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ORIGINAL RESEARCH

Dynamics of Herbicidal Potential: Effects of Two Bacterial Species and Five Aqueous Plant Extracts on Yield and Yield Components of Rice (*Oryza sativa L.*) and Surof (*Echinochloa crus-galli L.*)

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ABSTRACT: This research aimed of investigate the effects of foliar applications using different levels of herbicidal potential from two bacterial species and five aqueous plant extracts on the yield and yield components of *Oryza sativa L.* and *Echinochloa crus-galli L.* The study followed a split factorial design based on randomized complete block design (RCBD) with three factors and three replications during the 2017-2018 crop year. The primary treatments consisted of aqueous extracts from *Sorghum*, *Broccoli*, *Nettle*, *Eucalyptus* and Elderberry, as well as *Streptomyces sp-albos* containing thaxtomin, *Xanthomonas campestris*, and control s (without aqueous plant extracts and surfactant). The secondary factor involved foliar application treatments with varying levels of concentration, including zero (control), 5, 10, and 15 per thousand extracts and the third experimental factor included *Oryza sativa L.* and *Echinochloa crus-galli L.* The findings revealed that as the extract concentration increased, plant height, leaf area index (LAI), grain yield, biological yield, chlorophyll a, chlorophyll b, and total chlorophyll contents decreased. Conversely, grain starch content increased with higher extract concentrations, with the most pronounced effects observed at 15 per thousand extract concentration. It was also observed that the use of extracts led to reduced yield characteristics and components in both *Oryza sativa* L. and *Echinochloa crus-galli* L. Among the extracts, Elderberry extract exhibited the most significant negative impact. Comparatively, *Oryza sativa* L. demonstrated higher plant height, LAI, grain yield, biological yield, harvest index, chlorophyll content, grain protein content, and grain starch content when compared to *Echinochloa crus-galli* L. The results suggested that various extracts, particularly *Nettle*, along with 15 per thousand concentrations of Elderberry and *Nettle* extracts, hold potential for controlling *Echinochloa crus-galli* L. during the germination stage under field conditions.

KEYWORDS: Allelopathy, rice, aqueous extracts; chlorophyll, starch

1. Introduction

Oryza sativa L. belongs to the genus *Oryza* and the Gramineae family (Sreenivasulu, 2019). It stands as a pivotal crop, following wheat (*Triticum aestivum L.*), and contributes to over 40% of the nutritional energy for more than half of the global population (Gbanguba et al., 2011).

Echinochloa crus-galli L., an annual summer plant in the Gramineae family, propagates through seeds. This plant primarily thrives in warm temperate regions and exhibits a preference for moisture-rich environments. Notably, it is one of the most significant weeds found in *Oryza sativa L.* fields, not only in Iran but also in various other countries. Being a C4 plant, *Echinochloa crus-galli* L. displays rapid growth, vigorous tillering, and a tendency to swiftly dominate *Oryza sativa* L. fields (Khanh et al., 2018). Within *Oryza sativa* L. fields, *Echinochloa crus-galli* L. is a common presence that introduces new biological constraints on *Oryza sativa* L. productivity. Its presence also detrimentally affects the growth and yield of *Oryza sativa* L. grains (Kong et al., 2012). The widespread utilization of chemical herbicides and the subsequent heavy reliance on them have given rise to issues such as weed resistance and negative impacts on human health and the environment (Das, 2012). Thus, the search for an approach that ensures safety for both humans and the environment, while also being cost-effective for weed control, becomes imperative. Leveraging the allelopathic properties of certain plants to manage weeds holds special significance (Mubeen et al., 2012). Allelopathy constitutes a phenomenon where plants produce allelochemicals capable of influencing the growth of themselves and other plants. These substances can yield either detrimental or beneficial effects during germination, vegetative growth, and reproductive stages of other plants (Tantiado and Saylo, 2012). Allelochemicals are released into the surroundings through shoot leaching, volatilization, root exudation, and the decomposition of plant litter (Song and Liu, 2014). The effects attributed to allelochemicals encompass anatomical irregularities, hindered absorption, germination and bud growth, as well as phenomena like chlorosis and necrosis. The inhibitory impact of these substances hinges on the concentration of aqueous extracts from the plant (Mishra, 2015).

In a study by Dastres et al. (2015), it was discovered that allelochemicals present in aqueous extracts from the pagoda tree and creeping jenny led to reduced plant height and diminished wheat dry weight. Hatami Hampa et al. (2018) explored the allelopathic influence of aqueous extracts from Sorghum and Russian knapweed on seedling growth and the activity of antioxidant enzymes in plants like wheat, sugar beet, common lambsquarter, and redroot pigweed. Their findings indicated that Sorghum and Russian knapweed extracts hindered seedling growth while augmenting soluble carbohydrate levels, proline concentrations, and catalase activity in the studied plants, as compared to the control group. Sadat Asilan et al. (2015) demonstrated that certain cultivars of *Oryza sativa* L. displayed stimulatory effects, while others exhibited inhibitory effects on the growth of *Echinochloa crus-galli* L. Furthermore, an increase in the concentration of *Oryza sativa* L. cultivar extracts correlated with intensified inhibition or stimulation of *Echinochloa crus-galli* L. growth.

Weeds pose numerous challenges across diverse land uses. The escalating presence of herbicide-resistant weeds, coupled with restrictions on pesticide and herbicide application, has prompted the exploration of novel weed control strategies. In the past three decades, considerable attention has been directed towards leveraging bacteria, fungi, and viruses to tackle this issue. This approach holds several advantages, including diminished environmental repercussions, reduced developmental costs compared to conventional herbicides, and the discovery of fresh herbicidal mechanisms (Harding and Raizada, 2015). Utilizing bacteria for biological weed control has garnered particular interest due to their rapid growth, relatively straightforward propagation requirements, and suitability for genetic manipulation through mutagenesis or gene transfer (Mithila et al., 2011). The broader concept of biological control pertains to introducing organisms into an ecosystem to manage one or more undesirable species (Bailey et al., 2014). *Xanthomonas campestris*, a Gram-negative rod-shaped bacterium possessing a mobile polar flagellum, belongs to the Pseudomonas family and is a seed-borne plant pathogen (Zabot et al., 2012; Ya-Wen et al., 2011). On the other hand, the Streptomyces genus is a member of Actinomycetes and is commonly found in soil environments. This genus holds potential as a source of various antibiotics and secondary metabolites due to its ability to exude substantial quantities of extracellular enzymes (Valan Arasu et al., 2009).

Research on allelopathy and plant pathogens can be among the basic ways to control weeds in the sustainable agricultural system. Allelopathic weeds may cause positive, negative, or even neutral effects on crops by releasing chemical compounds, mainly secondary metabolites, through various ways such as root exudation, litter decomposition, leaching, and volatilization. Therefore, considering the importance of this issue in terms of protection and sustainable production, this study tried to take a step towards sustainable agriculture by implementing the above plan, reducing the application of pesticides and herbicides, and establishing non-chemical methods to improve the quality and quantity of crops by control ling weeds and moving from conventional agriculture to sustainable agriculture.

2. Materials and methods

2.1 Experimental design

A factorial experiment was carried out using RCBD with three replications on an arable land located in one of the villages of northern Iran, Amol, Mazandaran Province, in the 2017-2018 crop year. The first experimental factor included aqueous extracts of *Sorghum*, *Broccoli*, *Nettle*, *Eucalyptus*, Elderberry, as well as *Streptomyces sp-albos* containing thaxtomin, *Xanthomonas campestris*, and control's (without aqueous plant extracts and surfactant). The second experimental factor consisted of 0 (Control), 5, 10, and 15 per thousand of extracts; the third experimental factor included the local Tarom *Oryza sativa* L. of Fereydunkenar and *Echinochloa crus-galli* L.

The bacteria were purchased as lyophilized in coordination with Islamic Azad University and Center for Scientific and Industrial Research of Iran. Plates containing solid medium and inoculated bacteria were incubated at 30°C for 24-48 h for *Xanthomonas campestris* and 1-2 weeks for Streptomyces to provide the sufficient time for the bacteria to grow and produce their metabolites. After this period, the sterilized seeds of the studied plants were placed on the grown plates on the opposite sides. A plate containing the medium without bacterial culture with seeds should always be considered as the control.

According to the conditions, the leaves and stems of *Sorghum*, *Broccoli*, *Nettle*, *Eucalyptus*, and Elderberry were placed in an oven at 70°C for 48 h and, then, ground by a mill to prepare the aqueous extracts. To do so, the sterile distilled water was used. The aqueous extracts of the intended plants were obtained by pouring 50 g of the powdered aerial parts of each plant into an Erlenmeyer flask and adding 500 ml of distilled water, which had reached 70-80°C. The flask's neck was then covered and it was placed in a bain-marie at 60°C. After 24 h, the mixture inside the flask was squeezed and the obtained extract was filtered using a filter paper and funnel. The extract was then sprayed on 4-7-leaf plants and kept in a petri dish as liquid.

Land preparation operations including plowing, harrowing, and levelling were carried out in a desirable manner before planting, terracing the nursery, and sowing *Oryza sativa* L. and *Echinochloa crus-galli* L. seeds in the nursery. *Oryza sativa* L. and *Echinochloa crus-galli* L. seeds were prepared from Amol *Oryza sativa* L. Research Institute and disinfected with Vitavax fungicide before sowing. Three-four-leaf seedlings (25 days old) were used. Urea fertilizer (150 kg ha^{-1}) and phosphorus fertilizer were applied in three stages along with transplanting the seedlings to the main plot, tillering, and clustering. After transplanting the seedlings, the field was kept submerged for two weeks; since then, until two weeks before the harvest, the intermittent irrigation was carried out. All the preparation operations performed for experimental plots and the studied *Oryza sativa* L. and *Echinochloa crus-galli* L. seedlings were similar. The seeding density was 300 g m^{-2} in the nursery in the Japanese form and planting density was 9 plants m^{-2} in the main plot; then, the result was generalized. The experimental plot size was considered to be 1×1 m^2. A stack was considered between the experimental plots. Herbicides and pesticides were not used during the growing season.

Plant extracts were prepared according to the experimental protocol and kept in a graduated container along with two species of bacteria and sprayed on the *Oryza sativa* L. nursery in three stages after germination until the seedling stage. The seedlings were then transplanted to the main plot until ripening and harvesting, and sampling and measurements were performed.

2.2 Measurement and Analysis

After the growing period, complete physiological maturity, and 14% grain moisture content, harvesting was carried out. When the crops ripened, 10 plants were randomly sampled from each plot, the yield and yield components of which were examined.

Leaf area was measured using the leaf area meter. To determine chlorophyll content, 0.2 g of the leaf sample was extracted in 80% acetone. The extract was then filtered by the filter paper and the acetone was added until reaching the volume of 25 ml. Chlorophyll was completely extracted from the leaf tissue. The light absorption of chlorophylls a and b was read at 645 and 663 nm, respectively, and the total chlorophyll content was obtained using the following equation (Arnon, 1972):

$$A: (12.25*A663) - (2.79*A645) \quad (1)$$

$$B: (21.21*A645) - (5*A663) \quad (2)$$

TOTAL Chl (mg ml^{-1}) = chla+chlb (3)

The grain protein content was measured using Kjeldahl method by Kjeltec 1030 autoanalyzer (made in Sweden). The total nitrogen content was first obtained and, then, the grain protein content was estimated by multiplying the total nitrogen value by 6.25.

To determine the grain starch content, 0.1 g of the fully powdered sample was poured into 15 ml of 80% ethanol. After 20 sec, the vortex was filtered by the filter paper and placed in an oven at 50°C until the ethanol evaporated. To remove excess sediments and other compounds, 5 ml of 5% zinc sulfate solution and 4.7 ml of 0.3 N normal barium hydroxide solution were added. After centrifugation, 2 ml of the floating extract and 1 ml of 5% phenol solution were added. After the mixture was stirred well, 5 ml of the concentrated sulfuric acid was added; after 45 min, light absorption was read at 485 nm by the UV-VIS spectrophotometer (Sivakumar et al., 2000).

2.3 Statistical analysis

At the end of the experiments, the data were analyzed using SPSS statistical software and comparison of the means was performed by Duncan's test at the significance level of 5%.

3. Results and discussion

3.1 Plant height

The ANOVA results in Table 1 showed that the extract, extract concentration, plant, and interactions between the extract and plant as well as extract concentration and plant had significant effects on plant height ($P < 0.01$). The results of comparing the mean plant height under the impact of the extract indicated the plant height reduced compared to the control by applying the extract. The highest plant height (114.59 cm) was observed in the control group and the lowest plant height was measured as 78.79 cm by applying the Elderberry extract, suggesting a 31.24% reduction in plant height compared to the control (Table 2). With increasing the extract concentration, the plant height decreased compared to the control. The minimum plant height (84.63 cm) was obtained at the concentration of 15 per thousand of extracts, indicating the plant height decreased by 26.13% compared to the control (Table 3). Based on the results of comparing the mean plant height under the effect of the plant, the *Oryza sativa* L. and *Echinochloa crus-galli* L. plant height was 105.345 cm and 77.066 cm, respectively (Table 4).

The results of comparing the mean plant height under the influence of the extract and plant showed that the plant height reduced compared to the control by applying the extract. The lowest *Oryza sativa* L. and *Echinochloa crus-galli* L. plant height (93.81 cm and 65.46, respectively) was related to Elderberry extract (Table 6). The results of comparing the mean plant height under the impact of extract concentration and plant suggested that the plant height decreased with increasing concentration. The concentration levels of 5, 10, and 15 per thousand of extracts were in the same statistical group as the *Oryza sativa* L. plant, while the lowest *Echinochloa crus-galli* L. plant height with the mean values of 75.73 cm and 68.84 cm were related to 10 and 15 per thousand of extracts, respectively (Table 7).

Hormonal imbalance is among the most important reasons for decreased growth of

Table 1. ANOVA results of investigating the herbicidal potential of two bacterial species and five aqueous plant extracts in yield and yield components of *Oryza sativa* L. and *Echinochloa crus-galli* L.

Sources of changes	df	PH	LAI	GY	BY	Chla	Chlb	Total chl	GP	GS
Block	2	50.62ns	0.1ns	0.02ns	0.3ns	0.04ns	0.01ns	0.05ns	0.14ns	183.22ns
Extract (a)	6	684.55**	2.35**	1.79**	12.67**	1.68**	0.53**	4.11**	1.84**	182.27**
Extract concentration (b)	2	1536.5**	1.89**	3.39**	27.36**	3.68**	1.31**	9.58**	0.36**	119.83**
Plant (c)	1	18805.69**	26.15**	242.12**	2618.57**	81.18**	17.67**	175.52**	21.78**	685.3**
a*b	12	14.81 ns	0.06ns	0.09*	1.15ns	0.12*	0.06*	0.27**	0.38ns	29.83ns
a*c	6	218.91**	0.64**	0.68**	3.67**	0.8**	0.18**	1.63**	0.02ns	36.43ns
b*c	2	271.11**	0.29*	0.24**	1.72ns	0.003ns	0.04ns	0.04ns	0.02ns	5.87ns
a*b*c	12	23.77ns	0.04ns	0.07ns	0.44ns	0.12ns	0.03ns	0.17ns	0.02ns	11.29ns
Error	86	21.42	0.07	0.05	0.73	0.06	0.03	0.1	0.48	22.8
%CV		5	8.27	8.75	8.82	6.77	9.88	5.94	7.56	6.59

Note: GS-Grain starch content; GP-Grain protein content Total Cl-Total chlorophyll; Clb-Chlorophyll b; Cla, Chlorophyll a BY-Biological yield; GY-Grain yield; LAI-leaf area index; PH-Plant height df-Degrees of freedom (df), * and ** are not significant (ns) and significant at levels of 5% and 1%, respectively

Table 2. Comparing the mean herbicidal potential of two bacterial species and five aqueous plant extracts in yield and yield components of Oryza sativa L. and Echinochloa crus-galli L.

Extract	PH (cm)	LAI	GY (ton ha^{-1})	BY ton ha^{-1})	Chl a (mg g^{-1} FW $^{-1}$)	Chl b (mg g^{-1}FW $^{-1}$)	Total Chl (mg g^{-1} FW^{-1})	GP (%)	GS (%)
Control	112.74a	4.19a	3.36a	11.84a	4.69a	2.33a	7.02a	9.96a	61.35e
Sorghum	100.31b	3.78b	2.8b	10.73b	4.07b	1.94b	6.02b	9.33bc	68.74d
Broccoli	94.21c	3.17c	2.43cd	9.82c	3.52d	1.59de	5.11d	9.21bcd	72.62bc
Nettle	87.95d	2.87d	2.04e	9.34c	3.37d	1.52ef	4.89d	8.96cd	75.68ab
Eucalyptus	92.77c	3.22c	2.57c	9.74c	3.78c	1.7cd	5.48c	8.87cd	77.6a
Elderberry	80.47e	2.63e	1.89e	7.98d	3.15e	1.42f	4.57e	8.73d	75.03ab
Streptomyces sp-albos	94.23c	3.27c	2.53cd	9.6c	3.77c	1.75c	5.52c	9.67ab	70.65cd
Xanthomonas campestris	91.84c	3.28c	2.4d	10.04c	3.75c	1.73c	5.51c	9cd	70.94cd

Note: GS-Grain starch content; GP-Grain protein content Total Cl-Total chlorophyll; Clb-Chlorophyll b; Cla, Chlorophyll a BY-Biological yield; GY-Grain yield; LAI-leaf area index; PH-Plant height; Means with similar letters are not significantly different at 5% level according to Duncan's test.

plant shoots and roots. Some of the mechanisms of action of allelopathic substances are similar to those of plant hormones; for example, phenolic acids and polyphenols reduce auxin-stimulated growth by stopping its oxidative decarboxylation (Dastres et al., 2015). Auxin, as the hormone regulating cell longitudinal growth and its divisions, is among the factors affecting longitudinal growth. Therefore, any disturbance in the action of this hormone inhibits or reduces plant growth (Zuo et al., 2014).

Studies have shown that some of the allelopathic substances interfere with normal levels of the hormone by inhibiting auxin transport, leading to growth arrest and development of abnormal structure in the growing organ (Mirsky et al., 2013).

3.2 Leaf area index (LAI)

According to the ANOVA results, the extract, concentration, plant, and interactions between the extract and plant at the significance level of 1% as well as extract concentration and plant at the significance level of 5% had significant effects on LAI (Table 1). The LAI decreased compared to the control by applying the extract, so that the highest LAI (3.78) was observed in the control group and the lowest LAI (2.613) was obtained by applying the Elderberry extract, indicating the LAI decreased by 30.95% compared to the control (Table 2). With increasing the extract concentration, the LAI decreased compared to the control. The minimum LAI (2.97) was obtained at the concentration of 15 per thousand of extracts, suggesting a 21.42% decrease in the LAI compared to the control (Table 3). It was observed that LAIs were 3.686 and 2.667 in *Oryza sativa* L. and *Echinochloa crus-galli* L., respectively (Table 4). The results of comparing the mean LAI under the impact of the extract and plant showed that the LAI decreased compared to the control by applying the extract. The lowest LAIs in *Oryza sativa* L. and *Echinochloa crus-galli* L. were 3.28 and 1.96, respectively, which were associated with Elderberry extract (Table 6). The LAI decreased in *Echinochloa crus-galli* L. with increasing concentration; however, different extract concentrations showed no statistically significant difference in *Oryza sativa* L. LAI. The lowest *Echinochloa crus-galli* L. LAI with the average of 2.34 was related to 15 per thousand of extracts (Table 7). It seems that with increasing the extract concentration, the amount of allelopathic substances increased and prevented the full growth of the leaves; therefore, the leaves remained smaller and the leaf area decreased (Azizbeigi and Khara, 2014). Decreased leaf area can be due to the inhibitory effect of allelopathic substances, reduced cell division, and tissue development (Jabran et al., 2015).

3.3 Grain yield

Based on the ANOVA results, the extract, concentration, plant, interaction between the extract and plant, as well as the concentration and plant at the significance level of 1%, and interaction between the extract and concentration at the significance level of 5% had significant effects on grain yield (Table 1). The grain yield reduced compared to the control by applying the extract. The highest grain yield (3.232 t ha^{-1}) was obtained in the control treatment and the lowest grain yield (1.888 t ha^{-1}) was obtained by applying Elderberry extract, suggesting a 41.58%

decrease in grain yield compared to the control (Table 2). With increasing the extract concentration, the grain yield decreased compared to the control. The minimum grain yield (2.049 t ha^{-1}) was obtained at the concentration of 15 per thousand of extracts, showing that the grain yield decreased by 36.6% compared to the control (3.232 t ha^{-1}) (Table 3). Based on the results of comparing the mean grain yield under the effect of the plant, the grain yields in *Oryza sativa* L. and *Echinochloa crus-galli* L. were 3.855 t ha^{-1} and 0.86 t ha^{-1}, respectively (Table 4). The grain yield decreased with increasing extract concentration, so that the highest grain yield (3.3 t ha^{-1}) was observed in the control treatment and the lowest grain yield (1.6 t ha^{-1}) was related to 10 per thousand of Elderberry extract (Table 5). The results of comparing the mean grain yield under the influence of the extract and plant showed that, with the application of the extract, the grain yield decreased compared to the control, so that the lowest grain yield in *Oryza sativa* L. (3.16 t ha^{-1}) was related to Elderberry extract and the lowest grain yields in *Echinochloa crus-galli* L. were 0.68 t ha^{-1} and 0.61 t ha^{-1}, which were related to *Nettle* and Elderberry extracts, respectively (Table 6). The results of comparing the mean grain yield under the impact of extract concentration and plant indicated the grain yield decreased with increasing concentration. The concentration levels of 5, 10, and 15 per thousand of extracts were in the same statistical group in *Oryza sativa* L. plant and the grain yield was at the lowest level at these concentrations. The lowest grain yield of *Echinochloa crus-galli* L. with the average of 0.65 t ha^{-1} was related to 15 per thousand of extracts (Table 7). Allelopathic compounds affect nitrogen metabolism and, consequently, biochemical interactions, photosynthesis, dry matter accumulation in shoots, and grain yield components. However, changes in the nitrogen metabolism rate depend on the type of allelopathic compounds and plant species (Young et al., 2014). Probably over time, due to the decrease in leaf activity and their fall at the end of the growing season, the growth rate of the plant gradually decreases and becomes negative, which results in reduced yield (Sadat Asilan et al., 2015).

3.4 Biological yield

The ANOVA results in Table 1 showed that the extract, concentration, plant, and interaction between the extract and plant had significant effects on the biological yield ($P < 0.01$). The results of comparing the mean biological yield under the effect of the extract indicated the biological yield decreased compared to the control by applying the extract, so that the highest biological yield (11.175 t ha^{-1}) was observed in the control treatment and the lowest biological yield (7.991 t ha^{-1}) was obtained by applying Elderberry extract, suggesting a 28.49% decrease in biological yield compared to the control (Table 2). The biological yield decreased compared to the control with increasing the extract concentration, so that the minimum biological yield (8.605 t ha^{-1}) was observed at the concentration of 15 per thousand of extracts, indicating the biological yield decreased by 22.99% compared to the control (11.175 t ha^{-1}) (Table 3). Based on the results of comparing the mean biological yield under the effect of the plant, the biological yields in *Oryza sativa* L. and *Echinochloa crus-galli* L. were 14.56 t ha^{-1}

Table 3. Comparing the mean effect of extract concentration on yield and yield components of *Oryza sativa* L. and *Echinochloa crus-galli* L.

Extract concentration (Per thousand)	Plant height (cm)	LAI	Grain yield (ton ha $^{-1}$)	Biological yield (ton ha $^{-1}$)	Chlorophyll a (mg g $^{-1}$ FW $^{-1}$)	Chlorophyll b (mg g $^{-1}$ FW $^{-1}$)	Total chlorophyll (mg g $^{-1}$ FW $^{-1}$)	Grain protein content (%)	Grain starch content (%)
0	112.74a	4.19a	3.36a	11.84a	4.69a	2.33a	7.02a	9.96a	61.35b
5	98.34b	3.39b	2.69b	10.5b	3.94b	1.86b	5.81b	9.21b	71.08a
10	90.18c	3.16c	2.31c	9.39c	3.6c	1.62c	5.22c	9.03b	74.11a
15	86.53d	2.97d	2.14d	8.93d	3.35d	1.52d	4.87d	9.1b	73.9a

Means with similar letters are not significantly different at 5% level according to Duncan's test

Table 4. Comparing the mean effect of plant on yield and yield components of *Oryza sativa* L. and *Echinochloa crus-galli* L.

Plant	Plant height (cm)	LAI	Grain yield (ton ha $^{-1}$)	Biological yield (ton ha $^{-1}$)	Chlorophyll a (mg g $^{-1}$ FW $^{-1}$)	Chlorophyll b (mg g $^{-1}$ FW $^{-1}$)	Total chlorophyll (mg g $^{-1}$ FW $^{-1}$)	Grain protein content (%)	Grain starch content (%)
Oryza sativa L.	106.51a	3.74a	3.92a	14.78a	4.59a	2.09a	6.66a	9.61a	74.97a
Echinochloa crus-galli L.	78.77b	2.7b	0.93b	4.64b	2.8b	1.3b	4.1b	8.7b	70.03b

Means with similar letters are not significantly different at 5% level according to Duncan's test

and 3.88 t ha $^{-1}$, respectively (Table 4). The results of comparing the mean biological yield under the influence of the extract and plant showed that the biological yield decreased compared to the control by applying the extract.

The lowest biological yield in *Oryza sativa L.* (13.11 t ha $^{-1}$) was related to Elderberry extract and the lowest biological yields in *Echinochloa crus-galli* L. were 3.78, 3.56, 2.86, and 3.59 t ha $^{-1}$, which were associated with *Broccoli*, *Nettle*, Elderberry, and *Streptomyces sp-albos* extracts, respectively (Table 6). Decreased biological yield can be due to reduced nutrient and water uptake by roots, reduced leaves for photosynthesis, and decreased photosynthesis by allelochemicals, which resulted in reduced biomass production in plants (Mishra, 2015). The inhibitory effect of allelochemicals on plant growth is also related to the reduced photosynthesis. Photosynthesis reduction leads to the decreased amount of carbohydrates, which ultimately decreases dry matter accumulation in plant organs (Le-Thi et al., 2014).

3.5 Chlorophyll *a*

The ANOVA results showed that the extract, concentration, plant, and interactions between the extract and plant at the significance level of 1%, as well as extract and concentration at the significance level of 5% had significant effects on chlorophyll a (Table 1). The results of comparing the mean chlorophyll a under the impact of the extract indicated chlorophyll a reduced compared to the control by applying the extract. The highest chlorophyll a content (4.668 mg g^{-1} fresh weight) was observed in the control group and the lowest chlorophyll a content (3.16 mg g^{-1} FW^{-1}) was obtained by applying Elderberry extract, suggesting the 32.3% reduction in chlorophyll a compared to the control (Table 2).

The results of comparing the mean chlorophyll a under the effect of the extract concentration showed that, with increasing the extract concentration, chlorophyll a decreased compared to the control. The minimum content of chlorophyll a (3.317 mg g^{-1} FW^{-1}) was obtained at the concentration of 15 per thousand of extracts, indicating chlorophyll a decreased by 28.94% compared to the control (4.668 mg g^{-1} FW^{-1}) (Table 3). Based on the results of comparing the mean chlorophyll a under the influence of the plant, chlorophyll a content in *Oryza sativa* L. and *Echinochloa crus-galli* L. were 4.492 and 2.827 mg g^{-1} FW^{-1}, respectively (Table 4). The results of comparing the mean chlorophyll a under the effect of extract and concentration showed that chlorophyll a content decreased with increasing the concentration. The highest chlorophyll a content (4.68 mg g^{-1} FW^{-1}) was observed in the control group and the lowest chlorophyll contents were obtained as 2.95 and 3.08 mg g^{-1} FW^{-1} at the concentration levels of 10 and 15 per thousand of Elderberry extract (Table 5). The results of comparing the mean chlorophyll a under the effect of the extract and plant showed that, with applying the extract, chlorophyll a content reduced compared to the control. The lowest contents of chlorophyll a in *Oryza sativa* L. were 4.15, 4.11, and 4.22 mg g^{-1} FW^{-1}, which were related to *Nettle*, Elderberry, and *Xanthomonas campestris* extracts, respectively, and the lowest chlorophyll a content in *Echinochloa crus-galli* L. was 2.21 mg g^{-1} FW^{-1}, which was related to the Elderberry extract (Table 6)

3.6 Chlorophyll b

The ANOVA results showed that the extract, concentration, plant, and interactions between the extract and plant at the significance level of 1% as well as extract and concentration at the significance level of 5% had significant effects on chlorophyll b (Table 1). It was found that chlorophyll b reduced compared to the control by applying the extract. The highest chlorophyll b content (2.337 mg g^{-1} FW^{-1}) was observed in the control group and the lowest chlorophyll b content (1.379 mg g^{-1} FW^{-1}) was obtained by applying Elderberry extract, suggesting 40.98% reduction in chlorophyll b compared to the control (Table 2). With increasing the extract concentration, chlorophyll b decreased compared to the control. The minimum chlorophyll b content (1.512 mg g^{-1} FW^{-1}) was achievement at the concentration of 15 per thousand of extracts, which showed 34%

Table 5. Comparing the mean herbicidal potential of two bacterial species and five aqueous plant extracts in grain yield and photosynthetic pigments at different concentrations

Extract	Extract concentration (Per thousand)	Grain yield (ton ha^{-1})	Chlorophyll a (mg g^{-1} FW^{-1})	Chlorophyll b (mg g^{-1} FW^{-1})	Total chlorophyll (mg g^{-1} FW^{-1})
Control	0	3.3a	4.68 a	2.33 a	7.01 a
Sorghum	5	3.14ab	4.29 ab	2.07 ab	6.36 b
Sorghum	10	2.71b-d	4.27 a-c	1.83 b-e	6.1 bc
Sorghum	15	2.37c-i	3.72 c-e	1.95 bc	5.67 c-e
Broccoli	5	2.66c-f	3.74 b-e	1.69 c-g	5.43 d-g
Broccoli	10	2.44c-i	3.41 e-g	1.65 c-g	5.06 e-i
Broccoli	15	2.06h-l	3.3 e-g	1.4 f-h	4.7 ij
Nettle	5	2.28dj	3.65 d-f	1.72 c-f	5.37 d-h
Nettle	10	1.97i-l	3.31 e-g	1.48 e-h	4.79 g-j
Nettle	15	1.79kl	3.12 fg	1.33 hi	4.44 ij
Eucalyptus	5	2.7b-e	4.05 b-d	1.85 b-d	5.9 b-e
Eucalyptus	10	2.56 c-g	3.87 b-e	1.65 c-g	5.52 c-f
Eucalyptus	15	2.24 d-k	3.38 e-g	1.59 d-g	4.97 f-i
Elderberry	5	2.21 f-k	3.44 e-g	1.59 d-g	5.03 e-i
Elderberry	10	1.6 l	2.95 g	1.23 i	4.18 j
Elderberry	15	1.84 j-l	3.08 g	1.37 f-h	4.45 ij
Streptomyces sp-albos	5	2.79 bc	4.12 b-d	1.99 bc	6.11 bc
Streptomyces sp-albos	10	2.48 c-h	3.76 b-e	1.79 b-e	5.56 c-f
Streptomyces sp-albos	15	2.22 e-k	3.41 e-g	1.54 d-h	4.95 f-i
Xanthomonas campestris	5	2.81 bc	4.14 b-d	1.96 bc	6.14 bc
Xanthomonas campestris	10	2.32 c-j	3.71 c-e	1.67 c-g	5.42 d-g
Xanthomonas campestris	15	2.11 g-k	3.32 e-g	1.42 f-h	4.75 h-j

Means with similar letters are not significantly different at 5% level according to Duncan's test

decrease in chlorophyll b compared to the control (2.337 mg g^{-1} FW^{-1}) (Table 3). Chlorophyll b contents in *Oryza sativa* L. and *Echinochloa crus-galli* L. were 2.045 and 1.313 mg g^{-1} FW^{-1}, respectively (Table 4).

The results of comparing the mean chlorophyll b under the effect of extract and concentration showed that chlorophyll b content decreased with increasing the concentration. The highest chlorophyll b content (2.33 mg g^{-1} FW^{-1}) was observed in the control group and the lowest chlorophyll b content was obtained as 1.23 mg g^{-1} FW^{-1} at the concentration level of 10 per thousand of the Elderberry extract (Table 5).

Table 6. Comparing the mean herbicidal potential of two bacterial species and five aqueous plant extracts in yield and yield components of *Oryza sativa* L. and *Echinochloa crus-galli* L.

Extract	Plant	PHt (cm)	LAI	GY (ton ha^{-1})	BY (ton ha^{-1})	Chla (mg g^{-1} FW^{-1})	Chl b (mg g^{-1} FW^{-1})	Total chl (mg g^{-1} FW^{-1})
Control	*Oryza sativa L.*	120.72a	4.11ab	4.93a	16.02a	5.46a	2.77a	8.23a
Control	*Echinochloa crus-galli L.*	106.61bc	3.86a-c	1.66f	7d	3.91d	1.89c	5.8e
Sorghum	*Oryza sativa L.*	112.13b	4.26a	4.35b	15.32ab	4.92b	2.39b	7.31b
Sorghum	*Echinochloa crus-galli L.*	86.69ef	3.25ef	1.14g	5.46e	3.27e	1.51de	4.78f
Broccoli	*Oryza sativa L.*	110.39b	3.81a-d	4bc	15.38ab	4.43c	2c	6.43cd
Broccoli	*Echinochloa crus-galli L.*	78.38gh	2.5g	0.78gh	3.78f	2.53fg	1.17fg	3.7g
Nettle	*Oryza sativa L.*	100.68 cd	3.43c-e	3.34 de	14.46bc	4.15cd	1.82cd	5.97de
Nettle	*Echinochloa crus-galli L.*	73.97h	2.32gh	0.68h	3.56f	2.58fg	1.2e-g	3.77g
Eucalyptus	*Oryza sativa L.*	106.78bc	3.64b-e	4.1b	14.68ab	4.56bc	2.02c	6.57c
Eucalyptus	*Echinochloa crus-galli L.*	77.21gh	2.84fg	0.9gh	4.15ef	2.97ef	1.37ef	4.35f
Xanthomonas campestris	*Oryza sativa L.*	93.81de	3.28d-f	3.16e	13.11c	4.11cd	1.78cd	5.89de
Xanthomonas campestris	*Echinochloa crus-galli L.*	65.46i	1.96h	0.61h	2.86f	2.21g	1.02g	3.22g
Streptomyces sp-albos	*Oryza sativa L.*	112.59b	4.03ab	4.23 b	15.03ab	4.98b	2.33b	7.31b
Streptomyces sp-albos	*Echinochloa crus-galli L.*	73.33h	2.37gh	0.76gh	3.59f	2.54 fg	1.22e-g	3.77g
Xanthomonas campestris	*Oryza sativa L.*	100.17cd	3.43c-e	3.69cd	14.23bc	4.22 cd	1.91c	6.18c-e
Xanthomonas campestris	*Echinochloa crus-galli L.*	84.51fg	3.15ef	1.14g	5.52e	3.23e	1.46ef	4.68f

Note: PHt-plant height, LAI-leaf area index, GY-grain yield, BY-biological yield, chl-chlorophyll, means with similar letters are not significantly different at 5% level according to Duncan's test.

The results of comparing the mean chlorophyll b under the effect of the extract and plant showed that, with applying the extract, chlorophyll b content reduced compared to the control.

The lowest contents of chlorophyll b in *Oryza* *sativa L.* were 1.82 and 1.78 mg g^{-1} FW^{-1}, which were related to *Nettle* and Elderberry extracts, respectively, and the lowest chlorophyll b content in *Echinochloa crus-galli L.* was 1.02 mg g^{-1} FW^{-1}, which was related to the Elderberry extract (Table 6).

3.6 Total chlorophyll

The ANOVA results in Table 1 showed that the extract, extract concentration, plant, and interactions between the extract and concentration, as well as extract and plant had significant effects on total chlorophyll ($P<0.01$). The results of comparing the mean total chlorophyll under the impact of the extract indicated total chlorophyll reduced compared to the control by applying the extract.

The highest total chlorophyll (7.005 mg.g fresh weight) was observed in the control group and the lowest total chlorophyll (4.539 mg.g fresh weight) was investigated by applying the Elderberry extract, suggesting 35.2% reduction in total chlorophyll compared to the control (Table 2). The results of comparing the mean total chlorophyll under the effect of the extract concentration revealed that, with increasing the extract concentration, total chlorophyll decreased compared to the control. The minimum total chlorophyll (4.829 mg.g fresh weight) was achievement at the concentration of 15 per thousand of extracts, indicating the total chlorophyll decreased by 31.6306 compared to the control (7.005 mg.g fresh weight) (Table 3). Based on the results of comparing the mean total chlorophyll under the impact of the plant, the total chlorophyll contents in Oryza sativa L. and Echinochloa crus-galli L. were 6.54 and 4.14 mg g^{-1} FW^{-1}, respectively (Table 4). The results of comparing the mean total chlorophyll under the effect of extract and concentration showed that total chlorophyll decreased with increasing the concentration. The highest total chlorophyll (7.01 mg.g fresh weight) was observed in the control group and the lowest total chlorophyll (4.18 mg.g fresh weight) was obtained at the concentration level of 10 per thousand of the Elderberry extract (Table 5). The results of comparing the mean total chlorophyll under the effect of the extract and plant showed that, with applying the extract, total chlorophyll reduced compared to the control. The lowest contents of total chlorophyll in Oryza sativa L. were 5.97 and 5.89 mg.g fresh weight, which were related to Nettle and Elderberry extracts, respectively; the lowest contents of total chlorophyll in Echinochloa crus-galli L. were 3.7, 3.77, 3.22, and 3.77 mg.g fresh weight, which were related to Broccoli, Nettle, Elderberry, and Streptomyces sp-albos extracts, respectively (Table 6). When allelochemicals were stressed, the abscisic acid (ABA) and ethylene concentrations increased, and these hormones had a stimulating effect on the chlorophylase activity. Chlorophylase plays an important role in the process of chlorophyll degradation.

3.7 Grain protein content

The ANOVA results in Table 1 showed that the extract, concentration, and plant had significant effects on grain protein content ($P <0.01$). The grain protein content reduced compared to the control by applying the extract, so that the highest grain protein content (10.083 %) was observed in the control group and the lowest grain protein content was obtained as 8.654 % by applying the Elderberry extract (Table 2).The results of comparing the mean grain protein content under the effect of extract concentration revealed that the grain protein content decreased compared to the control with increasing the extract concentration.

Table 7. Comparing the mean herbicidal potential of extract concentration in yield and yield components of *Oryza sativa L.* and *Echinochloa crus-galli L.*

Extract concentration (Per thousand)	Plant	Plant height (cm)	LAI	Grain yield (ton ha^{-1})
0	*Oryza sativa L.*	120.72a	4.11a	4.93a
0	*Echinochloa crus-galli L.*	106.61b	3.86a	1.66d
5	*Oryza sativa L.*	110.26b	3.85a	4.23b
5	*Echinochloa crus-galli L.*	86.66c	2.93b	1.08e
10	*Oryza sativa L.*	103.07b	3.63a	3.75c
10	*Echinochloa crus-galli L.*	75.73d	2.62bc	0.84ef
15	*Oryza sativa L.*	102.33b	3.6a	3.54c
15	*Echinochloa crus-galli L.*	68.84d	2.34c	0.65f

Means with similar letters are not significantly different at 5% level according to Duncan's test

Concentrations of 5, 10, and 15 per thousand of extracts were in the same statistical group (Table 3). Based on the results of comparing the mean grain protein content under the impact of the plant, the grain protein contents in *Oryza sativa L.* and *Echinochloa crus-galli L.* were 9.662 % and 8.653 %, respectively (Table 4). Decreased protein content can be due to their high degradation or denaturation rate, changes in their three-dimensional structure and spatial shape, as well as disruption of the protein synthesis process during stress. Protein degradation and formation of molecules with lower weight as well as conversion of polypeptides into amino acids such as proline, glycine, and betaine have also been reported (Niromand et al., 2017).

3.8 Grain starch content

The ANOVA results showed that the extract, concentration, and plant had significant effects on grain starch content (Table 1). The grain starch content increased compared to the control by applying the extract, so that the highest grain starch contents were obtained as 75.6% and 76.97% by applying *Eucalyptus* extract and the lowest grain starch content was obtained as 61.697% in the control group (Table 2). The results of comparing the mean grain starch content under the effect of extract concentration revealed that the grain starch content increased compared to the control in the presence of the extract. Concentrations of 5, 10, and 15 per thousand of extracts were in the same statistical group (Table 3). Based on the results of comparing the mean grain starch content under the impact of the plant, it was observed that the grain starch contents in *Oryza sativa* L. and *Echinochloa crus-galli* L. were 73.93 % and 69.713 %, respectively

(Table 4). Some of the respiratory enzymes may be inactivated by allelopathic substances existing in the leaves of this plant. In this case, decomposition of soluble sugars in the treated plants decreases, which leads to the increased levels of soluble sugars (Song and Liu, 2014). Accumulating carbohydrates such as sugars (glucose, fructose, and sucrose) and starch play an important role in osmotic protection and regulation. The increase in carbohydrates is probably due to inhibiting respiratory enzymes, decomposing soluble sugars, and reducing cell energy levels (Aasifa and Badruzzaman, 2014).

Conclusion

Nowadays, weed control programs are designed to reduce toxin consumption and avoid the negative consequences of herbicide use including incomplete weed control, environmental pollution, adverse effects on human health, increasing weed resistance to herbicides, and elimination of natural enemies. The results indicated various extracts, especially *Nettle*, as well as 15 per thousand of *Nettle* and Elderberry extracts can be used to control *Echinochloa crus-galli L.* at the germination stage under field conditions. Allelopathy and plant pathogens can be among the basic ways to control weeds in the sustainable agricultural system

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