



Research



The impact of two bacterial species and five aqueous plant extracts on yield and yield components of rice (*Oryza sativa* L.) and suruf (*Echinochloa crus-galli* L.)

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Abstract

Chemical exudates from some plants to the environment have been reported as causative agents of adverse effect on growth of neighboring plants and thus affecting normal growth in their natural environment. The mode of action of Chemical exudates on plants involves a large number of biochemical reactions. This research aimed to investigate the effect of foliar application of different levels of two bacterial species and five aqueous plant extracts on the yield and yield components of *Oryza sativa* L. and *Echinochloa crus-galli* L. This research was conducted as a factorial based on randomized complete block design (RCBD) with three factors with three replications during the 2017–2018 crop year. The main factor included aqueous extracts of *Sorghum* (*Sorghum bicolor* L. Moench), *Broccoli* (*Brassica oleracea* var. *Italica*), *Nettle* (*Urtica dioica*), *Eucalyptus* (*Eucalyptus globulus* Labill), *Elderberry* (*Sambucus nigra* L.), and *Streptomyces sp.-albos* containing thaxtomin, *Xanthomonas campestris*, and control (without aqueous plant extracts and surfactant). The sub-factor includes foliar application treatment with different levels of zero (control), 5, 10, and 15% of extracts, and the third experimental factor included *Oryza sativa* L. and *E. crus-galli* L. The results indicated the plant height, leaf area index (LAI), grain yield, biological yield, chlorophyll a, chlorophyll b, total chlorophyll, and grain protein content decreased, and the grain starch content increased with increasing the extract concentration; the highest effect was obtained at 15% of extracts. The lowest value of leaf area index (3.13) were found in higher concentration of *X. campestris* followed by Elderberry extract (5%, 10% and 15%) and Nettle (10%) extracts. It was also found that using extract reduced yield characteristics and components of *Oryza sativa* L., *E. crus-galli* L., and Elderberry extract showed the highest negative effect. According to the results, *Oryza sativa* L. had higher plant height, LAI, grain yield, biological yield, harvest index, chlorophyll, grain protein content, and grain starch content than *E. crus-galli* L. In all extractions, control treatment had the highest number of panicles plant⁻¹ in both plants (rice and *E. crus-galli* L., 260.1 and 117.1 mg g⁻¹ FW). The results suggested that various extracts, especially *Nettle*, and 15% of Elderberry can control *E. crus-galli* L. at the germination stage under field conditions.

Article highlights

- The Elderberry and Nettle extracts had the highest impact on rice and suruf.
- The negative impact of extract reduced yield and yield components of rice.
- The reduction in grain weight correlated to in leaves and plant height.

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1 Introduction

Oryza sativa L., belongs to the *Oryza* genus and Gramineae family [1] and is the most important crop after wheat (*Triticum aestivum* L.), which provides about 40% of the nutritional energy of more than half of the world's population [2]. *Echinochloa crus-galli* L., *E. crus-galli* L., is an annual summer plant of the Gramineae family that seeds propagate. *Echinochloa crus-galli* L. grows mostly in warm temperate regions and is moisture-friendly. This plant is among the most important weeds grown in rice fields in Iran and other countries. *Echinochloa crus-galli* L. is a C4 plant and, therefore, has rapid growth and tillering and quickly dominates rice fields [3]. *Echinochloa crus-galli* L. is usually found in rice fields, imposes new biological limitations on rice productivity, and has adverse effects on rice grain growth and yield [4]. Widespread use of chemical herbicides and severe dependence on them has caused problems such as weed resistance to herbicides and adverse effects on human health and the environment [5]. Therefore, it is essential to find a way that is not only safe for the humans and environment but also cost-effective for weed control.

Using the allelopathic property of some plants to control weeds is of particular importance [6]. Allelopathy is a phenomenon, by which a plant produces allelochemicals and can influence the growth of itself and other plants. These substances can have negative or positive effects on the germination, vegetative, and reproductive stages of other plants [7]. Allelochemicals are released into the environment by shoot leaching and volatilization, root exudation, and plant litter decomposition [8]. Known effects of allelochemicals include anatomical abnormalities, decreased absorption, germination, and bud growth, chlorosis, and necrosis. The inhibitory effect of these substances depends on the aqueous extract concentration of the plant [9].

In their study, Dastres et al. [10] found that allelochemicals of aqueous extracts of pagoda tree and creeping jenny reduced plant height and wheat dry weight. Hatami Hampa et al. [11] investigated the allelopathic effect of aqueous extracts of *Sorghum* and Russian knapweed on the seedling growth and activity of antioxidant enzymes in the wheat, sugar beet, common lambsquarter, and redroot pigweed, and reported that *Sorghum* and Russian knapweed extracts reduced seedling growth and increased soluble carbohydrates, proline concentration, and catalase activity in the studied plants compared to the control. Sadat Asilan et al. [12] found that a number of rice cultivars

had a stimulatory effect, and some had an inhibitory effect on *E. crus-galli* L. growth. Increasing the concentration of rice cultivar extract increased the inhibition and stimulation of *E. crus-galli* L. growth [13–15]. A comprehensive list of allelochemicals and secondary metabolites present in different plant parts of sorghum (roots, stems, foliage and panicle) is documented by Hussain et al. [16]. *Broccoli* is well known for their allelopathic potential as most of them endogenously produce potent allelochemicals such as glucosinolates, allyl isothiocyanates, and brassinosteroids [17]. Extracts of fresh nettle (*Urtica dioica* L.) showed greater harmful effects than those from dried plants and generally reduced the germination of cereals from control values of 90–93 to 50–93% and decreased root length in barley from 426 to 455 to 162–397 mm [18].

Weeds cause many problems in different land uses. The growing number of herbicide-resistant weeds and prohibiting the use of pesticides and herbicides have provided a strong incentive to develop new weed control strategies. Using bacteria, fungi, and viruses to achieve this goal has received increasing attention over the last 3 decades. The benefits of this strategy include reducing environmental impacts, decreasing development costs compared to conventional herbicides, and identifying new herbicide mechanisms [19]. It has been recommended to use bacteria for the biological control of weeds due to the faster growth of biological antiseptics, relatively simple propagation requirements, and suitability for genetic modification through mutagenesis or gene transfer [20]. Biological control, as a general term, refers to introducing organisms into an ecosystem in order to control one or more undesirable species [21]. *Xanthomonas campestris* is a Gram-negative rod-shaped bacterium with a mobile polar flagellum belonging to the *Pseudomonas* family and seed-borne plant pathogens [22, 23]. The *Streptomyces* genus belongs to Actinomycetals and is commonly found in soil. This genus is considered a potential source for producing a variety of antibiotics and other secondary metabolites due to its ability to secrete large amounts of extracellular enzymes [24]. Chen et al. [25] determined the role of endophytic bacteria of *C. equisetifolia*, involved in synthesis of allelochemicals. The results of Abbas et al. [26] indicated that dry matter of broad-leaved dock, wild oat, little seed canary grass, and common lambs' quarter were reduced by strains of rhizobacteria and some strains suppressed the growth of wheat, some strains increased its dry matter and some strains remained ineffective.

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 controlling weeds and moving from conventional agriculture to sustainable agriculture.

2 Materials and methods

This factorial experiment was carried out using randomized complete block design with three replications an arable land located in one of the villages of northern Iran, Amol, Mazandaran province (52° 25' E, 36° 25' N, 110 m above sea level), in the 2017–2018 crop year. There were three experimental factors:

1. Aqueous extracts in seven levels: Sorghum (*Sorghum bicolor* L. Moench), Broccoli (*Brassica oleracea* var. *Italica*), Nettle (*U. dioica*), Eucalyptus (*Eucalyptus globulus* Labill), Elderberry (*Sambucus nigra* L.), and *Streptomyces sp.-albos* containing thaxtomin, *Xanthomonas campestris*, and control (without aqueous plant extracts and surfactant).
2. Concentration of extraction included: 0 (control), 5%, 10%, and 15% of pure extraction.
3. Two plants included the land-race rice (cv. Fereydunkenar) and *E. crus-galli* L.

The bacteria were purchased as lyophilized in coordination with Islamic Azad University and the 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 *X. campestris* and 1–2 weeks for *Streptomyces* to provide 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 opposite sides. A plate containing the medium without a 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 with sterile distilled water. 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, all the mixtures inside the flask were squeezed, and the obtained extract was filtered using filter paper and a funnel to get a stock solution with a concentration of 0.1 g ml⁻¹ (100%). From the stock solution, four concentrations; 5%, 10%, and 15%, were then made on the basis of percentage. The extract was then sprayed on 4–7-leaf plants and kept in a petri dish as a liquid.

Land preparation operations, including plowing, harrowing, and leveling were carried out in a desirable manner before planting, terracing the nursery, and sowing rice and *E. crus-galli* L. seeds in the nursery. rice and *E. crus-galli* L. seeds were brought from Amol rice Research Institute and disinfected with Vitavax fungicide before sowing. Urea fertilizer (150 kg ha⁻¹) and phosphorus fertilizer (90 kg P₂O₅ ha⁻¹ in the form of single superphosphate) 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 2 weeks; since then, until 2 weeks before the harvest, intermittent irrigation was carried out. All the preparation operations performed for experimental plots and the studied rice and *E. crus-galli* L. seedlings were similar. The seeding density was 300 g seeds m⁻² in the nursery in the Japanese form, the planting density was 9 plants m⁻² in the main plot; then, the result was generalized. 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 rice 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. After the growing period, complete physiological maturity, and 14% grain moisture content, harvesting was carryon out. When the crops ripened, 10 plants were randomly sampled from each plot, the yield and yield components of which were examined.

The 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 with the filter paper, and the acetone was added until reaching a volume of 25 ml. Chlorophyll was completely extracted from the leaf tissue. The light absorption of total chlorophylls was read at 645 and 663 nm, respectively, and the total chlorophyll content was obtained using the following equation (Eq. (1)) [27]:

$$\text{Total chlorophyll: } (12.7 \times A_{663}) - (2.69 \times A_{645}) \quad (1)$$

The grain protein content was measured using the Kjeldahl method by Kjeltex 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 s, the vortex was filtered by 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 [28]. At the end of the experiments, the data were analyzed using Statistical Package for the Social Sciences (SPSS) statistical software, and a comparison of the means was performed by LSD's test at the significance level of 5% [3].

3 Results

The analysis variance results (Table 1) indicated that the main effect of extraction concentration and plant were significant on all studied traits (Table 1). The interaction between extraction and concentration treatment was also statistically significant on all studied traits. Also, the interaction between extraction and plant was significant in all studied traits except grain protein content. In addition, the interaction between concentration and plant was significant on LAI, a number of grains panicle⁻¹, 1000-grain weight, grain yield, and biological yield. Based on analysis variance table, none of the parameters were affected by the main effect of year and the four-way interaction of year, extraction, concentration, and plant (Table 1).

3.1 Total chlorophyll

The two plants (rice and *E. crus-galli* L.) responded differently to extraction and their concentration (Table 2). In all extractions, the control treatment had the highest content of total chlorophyll in both plants (rice and *E. crus-galli* L., 8.38 and 5.69 mg g⁻¹ FW). Increasing in extract concentration decreased the content of total chlorophyll. The results show that the different concentrations (5–15%) of extraction significantly decreased the total chlorophyll content of rice as compared to untreated plants (0%), and high inhibitory impacts were observed in Nettle and Elderberry extraction. Maximum reduction in total chlorophyll of rice was in the highest concentration of *Eucalyptus* and *X. campestris* (15%), Nettle, and Elderberry with a

concentration of 10% that decreased 30–34% compared to the control. The results showed that there is *E. crus-galli* L. was a high total chlorophyll accumulation in control plants whereby the highest value was 5.69 mg g⁻¹ FW. However, the total leaf chlorophyll content was remarkably lowered to 3.07 mg g⁻¹ FW in Elderberry extract (15%) while in 15% of sorghum, Broccoli, Nettle, Eucalyptus, *S. sp-albos*, and *X. campestris*, the total chlorophyll content was reduced to 4.43, 3.26, 3.14, 4.07, 3.19 and 4.07 3.07 mg g⁻¹ FW which was lower than control (0%) by 22.1, 42.7, 44.8, 28.4, 43.9 and 28.4% respectively.

3.2 Plant height

Relative to the control, the lowest plant height of rice plants was observed at concentrations of 10% and 15% of Elderberry extraction, which was lower by average of 24% and 22% than the control. Averaged over the extract concentration (5%, 10%, and 15%), the plant height of rice was 113.6 cm, 111.1 cm, 101.8 cm, 108.1 cm, 95.4 cm, 113.8 cm, and 100.1 cm while rice plants were treated by sorghum, Broccoli, Nettle, Eucalyptus, Elderberry, *S. sp-albos*, and *X. campestris*, respectively and decreased by 6.9%, 9.0%, 16.6%, 11.4%, 21.8%, 6.7% and 17.9% at control treatment, respectively (Table 2). Regarding *E. crus-galli* L. and related with control plants which had the highest plant height (104.4 cm), the length of treated plants, was negatively affected by an increase in the level of extract concentration, the highest inhibitory effects were observed at the highest concentration of Elderberry, *S. sp-albos* and Nettle (57.56 cm, 67.62 cm, and 69.37 cm).

3.3 LAI

In comparison with the control plants, the LAI of rice and *E. crus-galli* L. plants were negatively influenced by extracts (Table 2). Concerning the rice plants, the highest LAI (4.5) was found in the control treatments related to the plants, which were treated with concentrations of extracts. The lowest value (3.13) were found in higher concentration of *X. campestris* followed by Elderberry extract (5%, 10% and 15%) and Nettle (10%) extracts which were at the same statistical level. The results showed that LAI was significantly reduced in *E. crus-galli* L. plants treated with different concentration of extract treatments (Table 2). High inhibitory effects were shown in Elderberry extract with 10% and 15% concentrations (2.05 and 1.68, respectively), followed by 15% concentration of Nettle (2.00). Averaged over the extract concentrations (5%, 10%, and 15%), the LAI of *E. crus-galli* L. was 3.32, 2.52, 2.32, 2.82, 1.99, 2.50 and 3.08 when *E. crus-galli* L. plants were treated by sorghum, Broccoli, Nettle, Eucalyptus, Elderberry, *S. sp-albos* and *X. campestris*, respectively and decreased by 14.8, 35.38, 40.5,

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.

SOV	df	Tchl	PH	LAI	No. of panicles m ⁻²	No. of grains panicle ⁻¹	1000-GW	GY	BY	GP	GS
Year	1	0.069 ns	78.5 ns	0.097 ns	200.1 ns	62.3 ns	1.38 ns	0.06 ns	0.87 ns	0.73 ns	40.9 ns
Year (Rep)	4	0.081	85.8	0.099	1506.1	87.4	8.64	0.19	3.22	2.36	79.6
Extract (E)	6	6.2**	1037.1**	3.55**	6896.2**	7803**	14.2**	2.7**	19.1**	2.79**	276**
Concentration (C)	3	75.6**	11471.2**	24.33**	49,125**	70,186**	118.5**	25**	142.3**	15.8**	3054**
Plant (P)	1	564.6**	55462.1**	76.1**	1,628,953**	1,985,241**	25,880**	789**	8059**	69.7**	2317**
Year*E	6	0.00004 ns	0.02 ns	0.00007 ns	0.17 ns	0.049 ns	0.0003 ns	0.00007 ns	0.0005 ns	0.00008 ns	0.006 ns
Year*C	3	0.00047 ns	0.28 ns	0.0006 ns	1.21 ns	0.44 ns	0.003 ns	0.0007 ns	0.0039 ns	0.00059 ns	0.074 ns
E*C	18	1.05**	135.1**	0.47**	1031.6**	1234.3**	3.79**	0.41**	3.68**	0.82*	70.8**
Year*E*C	18	0.000014 ns	0.003 ns	0.00001 ns	0.026 ns	0.008 ns	0.00009 ns	0.00001 ns	0.0001 ns	0.00002 ns	0.001 ns
Year*P	1	0.0033 ns	1.37 ns	0.001 ns	40.3 ns	12.3 ns	0.63 ns	0.018 ns	0.19 ns	0.002 ns	0.057 ns
E*P	6	2.45**	331.6**	0.97**	2106.9**	4759**	11.5**	1.022**	5.56**	0.02 ns	55.1*
Year*E*P	6	0.00001 ns	0.008 ns	0.00002 ns	0.052 ns	0.03 ns	0.0002 ns	0.00002 ns	0.0001 ns	0.000001 ns	0.0013 ns
C*P	3	0.13 ns	959 ns	1.46**	304.7 ns	29,805**	47.6**	0.71**	21.8**	0.02 ns	18.05 ns
Year*C*P	3	0.000019 ns	0.023 ns	0.00007 ns	0.007 ns	0.18 ns	0.001 ns	0.00001 ns	0.0007 ns	0.0000004 ns	0.00046 ns
E*C*P	18	0.5**	68.8**	0.16**	500.5**	735.3**	3.04**	0.20**	1.21*	0.032 ns	21.33 ns
Year*E*C*P	18	0.000007 ns	0.0017 ns	0.000007 ns	0.012 ns	0.005 ns	0.00007 ns	0.00001 ns	0.00003 ns	0.000002 ns	0.00053 ns
Error	220	23.6	19.9	0.073	132.9	246.03	1.08	0.042	0.69	0.47	23.5
CV (%)		5.7	4.58	7.87	7.42	9.08	8.05	7.76	8.15	7.33	6.89

Tchl, total chlorophyll; PH, plant height; LAI, leaf area index; 1000-GW, 1000-grain weight; GY, grain yield; BY, biological yield; GP, grain protein content; GS, grain starch content ns, * and ** are not significant (ns) and significant at levels of 5% and 1%, respectively

Table 2 Results of analysis of variance (mean of squares) of studied traits affected by three-way interaction of extracts and concentration of extracts in *Oryza sativa* L. and *Echinochloa crus-galli* L. plants

Extract	Concentration (%)	Total Chlorophyll (mg g ⁻¹ FW)		Plant height (cm)		Leaf area index		No. of panicles m ⁻²	
		<i>Oryza sativa</i> L.	<i>Echinochloa crus-galli</i> L.	<i>Oryza sativa</i> L.	<i>Echinochloa crus-galli</i> L.	<i>Oryza sativa</i> L.	<i>Echinochloa crus-galli</i> L.	<i>Oryza sativa</i> L.	<i>Echinochloa crus-galli</i> L.
<i>Sorghum</i>	0	8.38 a	5.69 a	122.1 a	104.4 a	4.5 a	3.90 a	260.1 a	117.1 a
	5	7.70 bc	5.03 c	116.84 bc	98.10 b	4.35 ab	3.59 b	231.1 bcde	110.1 ab
	10	7.42 cd	4.79 cd	110.80 fgh	87.52 c	4.26 b	3.38 bc	238.0 bc	99.6 bcd
	15	6.80 ef	4.43 ef	113.24 def	78.35 e	4.20 bc	3.00 de	218.4 ef	92.04 de
<i>Broccoli</i>	0	8.38 a	5.69 a	122.1 a	104.4 a	4.5 a	3.90 a	260.1 a	117.1 a
	5	6.85 e	4.12 gh	114.89 cde	84.31 c	4.01 cd	2.67 fg	224.3 de	82.04 ef
	10	6.44 fgh	3.72 i	110.87 fgh	76.57 ef	3.81 de	2.51 gh	237.8 bc	70.5 ghi
	15	6.29 hi	3.26 j	107.82 hij	73.58 efgh	3.72 ef	2.38 h	202.2 gh	68.7 hi
<i>Nettle</i>	0	8.38 a	5.69 a	122.1 a	104.4 a	4.5 a	3.90 a	260.1 a	117.1 a
	5	6.37 gh	4.48 ef	105.17 jk	86.04 c	3.56 fg	2.68 fg	221.4 e	72.1 fghi
	10	5.74 klm	3.79 i	101.10 lmn	69.43 hij	3.27 hij	2.30 hi	205.6 g	57.9 jkl
	15	5.90 jkl	3.14 jk	99.19 no	69.37 hij	3.47 gh	2.00 j	191.1 hi	52.6 kl
<i>Eucalyptus</i>	0	8.38 a	5.69 a	122.1 a	104.4 a	4.5 a	3.90 a	260.1 a	117.1 a
	5	7.40 cd	4.57 def	112.21 efg	86.08 c	3.76 ef	3.18 cd	243.6 b	96.4 cd
	10	6.75 ef	4.34 fg	103.32 klm	78.55 de	3.46 gh	2.84 ef	224.5 de	80.5 fg
	15	5.80 klm	4.07 gh	108.80 ghi	70.41 ghij	3.73 ef	2.45 gh	222.6 e	75.6 fgh
<i>Elderberry</i>	0	8.38 a	5.69 a	122.1 a	104.4 a	4.5 a	3.90 a	260.1 a	117.1 a
	5	6.36 gh	3.71 i	99.21 no	75.64 efg	3.37 ghi	2.24 hij	206.9 fg	64.9 ij
	10	5.49 m	2.88 k	92.16 q	65.70 j	3.26 hij	2.05 ij	165.4 k	48.07 l
	15	5.95 ijk	3.07 jk	94.92 pq	57.56 k	3.23 ij	1.68 k	176.1 jk	33.6 m
<i>Streptomyces sp-albos</i>	0	8.38 a	5.69 a	122.1 a	104.4 a	4.5 a	3.90 a	260.1 a	117.1 a
	5	7.97 b	4.33 fgh	120.07 ab	86.01 c	4.17 bc	2.71 fg	229.1 cde	78.3 fgh
	10	7.34 cd	3.63 i	115.85 cd	72.91 fghi	4.22 bc	2.52 gh	236.0 bcd	62.03 ijk
	15	6.71 efg	3.19 j	105.73 ijk	67.62 ij	3.78 ef	2.29 hi	206.8 fg	53.1 kl
<i>Xanthomonas campestris</i>	0	8.38 a	5.69 a	122.1 a	104.4 a	4.5 a	3.90 a	260.1 a	117.1 a
	5	7.32 d	5.30 b	104.46 jkl	94.58 b	3.91 cd	3.50 b	219.4 ef	103.4 bc
	10	6.21 hij	4.66 de	100.19 mn	83.72 cd	3.44 ghi	3.08 de	197.6 gh	97.4 cd
	15	5.55 lm	4.07 h	95.77 op	75.04 efg	3.13 j	2.67 fg	179.5 ij	80.9 fg
LSD value		0.37	0.27	3.47	5.34	0.22	0.28	12.6	6.04

Means having similar letters in each section and each column have no significant difference at 5% probability level by LSD test

27.6, 48.9, 35.8 and 21.02% at control treatment, respectively (Table 2). It should be noted that LAI of *E. crus-galli* L. (on average across extract) was 2.93, 2.66 and 2.35, at the 5%, 10%, and 15% and reduced by 24.8, 31.7 and 39.7%, at treated *E. crus-galli* L. plants with distil water (control), respectively (Table 2).

3.4 Number of panicles m⁻²

The two plants (rice and *E. crus-galli* L.) responded differently to extraction and their concentration (Table 2). In all extractions, control treatment had the highest number of panicles plant⁻¹ in both plants (rice and *E. crus-galli* L., 260.1 and 117.1 mg g⁻¹ FW, respectively). Increasing in

extract concentration decreased the number of panicles plant⁻¹. The results show that the different concentrations (5–15%) of extraction significantly decreased the number of panicles plant⁻¹ in rice as compared to untreated plant (0%) and high inhibitory impacts were observed in Elderberry extraction (10% and 15%, 165.4 and 176.1, respectively) which decreased by 36% and 32% compared the control. The results showed that in *E. crus-galli* L. there was a higher number of panicles plant⁻¹ in control plants whereby the highest value was 117.1. However, the total number of panicles plant⁻¹ was remarkably lowered to 33.6 in Elderberry extract (15%) while in highest extract concentration (15%) of sorghum, Broccoli, Nettle, Eucalyptus, *S. sp-albos* and *X. campestris*, the total number of

panicles plant⁻¹ was reduced to 92.04, 68.7, 52.6, 75.6, 53.1 and 80.9 which was lower than control (0%) by 21.4, 41.3, 55.0, 35.4, 54.6 and 30.9% respectively.

3.5 No. of grains panicle⁻¹

The decrease in number of grains panicle⁻¹ of rice associated with Elderberry at 10% and 15% (reduced to 72.8) followed by *X. campestris* at 15% (77.2) which were at same statistical level. Averaged over the extract concentrations (5%, 10% and 15%), the number of grains panicle⁻¹ of rice was 102.7, 89.7, 83.7, 97.7, 77.4, 102 and 81.8 when rice plants were treated by sorghum, Broccoli, Nettle, Eucalyptus, Elderberry, *S. sp-albos* and *X. campestris*, respectively and decreased by 7%, 18.7%,

24.2%, 11.5%, 29.9%, 7.6% and 25.9% at control treatment, respectively (Table 3). It should be noted that the number of grains panicle⁻¹ of rice (on average across extract) was 94.25, 89.98 and 88, at the 5%, 10% and 15% and reduced by 24.6%, 31.5% and 39.6%, at treated rice plants with distil water (control), respectively (Table 3). Relative to the controls, the maximum reduction in number of grains panicle⁻¹ of *E. crus-galli* L. was achieved at concentration of 10% and 15% of Elderberry extract and Nettle (15%) which were at same statistical levels and lower by 44.1% and 41.5% than control. Averaged across the extract treatments, the concentration of 15% in all extracts had the maximum inhibitory effects on number of grains panicle⁻¹ (Table 3). Among the extracts,

Table 3 Results of analysis of variance (mean of squares) of studied traits affected by three-way interaction of extracts and concentration of extracts in *Oryza sativa* L. and *Echinochloa crus-galli* L. plants

Extract	Concentration (%)	No. of grains panicle ⁻¹		1000-grain weight (g)		Grain yield (ton ha ⁻¹)		Biological yield (ton ha ⁻¹)	
		<i>Oryza sativa</i> L.	<i>Echinochloa crus-galli</i> L.	<i>Oryza sativa</i> L.	<i>Echinochloa crus-galli</i> L.	<i>Oryza sativa</i> L.	<i>Echinochloa crus-galli</i> L.	<i>Oryza sativa</i> L.	<i>Echinochloa crus-galli</i> L.
Sorghum	0	110.5 a	314.9 a	24.5 a	4.76 a	5.01 a	1.74 a	16.07 ab	7.72 a
	5	105.4 ab	295.4 ab	22.4 cd	4.48 b	4.89 ab	1.44 b	16.04 ab	7.11 a
	10	100.6 b	271.9 c	23.3 abc	4.33 bcd	4.38 de	1.17 c	15.59 abc	5.90 b
	15	102.2 b	233.6 de	22.1 cde	4.23 cdef	4.04 fg	0.92 efg	15.15 bcd	4.89 def
Broccoli	0	110.5 a	314.9 a	24.5 a	4.76 a	5.01 a	1.74 a	16.07 ab	7.72 a
	5	92.2 c	239.4 d	20.6 fg	4.12 defg	4.42 cd	0.99 def	15.74 abc	4.87 defg
	10	85.8 defg	217.02 ef	21.5 def	3.58 klmn	4.16 ef	0.82 ghi	15.16 bcd	4.20 fghij
	15	91.2 cd	217.01 ef	20.6 fg	3.68 jkl	3.61 i	0.60 kl	15.69 abc	3.55 jk
Nettle	0	110.5 a	314.9 a	24.5 a	4.76 a	5.01 a	1.74 a	16.07 ab	7.72 a
	5	85.8 defg	235.3 de	19.1 h	4.24 cde	3.69 hi	0.92 efg	15.41 abc	4.60 efgh
	10	80.6 gh	184.1 gh	20.1 gh	3.54 lmn	3.28 jk	0.71 ijk	14.31 def	4.25 fghij
	15	84.8 efg	202.4 fg	19.6 gh	3.78 ijk	3.15 k	0.53 lm	14.18 ef	3.54 jk
Eucalyptus	0	110.5 a	314.9 a	24.5 a	4.76 a	5.01 a	1.74 a	16.07 ab	7.72 a
	5	101.8 b	271.9 c	22.4 cd	4.32 bcd	4.45 cd	1.15 c	15.61 abc	5.58 bcd
	10	100.7 b	233.7 de	20.8 efg	4.02 efgh	4.34 de	0.91 fgh	14.19 ef	4.46 efghi
	15	90.6 cde	218.3 def	19.5 gh	3.44 mn	3.87 gh	0.77 hij	15.02 cde	3.85 ij
Elderberry	0	110.5 a	314.9 a	24.5 a	4.76 a	5.01 a	1.74 a	16.07 ab	7.72 a
	5	82.0 fgh	218.3 def	19.6 gh	4.00 fghi	3.64 hi	0.84 ghi	14.07 fg	4.15 ghij
	10	72.8 i	178.03 h	18.7 h	3.66 jklm	2.58 l	0.60 kl	12.10 i	2.63 lm
	15	77.5 hi	175.8 h	19.5 gh	3.19 o	3.25 jk	0.45 m	13.20 gh	1.98 m
<i>Streptomyces sp-albos</i>	0	110.5 a	314.9 a	24.5 a	4.76 a	5.01 a	1.74 a	16.07ab	7.72 a
	5	104.8 ab	230.8 de	22.9 bc	4.19 cdef	4.68 bc	1.05 cde	16.08 ab	5.05 cde
	10	108.9 a	202.4 fg	24.3 ab	3.89 hij	4.20 def	0.80 ghi	15.68 abc	3.92 hij
	15	92.5 c	180.7 gh	19.9 gh	3.35 no	3.87 gh	0.65 jkl	14.04 fg	3.07 kl
<i>Xanthomonas campestris</i>	0	110.5 a	314.9 a	24.5 a	4.76 a	5.01 a	1.74 a	16.07 ab	7.72 a
	5	87.8 cdef	284.07 bc	19.7 gh	4.73 a	4.23 def	1.41 b	16.28 a	7.11 a
	10	80.5 gh	262.5 c	19.9 gh	4.39 bc	3.43 ij	1.12 cd	13.96 fgh	5.75 bc
	15	77.2 hi	226.6 de	19.0 h	3.89 ghij	3.44 ij	0.83 ghi	13.10 h	4.31 efghi
LSD value		6.04	21.7	1.41	0.23	0.25	0.14	0.93	0.74

Means having similar letters in each section and each column have no significant difference at 5% probability level by LSD test

maximum reduction in number of grains panicle⁻¹ was observed in Elderberry (Table 3).

3.6 1000-grain weight

The 1000-grain weight of both plants treated with extracts decreased as the concentration of all extracts increased. The 1000-grain weight of rice was lowered to 18.7 g in treatment of Elderberry extract with 10% which was lower than control by 23.6% (Table 3). Averaged by concentration, the extract of sorghum, Broccoli, Nettle, Eucalyptus, Elderberry, *S. sp-albos* and *X. campestris* decreased 1000-grain weight of rice by 7.7%, 14.6%, 20.0%, 14.6%, 21.3%, 8.7% and 20.2%, respectively compared to control. All concentration of extract treatments reduced the 1000-grain weight of *E. crus-galli* L. The mean maximum and minimum 1000-grain weight of *E. crus-galli* L. (recorded in plants grown in control and plants treated with 15% of Elderberry extraction), were 4.76 and 3.19 g, respectively (Table 3). Averaged across the extract treatments, the concentration of 15% in all extracts had the maximum inhibitory effects on biological yield (Table 3). Among the extracts, maximum reduction in 1000-grain weight was observed in Elderberry, *S. sp-albos*, and Nettle which were found statistically at par with Elderberry (Table 3).

3.7 Grain yield

The decrease in grain yield of rice associated with Elderberry at 10% (reduced to 2.58 ton ha⁻¹) followed by Elderberry at 15% (3.25 ton ha⁻¹) and Nettle at 10% and 15% (3.28 and 3.15 ton ha⁻¹, respectively). Averaged over the extract concentrations (5%, 10% and 15%), the grain yield of rice was 4.43, 4.06, 3.37, 4.22, 3.15, 4.25 and 3.7 ton ha⁻¹ when rice plants were treated by sorghum, Broccoli, Nettle, Eucalyptus, Elderberry, *S. sp-albos* and *X. campestris*, respectively and decreased by 11.54, 18.9, 32.7, 15.7, 37.1, 15.1 and 26.1% at control treatment, respectively (Table 3). It should be noted that grain yield of rice (on average across extract) was 4.28, 3.76 and 3.60 ton ha⁻¹, at the 5, 10 and 15% and reduced by 14.57, 24.95 and 28.14%, at treated rice plants with distil water (control), respectively (Table 3). Relative to the control, the maximum reduction in grain yield of *E. crus-galli* L. was achieved at highest concentration of Elderberry and Nettle (15%) which were at same statistical levels and lower by 74.1% and 69.5% than control. Averaged across the extract treatments, the concentration of 10% in all extracts had the maximum inhibitory effects on grain yield (Table 3). Among the extracts, maximum reduction in grain yield was observed in Elderberry and Nettle (Table 3).

3.8 Biological yield

The capacity of both plants treated with extracts to accumulated biomass decreased as the concentration of all extracts increased. In rice plants, the biological yield was finally affected and significantly reduced ($P < 0.01$) in plants treated with all extracts (Table 1). High inhibitory effects were observed at 10% of all extracts. The biological yield rice of was lowered to 5.49 ton ha⁻¹ in treatment of Elderberry extract with 10% which was lower than control by 52.6% (Table 3). Averaged by concentration, the extract of sorghum, Broccoli, Nettle, Eucalyptus, Elderberry, *S. sp-albos* and *X. campestris* decreased biological yield of rice by 12.8%, 22.1%, 24.8%, 20.6%, 29.4%, 12.6% and 24.1%, respectively compared to control. All concentration of extract treatments reduced the biological yield of *E. crus-galli* L. The mean maximum and minimum biological yield of *E. crus-galli* L. (recorded in plants grown in control and plants treated with 15% of Elderberry extraction), were 5.69 and 3.07 ton ha⁻¹, respectively (Table 3). Averaged across the extract treatments, the concentration of 15% in all extracts had the maximum inhibitory effects on biological yield (Table 3). Among the extracts, maximum reduction in biological yield was observed in Nettle, *S. sp-albos* and Braccoli which were found statistically at par with Elderberry (Table 3).

3.9 Grain protein content

The results presented in Fig. 1 showed clearly the significant different ($P < 0.01$) of grain protein content in rice and *E. crus-galli* L. Regarding to rice, the grain protein content (9.82%) was significantly higher than *E. crus-galli* L. (8.91%)

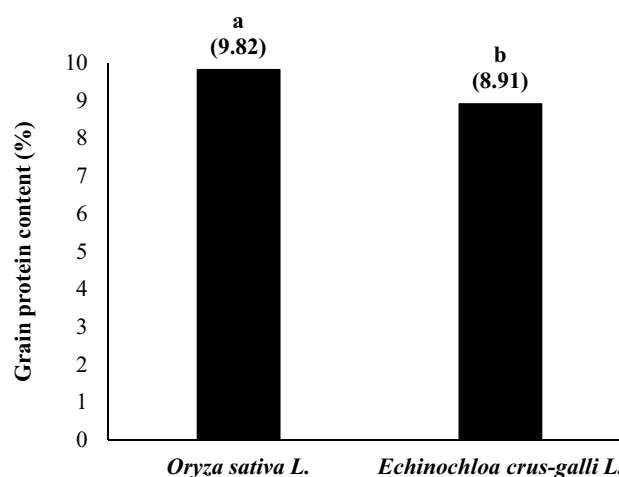


Fig. 1 Different between *Oryza sativa* L. and *Echinochloa crus-galli* L. in grain protein content. Means having similar letters, have no significant difference at 5% probability level by LSD test

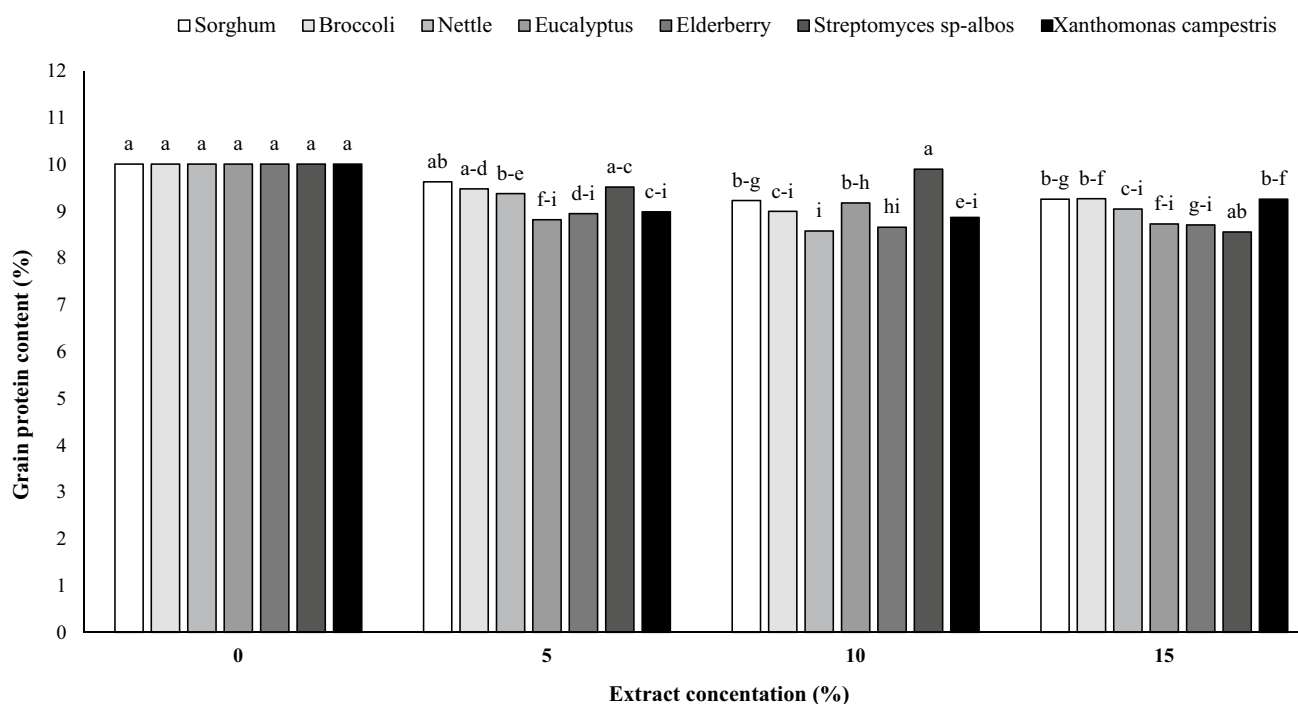


Fig. 2 Interaction between extract and extract concentration on grain protein content. Means having similar letters, have no significant difference at 5% probability level by LSD test

by averaged of 9.26%. The results showed that there was high grain protein content (10%) in control plants (Fig. 2). However, the grain protein content of both plants (rice and *E. crus-galli* L.) was significantly decreased for those plants treated with extract treatments. High inhibitory impacts were exhibited at 15% of *S. sp-albos* extract and 10% of Nettle extract (8.9%).

3.10 Grain starch content

The results showed that there was high grain starch content (61.7%) in control plants (Fig. 3). However, the grain protein content of both plants (rice and *E. crus-galli* L.) was significantly increased for those plants treated with extract treatments. High stimulate effects were exhibited at 10% of Elderberry (80.7%) and 15% of Nettle extract (79.4%). In addition, the results showed that there was high grain starch content in Nettle, Eucalyptus and Elderberry with content 74.87%, 76.8% and 74.62%, respectively (for rice) and 70.04%, 70.91% and 69.3%, respectively (for *E. crus-galli* L.). When compared with rice plant, the grain starch content of *E. crus-galli* L. decreased 7.16, 8.11, 6.45, 7.76, 7.12, 1.80 and 11.59% by application of sorghum, Broccoli, Nettle, Eucalyptus, Elderberry, *S. sp-albos* and *X. campestris* (Fig. 4).

4 Discussion

When allelochemicals were stressed, the abscisic acid (ABA) and ethylene concentrations increased [22], and these hormones had a stimulating effect on the chlorophyllase activity. Chlorophyllase plays an important role in the process of chlorophyll degradation. Allelopathic compounds may either inhibit chlorophyll synthesis pathways, stimulate chlorophyll consumption pathways, or cause both reactions, leading to reduced chlorophyll accumulation and, consequently, decreased photosynthesis and plant growth [29]. Another possible reason for the decreased chlorophyll in plants under allelopathic stress is a change in nitrogen metabolism. Allelopathic compounds cause glutamate, which is the common precursor of chlorophyll and proline synthesis, to be less involved in chlorophyll synthesis [30].

Hormonal imbalance is among the most important reasons for decreased growth of 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 [10]. 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 [30]. Studies have shown

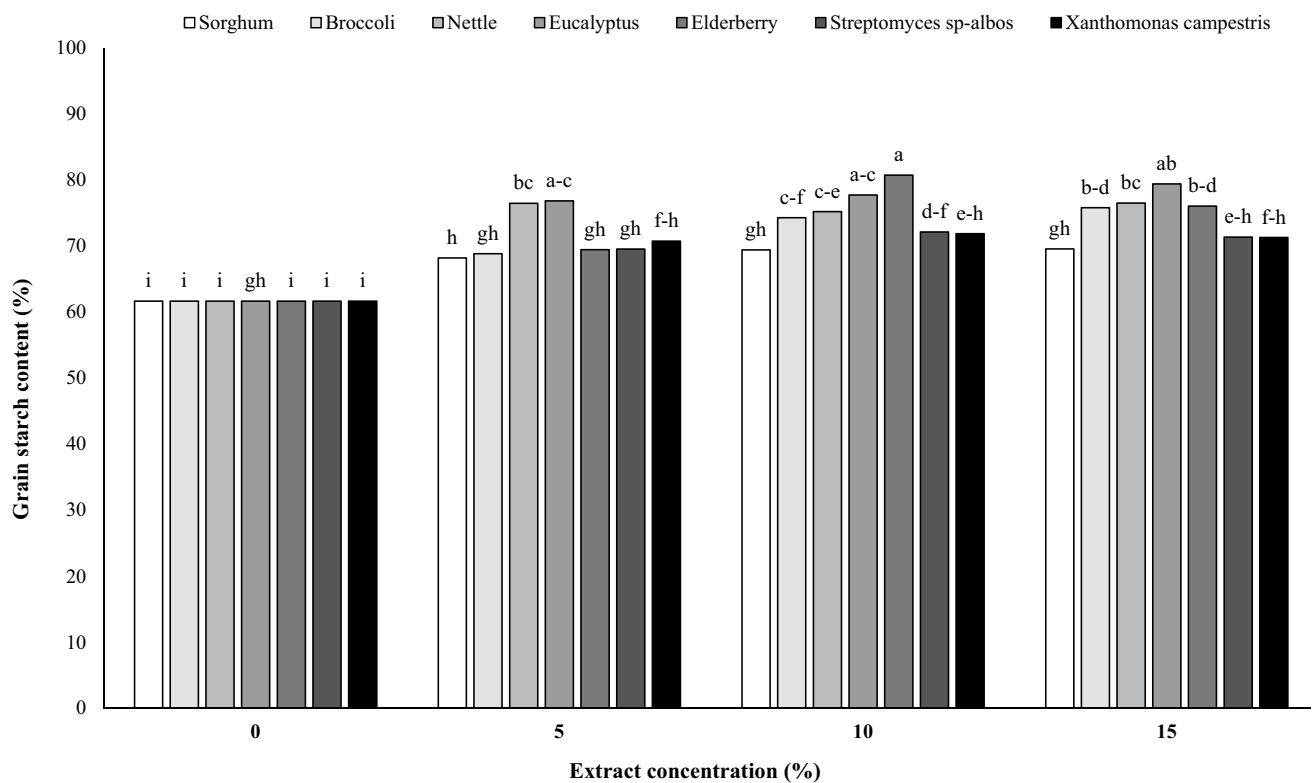


Fig. 3 Interaction between extract and extract concentration on grain starch content. Means having similar letters, have no significant difference at 5% probability level by LSD test

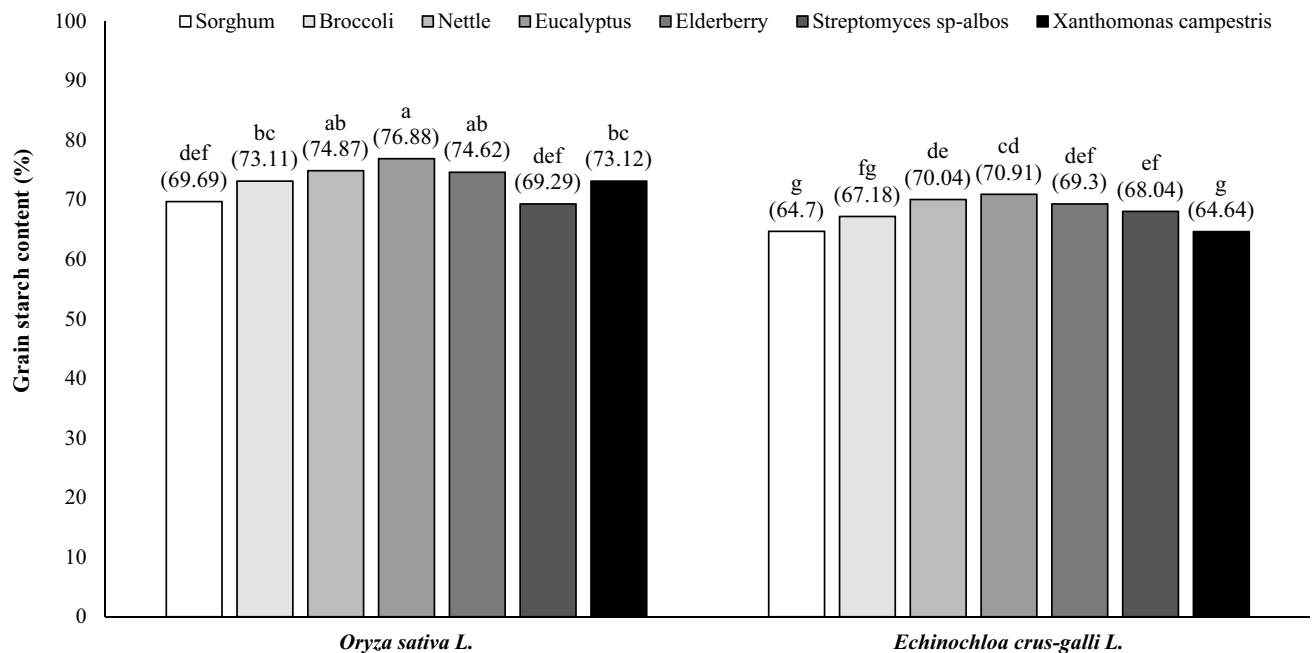


Fig. 4 Interaction between extract concentration and plant on grain starch content. Means having similar letters, have no significant difference at 5% probability level by LSD test

that some of the allelopathic substances interfere with normal levels of the hormone by inhibiting auxin transport, leading to growth arrest and the development of abnormal structures in the growing organ [31]. It seems that with increasing the extract concentration, the number of allelopathic substances increased and prevented the full growth of the leaves; therefore, the leaves remained smaller, and the leaf area decreased [32]. Decreased leaf area can be due to the inhibitory effect of allelopathic substances, reduced cell division, and tissue development [33]. Production of panicles m^{-2} was considerably reduced when treated with different concentrations of extraction which might be due to translocation of active ingredients of allelochemicals [7]. Further, the higher concentrations of extraction might have controlled or arrested the various metabolic processes in leaves causing lesser photosynthates [34] and thus decreased rate in assimilation of growth regulators, which might have checked the formation of panicles. Decrease in number of grains panicle $^{-1}$ of rice over control shows that stimulatory allelopathic compounds might be responsible for this increase. Furthermore, the decrease in number of grains panicle $^{-1}$ can also be attributed to decrease in LAI and length of shoots of rice over control [35]. Also, this inhibitory effect in number of grains panicle $^{-1}$ might be due to checking or inhibition of biosynthesis of gibberellins [36], which are responsible for cell division and cell-elongation. It is expected that the phenolics might have checked the increase of number of grains panicle $^{-1}$ by preventing the synthesis of protein, nucleic acid and other metabolites responsible for the synthesis of growth regulators [37]. The present findings agree with the report of Tibugari et al. [38]. The allelochemicals released from the extraction might have checked the synthesis and translocation and/or accumulation of plant hormones responsible for production of assimilate for grain filling. Grain setting inside the plant is another important factor of yield per plant. The development of grain depends on the photosynthates available in leaves and their translocation to grains [39]. The allelochemicals might have lowered 1000 grain weight. 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 [40]. 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 [12]. 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 [9]. 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 [41]. 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 [41]. 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 [42]. 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 [43–48].

5 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. Allelopathy and plant pathogens can be among the basic ways to control weeds in the sustainable agricultural system. Maximum reduction in total chlorophyll of rice was in the highest concentration of *Eucalyptus* and *X. campestris* (15%) that decreased 30% compared to the control. The lowest height of *E. crus-galli* L. was observed at highest concentration of Elderberry, *S. sp-albos* and Nettle (57.56 cm, 67.62 cm, and 69.37 cm). The lowest LAI (3.13) were found in higher concentration of *X. campestris*. It could be concluded that among the extractions, 15% of *Nettle* and Elderberry extracts can be used to control *E. crus-galli* L. at the germination stage under field conditions.

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Data availability The datasets used during the current study available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors declare no competing interests.

Plant Guidelines The authors confirm that the use of plants in the present study complies with international, national and/or institutional guidelines.

Permissions to collect the plants/plant parts All plant specimens collected with permissions.

Source of the plant used in your study All plants seeds name and he source of them are in the Methods section.

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