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Effects of Coumarin on Amylase Activity, Electrolyte Leakage and Growth of Wheat (*Triticum aestivum* L.), Barley (*Hordeum vulgare* L.) and Sesame (*Sesamum indicum* L.)

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ABSTRACT

Coumarin as a secondary metabolite widely found in plant species. Coumarin with a strong inhibitory effect on germination will be a good alternative for weed control. The mechanism of inhibition effects of coumarin has not yet been well understood. In this research, coumarin effects on amylase activity, electrolyte leakage and growth of wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.) and sesame (*Sesamum indicum* L.) were studied using factorial statistical design. Results showed that coumarin with a concentration-dependent pattern (0.2 to 0.5 mM) reduced all growth indices. Coumarin had the greatest effect on root length and leaf length of seedlings. The results showed that the species sensitivity and resistance to coumarin are significantly different. Coumarin had no significant concentration-dependent decrease in amylase activity. Coumarin (0.2 mM and 20 mM) increased the electrical conductivity of the solution around the seeds and roots of wheat seedlings. The mechanism of coumarin inhibition may be through its inhibitory effect on the expression of amylase genes. Increased electrical conductivity in the periphery of the living tissue can result in electrolyte leakage and damage to seed and root cell membranes which is called one of the mechanisms of coumarin inhibition of germination. Some allelopathic properties of coumarin may be attributed to its effect on amylase activity, cell membrane integrity, and stimulation of metabolite leakage in competing plants. Allelopathic studies of coumarin can be used in the field to weed and pest control and have a practical result.

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1. Introduction

Recently, consideration of natural compounds with allopathic properties has increased because of fewer side effects (Enteshari and Ahrabi, 2012). Allopathic relationships among plants and use of secondary natural metabolites can be a good alternative to harmful chemicals which are used to control weeds. Allelopathy interaction has the potential to impact the growth and development of neighboring plants in both natural and agricultural ecosystems (Palanivel *et al.*, 2021). Coumarins as a large group of secondary metabolites have a phenolic structure and more than 1,300 types (Nazari and Iranshahi, 2011; Venugopala *et al.*, 2013). These compounds are widely found in plant species such as Apiaceae, Rutaceae, Asteraceae and Fabaceae (Hussain *et al.*, 2018; Zobel and Brown, 1995). Coumarins have a phenylpropanoid structure with combination of benzene ring and alpha-pyron. Coumarin ($C_9H_6O_2$) (Fig. 1) prevents seed germination, reduces seedling growth and can inhibit the activity of certain enzymes such as amylase (Razavi, 2011). Coumarin effectively inhibits the germination of *Brassica parachinensis* (*B. parachinensis*) seeds (Chen *et al.*, 2021). The mechanism of this inhibition has not yet been well understood. Phenolic allelochemicals usually increase the cell membrane permeability, as a result, the cell's contents are leaked and lipid peroxidation increases so growth is reduced. In addition, phenolic allelochemicals usually reduce absorption of root and therefore affect normal growth.

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Phenolics in general can inhibit plant cell division, extensions and growth (Abenavoli *et al.*, 2006).

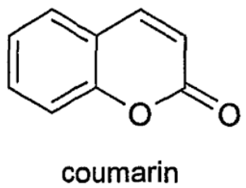

Figure 1. Chemical compound of simple coumarin, 2H-chromen-2-one, $C_9H_6O_2$

The inhibition of germination by coumarin is attributed to their ability to inhibit mitosis. The results showed that coumarin and 7-hydroxy-coumarin had a highly inhibitory effect on rye seed growth. Therefore, coumarin with a strong inhibitory effect on germination will be a good option for weed control (Lupini *et al.*, 2014). Various studies confirm the inhibitory effect of coumarin on seed germination and root growth. The effects of coumarin on plant growth depend on the species, the applied method and the concentration of coumarin (Chattha *et al*., 2018). Progress made in understanding mechanisms of allelochemical selectivity, physiological modes of action, as well as genetic regulation of biosynthesis should represent the basis for manipulation of germplasm resources to select for novel secondary metabolites, enhance production or regulate release to obtain season-long weed suppression either in living plants or in their residues. Allelopathic plants also offer a source for development of model herbicides and could provide germplasm for use to improve weed suppression traits. Similarly, particularly relevant are studies on the determination of genes responsible for allelopathic activities and use of transgenic techniques to transform wild ecotypes into commercial cultivars. In order to achieve the goal of sustainable agriculture the allelopathic potential of plants should be enhanced (Tesio and Ferrero, 2010).

Sesame seeds are one of the most nutritious oil seeds. Also, in addition to sesame seeds, which have a lot of nutritional and medicinal value, the products obtained from it, such as sesame oil and arde, are rich in nutritional and medicinal value (Fazeli Kakhki *et al.*, 2014; Mansouri and Soltani Najafabadi, 2004). Sesame seeds contain valuable oil that may reach 45-62% depending on the environmental conditions and type of variety, and due to the presence of an antioxidant phenolic compound called Sesamolin, its oil has good durability. (Arab *et al.*, 2022; Arooj *et al.*, 2023; Huang *et al.*, 2023; Wan *et al.*, 2023; Wei *et al.*, 2022). Consuming sesame and its products reduces the inflammation and intensity of uterine contractions, as well as reducing the intensity of pain during menstruation. This plant softens the stomach and intestines. Sesame reduces blood pressure. Infusion of sesame leaves cures dysentery. Sesame is anti-rheumatism (Arooj *et al.*, 2023; Wan *et al.*, 2023; Wei *et al.*, 2022).

Coumarin and its derivatives have a wide range of biological and medicinal activities. Presently no studies that demonstrate the allelopathic effect of coumarin differs from plant to plant. In other words, does the response of the studied plants to coumarin have a significant difference? Does coumarin affect amylase activity in germinated seedlings? How does coumarin affect ion leakage and electrical conductivity of root and seed in studied species? In this research, we seek to find the answers to these questions. In this study, allelopathic effects of coumarin on germination of wheat, barley and sesame and effects of coumarin on amylase activity and ionic leakage and electrical conductivity were also investigated.

2. Materials and methods

2.1. Allelopathic effects of coumarin

Certified seeds of wheat (*Triticum aestivum* L.) Pishgam cultivar, Barley (*Hordeum vulgare* L.) Abidar cultivar and Sesame (*Sesamum indicum* L.) were prepared from the Sanandaj Agriculture Research Center. At first, concentrations of coumarin (20, 10, 5 and 2.5 mM) and (0.2, 0.1, 0.05 and 0.025 mM) (Merck) were prepared. Wheat, barley and sesame seeds were selected with weights (0.053 ± 0.001) g, (0.047± 0.001) g and (0.073 ± 0.001) g respectively. the seeds were rinsed firstly with distilled water, and for 20 minutes in 70% ethanol and rinsed again with distilled water and then washed out with 1.5% sodium hypochlorite solution for 15 minutes to completely disinfect. The seeds were washed with distilled water and cultivated in five plates containing disinfected filter paper. Each plate contains eight seeds with approximately equal spacing. 2.5 ml of the solutions containing pre-prepared concentrations of coumarin were added to each plate. One plate was cultured with distilled water as a control. The plates were closed with parafilm and then placed in the growth chamber for eight days. The growth chamber (IKH.RH) was regulated with 12-hour light and 12 hours of darkness period, daily temperature of 25°C and night temperature of 18°C, and relative humidity of 27% (Fig. 2) (Farsi and Zolali, 2002). leaf length, radicle length, leaf weight and root weights of seedlings measured after eight days.

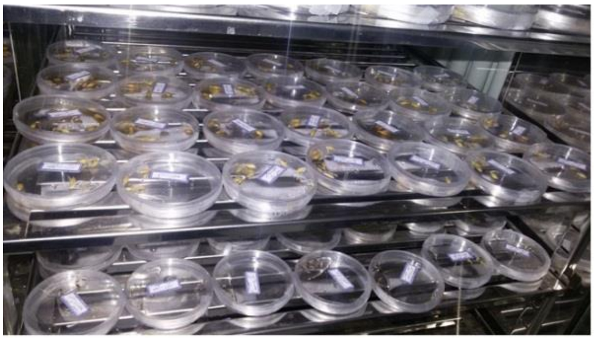

Figure 2. The growth chamber (IKH.RH)

2.2. Effect of coumarin on the activity of alpha-amylase in seedlings

In this experiment, the effect of different concentrations of coumarin on alpha-amylase activity of wheat, barley and sesame was investigated. This method requires starch solution, acetate buffer, amylase and buffer starch. Logol and starch form a dark blue color, which its absorption is directly proportional to the starch concentration. Absorption of each solution is read by spectrophotometer (Unico 2100) at 640 nm. Each treatment is performed in three replicates (Khavari nejad and Najafi, 1999).

2.3. Effects of coumarin on electrolyte leakage of seeds and root of wheat

The purpose of this experiment was to measure the ion leakage in the root and seed of wheat seedlings after exposure to different concentrations of coumarin. For the electrolyte leakage test, 1 g of wheat seeds (20 pcs) was disinfected with 1.5% hypochlorite solution, then washed with water several times and placed on the falcon. Three treatments including 0.2 mM coumarin, 20 mM coumarin and distilled water, were prepared. Each treatment was performed in three replicates. 20 ml of each treatment was added to the seeds in the Falcons. Then, the conductivity of the samples was measured by a conductivity meter (inolab-IDS, Multi9310) every 3 minutes, until 4.5 hours at a constant temperature of 25 ° C. After disinfection and washing with distilled water, in each petri dish, 20 seeds of wheat were placed in a growth chamber to grow completely. After full growth of the root, they were placed in a container so that only the root was exposed to the treatments (0.2 mM, 20 mM coumarin and distilled water) 20 ml was added to each dish. Then the conductivity of the samples was measured by a conductivity meter (inolab-IDS, Multi9310) every 3o minutes, until 4.5 hours at a constant temperature of 25°C (Abenavoli *et al*., 2006). In this research, experiments were designed using factorial statistical design and three replications. For each of the data, analysis of variance was performed to compare the means and Duncan's test was used to evaluate the significance of the difference between the means. Data analysis was done using Spss20 software.

3. Results and discussion

3.1. Allelopathic effects of coumarin

Coumarin at concentrations of (20, 10, 5 and 2.5 mM) completely inhibited the germination of wheat, barley and sesame (not shown) and in lower concentrations (0.2, 0.1, 0.05 and 0.0025 mM), reduced the length and weight of root radicle and leaf of seedlings. Results showed that coumarin with a concentration-dependent pattern (0.2 to 0.5 mM) reduced all growth indices. Coumarin had the greatest effect on leaf length (Fig. 3A) and root length (Fig. 3B) of seedlings. Indices that evaluate lengths such as leaf length (Fig. 3A) and root length (Fig. 3B) are more sensitive to coumarin than weight indices such as leaf weight (Fig. 3C) and root weight (Fig. 3D). Analysis of variance showed that both the coumarin concentration and the species had a significant effect at 1% and 5% levels on leaf weight, root weight, root length and leaf length variables. The Duncan test confirmed the significant difference in physiological responses of three plants to coumarin and showed that plants' sensitivity and resistance to coumarin differ significantly (Fig. 4).

3.2. Effect of coumarin on alpha-amylase activity

There was no significant difference in amylase activity in the control (0 mM) group (without coumarin) and the treatment groups (20, 10, 5, 2.5 mM concentrations of coumarin). There was no significant difference between different concentrations of coumarin in inhibition of amylase activity. Coumarin had no significant inhibitory effect on amylase activity (Fig. 5).

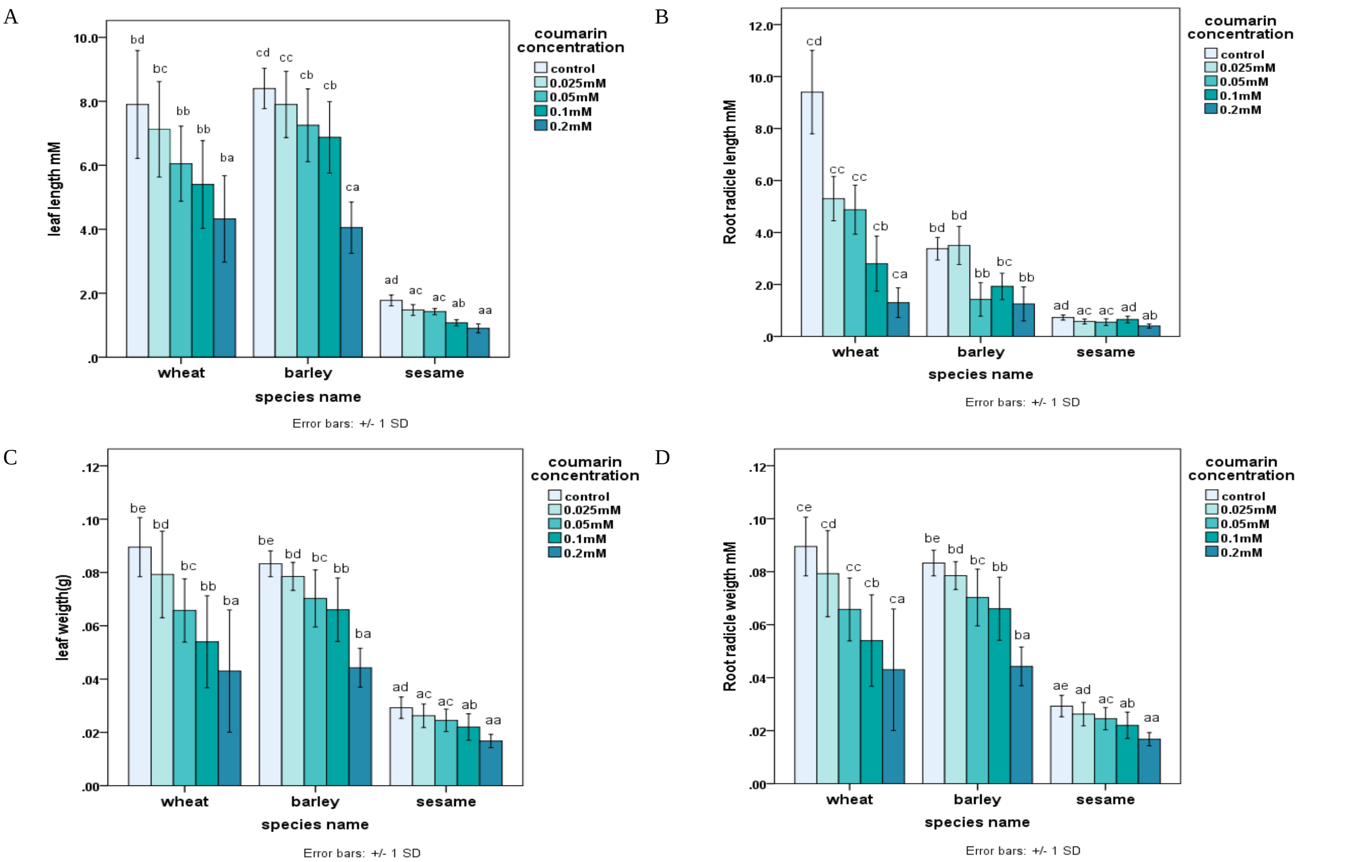

Figure 3. Effect of different concentrations of coumarin on leaf length, radicle and seminal root length, leaf weight and root radicle and seminal weight of wheat, barley and sesame seedlings

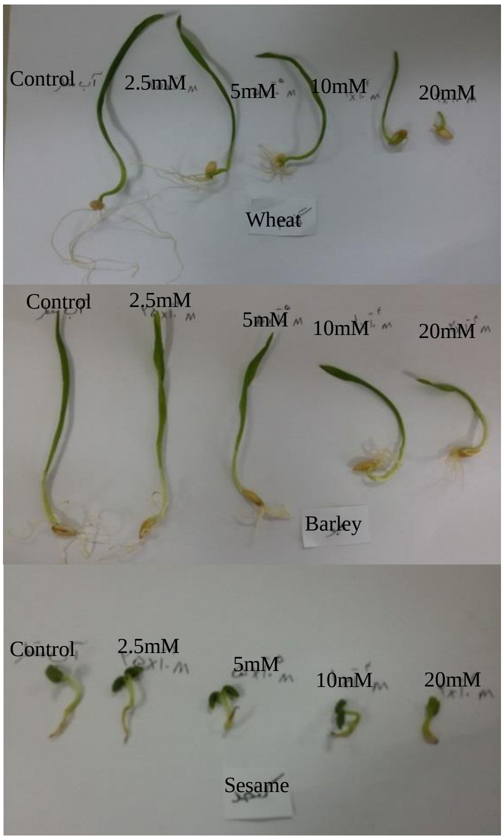

Figure 4. Effect of different concentrations of coumarin on leaf length, radicle and seminal root length, leaf weight and root radicle and seminal weight of wheat, barley and sesame

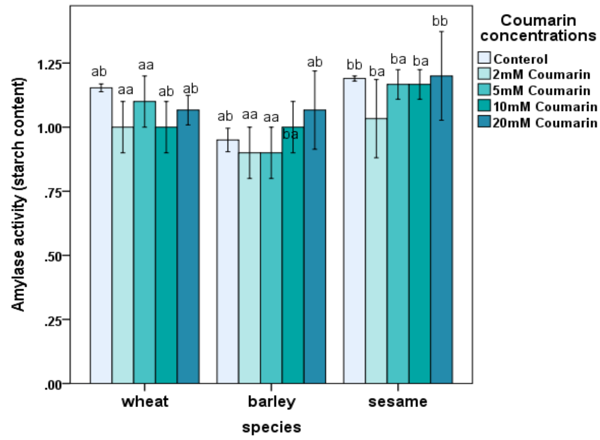

Figure 5. Effect of different concentrations of coumarin on amylase activity (starch content) of wheat, barley and sesame seedlings

3.3. Effects of coumarin on electrolyte leakage

Fig. 6 shows the effects of 20, 0.2 and 0 mM concentrations of coumarin on the electrical conductivity and electrolyte leakage of the peripheral solution of seed (Fig. 6A) and root of wheat (Fig. 6B). Diagrams a, b, show that both concentrations of coumarin (0.2 mM and 20 mM) increased the electrical conductivity of the solution around the seed and root of wheat.

A

B

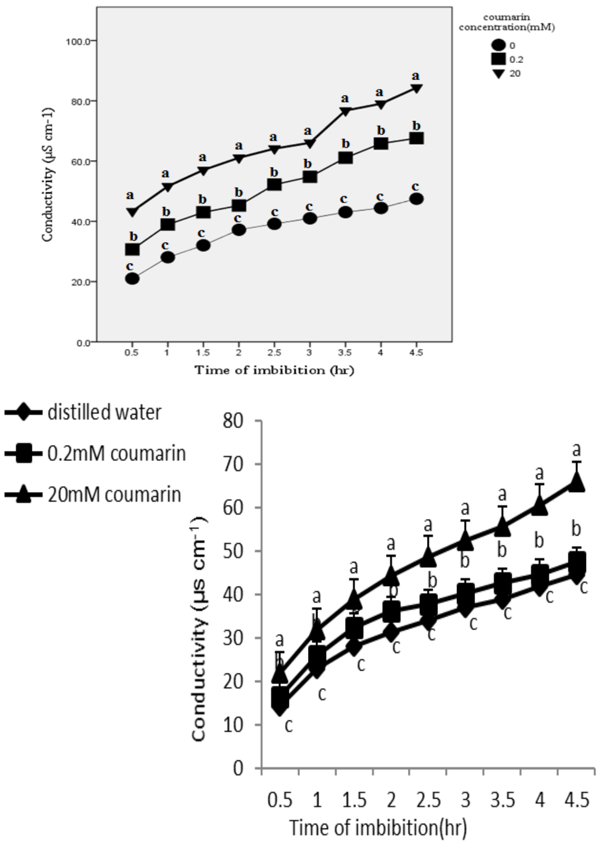

Figure 6. Effect of different concentrations of coumarin on seed (a) and radicle seminal root (b) of wheat electrolyte leakage

Allelopathy may represent a new frontier for the implementation of the practices applicable in integrated weed management strategies by using and selecting varieties with strong allelopathic potential to biologically reduce the intensity of weeds. Another important approach is the identification of genes with allelopathic activities, and the application of breeding and transgenic techniques to place allelopathic traits from wild accession into useful crops. Furthermore, using modern technology, allelochemicals with strong herbicidal potential may be identified from suppressive plants to produce bioactive herbicides and pesticides for use in sustainable agriculture (Tesio and Ferrero, 2010). It has measured (Zobel and Brown, 1995) the concentration of furanocoumarins in the fruits and seeds of different species of Apiaceae and showed that in some of these species psoralens act as inherent inhibitors.

It has investigated (Abenavoli *et al*., 2006) the inhibitory effect of coumarin on wheat germination. Moreover, it showed that the 200µM concentration of coumarin is the threshold concentration to inhibit germination of wheat seeds. In addition, no visible morphological changes were observed in the inhibited grains. It has isolated (Razavi, 2011) three coumarin derivatives; emperatorine, urapetin and 7-prenyl oxy coumarin from Zosima absinthifolia seeds and showed that these compounds reduce germination percentage as well as reduce the length of root and shoot of lettuce seedlings. It has investigated (Enteshari and Ahrabi, 2012) the effect of different concentrations of coumarin on germination and growth of canola seedlings and showed that with increasing coumarin concentration, seed germination and seedling growth decreased. Li et al. (2011) showed that 4-methyl amblyphrone (a coumarin derivative) inhibits the germination of Arabidopsis seeds. They also showed that at a 500 µM concentration of coumarin, the seeds could not germinate at all (Li *et al*., 2011).

It has investigated the effects of six phenolic compounds (cinnamic acid, ferulic acid, vanillic acid, p-coumaric acid, m-coumaric acid, and p-hydroxybenzoic acid) at different concentrations on germination and some physiological indices of Barnyard grass and showed that all of these compounds had inhibitory effects on germination and other physiological parameters such as root length and shoot length and there are also significant differences between the effects of these six allelochemicals (Esmaeili *et al*., 2012). It has investigated the effect of different concentrations of coumarin on eighteen plant species and showed that with increasing coumarin concentration, the percentage of cumulative germination decreased in all species (Zaeri *et al*., 2013).

It has investigated the effect of different concentrations of coumarin on early growth and some other physiological parameters in Faba bean and showed that except for the highest concentration applied (4 mM), other different coumarin treatments had no effect on germination. They also showed that increasing coumarin concentration up to 1 mM had no significant effect on root length (Saleh and Abu El-Soud, 2015). Our results showed that coumarin had a significant effect on wheat, barley and sesame growth indices. Coumarin inhibition is concentration dependent. Coumarin at concentrations higher than 0.2 mM completely inhibited all growth indices of wheat, barley and sesame. Also, coumarin showed certain deleterious allelopathic effects on the growth of these plants. At this concentration, no significant difference was observed in the sensitivity of wheat, barley and sesame to coumarin. In lower concentrations (0.1, 0.05 and 0.0025 mM) in some indices, light differences showed in the responses of wheat, barley and sesame to coumarin. The results of this study are in agreement with previous research that has shown a 1 µM concentration of coumarin is a threshold concentration for inhibition of germination of wheat seeds. The results of this study are also in agreement with previous research (Enteshari and Ahrabi, 2012) that shown that with increase in coumarin concentration, seed germination and seedling growth decreased. The results of this study showed significant differences in physiological responses of three species to Coumarin. This means that the species sensitivity and resistance to coumarin are significantly different. Other studies confirm this result.

The results of this study showed a significant difference between the studied growth indices for coumarin. This means that the effects of coumarin on radicle length, root weight, root length and leaf weight were significantly different. The effect of coumarin on some indices was greater and on some indices it was less. This comparison is not observed in other studies. Amylases (alpha-amylase, beta-amylase) are involved in seed germination. Decreasing and inhibiting the activity of these enzymes is likely to decrease seed germination. Mitosis reduction in the apical meristems, decrease in the activity of enzymes catalyzing plant metabolism and impair in absorption of inorganic ions which occurs in the presence of allelochemicals, eventually decreases the growth rate of seeds (Saleh and Abu El-Soud, 2015). The results showed that 0.2 mM concentration of coumarin had a significant effect on seed germination indices. Germination rate is the most appropriate index for, evolving, allelochemical effects on germination because this index, better than the other two indices, shows the effect of coumarin on seedlings. It seems that germination indices show wheat is more resistant and sesame is more susceptible to coumarin. It can be concluded that the response of different species to coumarin varies by species and is not the same. Germination indices in very few studies have been considered.

The results of our study showed that treatment of seed and root cells with Coumarin (20 and 0.2 mM) caused a temporary disturbance in membrane structure and electrolyte leakage into the environment of the growth medium. It has been observed (Abenavoli *et al*., 2006; Saleh and Abu El-Soud, 2015) that solute leakage was 20% higher at 1000 µM and concluded that Coumarin (1000 mM) can delay or even prevent the membrane's stable configuration repair. In the present study, it was observed that the electrolyte leakage due to coumarin (20 mM) was more than distilled water and the 0.2 mM concentration of coumarin. Phenolic compounds such as coumarin can affect cell membranes and lead to the release of electrolytes. In fact, phenolic compounds can cause depolarization and peroxidation of membrane lipids and increase electrolyte leakage. Coumarin concentrations of 20 mM had the greatest effect on the root membrane. It can be concluded that damage to the membrane and roots of the seeds may be one of the inhibitory mechanisms of germination by compounds such as coumarin. Further studies on the physiological and biochemical processes of seed germination should be performed. Increased electrical conductivity in the periphery of the living tissue can be the result of electrolyte leakage. Electrolyte leakage can also occur due to the disruption of the ion exchange equilibrium. Secondary metabolites with an adverse effect such as coumarin affecting the integrity of biological membranes may cause electrolyte leakage and alter the electrical conductivity of the peripheral environment of a growth medium.

It is reported that coumarin completely inhibited alpha-amylase synthesis in barley seeds at 340 µM (Saleh and Abu El-Soud, 2015). At concentrations above 200 µm, coumarin inhibits seed germination in a concentration-dependent manner. Inhibition occurred early during seed (phase I), and was rapid and irreversible. During phase I, coumarin inhibited water uptake, electrolyte retention capacity, and O_2 consumption. Later on, coumarin delayed the reactivation of peroxidases, enhanced the activity of superoxide dismutase, decreased the activities of selected marker enzymes for metabolic resumption, and repressed the transcription of molecular chaperons involved in secretory pathways. Insufficient and/or late seed rehydration caused by coumarin could have delayed membrane stabilization or decreased respiratory O_2 consumption, both of which are conducive to an overproduction of reactive O_2 species. Being unbalanced by an adequate upsurge of antioxidant defense systems, the resulting oxidative stress might have ultimately interfered with the germination program (Abenavoli *et al*., 2006). In the present study, amylase activity was measured using logol and based on starch content. The results showed that different concentrations of coumarin (20, 10, and 5, 2.5 and 0 mM) had no significant effect on amylase activity. Coumarin may inhibit amylase synthesis and secretion through inhibition of genes required for packaging and stability of secretory amylase proteins. Coumarin had the highest inhibition effect on the ryegrass (*Lolium perenne*) seed, where coumarin disturbed the hormone pathway by decreasing the content of gibberellic acid 3, resulting in the reduction of amylase activity and consumption of starch during the germination process of ryegrass seed (Yang *et al.*, 2023).

Our results showed that coumarin had no effect on the catalytic activity of amylase. Coumarin is a physiologically active plant phenolic compound. The mechanism of inhibition appears to be through the inhibitory effect on the expression of amylase genes. In this study, a 0.2 mM concentration of coumarin showed a severe inhibitory effect on germination and growth of the plant species. The results of this study showed that concentrations of more than 0.2 mM of coumarin completely stopped all growth indices of all three plants; wheat, barley and sesame species. At this concentration, no significant difference was observed in the sensitivity of the wheat, barley and sesame species to coumarin. At lower concentrations, there was a significant difference in physiological responses of the three species to coumarin. This means that the species sensitivity and resistance to coumarin are significantly different.

Coumarin (20 mM) increased the electrical conductivity of the solution around the seed and root of wheat. Increased electrical conductivity in the periphery of the living tissue can be the result of electrolyte leakage. Damage to seed and root cell membranes may be one of the mechanisms of coumarin germination inhibition. The results showed that coumarin (20, 10, 5, 2.5 and 0 mM) had no concentration-dependent significant effect on amylase activity. As the concentration of coumarin increased, its inhibition of amylase activity did not increase. The effects of coumarin on germination processes may be through inhibition of the expression of genes necessary for the synthesis, packaging and stability of secretory amylase proteins. Some allopathic properties of coumarin are attributed to its effect on amylase activity, cell membrane integrity, and stimulation of metabolite leakage in living tissues of competing plants. Allopathic studies of coumarin in the field of weed and pest control can have practical results. Also, improve understanding of the cellular mechanisms of coumarin's biological effects by investigating its effects on electrolyte leakage, electrical conductivity, and amylase activity.

4. Conclusion

Concentrations of more than 0.2 mM of coumarin completely stopped growth indices of wheat, barley and sesame species. At lower concentrations, the species sensitivity and resistance to coumarin are significantly different. Coumarin (20 mM) increased the electrical conductivity. Increased electrical conductivity in the periphery of the living tissue can be the result of electrolyte leakage. Damage to seed and root cell membranes may be one of the mechanisms of germination inhibition by coumarin. Coumarin had no concentration-dependent significant effect on amylase activity. As the concentration of coumarin increased, its inhibition of amylase activity did not increase. The effects of coumarin on germination processes may be through inhibition of the expression of genes necessary for the synthesis, packaging and stability of secretory amylase proteins. Some allopathic properties of coumarin are attributed to its effect on amylase activity, cell membrane integrity, and stimulation of metabolite leakage in living tissues of competing plants. Allelopathic studies of coumarin in the field of weed and pest control can have practical results on electrolyte leakage; electrical conductivity and amylase activity may be one of the cellular mechanisms of coumarin's biological effects.

Conflict of interests

All authors declare no conflict of interest.

Ethics approval and consent to participate

No human or animals were used in the present research.

Consent for publications

All authors read and approved the final manuscript for publication.

Availability of data and material

All the data are embedded in the manuscript.

Authors' contributions

All authors had an equal role in study design, work, statistical analysis and manuscript writing.

Informed consent

The authors declare not to use any patients in this research.

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