

Allelopathic effects of quinoa (*Chenopodium quinoa* Willd) residues on germination and growth of wild barley (*Hordeum spontaneum* C. Koch)

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ABSTRACT

Wild barley (*Hordeum spontaneum*) is a highly invasive weed in wheat fields. Quinoa (*Chenopodium quinoa*), a halophytic plant rich in phenolic compounds exhibited allelopathic potential. Its seeds contain a bitter, toxic outer layer of saponins. This study investigated the allelopathic effects of quinoa-derived saponin and plant residues on wild barley under laboratory, greenhouse and field conditions during 2022-2023. Results confirmed that both saponin and quinoa residues exerted significant allelopathic herbicidal effects on wild barley. There is little work on allelopathic effects of quinoa saponin on weeds or crops.

Key words: Allelopathy, *Chenopodium quinoa*, germination, growth, *Hordeum spontaneum*, quinoa, saponin, wild barley.

INTRODUCTION

Wild barley (*Hordeum spontaneum* C. Koch) is most invasive, dominant and damaging specie in Iran's major wheat-producing regions such as Khuzestan, Fars, Kermanshah and Razavi Khorasan, posing a serious threat to wheat production (6). It matures earlier than wheat and scatters seeds in the fields. So far, no herbicide controls wild barley (7), even sulfosulfuron at the recommended dose failed to control wild barley species (6). Hence, weed research and management strategies have shifted toward non-chemical methods, such as allelopathy. Allelopathy involves the chemical inhibition of one plant species by another, where allelopathic compounds released by a plant suppress the growth and development of neighboring species (8). These allelochemicals present in all plant tissues are released through leaching from live plants, decomposing residues, or microbial activity, ultimately influencing weed density and growth (20). However, research suggests that allelochemicals typically exert more severe inhibitory effects on seed germination than plant growth.

Different species of Amaranthaceae family have allelopathic effects due to their active terpene and phenolic compounds. Quinoa (*Chenopodium quinoa* willd) also belongs to this family. Phenolic substances in different parts of this plant are 71.7±5.5 mg per 100 g, which have allelopathic effects (3). Quinoa, a facultative halophyte, is a promising alternative crop for saline soils and highly saline water sources (2). Introduced to Iran in 2008, quinoa has since seen expanding research, cultivation and commercial production. Given its high nutritional value and the increasing salinization of water and soil in many

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regions, quinoa's role as a stress-resistant, adaptable crop is set to grow, hence, research is essential for optimizing its cultivation and use. According to Hosseini (13), all organs of quinoa exhibit inhibitory effects on the germination and growth of amaranth seedlings, with leaves showing the highest inhibitory activity and roots the lowest. Additionally, high concentrations of quinoa extract adversely affects the morphological traits of wheat (4). Furthermore, aqueous extracts from quinoa genotypes cultivated under rainfed conditions demonstrates higher allelopathic activity than those grown under irrigated conditions (12). The pot experiment revealed that oat growth was significantly inhibited by the phytotoxic activity of quinoa inflorescences, leaves and roots (10). Additionally, the aqueous extract of its residues had significant allelopathic effects on wheat seedlings, influencing electrolyte leakage, biochemical traits, antioxidant content and photosynthetic pigments (18).

MATERIALS AND METHODS

This study was conducted through 5-experiments (laboratory, greenhouse and field conditions) at the National Salinity Research Center, between 2022 and 2023.

Experimental Site: All experiments were done in the laboratory, greenhouse and farm of the National Salinity Research Center, Yazd. Geographical coordinates: 242245-3534549 and altitude: 1,214 m above sea level, Yazd is in central Iran dry desert climate with hot summers, low rainfall and high evaporation rates (maximum and minimum annual temperatures are 45 and -8 degrees C, respectively and the average annual rainfall is 60 mm).

Wild barley seeds were collected from wheat fields in Yazd and Fars provinces. To assess seeds viability, a tetrazolium test was conducted following the International Seed Testing Association guidelines (15). Wild barley seeds from Yazd and Fars provinces had 95 % and 85 % viability, respectively, as determined by embryo staining patterns. Before planting, the seeds were disinfected using sodium hypochlorite and Carbendazim fungicide. The field soil had salinity of 2.36 dS/m and a pH of 7.76.

The saponins used were obtained from quinoa saponin extraction facility, Fars province. The amount of saponin used in the experiments was determined by the Koziol method (16). We measured the saponin at 100 % concentration. The average height of saponins peak in this sample was 6.75 cm. Quinoa cultivar 'Titikaca' was cultivated to get material for extraction and powder preparation. Plants were harvested during inflorescence formation, separated, shade-dried and ground. We planted quinoa in March and harvested it in July.

Preparation of extract: To prepare the extract, 100 g powder was soaked in 1000 mL distilled water and agitated the mixture on a shaker for 24 h at 135 rpm. The solution was then filtered using Whatman filter paper. This procedure was repeated multiple times until the required extract volume was obtained. The final extract was stored in a refrigerator at 4 °C until further use. Saponin powder was extracted using the same method and subsequently sterilized in an autoclave. Serial dilutions were then prepared at concentrations of 0 %, 25 % and 50 % with distilled water.

Polyethylene glycol control: To distinguish the effects of osmotic pressure and allelochemicals in the extract solutions, a control treatment using polyethylene glycol 8000 (PEG) was included. First, the electrical conductivity (Ec) of the extracts was measured and the osmotic potential (Ψ) was calculated using the equation $OP = 0.036Ec$ (1), following the method described by Abedi (1). The required PEG concentration for each extract was determined as under:

$$PEG = \frac{4 - \sqrt{5.16 \Psi T - 560 \Psi + 16}}{(2.58 T - 280)}$$

, Ψ : Osmotic potential, T: Temperature in Kelvin (19).

The PEG control was prepared accordingly and evaluated with other treatments. The difference between the PEG control and the distilled water control was negligible, with no significant impact on results. Therefore, no data adjustments were necessary. For example, Table 1 presents the data from these two controls in the experiment assessing the allelopathic effects of saponin and quinoa residue extract on wild barley germination indices.

Table 1. Effects of distilled water control and PEG control on wild barley growth indices

Treatment	Germination (%)	Germination rate	MGT (day)	Epicotyl Length (cm)	Radicle Length (cm)	Seed vigour
PEG control	31.66	2.98	2.89	125.25	141.29	64.46
Distilled water control	30.92	2.94	2.77	122.20	139.88	63.20

MGT: Mean Germination Time

EXPERIMENTS

I. LABORATORY BIOASSAYS

Experiment (i): Effects of saponin and Quinoa residues extract on germination indexes of Wild barley

This factorial experiment was done in 9-cm Petri dishes under controlled laboratory conditions in a Growth Chamber in completely randomized design with two factors: (i). Plant organs 5 (Stem and leaf extract, root extract, a mixture of aerial organs (leaf, stem and inflorescence), saponin and a control (distilled water) and (ii). Quinoa residues extract concentrations 5 (0,10,25,50, 100 %) and 3-replications. Each Petri dish was lined with two layers of Whatman filter paper, 25 disinfected wild barley seeds were sown and 10 mL of aqueous extract of designated concentration was added. To maintain moisture, all dishes were placed in specialized trays covered with nylon. The trays were incubated in the growth chamber at 25 °C, 70 % humidity and a 14-h photoperiod with a light intensity of 500 lux.

Data collection began 48 h after setup and continued for 6- days. Germination was defined as emergence of 2 mm root length from seed (14). Key measured and analyzed

traits included germination (%) and rate, mean germination time, normal seedling percentage, stem length, root length and seed vigour.

(A). Germination (%): It was calculated using the standard formula (14).

$$GP = \left(\frac{n}{N}\right) \times 100$$

Where, GP: Germination (%), n: Total number of germinated seeds and N: Total number of sown seeds.

(B). Germination Rate (GR): It was calculated using the following equation.

$GR = \frac{\sum Si}{\sum di}$: Where, Si: Number of seeds germinated per day (in each count), di: number of days from onset of germination to the nth day and n: Number of counts.

(C). Mean germination time (MGT): It was calculated by following equation (14).

$$MGT = \frac{\sum (ni \times di)}{\sum ni}$$

Where, ni: Number of germinated seeds per count and di: Number of days after the start of germination.

(D). Seed vigour index was calculated from the following equation:

$$\text{Seed vigour index} = \frac{\text{Germination (\%)} \times \text{mean seedling length (mm)}}{100}$$

Experiment (ii): Effects of mixture of saponin and quinoa residues with soil on germination indexes of Wild barley

This factorial experiment was done in a seedling tray with a volume of 100 cubic centimeters per cell, under controlled laboratory conditions in a Growth Chamber in completely randomized design with two factors: (i). Plant organs 5 (stem, leaf and root residues, a mixture of aerial organs (leaf, stem and inflorescence) residues, saponin with soil, and a control (soil only) and residues concentrations 5 (0,2,5,10,20 %) and 3-replications. The prepared mixtures were added to seedling trays, with each cell holding 100 cubic cm; control cells contained only agricultural soil. Ten disinfected Wild Barley seeds were sown per cell, and the trays were placed in a growth chamber set at 25 °C, 70 % humidity and a 14-h photoperiod with 500 lux light intensity. Data collection began 48 h after planting and continued for 9- days. Key parameters measured were germination (%) and rate, mean germination time, stem length and seedling dry weight.

II. GREEN HOUSE STUDY

Effects of saponin and Quinoa residues extract on Wild barley growth indexes

This factorial experiment was done in randomized block design with two factors and three replications under greenhouse conditions with two factors. (i). Plant organs- 5 (stem and leaf extract, root extract a mixture of aerial organs (leaf, stem and inflorescence), saponin and a control (distilled water). (ii). Quinoa residues concentrations 5 (0,10,25,50, 100 %). The soil was sandy loam, with an EC of 2.36 dS/m and a pH of 7.76, while the irrigation water had an EC of 0.635 dS/m and a pH of 8.2. Initially, 60 pots (20 cm dia)

were filled with soil, watered and sown with 10 disinfected Wild Barley seeds per pot the following day. After germination, 3-plants per pot were kept. The prepared solutions at 20 ml per pot were applied twice (30 and 50 days after sowing) near the roots. Traits measured were: leaf area index (LAI), number of panicles per m², grains per panicle, grain yield per m², 1000-seed weight and dry weight per m².

III. FIELD STUDIES

Experiment (i): Effects of mixture of saponin and quinoa residues powder with soil on growth indexes of Wild barley

This factorial experiment was done in randomized block design, with two factors: (i). Plant organs 5 (stem, leaf and root residues, a mixture of aerial organs (leaf, stem and inflorescence) residues, saponin with soil and a control (soil only) and residues concentrations 5 (0,2,5,10,20 %) and 3-replications.

The field soil was sandy loam soil (EC of 2.44 dS/m and pH of 7.76), with no prior quinoa cultivation history and one year fallow. Based on soil analysis, Diammonium Phosphate and Potassium Sulfate were applied before planting at 250 Kg/ha. Plots size was 1 x 2 m. Disinfected wild barley seeds were sown in rows spaced 25 cm apart, plant to plant spacing was 20 cm. The first, four furrows were created along each plot and a mixture of saponin and quinoa residue with soil was poured into the furrows in the mentioned proportions according to the planting plan. Then, disinfected wild barley seeds were sown in these furrows and finally, the surface of the furrows was covered with field soil.

After germination, only one plant per cluster was retained. Irrigation was done with well water (EC = 2460 µmho/cm, pH = 7.74). Key measured traits included LAI, panicles per m², grains per panicle, yield, 1000-seed weight, dry weight and harvest index. Leaf area index (LAI) measurements were taken in late March before spike formation using a portable scanner. At harvest, 10 plants from the two central rows of each plot were collected.

Experiment (ii): Effects of foliar sprayed saponin and Quinoa residues extract on growth of Wild barley

The experiment was done in randomized block design with three replications, with soil and water specifications, preparation, sowing and harvesting methods similar to above experiment. However, unlike the previous experiment, saponin and quinoa residue powder were not incorporated into the soil before sowing. When plants were about 15 cm tall with saponin and quinoa residue extracts at 0, 10, 25, 55 and 100 % concentrations. Key growth parameters measured were: leaf area index (LAI), number of panicles per m², grains per panicle, yield, 1000-seed weight, dry weight and harvest index. This study aimed to assess the impact of foliar-applied saponin and quinoa residue extracts on Wild barley's growth and productivity.

Statistical Analysis

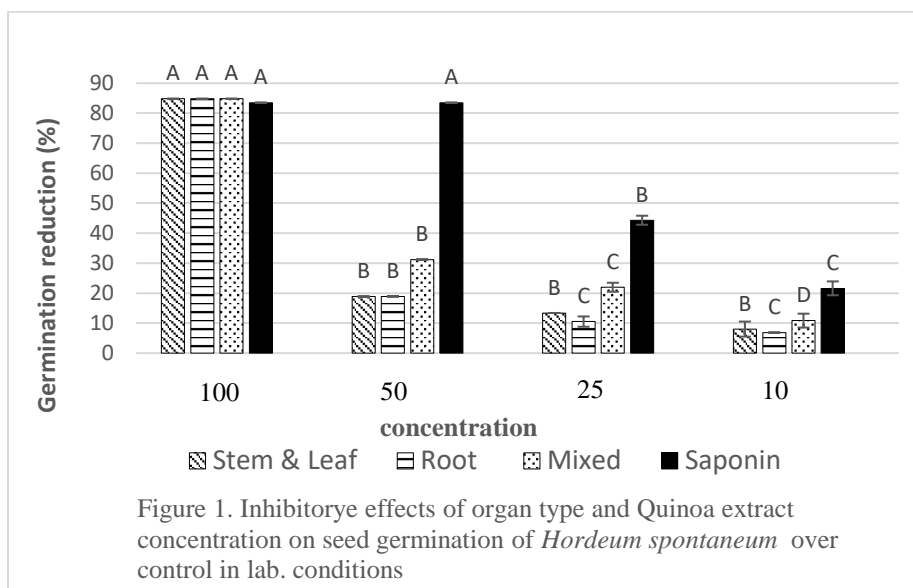
To analyze the data, initially the normality test of the data was examined using the Kolmogorov-Smirnov test and using SPSS software ver. 16. After testing for normality, the

data were analyzed using Analysis of Variance (ANOVA). Since the germination percentage and other indices at 100 % and 50 % extract concentrations, as well as the 20 % mixture of saponin powder and quinoa residues with soil, were zero, the data required transformation before analysis. The germination and normal seedling percentages (%) were subjected to an arcsin $\sqrt{(y + 0.1)}$ transformation, while the remaining traits were transformed using $\sqrt{(y + 0.5)}$ (24). The means were also compared using the LSD (Least Significant Difference) test. For statistical analyses, SAS V9 (Statistical Analysis System) software was used and the graphs were plotted using Microsoft Excel. Interaction effects were compared using the standard physical slicing method. Interaction effects were compared using the standard physical slicing method (22).

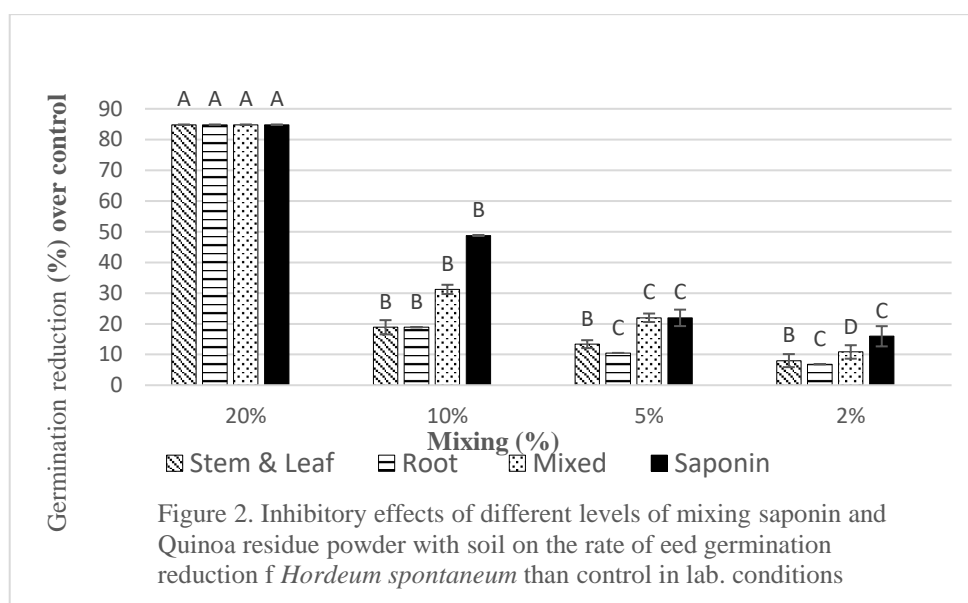
RESULTS AND DISCUSSION

I. LABORATORY BIOASSAYS

Experiment (i): The aqueous extracts of saponin and quinoa residues, along with their concentration and interactions, significantly inhibited all germination indices of wild barley at 1 % probability level. At 50 % and 100 % concentrations, germination (%) and other indices were suppressed in all treatments. The stem and leaf aqueous extract exhibited the most robust allelopathic effects, reducing germination (%), germination rate, mean germination time, stem length, radicle length and seed vigor by 47 %, 39 %, 17 %, 52 %, 54 % and 59 %, respectively, than control (Figure 1). Additionally, saponin and quinoa residue extracts induced abnormalities in germinated seeds, including stunted or slowed stem and root growth, short and curly roots and brown, necrotic root tips, more severe symptoms were observed in the saponin treatment.



Experiment (ii): The inhibitory effects of mixing saponin and quinoa residues with soil, as well as the mixing percentage and their interactions, significantly influenced the germination indices of wild barley at 1 % probability level. In the mean comparison, the root powder mixture had least inhibitory effects compared to other treatments. Notably, at the highest mixing percentage (20 %), germination (%) and all other germination indices were suppressed in all treatments, demonstrating the strong allelopathic potential of saponin and quinoa residues on wild barley weeds (Figure 2).



Laboratory studies demonstrated that saponin and various quinoa organs exhibited allelopathic effects on wild barley weed, with concentration and the interactions between organ type and concentration showing significant effects ($p \leq 0.01$) on most examined traits. While prior research has documented the allelopathic properties of quinoa organs on crops such as wheat, barley, onion and fava bean, as well as weeds like amaranth, oat and *Phalaris* (4,9,10,12,13,17,18,21). These studies predominantly focused on seed germination. Notably, no existing literature reports the allelopathic influence of quinoa saponins on weeds or crops, suggesting this may be the first study to highlight its impact on plants-particularly Wild barley weed. Recent investigations into saponin's antifungal and antibacterial properties further underscore its bioactive potential, though its role in allelopathy remains underexplored (11).

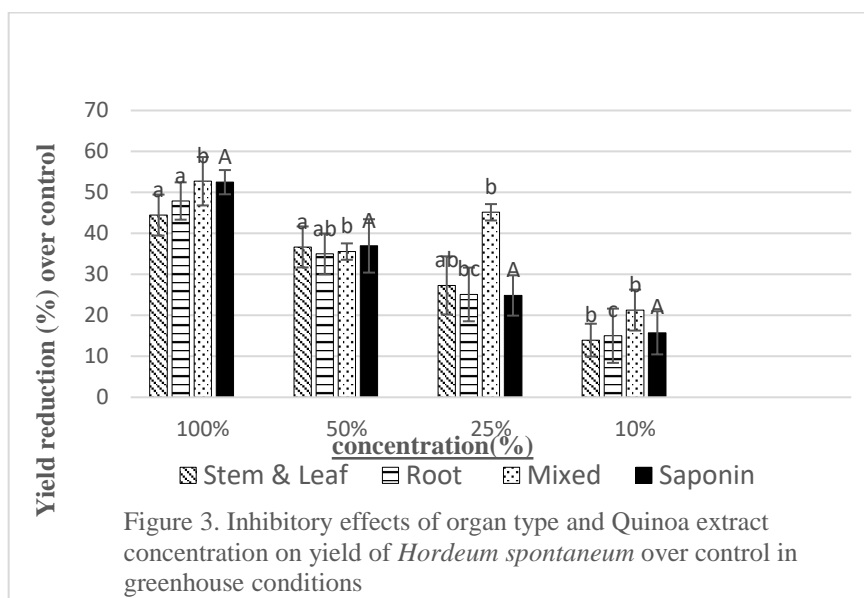
Saponins, (including a diversified family of steroidal glycoalkaloids, steroids, or triterpenoids,) originate widely in plant species and mainly provide the antimicrobial defence systems of plants (5) and act as potential chemical barriers against pathogens (25). Wild barley (*Hordeum spontaneum* C. Koch) is most invasive and damaging weed in wheat fields across Iran. It has become the dominant narrow-leaved weed in major

wheat-producing regions of the country and if left unmanaged, it will threaten sustainable wheat production in future (6).

Regardless of the severe environmental consequences of synthetic herbicide overuse-including the emergence of herbicide-resistant weeds, shifts in weed populations and risks to human health and ecosystems-no herbicide applied at recommended doses has successfully controlled wild barley (7). Given its resilient biological characteristics and the inadequacy of conventional herbicides, current research and management strategies now prioritize non-chemical control methods. On the other hand, due to the gradual salinization of water and soil resources in most of the country's plains, the area under quinoa cultivation as a saline plant resistant to environmental stresses with very high nutritional value and compatible with the water and soil conditions of most provinces of the country is increasing and a high volume of saponins and quinoa residues will be available, which can be used in the biological control of wild barley.

II. GREEN HOUSE STUDY

The aqueous extracts of saponin and different quinoa organs significantly inhibited the leaf area index (LAI), number of panicles per m², grain yield per m² and 1000-seed weight at the 1% probability level. Additionally, the concentration of the extract had a significant effect ($p < 0.01$) on all examined growth indices. However, the interactions between organ types and concentrations were only significant for LAI and 1000-seed weight ($p < 0.01$). These findings, consistent with laboratory results, confirm the allelopathic properties of quinoa saponin and organ extracts on wild barley weeds. At the highest concentration, the extracts reduced LAI, panicles per m², grain yield per m² and 1000-seed weight by approximately 43 %, 16 %, 28 % and 23 %, respectively (Figure 3).



III. FIELD STUDIES

Experiment (i): The mixture of saponin and different quinoa organs with soil significantly inhibited several growth parameters including leaf area index (LAI), panicles per m², grains per panicle, yield, 1000-seed weight, dry weight and harvest index at 1 % probability level. Consistent with laboratory and greenhouse findings, these results confirmed the allelopathic effects of saponin extract and quinoa organs. The mixed treatment of aerial organs exhibited the most robust allelopathic impact, reducing yield by ~30 % and dry weight by ~27 % than control. Mixing percentage also significantly influenced all traits (except 1000-seed weight), with higher proportions intensifying the inhibitory effects. Mean comparisons showed that soil-applied saponin and quinoa parts reduced LAI, panicles/m², grain yield/ha, dry weight and harvest index by 26 %, 29 %, 30 %, 26 % and 9 %, respectively (Figure 4). Notably, a 20 % saponin-powder mixture caused the most severe yield reduction (~48 %).

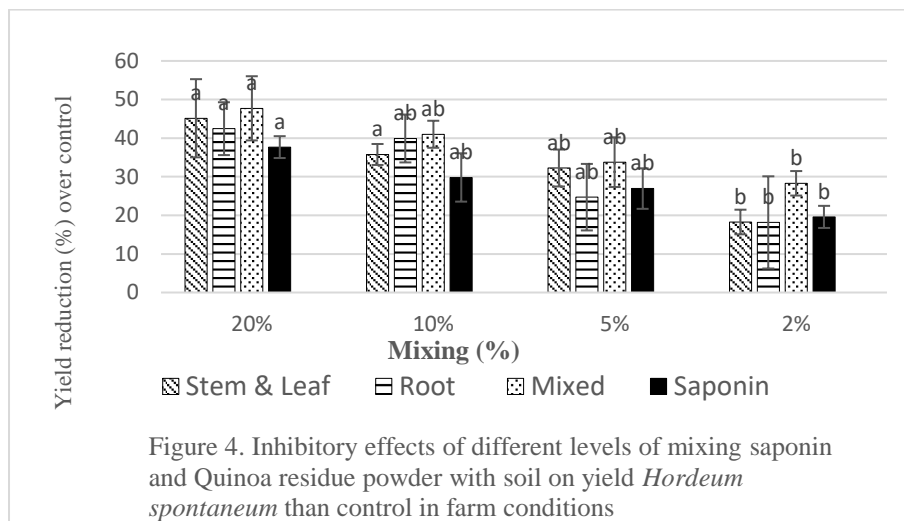
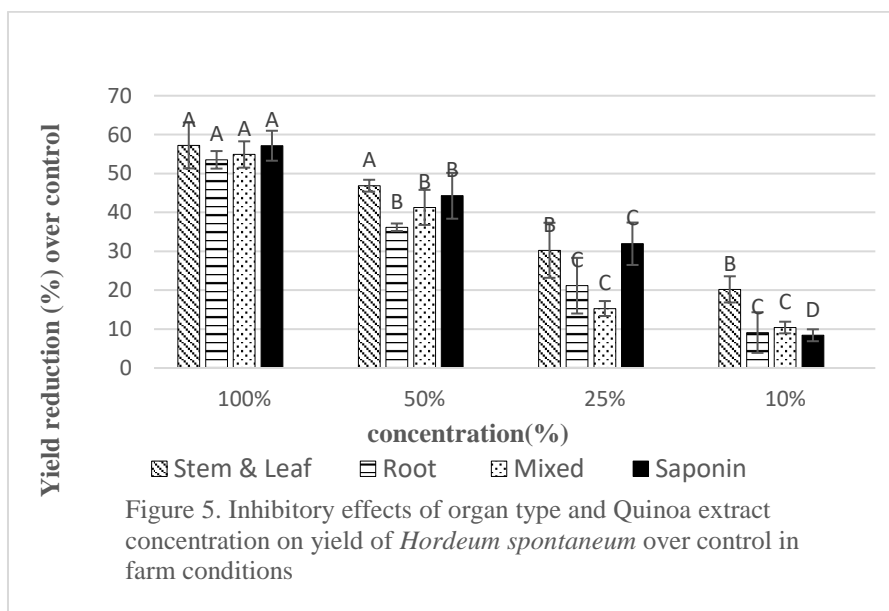


Figure 4. Inhibitory effects of different levels of mixing saponin and Quinoa residue powder with soil on yield *Hordeum spontaneum* than control in farm conditions

Experiment (ii). The foliar application of saponin aqueous extract and quinoa organ extracts significantly inhibited wild barley weed growth, affecting all measured traits except 1000-seed weight and seeds per panicle at 1 % probability level. These findings align with prior experiments, confirming the allelopathic potential of quinoa-derived saponins and organ extracts. Mean comparisons revealed that foliar treatment reduced leaf area index, panicles per m², grain yield, dry weight per hectare and harvest index by approximately 38 %, 19 %, 28 %, 25 % and 11 %, respectively, than control (Figure 5). Concentration effects were significant ($p < 0.01$) for all traits except 1000-seed weight, with higher extract concentrations amplifying inhibitory effects. Additionally, the interactions between organ type and concentration significantly influenced leaf area index, panicle density, yield and dry weight. Notably, 100 % saponin extract caused the most severe yield reduction (57 %), showing its potent phytotoxic impact.



Another key finding of this research was that the inhibitory effects of saponin and various quinoa organs on wild barley were weaker in most growth traits under greenhouse and field conditions compared to laboratory experiments. This aligns with the report by Weir *et al.* (23), which stated that allelochemicals typically exert a more robust influence on germination than on later growth stages, with most allelopathy studies focusing on seed germination and early seedling development. The reduced inhibitory effects may result from decreased allelopathic compound concentrations in quinoa residues or their degradation between quinoa harvest and subsequent crop planting. Further research is necessary to explore this phenomenon in greater detail. In this study, the analysis of saponin and quinoa residues was not performed due to laboratory limitations and time constraints. Notably, this critical gap persists in other published research within the field. Future complementary studies should address this limitation by identifying and investigating the active components in saponins and quinoa residues responsible for their allelopathic effects, thereby enhancing understanding of their mechanisms.

CONCLUSIONS

This study demonstrated that saponins and quinoa components exhibit potent inhibitory effects on wild barley, particularly during germination. These findings provide a promising and practical foundation for integrated weed management. Given these results, expanding this research into a comprehensive program is recommended as a viable alternative strategy to control this invasive weed, ultimately supporting the production of healthy, organic crops.

DECLARATION

We declare that all authors of this manuscript have made substantial contributions. We did not exclude any author who substantially contributed to this manuscript. We have followed the ethical norms established by our respective institutions.

AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration with all authors. All authors finally approved and drafted the manuscript.

CONFLICT OF INTEREST

The authors announce that they have no conflict of interest.

ETHICAL APPROVAL

The authors declare that the study was carried out following scientific ethics and conduct. However, this study did not involve any use of animals, hence no ethical approval has been obtained from the concerned committee.

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