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# Comparative allelopathic effect of *Thymus kotschyanus* on germination and early growth of *Achillea millefolium* under Laboratory and Pot conditions

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**ABSTRACT**

*The allelopathic effects of Thymus kotschamus plant parts on germination and early growth of A. millforlium was compared under laboratory and pot house conditions. Seeds germination was stimulated by 0.1%KNO3. Seed germination and growth was monitored daily for germination (%), rate of germination, mean germination time and germination inhibition. The root and stem portions were studied to determine the effect of T. kotschyanus on plant vigour. The data obtained was subjected to Duncan's test. The results showed that thymus plant parts had concentration dependant negative effects on seed germination and seedling growth of A. millefolium.*

**Key words:** *Achillea millefolium*, allelopathy, germination, growth inhibition, *Thymus kotschyanus*

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## INTRODUCTION

Mollish in 1937 (11) was the first to describe allelopathy as a harmful effect of one species of higher plants on the germination, growth and development of plants of other species (5). This affects the growth and development of forest and pasture plants (20). The awareness of this phenomenon is necessary to know its effect in improving the pastures, production of crops and medicinal plants. Matizha et al (9) have ascribed the low vitality of plants in arid and semiarid habitats to allelopathy. Peneva et al (14) studied the allelopathic effects of aqueous extracts of Coffee arabica seed powder on the growth characteristics of Xanthium strumanrium. His results showed that increased amount of plant extracts and powdered biomass, had no significant effect on seed germination but decreased the vegetative bud formation. Safari et al (15) have examined the allelopathic effect of T. kotschyanus plant parts on the germination and initial growth characteristics of T. repens and B. tomentellus and found that by increasing the concentration of T. kotschyanus, the germination parameters of both plants were reduced. The adverse effects of T. kotschyanus on both plant species increased with the increasing concentration of plant material.

The herbs Thymus and Achillea are two medicinal plants grown in Iran. T. kotschyanus is grown on large areas of range lands and is an important medicinal plant species of rangelands. Understanding the plant-plant interaction between these two plants is needed to improve their production as there is an increasing demand for these herbal plants. It is necessary to study the interaction of these species in order to rationalize their use and exploitation. Understanding the alleleopathic effect of T. kotschyanus on A. millefollium would help in improvement and proper management of this valuable medicinal plant in the rangelands. With this in view, the present study was conducted

to determine the effect of T. kotschyanus plant parts on the germination and growth of Achilliea millefolium under laboratory and pot house conditions. This research can be used in improvement and reclamation of rangelands in studied region. The results of this study can be used to enhance the exploited potential of studied medicinal species. Considering the importance of medicinal plants in this region, it seems that it is essential to survey mutual relationships between these species. Accurate and scientific comparison of allelopathic effects in the field and laboratory environments is an advantage of this study in comparison with previous studies. The aim of this study was to compare the allelopathic effects of Thymus kotchyanus on germination properties and initial growth of Achillea millefolium in term of simulation or no simulation of A. millefolium in both laboratory and field condition.

## MATERIALS AND METHODS

This research performed in Bijar protected region that is located in north Bijar city in Kurdistan province of Iran. Mean relative humidity is 28.6%. Maximum and minimum heights in region are 2187m and 1533m respectively. Mean precipitation is 337mm and mean annual temperature is 12.9° c. The soil for pot culture and seeds of A. millefolium and plant parts of T. kotschyanus were collected from the protected Rangelands of Bijar at the end of growing season. T. kotschyanus plants parts were dried in the shade and then were convertor to powder with less than 2mm in size. The field soil was powdered and 1.7 kg soil was placed in each pot that the content of each pot is 2 liter. Physicochemical properties of used soil in this study are presented in table 1 and 10gr soil was placed in each Petri plate.

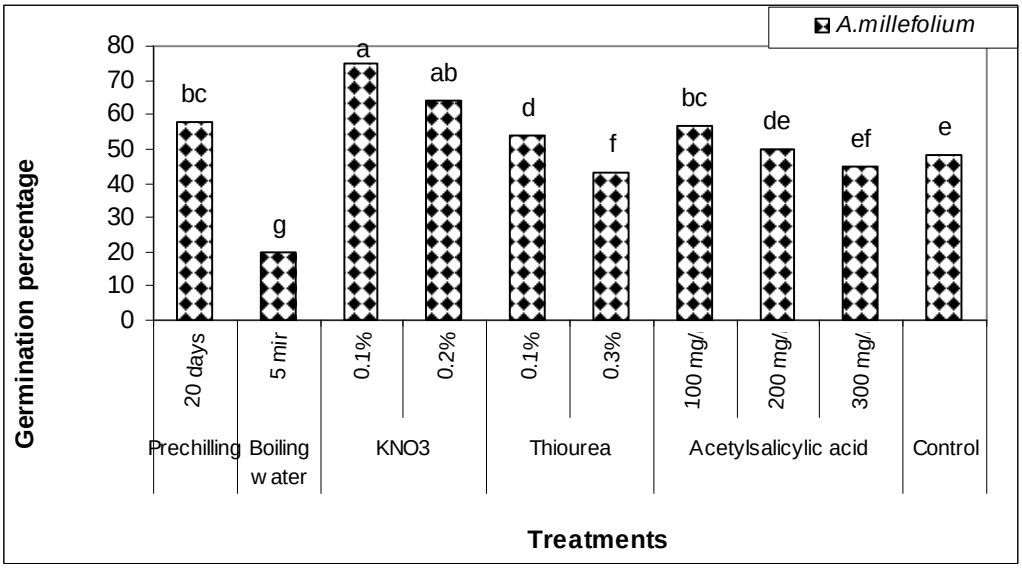
**Table1: Physicochemical properties of used soil in studied region**

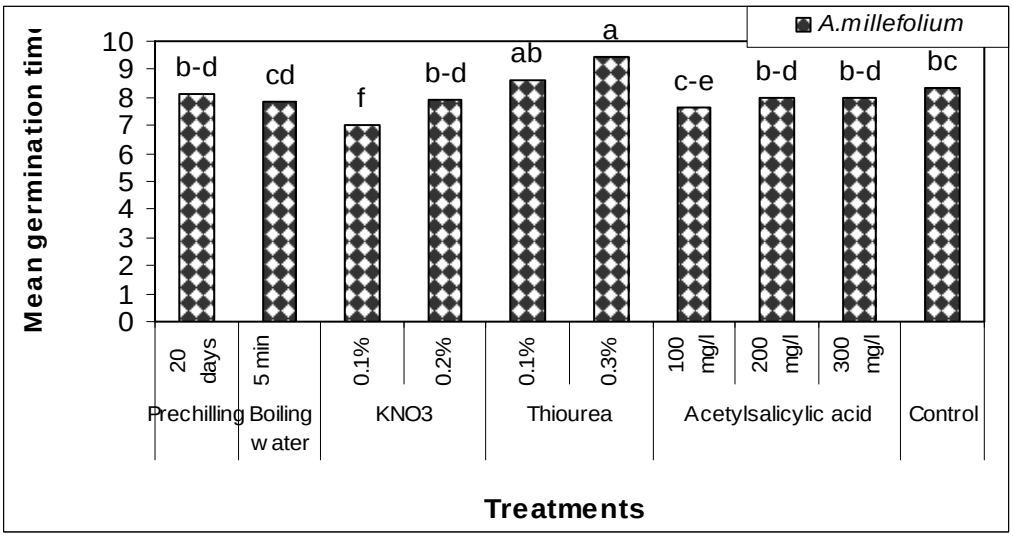
| Factor | Sand (percent) | Clay (percent) | Silt (percent) | pH | EC (ds/m) | Lime (percent) | Organic matter (percent) | Nitrogen (percent) | Phosphor ($mgkg^{-1}$) | Potassium (ppm) |
|---|---|---|---|---|---|---|---|---|---|---|
| Value | 20 | 47 | 33 | 7.7 | 0.22 | 4.58 | 1.48 | 0.12 | 14 | 221 |

The allelopathic effects of T. kotschyanus plant material on the germination and growth of A. millefolium were tested both in laboratory using the soil in Petri plates with 10 centimeter in size and in pots. For preparing 10% aqueous extract, 10 gr of T.kotschyanus powder mix in 100ml water for 24h and then passed through the filter paper. 5cc from this extract selected and together with 95cc of distilled water formed 5% extract for our experiments. Other concentrate of extract was performed similarly. There were 7-concentrations of T. kotschyanus plant extract (0,5,10,15,20,25, 30 % w/v) for petri-plate bioassay and 7-doses of powdered Thymus plant biomass (0,85,170,255,340,425,520 g per pot) for pot culture. These values were considered with create a proportion and relation to powder of T.kotschyanus and pot soil amount that these values are equal to amount of extract concentration.

In both laboratory and pot studies, to stimulate early germination seed dormancy was broken by treatment with chemicals.

To determine the best treatment for breaking the A. millefolium seeds dormancy, Germination test was conducted by four replications and 9 different treatments. Ten seeds for each treatment were put in 10 centimeters Petri dishes.
1. Soaking in 0.1 and 0.2% KNO3 solution for 72 h
2. Soaking in Acetylsalicylic acid 100,200 and 1000 mg/l solution for 72 h
3. Pre chilling at 4C for 20 days in refrigerator
4. Heating in water at 80C for 5 min
5. Soaking in 0.1 and 0.3% thiourea solution for 72 h and
6. Control treatment (soaking in distilled water for all of the experimental times)




In each petri plate 10 seeds were sown. The randomized block design was used. Seed germination was monitored daily and the rate and germination (%), mean germination time and the inhibition (%) were calculated for the experimental period.

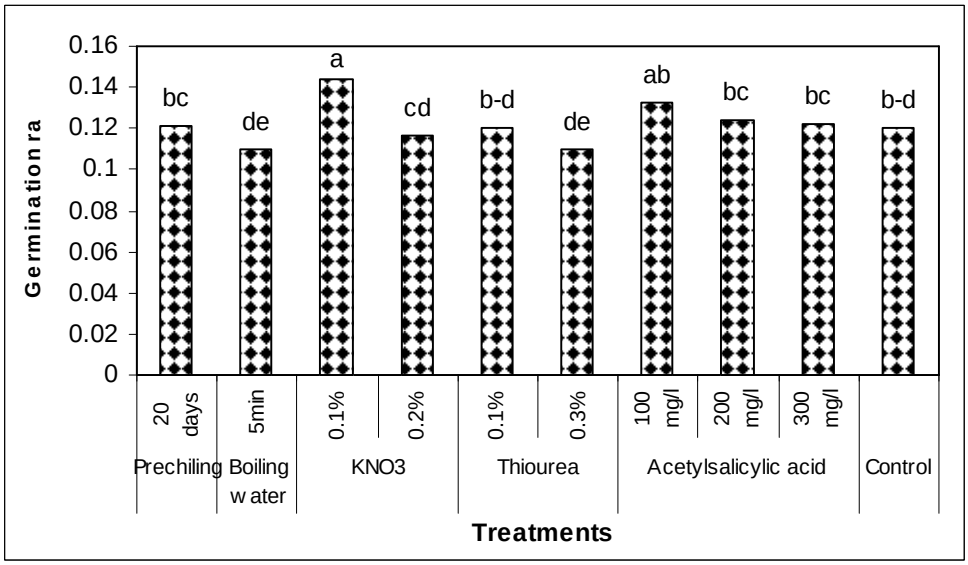
The experiments were terminated after 4-weeks. We complete our experiments (petri plate and pot culture) in August and September of 2011 in Kurdistan province of Iran. At the end of the experimental period, the seedlings from each pot were removed, cleaned free of soil. The roots and stem parts were separated and the length of each part recorded.

Mean germination time (MGT) was calculated as per the equation (6),

$$MGT = \frac{\sum D.N}{n}$$

Where, N: Number of seeds germinated on day
n: Total number of seeds sown
D: Number of days from the sowing date

At the end of experiment when final germination period was recorded, the germination rate was found inverse of MGT




The shoot and root length of each seedling was measured and the vigour index (VI) was calculated following modified formula of Abdul-Baki and Anderson (1):

VI = Germination percentage x seedling length (shoot + root length $_{cm}$)

The inhibitory percentage (PI) was calculated by the following formula:
PI =100- (FG % x Thymus tissue powder/FG % of control (without thymus powder x 100),
Where, FG: Final germination (%). Tavili *et al* (16)

Experimental data was then analyzed by MSTAT-C programme (11) and the difference between the means was compared using Duncan's multiple range test at (p<5%).

## RESULTS

It was found that soaking in 0.1% $KNO_3$ for 72 h was found the best for breaking the dormancy

**Effect of breaking dormancy under Petri dish and Pot conditions**
The thymus plant material stimulated the germination and growth of *A. millefolium* in both Petri dish and pot conditions at various Doses of plant material (Table-2). In table 2, considered mean of treatments for studied vegetative properties and are presented for comparison of four experiments modes. The values in each germination properties are the mean of powder or extract treatments.

**Table 2: Mean square of the allelopathic effects of *Th.kotchyanus* on seed germination and early growth of *A. millefolium* under Petri dish and pot conditions**

| Conditions | Germination (%) | Mean Germination Time (MGT) | Germination Rate(day) | Root(cm) | Shoot(cm) | Seedling (cm) | Vigority index | PI (%) |
|---|---|---|---|---|---|---|---|---|
| **Lab & no stimulation** | 57.93$^{b}$ | 15.00$^{b}$ | 0.06825$^{c}$ | 52.21$^{c}$ | 33.36$^{bc}$ | 85.57$^{c}$ | 51.21$^{b}$ | 22.62$^{a}$ |
| **Lab & stimulation** | 64.51$^{a}$ | 9.531$^{c}$ | 0.1103$^{a}$ | 50.50$^{c}$ | 31.39$^{c}$ | 81.89$^{c}$ | 54.33$^{a}$ | 18.80$^{a}$ |
| **Pot & no stimulation** | 48.24$^{c}$ | 18.74$^{a}$ | 0.05421$^{d}$ | 58.43$^{b}$ | 34.89$^{ab}$ | 93.32$^{b}$ | 46.19$^{c}$ | 20.92$^{a}$ |
| **Pot & stimulation** | 57.77$^{b}$ | 14.66$^{b}$ | 0.07443$^{b}$ | 61.00$^{a}$ | 37.04$^{a}$ | 98.04$^{a}$ | 57.91$^{a}$ | 17.23$^{a}$ |

Breaking dormancy by $KNO_3$ treatment improved the germination (%) marginally and increased the speed of germination in both laboratory and pot conditions. The germination rate was significantly increased with the $KNO_3$ treatment under both conditions.

The growth of seedlings in pot conditions was better than in Petri dish conditions because seedlings are established better in pots method. The vigour index was superior when germination was stimulated by breaking dormancy. The inhibition (%) is more when no stimulation treatment was given but showed less number of seeds germinated or the slower germination rate.

**GERMINATION OF *A. MILLEFOLIUM* SEEDS**

The effect Thymus plant material on the germination of stimulated *A.millefolium* seeds was tested in the laboratory using soil in petri dishes with varying levels of Thymus plant material. (Table3). The stimulation with KNO3 allowed quicker germination and faster germination rate. Increasing the dose of thymus plant material increased the time required for germination of Achillea seeds and the rate of germination has decreased (**Table 3**).

**Table 3: Mean square of interaction of allelopathic effects of *T. kotschyanus* on *A. millefolium* germination in Petri plate and pot conditions**

| **Conditions** | Concentration of Thymus organs(g) | Mean Germination Time (MGT) | Germination Rate | Conditions | Concentration of Thymus organs(g) | Mean Germination Time (MGT) | Germination Rate |
|---|---|---|---|---|---|---|---|
| **Lab & no stimulation** | 5 | 12.79 $^{ij}$ | 0.07850 $^{hi}$ | Pot & no stimulation | 5 | 16.54 $^{ef}$ | 0.06075 $^{lmn}$ |
| | 10 | 13.84 $^{hi}$ | 0.07250 $^{jk}$ | | 10 | 17.51 $^{de}$ | 0.05725 $^{no}$ |
| | 15 | 14.58 $^{gh}$ | 0.06875 $^{k}$ | | 15 | 19.95 $^{bc}$ | 0.05000 $^{pq}$ |
| | 20 | 15.68 $^{fg}$ | 0.06425 $^{l}$ | | 20 | 20.75 $^{ab}$ | 0.04850 $^{q}$ |
| | 25 | 17.17 $^{def}$ | 0.05850 $^{mno}$ | | 25 | 20.19 $^{b}$ | 0.04975 $^{pq}$ |
| | 30 | 18.56 $^{cd}$ | 0.05400 $^{op}$ | | 30 | 20.56 $^{ab}$ | 0.04900 $^{q}$ |
| | control | 12.38 $^{ij}$ | 0.08125 $^{h}$ | Pot & stimulation | control | 15.65 $^{fg}$ | 0.06425 $^{l}$ |
| **Lab & stimulation** | 5 | 7.70 $^{no}$ | 0.1308 $^{b}$ | | 5 | 10.66 $^{kl}$ | 0.09425 $^{f}$ |
| | 10 | 8.00 $^{no}$ | 0.1260 $^{c}$ | | 10 | 11.47 $^{jk}$ | 0.08725 $^{g}$ |
| | 15 | 8.82 $^{mn}$ | 0.1143 $^{d}$ | | 15 | 13.11 $^{hi}$ | 0.07650 $^{ij}$ |
| | 20 | 10.04 $^{klm}$ | 0.1000 $^{e}$ | | 20 | 16.07 $^{efg}$ | 0.06225 $^{lm}$ |
| | 25 | 11.45 $^{jk}$ | 0.08800 $^{g}$ | | 25 | 21.89 $^{a}$ | 0.04625 $^{q}$ |
| | 30 | 13.48 $^{hi}$ | 0.07425 $^{ij}$ | | 30 | 19.71 $^{bc}$ | 0.05075 $^{pq}$ |
| | control | 7.22 $^{o}$ | 0.1388 $^{A}$ | | control | 9.67 $^{lm}$ | 0.1038 $^{e}$ |

Similar observations were also made under the pot conditions. The germination (%) decreased gradually up to 30% and the mean germination time also increased with increasing concentration of Thymus plant material in the Petri dish soil. These results showed that the thymus plant material exerted an effect on the germination time and rate of germination of *A. millefolium* seeds.

**Table 4: Mean square of the allelopathic effect of *T. kotschyanus* on *A. millefolium* germination and early growth characteristics under various treatment**

| Concentration of Thymus organs(g) | Germination (%) | Mean Germination Time (MGT) | Germination Rate(day) | Root(cm) | Shoot(cm) | Seedling (cm) | Vigority index | PI (%) |
|---|---|---|---|---|---|---|---|---|
| **5** | 67.77 $^{ab}$ | 11.92 $^{e}$ | 0.09106 $^{b}$ | 64.94 $^{b}$ | 39.19 $^{ab}$ | 104.1 $^{b}$ | 70.06 $^{b}$ | 2.010 $^{d}$ |
| **10** | 63.28 $^{bc}$ | 12.71 $^{d}$ | 0.08575 $^{c}$ | 60.63 $^{c}$ | 36.75 $^{bc}$ | 97.38 $^{c}$ | 61.30 $^{c}$ | 7.77 $^{cd}$ |
| **15** | 59.36 $^{c}$ | 14.12 $^{c}$ | 0.07738 $^{d}$ | 56.56 $^{d}$ | 34.81 $^{cd}$ | 91.38 $^{cd}$ | 54.03 $^{d}$ | 13.89 $^{c}$ |
| **20** | 52.49 $^{d}$ | 15.64 $^{b}$ | 0.06875 $^{e}$ | 52.56 $^{e}$ | 32.81 $^{d}$ | 85.38 $^{d}$ | 44.62 $^{e}$ | 26.67 $^{b}$ |
| **25** | 44.26 $^{e}$ | 17.67 $^{a}$ | 0.06063 $^{f}$ | 43.63 $^{f}$ | 27.19 $^{e}$ | 70.81 $^{e}$ | 31.22 $^{f}$ | 43.39 $^{a}$ |
| **30** | 43.21 $^{e}$ | 18.08 $^{a}$ | 0.05700 $^{g}$ | 40.81 $^{f}$ | 27.31 $^{e}$ | 68.13 $^{e}$ | 29.29 $^{f}$ | 44.77 $^{a}$ |
| **control** | 69.43 $^{a}$ | 11.23 $^{e}$ | 0.09700 $^{a}$ | 69.63 $^{a}$ | 41.13 $^{a}$ | 110.8 $^{a}$ | 76.35 $^{a}$ | 0.00 $^{d}$ |

**GROWTH OF *A. MILLEFOLIUM***

The effect of varying concentration of thymus plant material on the germination and growth of seedlings was studied under various treatments (Table 4). It was found that increasing concentration of thymus from 5-30% decreased the germination rate and germination (%). The mean germination time was also reduced significantly. Similarly, the root length, the shoot length were also significantly affected. While in the control it was 110.8, the length decreased steadily and at 30% concentration, it was reduced to 68.13. This is reflected both in the vigour index, which is 76.35 in control and it increased in all treatments containing thymus plant material. At 30% Thymus plant material, the root length was only 68.13 as opposed to 110.8 in the control. This also reflected in the Inhibitory

index that increased from 0 in control to 44.77% in 30% concentration. These results show that increasing concentration of thymus in the soil reduces the germination and growth of *A millefolium* plants significantly.

## DISCUSSION AND CONCLUSION

From the results it is clear that Thymus plant material significantly inhibited the germination and growth of *A. millefollium* plants. The inhibitory effect is noticed even at 5-10%/1.7Kg soil. These results are in agreement with Safari *et al* (15) who reported that increasing concentration of *T. kotschyanus* in the soil decreased the germination characteristics of both *Trifolium repens and Bromus tomentellus*. Our studies are consistent with those reported by several others (7, 13, 15, and 16). This effect on the growth of plants by *T. kotschyanus* is probably because of the effect of the allelochemicals in this plant on the cell division or susceptible plants. (2) Allelochemicals are known to reduce the auxin inducing root growth (16) and also affects the respiration, oxidative phosphorylation and thus reduces the growth. It therefore appears that the effects observed in the present study on germination and growth of *A. millefolium* plants due to the effects of allelochemicals present in the *T. kotschyannus* plant material on *A. millefolium*. In recent years research has shown that (3) ethanol extracts of *T. kotschyannus* can limit or stop the seed germination and early growth of Achillea medicinal plants, pig weed, wild lettuce and purslane. The results of these studies on other plants are consistent. These studies also show that in rangeland ecosystems of Bijar, the A.millefolium is reduced with increasing the population of *T.kotschyanus*.

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