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The Allelopathic Effect Of The Mentha Longifolia Aqueous Extract On The Germination And Growth Of Cicer Arietinum L

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1. Abstract

In this study an experimental investigation has been conducted at the Diyala University / Biology department laboratories to investigate the allelopathic effect of the Mentha longifolia cold aqueous extract on the germination and grow of the Cicer arietinum L. plant. The study was performed according to the Completely Randomized Design (C.R.D) with three replications. The Cicer arietinum L seeds were first sterilized with sodium hypochlorite solution (1%), before they were planted in Petri dishes, and soaked in different concentration of the Mentha longifolia aqueous extract (0, 0.5, 1, and 1.5 g/l). Special detection methods were used to detect the secondary metabolic compounds, such as alkaloids, phenols and tannins, in the Mentha longifolia extract. The results revealed reduction in all of the Cicer arietinum L. considered properties due to the treatment with the Mentha longifolia aqueous extract. The reduction is significant at higher Mentha longifolia extract concentration; maximum reduction, in most of the considered properties, was reported when the concentration was 1.5 g/l. However, the reduction was not significant in all of the investigated properties except the dry weight of the radicle. This property showed maximum reduction at 1.5 g/l concentration, which represents the lowest average (0.0086 g) compared to the control treatments that demonstrated average minimum reduction of 0.0159 g.

Keywords: Mint**,** Chickpea**,** Secondary Metabolites, Allelochemicals, Allelopathy

2. Introduction

The term “Allelopathy” includes the plants’ or microbes’ positive and negative effects on other living organisms by means of chemicals (Hickman et al., 2021). These chemicals are secondary metabolites called allelochemicals, they have a significant role in the ecosystem balancing (Muhammad and Majeed, 2020). Roots and leaves are the main parts that contain the Allelopathic compound, though, this compound also exist in other parts of the plants (Rietveld, 1983). Scholars have considered the Allelopathy role, and reported the Allelopathic effect on the crops growth. The studies also revealed that these secreted substances may affect the plants their self or other neighboring plants. Moreover, their effect is not always inhibiting, but sometimes they exhibit growth motivational effect (Thiébaut et al., 2019).

Cicer arietinum L. is an important food crops, it is part of the Fabacea family, and it plays important role in soil fertilizing due to its contribution to nitrogen fixation with the help of streptococcus bacteria. It is an old crop that it has been grown in dry and semi-dry regions of more than 50 countries, mainly in Asia, Africa, Europe, Australia, North and South America (Rani et al., 2020). It has been grown for more than 7000 years, and it represents the second largest legume crop, after beans, in terms of consumption in Iraq (Singh and

Saxena, 1999). Its average production in Iraq in 2020 was 250 Kg/acres (Central Statistical Organization of Iraq ,2020).

Cicer arietinum L. seeds are of high nutritional value, they are good source of carbohydrates, essential amino acids, protein, many water-soluble vitamins, and minerals (Dhankhar et al., 2021). Its seeds, also, contain a variety of bioactive compounds that show anti-oxidant, anti-diabetic, and anti-inflammatory properties (Ramadhani et al., 2020).

Mentha longifolia is one of the most important aromatic perennial plants; it belongs to the Lamiaceae family, and it is widely distributed worldwide especially in the tropical and subtropical regions. Many species of the Mentha genus are described as industrial crops and a good source of essential oils. Worldwide, India is the number one in mint oil production followed by China and Brazil. The useful parts of the mint plant are the floral tops and leaves, due to the importance of its oil, among other oils, it is considered as number one plant from which the oil is extracted (Brahmi et al., 2017). Phytochemical analyses revealed that the Mentha longifolia contains many secondary metabolic compounds, such as menthol (50%), menthone, terpenes and terpene derivatives, tannins, nicotinic acid, flavonoids and their glycosides to which the biological activity of the mint plant is attributed (Hanafy et al., 2020).

Due to the increased population and the lack of the leguminous crops production, including Cicer arietinum L., compared to market requirements, it has become essential to investigate the effect of some plants that contains effective substances, such as mint, on the germination and growth of this crop. Therefore, this study was conducted to determine the allelopathic effect of the Mentha longifolia extract on the germination and growth of Cicer arietinum L.

3. Materials and Methodology

3.1 Aqueous extract preparation

The experimental work was conducted at the plant laboratory at the biological sciences department at the College of Education for Pure Sciences during the autumn season of 2021. Mentha longifolia leaves were obtained from the local market at Diyala Governorate, they were thoroughly washed with tap water and then distilled water before they were prepared by drying and grinding according to (Harborne, 1984). Four concentrations of the Mentha longifolia extract (0, 0.5, 1, 1.5 g/l) were prepared with distilled water. Each concentrate was mixed separately by manual mixing for 30 minutes, then it was left for 1-hour before it was filtered for several times by three layers of gauze. After that, they became ready for Cicer arietinum L. soaking process.

3.2 Chemical reagents and diagnostic solutions for secondary compounds in the Mentha longifolia leave aqueous extract:

1. Saponins detection: according to Atlas et al. (1995)
2. Glycosides detection: according to Shihata (1951)
3. Tannins detection: according to Harborne (1984)
4. Resins detection: according to Atlas et al. (1995)
5. Alkaloids detection: according to Harborne (1984)
6. Phenols detection: according to Harborne (1984)

3.3 Preparation and sterilization of the treated seeds

Cicer arietinum L. seeds were brought from the local markets of Diyala Governorate. First, affected ones were excluded before they were sterilized with 0.5% sodium hypochlorite and then washed with distilled water for several times (Joshi and Gupta, 1980). Then, they were soaked in the solutions for 24 hours before they were dried with air at 25°C laboratory temperature.

3.4 Seeds planting in Petri dishes

The experiment comprised 4 treatments, each treatment had 3 replicates. 10 cm Petri dishes were prepared by placing pieces of gauze in them before 10 Cicer arietinum L. seeds were put in each dish. After that, 20 ml of each of the previously prepared extracts was added to each dish according to its treatment, though for the control treatment only distilled water was added. Later, the dishes were closed and kept in the laboratory at 25°C. All of dishes were irrigated by adding 5 ml of the extracts. The experiment lasted for 10 days during which the following parameters were calculated:-

3.4.1 Germination percentage

After 10 days of the seeding, the number of germinated seeds was counted and the germination percentage was calculated according to Ellis and Roberts (1981) as given in Equation (1):

$$Germination\ percentage = \frac{The\ number\ of germinated\ seeds}{The\ total\ number\ of\ seeds} \times 100\% \quad (1)$$

3.4.2 Germination speed percentage

Throughout the planting period, the number of germinated seeds, in each dish, was calculated on a daily base, then the germination speed percentage was calculated according to Batish et al. (2001) as in Equation (2):

$$Germination\ rate\ factor\ percentage = \frac{The\ sum\ of\ (the\ number\ of\ germinated\ seeds\ on\ a\ given\ day \times The\ number\ of\ that\ day\ \ starting\ from\ \ germination\ day\ )}{The\ total\ \ number\ of\ germinated\ seeds} \times 100\% \quad (2)$$

The measurement the of the radicle and plumule average length

After 10 days of germination the length of all the growing plants was measured and the average was calculated according to (ISTA, 2008).

3.4.3 Measuring the average of fresh and dry weight of the radicle and plumule

After 10 days of the germination, the fresh weight of the plumule and radicle was measured using electric balance. Then, they were dried at 60-65 °C until the dry weight became stable. The dry weight was then measured for each of them and for the same plants, and the average was calculated as given in Shettel and Balke (1983).

4. Statistical analysis

The data were statistically analyzed using the variance analysis method for a factorial experiment in a completely randomized design (C.R.D) for a factorial experiment with three replications for each treatment. Duncan test was used to compare the mean values at 5% probability level, and the SPSS software was used to analyze the data (Mahmoud and Aziz, 1980) and (Duncan, 1955).

5. Results

Table 1 shows the existence of various active compounds in the cold aqueous extract of the Mentha longifolia leaves for which the biological activity is attributed. The detection results indicate the presence of saponins, tannins, alkaloids, phenols and resins, while the glycosides had no significant presence.

Table 1 The allelopathic compounds detected in the aqueous extract of the Mentha longifolia.

The allelopathic compounds	Detection result
Saponins	++
Glycosides	-
Tannins	++
Resins	+
Alkaloids	++
Phenols	++

+ : Indicates the existence of the compound in the Mentha longifolia extract

++ : Indicates the existence of the compound with high concentration in the Mentha longifolia extract

- : Indicates no existence for the compound in the Mentha longifolia extract

Table 2 show no significant differences between the averages of germination percentage and the germination speed percentage of the Cicer arietinum L. seeds, grown in laboratory conditions, due to the treatment with different concentrations of Mentha longifolia extract. The data also indicate a reduction in germination and germination speed percentages due to the treatment with different concentrations extract (0.5, 1, 1.5 g/l) compared to the control treatment (0 g/l). The treatment with 1.5 g/l extract caused highest reduction in the averages of these two properties. Averages of 46.666% and 2.276% were recorded for each of the germination percentage and the germination speed percentage. Whereas, the highest obtained averages were from the control treatment (0 g/l), which were 53.333% and 2.6000% for the two properties respectively.

Table 2 also depicts non-significant differences between the different concentrations in regard to the plumule and radicle length. The treatment with the 1 g/l extract gave the highest decrease in plumule length with an average of 0.763 cm. Though, the treatment with concentration 0.5 g/l gave the highest reduction in the radicle length with an average of 1.55 cm. However, control treatment gave the lowest reduction in both properties, and averages of 1.3267 cm and 2.1833 cm were recorded for the two properties respectively.

Table 2 The effect of the Mentha longifolia extract on the germination percentage and germination speed percentage (%) and the length of plumule and radicle (cm)

Used extract concentration (g/l)	Germination percentage (%)	Germination speed percentage (%)	plumule length (cm)	Radicle length (cm)
0	53.333 a	2.6000 a	1.3267 a	2.1833 a

0.5	53.333 a	2.5000 a	0.9467 a	1.5500 a
1	56.666 a	2.9100 a	0.7633 a	1.8000 a
1.5	46.666 a	2.2767 a	0.8400 a	1.7767 a

Table 3 shows that there is no significant difference between the concentrations in terms of the plumule and radicle fresh weight. The treatment with 1.5 g/l extract gave lowest average fresh weight for the radicle and plumule which was 0.111 g and 0.030 g respectively. However, the control treatment exhibited the highest average of 0.381 and 0.066 for the two properties. The table also shows significant differences between the treatments in terms of the radicle dry weight. The control treatment gave the highest value of the average dry weight of the radicle which was 0.015 g. This value differs significantly from the 0.5 and 1.5 g/l treatments; however, it did not differ considerably from the 1.0 g/l treatment. The 1.5 g/l treatment gave the lowest average radicle dry weight of 0.0086 g. The data in Table 3 did not show significant differences between the treatments in terms of the plumule dry weight. The averages, also, decreased with the increase in the used concentrations. The lowest value was 0.0027 g which was achieved from treatment with 1.5 g/l extract compared to the control treatment, which gave the highest value of 0.0072 g.

Table 3 The effect of the Mentha longifolia extract on the fresh and dry weights of the plumule and the radicle

Used extract concentration (g/l)	Radicle fresh weight (g)	plumule fresh weight (g)	Radicle dry weight (g)	plumule dry weight (g)
Distilled water (0)	0.3813a	0.0667 a	0.0159 a	0.0072 a
0.5	0.1734 a	0.0373 a	0.0105 bc	0.0036 a
1	0.1427 a	0.333 a	0.0140 ab	0.0068 a
1.5	0.1110 a	0.0307 a	0.0086 c	0.0027 a

6. Discussion

The results that have been obtained in this study confirmed that the Mentha longifolia cold extract has significant effect in reducing the germination and growth of the Cicer arietinum L. seeds. This results conform to the findings reported by MoŻDŻEŃ et al. (2019), Ahmed et al. (2021), and Pukclai and Kato-Noguchi (2011). In these studies, significant reduction in seed germination percentage due to treating with different concentrations of Mentha longifolia extract was specified. This reduction was found to be directly proportional to the extract concentration. This is attributed to the fact that the Mentha longifolia extract contains high concentrations of effective compounds, such as phenols, alkaloids and tannins. These compounds have an ability to inhibit the germination and growth of the plants, as explained in Table 3. The presence of these allelochemical compounds in low concentrations can stimulate the growth of plants, thugh at high concentrations it declines in plant growth (Orcutt and Nilsen, 2000) and (Liu et al., 2011). This is due to its role in reducing the cell division and elongation which are essential for growth (John and Sarada, 2012). Another reason for growth declination is its impedance to enzymes activity (Siyar et al., 2017), such as food substance decomposition enzymes that are found in the endosperm of the seeds. The reduction in the enzymes' activity leads to shortage in food substances that delivered to the active seed tissues, like the plumule and the radicle. In addition to that, the extract effective compounds impedes the breathing process (Keating, 1999), plant

hormones and protein synthesis (John and Sarada, 2012), and ion absorption process (Qasem and Hill, 1989). Moreover, it has indirect role in cell death by producing reactive oxygen which causes lipid oxidation and damages the cell membranes (Mutlu et al., 2011). Consequently, this leads to plant growth stunting and death.

7. References

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