

Research Article

Allelopathic activity of *Cyclamen rohlfsianum* on germination and growth of two *Vicia faba* cultivars

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Abstract

Cyclamen rohlfsianum belonging to Primulaceae family, a medicinal herb tuberous, and is documented as a logo for Libyan Flora Encyclopedia, However, the allelopathic activities of this plant yet have not been examined. So, the present study was done to evaluate the allelopathic activity of *C. rohlfsianum* (Tubers-Leaves) aqueous extracts was tested in a dishes experiment at concentrations of 10, 15 and 20% , and as crude powder in soil at concentrations of 2, 4 and 8% in pots experiment on seeds germination and seedlings of two *Vicia faba* cultivars (Local and Massa). The results of a dishes experiment showed highly significant differences in the reduction of germination percentage, and reduction of radicle and plumule lengths for two cultivars compared with a control. The results of a pot experiment showed also reduced emergence percentages of seedlings, root and shoot systems, and fresh weights. Most of the concentrations used caused a decrease of content chlorophyll (a, b, and Total pigments) and an accumulation of carotenoids of two *V.faba* cultivars. The tubers had the most toxicity in inhibiting, while Massa cultivar is the most sensitive.

Introduction

Weeds are among the most important pests that hinder access to high-quality agricultural production [1], consequences effects caused by these weeds, especially wild ones a direct or indirect effect around agricultural fields known as allelopathy, by producing chemical compounds that leak into the environment, Allelochemicals is weed metabolites products, which may affect plants at different stages of growth, such as seed germination, seedling growth, flowering, and fruiting [2; 3 ;4]. Allelochemicals affect the germination and growth of neighboring plants by disrupting various physiological processes including photosynthesis, respiration, mineral nutrition, and hormonal balance {Formatting Citation} . *Vicia faba* L. is one of the most important leguminous crops in Libya, being a cheap source of protein, and minerals, in addition to its contribution to soil fertility [6]. However, the *Vicia faba* crop is subjected to many biotic and abiotic stresses, especially allelopathic stress [1] . *Cyclamen rohlfsianum* an endemic strain, of the most famous native plants in Al-Jabal Al-Akhdar region, belongs to the Primulaceae family, known locally as Al-Rakhf, perennial tuberous herbaceous

Materials and Methods

- Sample Collection

Samples of *Cyclamen rohlfsianum* (leaves-tubers) were collected from Al-Bayda city, it is defined to classified according to (7) ,washed with distilled water, then dried under natural conditions, grind with an electric grinder, and finally preserved for use.

- Petri dish Experiment

Seed Selection

The homogeneous seeds of *Vicia faba*, were selected, cleaned of impurities, and viability was tested by soaking in distilled water to get rid of empty seeds floating on the surface, soaked in 1% of sodium hypochlorite solution

plant, documented as a logo for Libyan Flora Encyclopedia ,[7] . Also, allelochemical interactions with *Vicia faba* have been documented in a variety of Weeds, especially medical. for example, a study done in Libyan showed that *Arum cyreniacum* added to soil has negative effects on root and shoot system lengths, fresh and dry weights, chlorophyll (a,b), and carotenoids, from *Vicia faba* [7] . In addition, a study done in Saudi Arabia revealed that chloroform and methanol extracts of the *Citrullus colocynthis*, did not have an inhibitory effect on seed germination, but it reduced the leaf surface area and carbohydrates content and led to the accumulation of proteins of *Vicia faba* [8] . On the other hand, the toxic mutagenic effect of *Zygophyllum simplex* L. extract on *Vicia faba* germination was also confirmed. . The purpose of this study was to see if aqueous extracts of *Cyclamen rohlfsianum* leaves and tubers at different concentrations (10, 15 and 20%), and adding the crude powder to the soil at a concentration of (4, 6 and 8 %), (tubers-leaves) had an allelopathic activity on the germination of seeds and the development of seedlings of *Vicia faba* cultivars (Local and Massa).

for 3 minutes and washed with distilled water [10].

Preparation of the Aqueous Extract

The aqueous extract (leaves- tubers) was prepared separately by adding 200 g of air-dried powder to 1000 ml of distilled water for 24 h, after that the extract was filtered through filter paper and placed on a Shaker for 24 hours. Then it was centrifuged at the speed of 2000 rounds per minute for 15 minutes. The extract was passed through Whatman filter paper No.1. The obtained extract concentration was considered as the stock solution (20%) [11]. Then it was appropriately diluted with distilled water to give final concentrations of 10, 15, and 20%.

Seed germination:

Normally, 10 seeds per Petri dish, were lined with two Whatman No.1 filter papers, incubated at room temperature, three replications for each treatment, dishes were subjected to daily observation for 10 days and follow-up of germination in terms of addition of extracts to the treated dishes. Distilled water was added to control as needed for each dish [12], germination was calculated by recording a number of germinated seeds in all treatments starting from the second day, which the first germination occurred, germination criterion is the appearance of radical outside seed cover [13]. At the

- Pots Experiment

The soil samples were finally sterilized at (90°C for 48h) to remove any microorganisms and weed seeds. Ten seeds of *Vicia faba* cultivars (local - Massa) were sown in plastic pots (16 cm in diameter) in pure culture practices with about 1000 g of sandy loam soil thoroughly mixed (w/w) with 2, 4, 8 % of electrically crushed crude powder of leaves and tubers of *C. rohlfsianum*. One treatment was run as a control with zero percent of crude powder with three replicates. The plants were watered every two days on average with normal tap water. The experiment was performed under normal laboratory conditions. After 21 days, the homogenous seedling was carefully collected from each treatment, washed with tap water to remove

Statistical Analysis:

The Experiments were designed according to the completely randomized design (CRD). The statistical analysis was performed using

experiment, results of following traits were taken:

- 1- Germination percentage (%) = number of germinated seeds / total number of seeds × 100 [14].
- 2- Mean germination time = the total number of germinated seeds per day / total number of germinated seeds at end of the experiment [15].
- 3- radical and plumule lengths: The root and plumule lengths were taken using a graduated ruler, the averages were calculated by taking 5 seedlings from each plate.

the adhering soil particles, and then, by distilled water, gently blotted with filter paper, recorded the final results .

- Seedling emergence rate (%) = (number of emerged seedlings/total number of seeds) × 100 [16].
- Shoot and Root Length (cm): Stems and roots were separated, and measurements were taken using a graduated ruler, the averages and calculations were sampled in three replicates.
- Fresh weight of seedling (g): The weight of the seedlings of each pot was taken and the averages were taken.
- photosynthetic pigments (chlorophyll a , b and carotenoids) were determined spectrophotometrically according to [17].

(Minitab 17) program and ANOVA variance analysis tables were used to compare averages with the least significant difference (LSD) at 0.05.

Results:

The results shown in Table (1) indicated that there were highly significant differences between *Cyclamen rohlfsianum* extracts and the tested concentrations on the growth of two *Vicia faba* cultivars (Local and Massa), in reducing germination percentage, delay means germination time, reducing radical and plumule length, after 10 days from the beginning of the experiment compared with the control. The concentration of 10% for the local cultivar recorded a germination rate of (93, 60%), and (70, 40%) for the cultivar Massa of leaves and tubers extract, respectively, compared to the control. The rates of decline increased with the increase in concentration, as a concentration recorded

15% for the Local cultivar germination rate of (56, 20%), and (40, 13%) for the cultivar Massa, for leaves and tubers extract, respectively, compared to the control. This was followed by an apparent delay in mean germination time, at rates that ranged between (4.2 - 6 days), for the two *Vicia faba* cultivars, all this, in turn, led to a decrease in radical lengths at rates ranging from (1.0 - 3.1 cm) compared to the control, for the two cultivars, also observed that no appearance of the plumule was recorded except for a 10% concentration of leaves extract at a rate of (2.9 -2.0 cm) for the two cultivars local and Massa, respectively, compared to the control. Finally, the 20% leaves and tubers extract concentration did not show any seed germination of *Vicia faba* cultivars.

Table (1): Effect of *Cyclamen rohlfsianum* extracts on seeds germination of *Vicia faba* cultivars

cultivars	Conc. (%)	Germination (%)		Mean Germination time		Radical length (cm)		Plumule length (cm)	
		leaves	tubers	leaves	tubers	leaves	tubers	leaves	tubers
Control		100.0		2.6		6.4		5.2	
Local	10%	93.3	60.0	4.8	4.3	3.1	1.5	2.9	0.0
	15%	56.6	20.0	5.7	5.5	1.8	0.0	0.0	0.0
	20%	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Control		100.0		2.9		4.2		4.1	
Massa	10%	70.0	40.0	4.5	4.2	2.5	1.3	2.0	0.0
	15%	40.0	13.3	6.0	5.6	1.0	0.0	0.0	0.0
	20%	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
L.S.D		3.2537		0.4163		0.1150		0.0614	

As shown in Table (2) and Fig. (1, 2) the results of adding crude powder (leaves, tubers) of *C. rohlfsianum* to soil at a concentration (2, 4 and 8%) on seedling emergence, Shoot and root length, and fresh weight of two *Vicia faba* cultivars after 21 days from agriculture at pots. It was found that the concentration of crude leaves powder (2 and 4%) had no significant effect on the seedling emergence rate of a Local cultivar, while this proportion for the Massa cultivar decreased to (70, 50%), respectively previous

concentrations. Increasing leaves powder in the soil to 8% increased seedling germination inhibition to (50, 20%) for two cultivars, Local and Massa, respectively. The data of adding crude tubers powder also recorded the highest inhibition rates, which amounted to (80, 60 and 20%) for the Local cultivar and (60, 20 and 10%) for the Massa cultivar with a concentration of 2, 4, and 8%, respectively. The results also indicate a decrease in shoot and root length, and fresh weight of two cultivars of *V. faba* (Local and Massa).

Table (2): Effect of *Cyclamen rohlfsianum* (Crude powder) on growth rates of *Vicia faba* cultivars at pots.

cultivars	Con (%)	Number seedling (%)		Shoot length (cm)		Root length (cm)		Fresh weight (g)	
		leaves	tubers	leaves	tubers	leaves	tubers	leaves	tubers
	Control	100.0		26		22		62.8	
Local	2%	100	80	21	16	17	11	53.8	33.6
	4%	100	60	17	11	13	7	41.5	15.4
	8%	50	20	12	6	6	3.5	13.2	7.0
	Control	100.0		23		18		48	
Massa	2%	70	60	18	12	14	12	39.0	21.0
	4%	50	20	13	5	9	6	23.9	11.1
	8%	20	10	5	3	4	2	8.2	5.0
	L.S.D	4.8612		1.1585		0.7778		0.8696	

As shown in table (3), the results of an effect of adding the dry crude powder of *C. rohlfsianum* (tubers - leaves) to soil at a concentration (2, 4 and 8%) on the content of chlorophyll (a, b, carotenoids and Total pigments) of two cultivars of *Vicia faba*

(Local and Massa), where the results showed a decrease of content chlorophyll (a, b and total pigments) and an accumulation of carotenoids of two *Vicia faba* cultivars leaves, compared to the control.

Table (3): Effect of *Cyclamen rohlfsianum* (Crude powder) on content of photosynthetic pigments of *Vicia faba*.

cultivars	Conc. (%)	Chlorophyll a mg/g		Chlorophyll b mg/g		carotenoids mg/g		Total pigments	
		leaves	tubers	leaves	tubers	leaves	tubers	leaves	tubers
		Control	13.7		11.5		2.3		27.5
Local	2%	11.5	6.3	7.4	3.7	3.2	3.9	22.1	13.9
	4%	8.2	3.4	5.9	3.0	3.3	4.0	17.4	10.4
	8%	6.0	1.6	4.0	2.5	3.0	4.5	13.0	8.6
Control	9.8		10.5		2.0		22.3		
Massa	2%	9.0	6.4	6.0	4.0	2.2	3.6	17.2	14.0
	4%	5.7	2.0	3.5	1.1	3.0	3.9	12.2	7.0
	8%	2.5	0.9	2.0	0.5	3.1	4.2	7.6	5.6
L.S.D	0.3765		0.3012		0.1609		0.4957		



Fig (1): Effect adding of a Crude powder of *Cyclamen rohlfsianum* on growth rates of *Vicia faba* (Local).



Fig (2): Effect adding of a Crude powder of *Cyclamen rohlfsianum* on growth rates of *Vicia faba* (Massa).

Discussion

Weeds stunt crop growth by competing with plants for water, nutrients, and sunlight, causing significant losses in crop production. In addition to its allelopathic negative effects [18; 19]. So this study was conducted to test allelopathic effects of aqueous extracts and the crude powder added to the soil from *Cyclamen rohlfsianum*. which showed that the aqueous extracts of (leaves and tubers) have an allelopathic effect against germination rates of *Vicia faba*. This result agreed with the previous findings [8] [20] Where allelopathic compounds present in the aqueous extracts of many wild weeds had high toxicity against growth *V. faba*. The powder added to the soil also had negative effects on the growth of *V. faba* seedlings, and of a decrease of content chlorophyll a, b and Total pigments, and

Conclusions

It can be concluded from this study that *C. rohlfsianum* has clear allelopathic effects

accumulation of carotenoids of two *V. faba* cultivars. These results were supported by [21] ,who found inhibition on germination and seedling growth of *V. faba* when using *Arum cyreniacum* powder added to the soil, through various mechanisms such as decreased mitotic activity, decreased ion uptake rate and inhibition of photosynthesis. The results also showed that tubers are more toxic than leaves, possibly because they contain phenolics, triterpenoids, saponins, and steroidal compounds. in addition Kaempferol, Genistein, Hesperetin, Oleanolic acid and 7, 8, 4-Trihydroxyflavone [22] . In general, Massa cultivar was found to be the most sensitive cultivar to allelopathic stress, this may be due to the different genomic properties of the cultivars [23].

against seed germination and seedlings growth of *Vicia faba*. The tubers were the most toxic than leaves, so , excluding of *C. rohlfsianum* and limiting its spread near *V. faba* crops is recommended.

References

1. Alaila A.K. Allelopathic Effects of *Arum cyreniacum* on Germination and Growth of Two Varieties of *Vicia faba*. Al-Mukhtar J Sci. 2022;37(1):57–67.
2. Scavo A, Restuccia A, Mauromicale G. Allelopathy: principles and basic aspects for agroecosystem control. Sustain Agric Rev 28 Ecol Agric. 2018;47–101.
3. Hayyat M.S., Safdar M.E, Asif M, Tanveer A, and Ali L. Allelopathic effect of waste-land weeds on germination and growth of winter crops. Planta daninha. 2020;38.
4. Shekari , F., Shekari, F., Najafi ,J., Abassi, A., Radmanesh ,Z.,and Bones A.M. Phytotoxic Effects of Catnip (*Nepeta meyeri* Benth.) on Early Growth Stages Development and Infection Potential of Field Dodder (*Cuscuta campestris* Yunck). Plants. 2022;11(19):2629.
5. Kengar Y.D. Allelopathic Potentiality of *Celosia argentea* L Review. Curr Investig Agric Curr Res. 2018;1(2):36–9.
6. El-Ammari ,A. Plant Fungal Diseases of Faba bean in Benghazi [Version 1; awaiting peer review]. ContROL. 2017;1:15.
7. Abdulrazziq A.A, and Salih S.M. Activity of *Cyclamen rohlfsianum* against two Species of *Xanthomonas*. Sci J Fac Sci Univ Vol. 2022;2(1):71–5.
8. Salama H.M, and Al Rabiah, H.K. Physiological effects of allelopathic activity of *Citrullus colocynthis* on *Vicia faba* and *Hordeum vulgare*. Eur J Biol Res. 2015;5(25):e35.
9. Haroun S.A, and Abualghaith A.S. Evaluation of the Allelopathic Effect of Aqueous Extract of *Zygophyllum simplex* L. on *Vicia faba* L. Plants. Cytologia (Tokyo). 2015;80(3):363–71.
10. Dafaallah ,A.B, Mustafa W.N, and Hussein Y.H. Allelopathic effects of jimsonweed (*Datura Stramonium* L.) seed on seed germination and seedling growth of some leguminous crops. Int J Innov Approaches Agric Res. 2019;3(2):321–31.
11. Al-Hawas ,G.H.S, and Azooz ,MM. Allelopathic potentials of *Artrmisia monosperma* and *Thymus vulgaris* on growth and physio-biochemical characteristics of pea seedlings. Pak J Biol Sci. 2018;21(4):187–98.
12. Othman, B., Haddad, D., and Tabbache, S. Allelopathic effects of *Sorghum halepense* (L.) Pers. and *Avena sterilis* L. Water extracts on early seedling growth of *Portulacca Oleracea* L. and *Medicago sativa* L. Int J Med Sci. 2018;5(10):7–12.
13. Ganatsas, P., Tsakaldimi ,M., and Thanos, C. Seed and cone diversity and seed germination of *Pinus pinea* in Strofylia Site of the Natura 2000 Network. Biodivers Conserv. 2008;17:2427–39.
14. Yousif M.A.I, Wang ,Y.R, and Dali ,C. Seed dormancy overcoming and seed coat structure change in *Leucaena leucocephala* and *Acacia nilotica*. Forest Sci Technol. 2020;16(1):18–25.
15. Das, M., Sharma ,M, and Sivan, P. Seed germination and seedling vigor index in *Bixa orellana* and *Clitoria ternatea*. 2017;
16. Huang ,S., Jia ,Y., Liu ,P., Dong, H., and Tang, X. Effect of ultrasonic seed treatment on rice seedlings under waterlogging stress. Chil J Agric Res. 2020;80(4):561–71.
17. Metzner, H., Rau, H. and Senger, H. Untersuchungen zur synchronisierbarkeit Einzelner Pigmentmangel-mutanten Von chlorella. planta (berl). 1965;65(bd 2 h):186–94.
18. Monteiro, A., Santos, S. Sustainable approach to weed management: The role of precision weed management. Agronomy. 2022;12(1):118.
19. Zhu, X., Li, W., Shao ,H., and Tang ,S. Selected aspects of invasive *Solidago canadensis* with an emphasis on its allelopathic abilities: a review. Chem Biodivers. 2022;19(10):e202200728.
20. Elqahtani ,M.M., El-Zohri, M., Galal, H.K., and El-Enany ,A.E. GC-MS analysis of crude extracts from *Heliotropium bacciferum* L. and their allelopathic effects on *Zea mays* L. and *Vicia faba* L. Allelopath J.

- 2017;41(1):51–64.
21. Salih,S. M., Alaila, A . and Abdulrazziq A.A. Allelopathy effect of aqueous extracts of *Arum cyreniacum* on germination of seeds of some leguminous crops. Libya J bayan. 2022;(11):175–80.
 22. Elabbar, F.A, Habel ,A.M., Bozkeha, N.M.A, and Awina, T.M. Isolation and Identification of Some Compounds From *Cyclamen Rohlfsianum* (Primulaceae) From Libya. Sci Rev Chem Commun. 2014;4(1):1–10.
 23. Laizer, H.C., Chacha, M.N., and Ndakidemi, P.A. Allelopathic effects of *Sphaeranthus suaveolens* on seed germination and seedling growth of *Phaseolus vulgaris* and *Oryza sativa*. Adv Agric. 2021;2021:1–9.