

Effect of Aqueous and Alcoholic Extracts of *Melissa Officinalis* on Germination, Growth and Yield of Cultivated Red Radish (*Raphanus Sativus* L.)

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Abstract

Radish (*Raphanus sativus* L.) is a member of the Brassicaceae family of root vegetables and both the roots and the leaves are consumed for their natural therapeutic value and health promoting effects owing to the presence of phytochemicals. Thus, the effect of three different solvents (Cold, hot and alcoholic) of *Melissa officinalis* (lemon balm) extracts on germination and growth of radish was studied. A field experiment was carried out during October to December 2021 season with Randomized Complete Block Design (RCBD). Alcoholic, hot water and cold water extraction at a concentration of 1 % showed a higher germination percentage compared to other concentrations, while Distilled water showed a major germination percentage 80% compared to other extractions. Regarding the chlorophyll content, alcoholic extraction at a concentration of 0.5 % showed higher Chlorophyll content of 19.5 compared to other treatments and even the control. The root length of radish for alcoholic extraction at a concentration of 1 % showed the highest root length. Alcoholic extraction at a concentration of 0.5 %, hot water extraction at a concentration of 3% and cold-water extraction at a concentration of 5% showed the highest stem diameter. Alcoholic extraction of lemon balm at concentration of 1 presented the biggest leaf diameter. This indicated that the *Melissa officinalis* extracts as a medicinal plant had different effects on radish germination and growth. Further research should evaluate radish development in different circumstances to see whether this strategy may boost the output of this vital crop.

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Introduction

Raphanus sativus L., often known as radish, is a member of the Brassicaceae family of root vegetables and a major vegetable crop across the globe (Manivannan *et al.*, 2019; Tsouvaltzi and Brecht, 2014) and belongs to the genus *Raphanus* (Nishio, 2017). Although radishes come in a wide range of colors on the outside (from white in Asia to red in Europe to purple green and black), the flesh of most European and Asian varieties is always

white (Gamba *et al.*, 2021; Nishio, 2017), while in Iraq and Kurdistan region both types are available and people ate daily in the meal. The edible part of radish vegetable are the root, leaves and sprouts which are most is often used raw in salads, although it may also be cooked or salted in combination with other vegetables (Manivannan *et al.*, 2019). The marketable part of the radish plant often is used the root part; otherwise, the leaves of the radish are used for the freshness of the vegetable. The chemical composition of radishes has been proven to

have exceptional therapeutic and nutritional potential. Because of this, it was proposed as a potential therapy for a wide range of illnesses, such as hyperlipidemia, cardiovascular disease, and cancer (Curtis, 2003). Calcium in leaves and potassium in roots was found to be significant minerals. Leaves showed a higher percentage of protein, ash and fiber than roots and total phenolic and flavonoid content in leaves was four times higher than in roots, and also produce the oil which is extracted from the seeds (Goyeneche *et al.*, 2015).

Red radish (*Raphanus sativus* L.) contains anthocyanins, which are utilized as natural food colors due to their great durability and similarity to Food Red No. 40 (Rodriguez-Saona *et al.*, 1999).

Previous studies were conducted on radish growth affected by salt stress (Scialabba *et al.*, 1990). Shoot fresh weight, chlorophyll contents, and soluble proteins were all negatively impacted by 40 days of exposure to NaCl 0, 80, or 160 mM, but proline levels and the activity of superoxide dismutase, peroxidase, and catalase were all raised (Noreen and Ashraf, 2009). Lemon balm (*Melissa officinalis*) is utilized internationally for its therapeutic benefits with many biologically active compounds (Petrisor *et al.*, 2022). The germination and development of *Amaranthus caudatus* L., *Lepidium sativum* L., *Digitaria sanguinalis* L., *Phleum pratense* L., *Lactuca sativa* L., and *Lolium multiflorum* Lam were all stymied by an aqueous acetone extract of lemon balm (Kato-Noguchi, 2001). The effects of priming treatments on radish growth showed that priming with 0.01 % ascorbic acid (AA) and 1 % KNO₃ enhances radish germination, growth, vigor index, and leaf water content. The priming agent AA reduced malondialdehyde (MDA) levels relative to unprimed seedlings. Hormoprimering with indole-3-acetic acid (IAA) boosted photosynthetic pigments and total soluble leaf proteins. After hormoprimering with 1 mM IAA and haloprimering with 1 % MgSO₄, total

phenolic compounds, including flavonoids, were greatest. Based on DPPH radical inhibition, IAA and AA may boost radish seedlings' antioxidant activity (Kanjevac *et al.*, 2022). Seed germination and early seedling development of 10 summer annual species were tested in Petri dishes and pot trials to investigate how aqueous extracts of the fragrant herb *Melissa officinalis* affected these processes. Extracts at 5 and 10 % were the most potent inhibitors for the entire weed species tested in in vitro trials using aqueous extracts from *M. officinalis* on seed germination. When compared to a control group of untreated seeds of the same species, aqueous extracts inhibited germination by as much as 54%. While several weed species (*P. minor*, *S. nigrum*, *P. angulata*, and *C. albida*) had a fairly stimulatory impact at 1 % concentration, germination decrease ranged from 1 % to 11 % at this level. While the allelopathy RI of lemon balm aqueous extracts was negative for the most part, at a concentration of 10 %, emergence was decreased by 58 % for *X. strumarium*, 54 % for *C. album*, 48 % for *S. faberi*, 46 % for *C. canadensis*, and 43 % for *C. bonariensis* (Kanas, 2020). The production of enough nutritious food for the world's ever-increasing population is now one of horticulture's greatest difficulties. In spite of decreasing crop varieties and shrinking farmland, the only way to accomplish this is to boost agricultural yields while protecting them from environmental dangers. To safeguard people from being hungry and malnourished, it is crucial to increase the production of high-quality nourishing food and to increase the yield of radish, the fertilizer application from gardening increased both macro and micro minerals (Godlewska *et al.*, 2021; Povero *et al.*, 2016). Thus, our aim is to know the effect of different extracts of *Melissa officinalis* on germination, growth and yield of cultivated red radish (*Raphanus sativus* L.).

Methods and Materials

A field experiment was carried out at the laboratory of Halabja Technical College of Applied Science during October to December 2021 season. The experiment aims to investigate the effectiveness of water and alcoholic *Melissa officinalis* extracts on the radish variety (findik trup) in terms of germination, growth and yield. The Factorial Experiments within Randomized Complete Block Design (RCBD) with three replicates were used. The experiment was divided into 45 pots (9 pots for each type of radish seeds) and three seeds were sown in each pot.

Cold Aqueous Extract

To prepare the extraction solution 20 g of dry *Melissa officinalis* was taken and then added to distilled water at a temperature 20-25 °C until the volume became 200 ml and remained for half an hour using a horizontal shaker device (Horizontal shaker) Medium velocity. After an hour the solution was filtered with three layers of gauze to separate the solid plankton (Harborne, 1984) Five concentrations were prepared 0, 0.5, 1, 3, and 5 % from the final solution.

Hot Aqueous Extract

The same steps were followed for Cold Aqueous Extract, Only replacing the cold water with boiling water at 100 °C.

Alcoholic Extract

The same steps were followed for Cold Aqueous Extract, Only replacing the cold water with ethyl alcohol with a concentration of 98 %.

Cultivation

Germination of seeds in Petri dishes

The laboratory experiment consisted of 15 treatments and it was repeated three times, thus we got 45 treatments. 10 Seeds of radish were placed in Petri dishes with a diameter of 10 cm after placing by filter paper (Wathmann No.1). Then added to

each dish 20 ml of each of the extracts prepared previously in the known concentrations, and the seedlings were watered with the extracts at the time of watering, as for the comparison treatment, only distilled water was added to it. The dishes were placed in the laboratory at a temperature of 25 °C, according to the method of (Ahmed, 2018). The experiment lasted 20 days, during which the following were calculated:

The percentage of germination

Seed numbers were calculated 10 days after the sowing date and converted to a percentage by the following equation cited by Kadiem and Nadir, (2019).

Germination percentage =

$$\frac{\text{number of germinated seeds} \times 100}{\text{Total number of seeds}} \dots\dots (1)$$

Percentage coefficient of germination speed

Germination Speed =

$$\frac{(a) n_1 + (a + b) n_2 + (b + c) n_3 + (c + d) n_4 + \dots \times 100}{a+b+c+d} \dots\dots (2)$$

a, b, c, d = the number of germinated seeds in each count.

n₁, n₂, n₃, n₄ = the time between counting (daily).

The average lengths of both the rootstock and the peduncle

After 20 days from germination, the average lengths of the rootstock and the stalk were measured for each plate, by measuring the lengths of three plants in each plate, and then taking their average. The experiment included 15 treatments and was repeated three times, on 11/8/2021, after using 45 12-liter pots and adding 5 kg of preparation soil inside the pot and with a void of (1) gm for phosphorous fertilizer and planted seeds Radish plant 3 seeded in each pot, then on 23/11/2021 1.5 g of phosphorous fertilizer was added as well.”

Nitrogenous fertilizer was added at a rate of 1 gm per pot on 5/12/2021, with a difference of 7 days between the first and second batch, and 10 days after the last batch was added of nitrogen fertilizer. The three plant extracts (cold water extract, hot water extract, and hot water extract) were added. Cold alcohol) in concentrations 0, 0.5, 1, 3, 5 % and in three batches, at a rate of 20 ml for each batch, with a difference of 5 days between one batch and another, and after 15 days have passed after the last addition. One plant was extracted from each experimental unit, and the following traits were studied: chlorophyll measurement was done according to (Ebadi *et al.*, 2022), leaf length

measurement (cm), leaf width (cm) fresh weight of shoots (gm) before drying, dry weight after drying (gm), root length (cm) root diameter (cm) and reduced after germination to 1 plants per pot.

Experimental Design

The Factorial Experiments within Randomized Complete Block Design (RCBD) with three replicates were used. The experiment was divided into 45 pots (9 pots for each type of radish seeds) and three seeds were sown in each pot. Then, the data were taken from the experiment analysis by the statistix-10 program for window- 10. In addition, used LSD comparison at $p > 0.05$

Table 1. Some chemical and pH property of the soil after harvesting

Treatment	Con.	N	P	K	EC	PH
T ₁ D.W CONTROL	-	55.1	22	312	0.000569	7.9
T ₂ Alcohol	0.5	71.4	25	371	0.000592	7.62
T ₃ Alcohol	1	70.8	23	357	0.000612	7.61
T ₄ Alcohol	3	72.3	24	364	0.000615	7.65
T ₅ Alcohol	5	82.8	21	318	0.000623	7.69
T ₆ Hot water	0.5	34.4	40	205	0.000419	7.60
T ₇ Hot water	1	46.3	31	201	0.000459	7.59
T ₈ Hot water	3	41.2	27	225	0.000474	7.56
T ₉ Hot water	5	50.6	21	350	0.000506	7.54
T ₁₀ Cold water	0.5	60	11	234	0.000575	7.54
T ₁₁ Cold water	1	47.4	15	212	0.000635	7.64
T ₁₂ Cold water	3	52.1	14	223	0.000596	7.57
T ₁₃ Cold water	5	32	20	231	0.000726	7.62

Con. = concentration, D.W. = Distilled water, H.W. = Hot water, C.W. = cool water.

Results and Discussion

This study investigated the effects of different water and alcoholic *Melissa officinalis* extracts on the radish variety germination, growth and yield. The results showed variable germination percentages of radish as shown in (Table 2). Alcoholic, hot water and cold water extraction at a concentration of 1 % showed a higher germination percentage compared to other concentrations 30, 73.33, and 75 % respectively, while Distilled water showed

major germination percentage of 80 % compared to other extractions. Regarding the Chlorophyll content of SPAD, Alcoholic extraction at a concentration of 0.5 % showed a higher Chlorophyll content of 19.5 compared to other treatments and even the control. Hot and cold water extraction at a concentration of 3 % showed higher Chlorophyll content of 18.367, and 15.033 respectively compared to other different concentrations. There are no much significant differences observed between studied treatments. It can be seen that the

root length of radish for alcoholic extraction at a concentration of 1 % showed the highest root length of 5.666 cm, while for hot water extractions at a concentration of 0.5 % and for cold water extractions at a concentration of 1 % showed the length of 4.833 and 4.033 cm correspondingly. In terms of stem

diameter, alcoholic extraction at a concentration of 0.5 %, hot water extraction at a concentration of 3 % and cold-water extraction at a concentration of 5 % showed the highest stem diameter at 0.05, 0.03, and 0.033 mm respectively.

Table 2. Germination percentage, chlorophyll content, root length before dry and stem diameter of radish

Treatment		Con.	GR%	Chlorophyll	RLBD (cm)	SD(mm)
T ₁	D.W CONTROL	-	80.00 ^a	13.800 ^{ab}	3.533 ^e	0.0063 ^c
T ₂	Alcohol	0.5	28.33 ^{de}	19.500 ^a	5.00 ^{abc}	0.0500 ^a
T ₃	Alcohol	1	30.00 ^d	15.167 ^{ab}	5.666 ^a	0.0250 ^b
T ₄	Alcohol	3	6.66 ^{ef}	13.600 ^b	5.360 ^{ab}	0.0250 ^b
T ₅	Alcohol	5	0 ^f	15.733 ^{ab}	5.00 ^{abc}	0.0263 ^b
T ₆	Hot water	0.5	66.66 ^{ab}	17.800 ^{ab}	4.833 ^{abcd}	0.0250 ^b
T ₇	Hot water	1	73.33 ^a	17.233 ^{ab}	4.166 ^{bcde}	0.0257 ^b
T ₈	Hot water	3	45.00 ^{bcd}	18.367 ^{ab}	4.333 ^{bcde}	0.0300 ^b
T ₉	Hot water	5	41.66 ^{cd}	15.700 ^{ab}	4.500 ^{abcde}	0.0277 ^b
T ₁₀	Cold water	0.5	73.33 ^a	14.233 ^{ab}	3.433 ^e	0.0277 ^b
T ₁₁	Cold water	1	75.00 ^a	14.033 ^{ab}	4.033 ^{cde}	0.0290 ^b
T ₁₂	Cold water	3	46.66 ^{bcd}	15.033 ^{ab}	3.600 ^{de}	0.0290 ^b
T ₁₃	Cold water	5	63.33 ^{abc}	13.367 ^b	3.2667 ^e	0.0327 ^{ab}
Alpha 0.05 S.E.±			10.958	2.898	0.616	0.0087
Critical Value			22.525	5.956	1.267	0.018

Con.= concentration, D.W. = Distilled water, H.W. = Hot water, C.W. = cool water, RLBD= Root length before dry, SD= stem diameter, GR % = percentage of germination rate, mean of the column are not significant different at the alpha 0.05 level by LSD comparison, and different letter shows different between treatment.

Table 3 shows the results of some morphological parameters at different concentrations. As can be seen, after application of alcoholic extraction of lemon balm at concentration 1 presented the biggest leaf diameter of 8.46 cm, while hot water extraction at concentration 5 showed 7.16 cm and cold water extraction at both concentrations 1 and 5 showed the same

level 6.16 cm. In terms of leaf length, alcoholic extraction at concentration 0.5 showed the highest length 20.667 cm, while hot water extraction at concentration 5 and cold water at concentration 0.5 showed the highest length leaf length 18.833, and 16.333 cm respectively. There is no difference in terms of leaf number among studied parameters. Alcoholic and cold water extraction at concentration 1 showed the highest fresh weight of 13.407, and 7.107 g respectively, while hot water extraction at concentration 5 showed the highest fresh weight of 11.5 g. The results of leaves weight after dry ranged from 0.703 to 1.6 g.

Table 3. Leaf diameter before dry, leaf length before dry, leaf length after dry, leaf number, leaf weight before dry and leaf weight after dry

Treatment		Con.	LD (cm)	LL (cm)	L No.	LWBD (g)	LWAD (g)
T1	D.W. CONTROL	-	6.66abc	17.833abcde	2.0a	9.293abc	1.140abc
T2	Alcohol	0.5	7.50ab	20.667a	0.66b	11.620ab	1.206abc
T3	Alcohol	1	8.46a	19.833abc	2.0a	13.407a	1.273abc
T4	Alcohol	3	7.00abc	20.333ab	2.0a	13.397a	1.253abc
T5	Alcohol	5	6.50abc	20.00ab	2.0a	9.777abc	1.600a
T6	Hot water	0.5	7.16abc	18.500abcde	2.0a	11.270ab	1.253abc
T7	Hot water	1	7.16abc	18.667abcd	2.0a	11.367ab	1.253abc
T8	Hot water	3	5.33c	15.833cde	2.0a	6.890bc	0.906bc
T9	Hot water	5	7.16abc	18.833abcd	2.0a	11.500ab	1.430ab
T10	Cold water	0.5	5.50bc	16.333bcde	2.0a	6.993bc	0.993abc
T11	Cold water	1	6.16bc	14.833de	2.0a	7.107bc	0.836bc
T12	Cold water	3	5.33c	14.500e	2.0a	5.633c	0.703c
T13	Cold water	5	6.16bc	15.667de	2.0a	5.823c	0.773bc
Alpha 0.05 S.E.±			0.978	1.955	0.235	2.335	0.317
Critical Value			2.0113	4.020	0.481	4.80	0.652

Con.= concentration, D. W. = Distilled water, H.W. = Hot water, C.W. = cool water, LD= leave diameter, LL = Leave length, L No. = leave number, LWBD = Fresh leaves weight (before dry/ gram), LWAD = Leave dry weight (after dry/gram), S.E = Standard Error; Mean of the column are not significant different at the alpha 0.05 level by LSD comparison.

Root tall results showed variable numbers ranging from 2.390 to 7.674 cm

Table 4. Alcoholic extraction at concentration 5, hot water and cold water at concentration 0.5 showed the highest root tall 7.674, 6.955, and 6.894 cm respectively. Regarding root weight before dry, alcoholic extraction at concentration 1, hot water at concentration 0.5 and cold water at concentration 3 resulted in higher root weights of 52.410, 48.047, and 30.163 g. This trend was also noted for root weight after drying and root diameter.

Table 4. Root Tall, Root weight before dry, Root weight after dry, and Root diameter

Treatment		Con.	RT (cm)	RWBD (g)	RWAD (g)	RD (mm)
T1	D.W. CONTROL	-	9.050a	27.927c	1.776ab	39.190ab
T2	Alcohol	0.5	2.390f	42.490abc	1.356abc	36.443b
T3	Alcohol	1	4.377def	52.410a	1.923a	38.893ab
T4	Alcohol	3	5.816bcde	41.563abc	1.320abc	35.327b
T5	Alcohol	5	7.674ab	30.043bc	1.483abc	32.637b
T6	Hot water	0.5	6.955abc	48.047ab	1.273abc	46.040a
T7	Hot water	1	6.358bcd	29.337bc	0.926c	35.800b
T8	Hot water	3	6.377bcd	28.900c	0.973c	35.400b
T9	Hot water	5	6.894abc	38.973abc	1.056bc	40.947ab
T10	Cold water	0.5	6.894abc	25.337c	1.323abc	38.313ab
T11	Cold water	1	4.925cde	25.060c	1.416abc	37.473b
T12	Cold water	3	4.097def	30.163bc	1.506abc	40.367ab
T13	Cold water	5	3.445ef	23.653c	1.346abc	38.130ab
Alpha 0.05 S.E.±			1.224	9.264	0.367	4.079
Critical Value			2.516	19.043	0.755	8.7385

Con. = concentration, D.W. = Distilled water, H.W. = Hot water, C.W. = cool water, RT = Root tall, RWBD = Root weight before dry/gram, RWAD = Root weight after dry/gram, RD = Root Diameter; Mean of the column are not significant different at the alpha 0.05 level by LSD comparison.

Radishes plant, which belong to the Brassicaceae vegetable family and are scientifically known as *Raphanus sativus* L., contain several different types of phytochemicals that are physiologically active (Hanlon and Barnes, 2011). The

roots of the radish, which are edible and fleshy, are the primary reason for the plant's cultivation. It is an excellent source of a number of essential nutrients, including potassium, calcium, copper, manganese, magnesium, vitamin B6, and vitamin C (Gamba *et al.*, 2021). Radish root, in addition to possessing protein, vitamins, and polysaccharides, has a large number of phenolics (kaempferol, luteolin, gentisic acid, vanillin acid, cyanide, hydrocinnamic acid, myrcetin, and quercetin) (Jakmatakul *et al.*, 2009). In this study, we applied three different *Melissa officinalis* extracts on

radishes to observe germination and growth. The highest germination was obtained at the concentration of 1 compared to other concentrations, while at concentration of 5 showed more inhibitory germination. This means that the *Melissa officinalis* extracts had stimulatory germination in low and inhibitory germination in high concentration. This was also mentioned by (Ahmed *et al.*, 2022) who indicated that plants could have phytotoxicity either stimulatory or inhibitory. On the other hand, in another study by (Kato-Noguchi, 2001) showed that *Melissa officinalis* water extracts inhibited greatly the growth of cress, cockscomb, lettuce, crabgrass, timothy and ryegrass than (n-hexane-, acetone). They also showed that shoots were affected mostly than roots by increasing the concentration. This is probably due to active compounds and allelochemicals which was present in many medicinal and aromatic plants extracts (Ahmed, 2018; Ahmed *et al.*, 2022). Plasma treatment boosts growth more for radish sprouts harvested in 2017 than for 2018 sprouts. Grey sprouts responded better to plasma treatment than brown radish sprouts (Attri *et al.*, 2021). (Zuverza-Mena *et al.*, 2016) found that exposing radish sprouts to silver nanoparticles (nAg) changed their development (seedling), nutritional content, and macromolecule structure in ways that are not known their harmful to human health. While in our study variable results were obtained, for example, alcohol extraction at a concentration of 0.5, hot water extraction at a concentration of 1, 3, and cold water extraction at a concentration of 5 showed a major reduction in root tall. Silver nanoparticles (AgNPs) application on radish showed that the roots of radishes treated with AgNPs were longer and the fresh weight of their shoots was greater. The accumulation of chlorophylls was greatest after treatment with 50 mg/L, whereas the accumulation of carotenoids was lowest after treatment with 100 mg/L (Tymoszuk, 2021). In our study, chlorophyll at a concentration of 0.5, 3 showed the

highest by application of alcohol, hot and cold water.

Conclusion

In this study, the impact of three different solvents of *Melissa officinalis* extracts on radish germination and growth showed that at a concentration of 1 %, alcohol, hot water, and cold water extraction exhibited a larger germination percentage than other concentrations, whereas distilled water showed a significant germination rate. Moreover, alcoholic extraction exhibited greater chlorophyll content and the longest radish root. This indicated that *Melissa officinalis* extracts affected radish germination significantly.

Conflict of Interest

There is no conflict of interest between authors.

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