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Evaluation of the bio-herbicidal potentiality of red radish on lupine plant and associated weeds

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Abstract

Pot experiments were performed throughout two winter seasons of (2019/2020) and (2020/2021) at the greenhouse of National Research Centre, Giza, Egypt. The experiment was performed to evaluate the bio-herbicidal potentiality of aqueous extract of root and leaf of red radish at 1.5%, 3.0%, 4.5% and compare them to mixed two chemical herbicides (Topik 240 EC (Clodinafop-Propargyl) and broad weed Basagran (Bentazon 48 EC)) at recommended doses 140 g fed^{-1} and 1 L $fed^{-1,}$ respectively) in controlling weeds (*Phalaris minor* and *Malva parviflora*) associated with lupine plants. Results show that the dry weight of the two weeds under investigation was significantly reduced by all applied aqueous extracts (either radish root or leaf). Leaf aqueous extract was more effective than root aqueous extract in controlling weeds. It is worthy to mention that the two mixed applied herbicides gave complete eradication of both weeds. In addition, all applied aqueous extracts significantly increased growth, photosynthetic pigments, seed yield and yield components of lupine plants. The inhibitory effect of radish aqueous extracts on weeds or its stimulatory effect on lupine plants was increased by increasing concentration of the extracts. The leaf aqueous extract and root aqueous extract at 4.5% were the most optimum treatments in increasing growth and seed yield of lupine accompanied by the highest decrease in weed dry weight relative to unweeded treatment. The results suggested using the aqueous extracts of radish leaf and root especially at highest concentration (4.5%) in controlling weeds associated with lupine plants.

Keywords: Allelopathy, weed control, bio-herbicides, lupine, *Phalaris minor*, *Malva parviflora*, radish.

1. Introduction

Lupine (*Lupinus termis* L.) seeds are good source of nutritive contents (proteins, lipids, carbohydrates), and functional components (tocopherols, carotenoids, oligosaccharides, polyphenols, dietary fibers). Lupine is exploited for human consumption and animal feed as well as for pharmaceutical applications [1]. Lupine is a poor weed competitor owing to its slow vegetative growth and development. However, slow development enables light penetration, weed seeding growth and loss of lupine yield owing to competition [2].

Weeds are harmful for plant growth and development because of competition with the main crop for nutrients, light and water [3].Chemically synthesized herbicides are well known for their effectiveness in reducing weeds, but they can also cause crop damage, human and animal health issues, soil and water pollution, and herbicide resistance in weeds [4]. As a result, a novel approach is being developed to use bio-herbicides produced from plants as an alternative tool for crop development due to their environmental benefits over chemical herbicides [5]. Allelopathy is a strategy to minimize environmental pollution and weed resistance to herbicides. The use of allelopathy in agriculture is a very efficient strategy to decrease the detrimental effects of herbicides on the human health and the environment, as well as to eradicate the problem of herbicide resistance in plants [6]. Allelopathy is the release of allelochemicals (allelopathic substances) from various plant species into the environment through a variety of mechanisms such as leaching or volatilization from aerial parts, secretion from roots, and degradation of plant wastes in soil [7, 8]. Allelochemicals and associated breakdown metabolites may have a positive or negative impact on nearby plants' physiological functions, germination, growth, and development, based on concentration. [9-13]. A better understanding of how allelochemical substances can be used as plant defense strategies to protect and manage developing crops, limit the spread of invading weeds, maintain local plant stands, and develop strategies for

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allelochemical production and application as new pesticides [8]. Several researchers have used natural extracts of several plants to suppress weeds in crop plants [3, 14-18]. Since the Brassicaceae family is identified by the existence of glucosinolates, a sulfur-containing secondary metabolite group, so several members of the Brassicaceae family have been studied for allelopathic impact on plant seed germination and growth [19-22].

The concentration of glucosinolates in plants varies depending on the species, tissue type, developmental stage, and environmental conditions [23-24]. Moreover, myrosinase enzyme degrades glucosinolates to isothiocyanates (ITCs), which are responsible for the allelopathic action. In addition to ITC's, glucosinolate hydrolysis products include thiocyanates, nitriles, and oxazolidinethiones, as well as organic cyanides, which are highly reactive degradation products [25-27].

Other allelochemicals include phenolic acids and related compounds, which are the most prominent growth inhibitors produced by living plants or liberated by microbial action or leaching from decaying plant portions.

Radish (*Raphanus sativus L.*) is a member of the Brassicaceae family that can inhibit the growth of weeds and a variety of crops [22; 28-29]. Radish has an allelopathic impact on several studied plants [5; 30] owing to allelochemical substances as p-hydroxybenzoic acid and isothiocyanates (isothiocyanate benzyl, Isothiocyanateallyl) [28]. It was recorded that several agricultural crops and weed have been found to be sensitive toward extracts of radish plant [31-32]. Aqueous extract of radish roots contains vanilic acid and ferulic acid, according to Ali [5].Vanillic acid (4-hydroxy-3-methoxybenzoic acid) suppresses shoot and root of wheat, annual bluegrass and rice, as well as reduces lettuce germination and growth parameters [33-34]. Likewise, ferulic acid (4-hydroxy-3-methoxycinnamic acid) suppresses soybean germination and growth because of its inhibitory impact on protein synthesis [35]. The same effect was reported on cucumber and lettuce plants [36-37].

This study aimed to evaluate the bio-herbicidal effect of aqueous extract of the root and leaf of red radish on lupine plants and its associated weeds.

Materials and methods

Preparation of plant materials

Red radish *(Raphanus sativas* L.) plants were gathered from the Egyptian fields then thoroughly cleaned with tap water. Leaves and underground root portions were detached and left to dry at room temperature in shadow places for many days. To ensure complete dehydration, plant materials were put in furnance at 30°C and weight it regularly until achieves the constant weight. An electric mill was used to grind dried plant tissues into a fine powder.

Preparation of aqueous extract

One hundred grams from each dried plant material (leaves and roots) was placed in 2 L Erlenmeyer flask and 1L of distilled water was added to each. Two flasks were wrapped with parafilm and shaken at room temperature for 24 hours at 200 rotations per minute. Both prepared mixtures were filtered through a fine mesh and squeezed thoroughly for complete extraction. To obtain clear stock solutions, the two obtained filtrates were filtered again using Whatman No. 1 filter paper. Using distilled water, 1.5%, 3% and 4.5% (w/v) concentrations were made from each stock solution. The extraction process was repeated as needed to ensure that the extract was fresh.

Experimental procedure

Two pot experiments were performed throughout two winter seasons of (2019/2020) and (2020/2021) at the green house of National Research Centre, Giza, Egypt. The experiment was performed based on a complete randomized block design with six replicates. Clay pots (30 cm in diameter) were filled with equal quantity of sieved sandy-loam soil.

Seeds of lupine (cv. Giza1) were obtained from Agricultural Research Centre, Egypt. Six lupine seeds and weed seeds (0.1 g of *P. minor* and 0.3 g of *M. parviflora*) were sown at a depth of 2 cm from the soil surface in each pot. In this study, nine treatments were performed including an unweeded treatment, a lupine-free weed treatment, and two mixed herbicidal treatments as controls. The other six treatments were 1.5%, 3% and 4.5% (w/v) aqueous freshly prepared extracts of both leaves and roots of red radish. Aqueous extracts were sprayed twice on lupine plants and its associated weeds using a hand sprayer at a rate of 50 mL pot^{-1} at 14 and 21 days after sowing (DAS).Two mixed herbicidal treatment of grass weed Topik 240 EC (Clodinafop-Propargyl) and broad weed Basagran (Bentazon 48 EC) were sprayed at the recommended rate 140 g fed^{-1} and 1 L fed^{-1} respectively at 30 DAS. All treatments were kept under greenhouse conditions and all cultural practices were applied especially irrigation and fertilization.

Recorded data

Weeds

Three replicates from each treatment were collected at 45 DAS and at the harvest. Weeds were dried in an oven at 40°C for 48 h to record dry biomass (g pot^{-1}).

Vegetative growth parameters of lupine

Three replicates of lupine were collected from each treatment at 45 DAS to determine the vegetative growth parameters (plant height, number of leaves/plant as well as fresh and dry biomass/plant).

Yield and yield attributes

At harvest, three replicates of lupine plants were collected from each treatment to determine plant height, number of pods/plant and seeds weight/plant.

Chemical analysis

Glucosinolate content of either leaf or root of red radish tissues was determined according to method described by Nasirullah and Krishnamurthy [38]. Photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids) in fresh leaf tissues of lupine at 45 DAS were estimated as the method reported by Moran [39].

Statistical analysis

The experiments were carried out in a complete randomized block design. All obtained data were subjected to proper statistical of variance according to Snedecor and Cochran [40].

Results

First of all, it is worthy to mention that glucosinolate content of root and leaf radish was 96.21 and 152.76 umole/g dry weight respectively. Table 1 shows that aqueous extract of both root and leaf parts at different concentrations significantly reduced dry weight of both weeds at 45 DAS and at harvest. Aqueous leaf extract was more effective in reducing both weeds than aqueous root extract. It was observed that the inhibitory effect of aqueous root extract and aqueous leaf extract was increased by increasing concentrations. The most pronounced treatments in decreasing weeds dry weight are aqueous leaf extract and aqueous root extract at 4.5%. Since, at 45 DAS, aqueous leaf extract at 4.5% reduced broad weed by 75.16% and narrow weed by 55.32 % relative to unweeded treatment. Whereas, aqueous root extract at 4.5% reduced broad weed by 62.5% and narrow weed by 51.06% relative to unweeded treatment. Furthermore, at harvest, aqueous leaf extract at 4.5% reduced broad weed by 82.33% and narrow weed by 80.91% relative to unweeded treatment. Whereas, aqueous root extract at 4.5% reduced broad weed by 69.98% and narrow weed by 73.71% relative to unweeded treatment. It is worthy to mention that the two mixed applied herbicides gave complete eradication of both weeds.

Table (2) shows that all applied treatments caused significant increases in plant height, fresh and dry weight of plant at 45 DAS relative to unweeded treatment. It was noted that aqueous leaf extract was more pronounced than aqueous root extract in increasing all growth parameters under investigation than unweeded treatment. The most effective treatment was 4.5% aqueous leaf extract followed by 4.5% aqueous root extract. All applied treatments were more effective than the two mixed herbicides except 1.5% water root extract.

Table 1. Effect of aqueous extract of red radish on dry weight of associated weeds with lupine

Treatments	At 45 DAS		At harvest	
	M. parviflora	*P.minor*	*M. parviflora*	*P.minor*
Lupine only	**0.0**	**0.00**	0.00	0.00
Mixed herbicides	**0.00**	**0.00**	0.00	0.00
Unweeded	**17.6**	**4.7**	**40.25**	**44.27**
Root extract at 1.5%	**8.4**	**3.9**	21.94	22.86
Root extract at 3.0%	**7.4**	**2.8**	15.45	16.28
Root extract at 4.5%	**6.6**	**2.3**	**12.08**	**11.64**
Leaf extract at 1.5%	**8.2**	**3.4**	20.11	13.05
Leaf extract at 3.0%	**5.6**	**2.7**	10.42	12.8
Leaf extract at 4.5%	**4.9**	**2.1**	**7.11**	8.**45**
LSD 0.05	**1.94**	**1.15**	**3.15**	**2.99**

Table 2. Effect of aqueous extract of red radish on growth parameters of lupine

Treatments	**Plant height (cm)**	**Leaves number/plant**	**Plant fresh weight (g)**	**Plant dry weight (g)**
Lupine only	**28.3**	**8.30**	**14.81**	**2.83**
Mixed herbicides	**26.0**	**6.66**	**9.60**	**1.43**
Unweeded	**20.30**	**6.00**	**7.83**	**0.95**
Root extract at 1.5%	**24.00**	**6.99**	**9.49**	**1.17**
Root extract at 3.0%	**26.10**	**7.16**	**10.43**	**1.47**
Root extract at 4.5%	**27.30**	**7.83**	**13.96**	**2.32**
Leaf extract at 1.5%	**26.66**	**7.00**	**11.94**	**1.57**
Leaf extract at 3.0%	**27.00**	**7.50**	**13.38**	**2.23**
Leaf extract at 4.5%	**29.66**	**8.33**	**14.20**	**2.53**
LSD 0.05	**2.23**	**1.20**	**1.05**	**0.15**

It was noted that all applied treatments caused significant increases in all components of photosynthetic pigments (Table 3) relative to unweeded treatment. Aqueous leaf extract and aqueous root extract at 4.5% were the most effective treatment in increasing total photosynthetic pigments than the two mixed herbicides. Aqueous leaf extract at 4.5% was the most pronounced treatment, since it increased total photosynthetic pigments by 127% followed by aqueous root extract at 4.5% that increased total photosynthetic pigments by 120 % relative to unweeded treatment. Whereas, two mixed herbicides increased total photosynthetic pigments by 98% relative to unweeded treatment.

Table 4 shows that all applied treatments caused significant increases in lupine seed yield and its components relative to unweeded treatment. Aqueous leaf extract at 4.5% was more effective in increasing seed yield by 132.54% followed by aqueous root extract at 4.5% that increased seed yield by 94.23% relative to unweeded treatment. It is worthy to mention that the effect of aqueous leaf extract and aqueous root extract at 4.5% were more pronounced than the two mixed herbicides that increased seed yield by 54.57% relative to unweeded treatment.

Table 3. Effect of aqueous extract of red radish on photosynthetic pigments (mg/g fresh leaf tissues)of lupine

Treatments	Chlorophyll a	Chlorophyll b	Carotenoids	Chlorophyll a+ Chlorophyll b	Total photosynthetic pigments
Lupine only	1.79	0.48	0.31	2.27	2.63
Mixed herbicides	1.42	0.34	0.22	1.76	1.98
Unweeded	0.71	0.19	0.096	0.91	1.00
Root extract at 1.5%	1.44	0.33	0.21	1.77	1.98
Root extract at 3.0%	1.52	0.36	0.26	1.88	2.14
Root extract at 4.5%	1.54	0.40	0.26	1.94	2.20
Leaf extract at 1.5%	1.43	0.32	0.22	1.75	1.99
Leaf extract at 3.0%	1.47	0.35	0.24	1.82	2.06
Leaf extract at 4.5%	1.54	0.46	0.27	2.00	2.27
LSD 0.05	0.30	0.05	0.01	0.35	0.36

Table 4. Effect of aqueous extract of red radish on seed yield an yield components of lupine

Treatments	Plant height (cm)	Pods number/plant	Weight of seeds /plant (g)
Lupine only	49.33	6.66	6.13
Mixed herbicides	46.66	4.33	4.56
Unweeded	32.66	3.00	2.95
Root extract at 1.5%	42.66	3.00	4.74
Root extract at 3.0%	42.00	5.33	4.92
Root extract at 4.5%	45.66	6.33	5.73
Leaf extract at 1.5%	43.66	4.33	4.20
Leaf extract at 3%	45.33	4.33	5.44
Leaf extract at 4.5%	49.33	6.66	6.86
LSD 0.05	2.51	1.06	0.99

Discussion

There have been significant efforts in developing weed management approaches that use allelopathic substances as bio-herbicides to repress undesirable weeds due to concerns environmental, and health problems, as well as an increase in the number of herbicide-resistant weeds as a result of using synthetic herbicides. Bio-herbicides are also easily biodegradable, making them much safer than synthetic chemical herbicides [41-42].

The results in Table 1 reveal that the two mixed herbicides completely eradicated both of the weeds under investigation. Regarding powerful effect of two mixed chemical herbicides (Topik for controlling grassy weeds and Basagran as a member of the benzothiadiazole group of herbicides for controlling broad weeds), Topik interacts with [acetyl co-enzyme A carboxylase (Accase)] and inhibits it, which is necessary for the synthesis of lipids (fatty acids) required for plant growth [43]. Whereas, Basagran inhibited photosynthesis II [44]. These results are consistent with those of Baghestani et al. [45] and Aboali and Saeedipour [46], who revealed that Basagran provides a significant increase in crop production in relation to its weed control spectrum. Several studies have demonstrated that controlling weeds by various types of herbicides decreased weed/plant competition and consequently, enhanced product income [47-49]. It is well known that the bio-herbicides extracted from plants act as an alternative tool for crop production due to its eco-friendly characteristics when compared to chemical herbicides [3,50].

On the other hand, root and leaf aqueous extract of red radish at all concentration caused significant decreases in dry weight of two weeds if compared to harmful effect of synthetic chemical herbicide (Table 1). Water leaf extract was more effective in reducing both weeds than water root extract. These results may be attributed to the level of glucosinolates, since radish leaves were characterized by higher value of glucosinolate rather than radish root. Chon et al. [51] mentioned that the existence of several causative allelochemicals in plant natural extracts may be responsible for the prevention of weed growth. According to previous research, leaves and roots of radish are considered the main sources of allelochemicals [5; 28; 30]. Likewise, some studies have found that the leaves are the organs with the highest concentration of allelochemicals and hence have a greater ability to inhibit weed seedling development [52]. Radish has the ability to suppress the growth of weeds and a number of crops [5; 22;28-30] due to its allelochemicals compounds such as p-hydroxy benzoic acid and isothiocyanates (isothiocyanate benzyl, Isothiocyanateallyl) [28].The decreases in dry weight of weeds under the effect of aqueos extract of either root or leaf of red radish may be due to the efficient role of various allelochemicals in red radish via exerting different mechanisms such as reduction in mitotic activity, photosynthesis, nutrient uptake, permeability of cell membrane, assimilation rate as well as inhibition of enzyme action and protein formation [6; 9; 53]. Allelopathic compounds may have a suppressive effect at high concentrations, but a promoting effect at low concentrations, acting on the enzyme activity and/or physiological activity of the plant and enhancing plant metabolism activity [54]. According to Filho [55], the level of suppression provided by allelochemicals varies depending on their concentration, at low concentrations could not be inhibitors for certain species or even having a stimulant effect, while at high concentration may be highly inhibitory. Norsworthy [31] indicated that wild radish aqueous extract or incorporated residues (or both) suppress seed germination, radical growth, seedling emergence and seedling growth of certain crops and weeds and these responses are attributed to an allelochemical effect. Moraes et al. [56] observed that the extract of *Raphanus sativus*, canola (*Brassica napus*) and arrow leaf clover (*Trifolium vesiculosum)*, had an allelopathic influence on the germination and early development of blackjack plantlets (Bidenspilosa). Ali [5] revealed that radish root portions contain powerful biochemicals that can be extracted, purified to suppress or eradicate the prevalence of noxious weeds in crop fields without causing pollution or environment damages compared to synthetic herbicides due to its eco-friendly characteristics. Different concentrations 5%, 10%, 15% and 20% aqueous extracts of radish imposed herbicidal effect on studied weed plants (*Avina fatua ,Phalaris minor* and, *Brassica napus*). In a field experiment, incorporated roots of *R. sativus* or whole plants resulted in a significant reduction of the numbers of shoots throughout the season. At comparable rates, whole plants had a stronger inhibitory effect than roots alone. The number of weed shoots was reduced to >50% at all treatments with *R. sativus* compared to controls [57].

Regarding lupine plant, it was noted from data recoded in Tables 2, 3 and 4, that all aqueous extracts of root and leaf of radish caused significant increases in growth parameters, photosynthetic pigments, seed yield and its components. Sometimes an allelochemical substance produced by one organism or plant is harmful or beneficial to another organism or plant [58-61]. In this regard, the allelochemicals may be acting directly on the metabolism of the target plant, impacting the cellular structure, photosynthesis, hormonal management, electron transport chains, membrane permeability, nutrients availability, enzyme activity, DNA and protein synthesis, among other changes [29;62-63]. According to Maraschin-Silva and Aquila [64], the presence of allelochemicals in plant extracts could restrict or stimulate the plantlets growth, which caused changes in membrane structure, absorption of nutrients and water, photosynthetic activity and respiration. Rigon et al. [65] studied the allelopathic effect of aqueous extracts of castor leaves on radishes and noted that extracts at low concentrations stimulated the initial development of seedlings development. According to the authors, this occurs as a defense mechanism of the seedlings; it increased cell division, and there is an increased sensitivity of the tissues.

It could be concluded that using water extract of either leaf or root of red radish and two mixed chemical herbicide treatments led to good control of weeds associated with lupine plant which consequently minimized weed competition and increased lupine growth, seed yield and yield attributes.

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