.57	0.49 ± 0.05	3.31 ± 1.13	0.86 ± 0.07
T4	1.05 ± 0.23	2.8 ± 0.54	0.38 ± 0.04	3.48 ± 1.21	1.05 ± 0.12
T5	1.32 ± 0.26	1.9 ± 0.29	0.69 ± 0.07	3.02 ± 1.06	0.82 ± 0.07

Table 2: Effect of water extracts of root exudate of five weeds on seedling growth of maize after 10 days incubation (mean ± SD).

Treatments	Radicle length (cm)	Plumule length (cm)	Radicle/ Plumule ratio	Fresh weight (g.Seedling^{-}1)	Dry weight (g.Seedlingt^{-}1)
T0	5.51 ± 1.38	7.37 ± 1.45	0.75 ± 0.05	7.22 ±1.42	2.02 ±0.46
T1	2.80 ± 0.27	1.83 ± 0.46	1.53 ± 0.10	4.10 ± 1.15	1.42 ± 0.02
T2	4.15 ± 1.12	2.92 ± 0.53	1.43 ± 0.09	5.23 ± 1.32	1.94 ± 0.08
T3	4.61 ± 1.23	3.82 ± 1.17	1.20 ± 0.07	5.45 ± 1.37	1.98 ± 0.08
T4	2.76 ± 0.15	1.61 ± 0.18	1.73 ± 0.09	4.21 ± 1.10	1.65 ± 0.04
T5	3 .01 ± 1.12	2.46 ± 0.75	1.25 ± 0.04	4.43 ± 1.17	1.72± 0.08

The radicle length ranged from 1.31 to 1.32 cm and the hypocotyl length from 2.12 to 1.90 cm in yard long been treated with T1 to T5 root exudates (Table 1). Maize, on the other hand, was not the same. Maize had a more excellent range of radicle (2.80 cm to 3.01 cm) and plumule (1.83 cm to 2.46 cm) than control (Table 2). In yard long bean, T3 (95.55%) showed the highest inhibition effect whilst the other exudate extracts showed moderate inhibitory effect for radicle. Cynodon dactylon corresponding to T5 showed in 48.42% inhibitory effect in hypocotyl. In comparison, radicle showed more inhibitory effect than hypocotyl. In maize, shoot inhibition was greater than roots. Root exudate of T4 exhibited 78.29% followed by T1 (76.32%) T5 (67.44%), T1 (60.65%), and T2 (60.65%). In terms of root, the highest inhibition effect was found in T1 (49.18%) and T4 (49.91%) and the lowest was found in T3 (16.52%). T2 showed 24. 68% inhibition (Table 3).

Table 3: Inhibitory effect (%) of root exudate of five weeds on the growth of radicle/root and hypocotyl/plumule of yard long bean and maize (mean ± SD).

Treatments	Yard long bean		Maize	
	Radicle inhibition	Hypocotyl inhibition	Root inhibition	Shoot inhibition
T1	61.71 ± 4.10	42.12 ± 3.11	49.18 ± 3.43	76.32 ± 5.09
T2	65.53 ± 4.36	28.83 ± 1.87	24.68 ± 1.56	60.65 ± 3.74
T3	95.55 ± 6.32	31.55 ± 2.64	16.52 ± 1.22	48.16 ± 3.07
T4	67.52 ± 4.34	23.33 ± 1.76	49.91 ± 2.78	78.29 ± 5.14
T5	61.1 1 ± 4. 58	48.42 ± 2.87	45.55 ± 2.91	67.44 ± 4.16

The fresh and dry weights of seedlings are essential characteristics, and the buildup of dry matter in plants can be beneficial to plant health. The fresh and dry weights of seedlings were consistent with the height of radicle and hypocotyl growth in this investigation. A similar outcome found during seed germination trials in wheat (*Triticum durum* L.) (Leela et. al., 2014). Increased concentrations of root exudate of switchgrass (*Panicum virgatum* L.) inhibited lettuce (*Lactuca sativa* L.) seedling growth, which was consistent with ours (An et al., 2013).

Seedling emergence, root to shoot ratio, fresh and dry weight of test crops were all reduced, indicating that the infested weeds in the crop fields may have contained phytotoxins that harmed the target crops (Tanvir et al., 2010). When wild rice root exudate was treated to *Sisybrium officinale,* the same results were obtained (Mahmoodzadeh et al., 2011). Water-soluble root exudate from *Tithonia diversifolia* was similarly found to reduce radicle length in *L. esculentum* (Otusanya et al., 2008). In addition, aqueous extracts of *Galinsoga paviflora* and *Bidens pilosa* reduced seed germination inhibition, plumule and radicle length (Singh et al., 1996). Allelochemicals in root exudates may cause deep oxidative stress in target tissues, compromising antioxidant mechanisms, or chemical compound accumulation that delays or obstructs the arrangement of microtubules during cell division, resulting in decreased hormonal activity, ion uptake, inhibition of protein and enzyme activation, and cell permeability (Sitthinoi et al., 2017). As a result of these actions, crop radicle and hypocotyl lengths may be shortened (Aenavoli et al., 2006). A group researchers found that at 1mM, another phenolic component, p-coumaric acid, has a significant impact on canola root development and fresh weight (*Brassica napus*) (Baleroni et al., 2000).

According to benzoic acid, a possible allelochemical, reduced mustard development by causing aberrant and disorganized tissues and disrupting cell division as concentrations increased (Kaur et al., 2005). Phenolic component such as p-coumaric acid, has a significant effect on canola root development and fresh weight on Brassica napus at a concentration of 1 mM (Baleroni et al., 2000). Benzoic acid, a possible allelochemical, reduced mustard development by causing aberrant and disorganized tissues and disrupting cell division as concentrations increased (Kaur et al., 2005). Extract of Bermuda buttercup root exudates supresses tomato, oat, and

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lettuce plant biomass production indicating that it may have allelopathic potential for these crops (Travols, et al., 2008). Catechol, gallic acid, and pyrogallic acid dramatically reduced the germination %, radicle and hypocotyl development, and fresh and dry weight of cucumber seedlings in one experiment. However, several researchers found that the aqueous weed extract did not suppress the crop plant in the field but instead acted as a bio stimulant (Raj and Syriac, 2016). Those findings are incongruent with our current conclusion.

3.2 Contents of chlorophyll

The results showed a decreasing trend in chlorophyll levels on both tested crops in the presence of five weed root exudate extracts (Figure 4). In comparison to control, the reduction factor is below 0.9 in yard long bean while Ti and T5 showed lowest one. However, The results revealed that maize control plants had high chlorophyll content (1.9 mg/f.wt) accumulation. Similarly, the reduced amount of chlorophyll content shown in all the treatments in maize (Figure 4). The decrease in chlorophyll content observed in this study after using aqueous extracts exudates of the weed plants studied is consistent with previous studies (Akter et al., 2019).

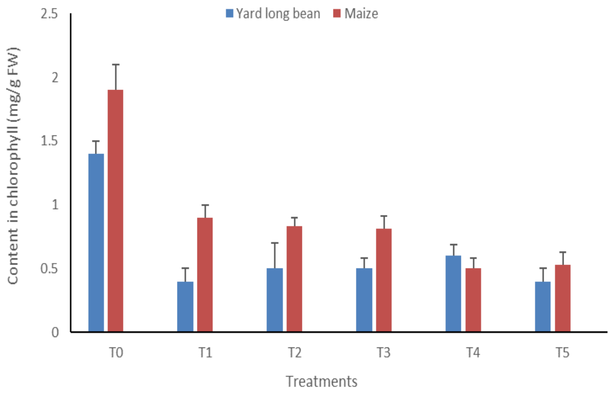

Figure 4: Effect of water extracts of root exudate of five weeds on total chlorophyll content of yard long bean and maize after 10 days incubation.

The most critical pigments in the leaf are chlorophyll, which ensure the absorption of solar radiation, which is the first step in the photosynthetic pathway (Sing et al., 2013). Changes in chlorophyll content, for example, could be due to secondary metabolites such 3-3'-5-Trihidroxi-4'-7-dimethoxyflavone and 3-3'-5-Trihidroxi-4'-7-dimethoxyflavone-3-O-sulphate, which could be playing a large role in changing the pigment concentration in crop plant (Devi et al., 2014). A group researcher also found variations in chlorophyll content in *Triticum durum* (Abu-Rommann et al., 2010). Other researchers found a reduction in photosynthetic pigments in *Amaranthus cruentus* L. and *Oryza sativa* L. treated with *Tithonia diversifolia* aqueous extract (Ilori et al., 2007). The extracts of root exudates of *Ageratum conyzoides*, *Leucas aspera* , *Scoparia dulcis* , *Spilanthes acmella* and *Vernonia patula* root exudates decreased the chlorophyll content of *Raphanus sativus* and *Cucumis sativus*) (Akter et al., 2019). The allelochemicals found in *Datura stramonium* hindered chlorophyll production, resulting in a decrease in total chlorophyll concentration, consistent with our findings (Elisante et al., 2013). Allelochemicals like sorgoleone (SGL), a p-benzoquinone from *Sorghum bicolor* root exudates, acts as a potent inhibitor of chlorophyll production, indicating that photosynthesis in soybean and pea is effectively prevented, resulting in a reduction in plant development (Einhellig et al., 1993). However, the decrease in chlorophyll pigments could be due to the formation of a proteolytic enzyme (chlorophyllase), which is responsible for chlorophyll degradation, or it could be due to a reduction in synthesis due to a change in the thylakoid membrane structure (Ding et al., 2016).

3.3 Cell metabolic activity

Figure 3 shows the formazan concentration in roots treated with various root exudate of weed extracts, given as a percentage of the control. These contents represent cellular metabolic activity, primarily dehydrogenase enzyme activity and thus mitochondrial respiration. When comparing the effects of different exudate extracts to the control, the results showed a decrease in formazan production in both tested crops. In seeds exposed to extracts of yard long bean at the germination stage, this content was 20, 25, 28, 23 and 20 percent, respectively. On the other hand, these values were 37, 64, 55, 50 and 40 percent (Figure 3). During germination, cellular respiration provides a source of ATP to the embryo, allowing it to continue its metabolic activities. Reduced respiration in seeds exposed to plant extracts could explain why germination halted in their presence. Plant extracts or allelochemicals have influenced breathing. Therefore, the decrease in dehydrogenase activity could be a consequence of cell damage caused by exposure to allelochemicals found in extracts (Sampietro et al., 2006; Rashid et al., 2010; Ladhari et al., 2014). Our findings revealed that membrane damage to tested seeds differed depending on the extracted source. The yard long bean was more damaging to biomass, than maize. The chemical composition of the aqueous exudate extracts may affect) and the likely excessive accumulation that causes membrane damage in both tested roots (Omezzine et al., 2014).

4. CONCLUSION

The study's observations and experimental results reveal that the root exudates of five weed species have a detrimental influence on seed germination and seedling growth of examined crops (Yard long bean and maize). This interaction between weeds and crops established that the observed effect was caused by the presence of allelochemicals in the weeds' root exudates. Crops and weeds fight for nutrients in the ecological niche, which can have an antagonistic effect in some situations. Thus, the existence of these weeds adjacent to crop fields should be monitored and eradicated during the seedling stage prior to cultivation.

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