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Full Length Research Paper

Effect of nano-silver on stages of plant growth and yield and composition of essential oil of *Thymus kotschyanus* Boiss. & Hohen

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***Thymus* plant is well known as medicinal plants because of their biological and pharmacological properties. In recent years, some effects of nano-silver solution on some of the plants have been studied. This study investigated the effect of different levels of nano-silver on some of the plant growth profiles and essential oil of *Thymus kotschyanus.* The study was carried out in a randomized block design with 15 replications in six levels of nano-silver ion (0, 20, 40, 60, 80, and 100 ppm). Different parameters such as survival rate, plant height, diameter of canopy area, days to flowering, yield, the amount and compositions of essential oil were determined. Moderate levels of nano-silver (20, 40, 60 ppm) had the highest positive impact on survival rate, diameter of canopy area, days to flowering, yield and essential oil amount; however 100 ppm had the minimal positive impact on them. One of the interesting results in this study was the increase in one of the compositions of essential oil, α-terpinyl acetate (14.2%) in 60 ppm level of nano-silver, however in 100 ppm treatment there was a significant reduction of thymol in comparison with other treatments. In conclusion, nano-silver can be used to change the stages of plant growth and the amount of secondary metabolites; however, the effect of nano-silver on plants needs more researches. These particles can be harmful to the environment if an exceedingly high amount is used.**

Key words: Nano-silver, *Thymus kotschyanus,* survival rate, plant height, diameter of canopy area, days to flowering, essential oil.

INTRODUCTION

The genus *Thymus* L. is represented in Iran by 14 species, 4 of which are endemic (Rechinger, 1982). *Thymus* species are well known as medicinal plants because of their biological and pharmacological properties. In traditional medicine, leaves and flowering parts of *Thymus* species are widely used as tonic and herbal tea and have antiseptic, antitussive, and carminative as well as treating colds properties (Amin, 2005; Zargari, 1997).

Thymus oils and extracts are widely used in pharmaceutical, cosmetic and perfume industry, also as flavoring and preservation of several food products (Bauer et al., 1997). Different species of *Thymus* have different types of components. Generally they contain thymol and carvacrol as main essential oil constituents. Some studies have been carried out on the quantity and quality of essential oils of *Thymus kotschyanus* Boiss. & Hohen from different regions in Iran (Rustaiyan et al., 1999; Nickavar et al., 2005; Semnani et al., 2006; Aberoomand et al., 2010).

There are some reports on the essential oils of *T. kotschyanus* from Turkey (Meriçli, 1986; Bagci and Baser, 2005). The effect of distillation methods and stage of plant growth on the essential oil content and composition

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of this species have been investigated (Sefidkon et al., 2000). The essential oil components in wild and cultivated populations of *T. Kotschyanus* have been determined and compared (Pirigharnaei et al., 2012).

The effect of altitude on essential oil and components in wild thyme (*T. kotschyanus*) from Taleghan region in Iran has been studied (Habibi et al., 2006). The comparison of volatile organic compounds of this species using hydrodistillation and headspace solid phase microextraction has been carried out (Nezhadali et al., 2010).

Nano-silver solution consisting silver ions in the size range of 10 to 100 nm has more stability in comparison to other solutions. Nano-silver particles, also, have more surface area in contact to outer space due to their small size. Thus, the amount of adhesion to the cell surface increases which leads to their higher efficacy (Shah and Belozerova, 2008). The effect of nano-silver in the control of citrus bacterial canker and in delaying the abscission (Samavi et al., 2009) and delaying the time of seed abscission in borage plant has been studied (Seif Sahandi et al., 2011). Also the efficacy of nano-silver as an antibactericidal agent in extending the vase-life of cut flowers of carnation has been reported (Basir et al., 2011). So far, there is no report in literatures about influence of nano-silver on yield and composition of essential oil. Accordingly, present study has been carried out to assess the effect of nano-silver ion on stages of plant growth, yield and composition of essential oil of *T. kotschyanus* Boiss. & Hohen from central Iran.

MATERIALS AND METHODS

Experiment was carried out based on a completely randomized design (CRD) with 15 replications, after disinfection of seeds with fungicides Mancozeb than one in a thousand. The seeds were soaked in six levels of nano-silver ion (0, 20, 40, 60, 80, and 100 ppm) for three hours and then cultured in pots. The experiment was conducted in a greenhouse. The combination of potting soil was 25% clay, 25% sand and 50% composted manure. The number of pots was 90 and they had a diameter of 20 cm and 8 holes for drainage.

The nano-silver solution with average particle diameter of 32 nm was obtained from Nanocide Company of Iran. The data of this study was analyzed by SAS software and the mean comparison was done according to Duncan test. Different parameters such as survival rate in the start and end of the experiment, plant height, diameter of canopy area, days to flowering, yield, essential oil yield and compositions of essential oil were determined.

Six months after planting, for evaluating the essential oils, areal parts of the plants at the flowering stage (50% flowering) were dried in the laboratory conditions. The samples were then powdered by a mill and the essential oil was extracted from 50 g of the resulting powder using Clevenger apparatus based on hydro-distillation method. After being dehydrated by sodium sulfate anhydrous, the essential oils were preserved in sealed sterile glass vials in the dark at 4°C for quantitative and qualitative analyses (British Pharm, 1988).

The isolated essential oils were analyzed using gas chromatography – flame ionization detector (GC/FID) for quantitative analysis and gas chromatography–mass spectrometry (GC/MS) for qualitative analysis on a HEWLETT-PACKARD 6890 gas chromatograph coupled with a mass detector (HEWLETT-PACKARD model 6973 HP). A fused silica HP-5 column, 60 m length, 250 µm I.D., and 0.25 µm film thickness was used as the oil analyser. The mass spectra were obtained by electron ionization at 70 eV. The oven operated isothermally with its temperature maintained first at 60°C for 30 min. It was then raised to 250°C at a rate of 5°C/min. The injection temperature was 250°C. The carrier gas (helium) flow rate was 1 ml/min. The sample (1 µl) was injected with a split ratio of 1/90. Retention indices were calculated for all components using a homologous series of n-alkanes injected in conditions equal to those of the samples. The compositions of the essential oil were identified by comparison of their retention indices (RI) and mass spectra with those of authentic samples in Wiley library (Adams, 2011).

RESULTS AND DISCUSSION

Descriptive evaluation of the traits showed the lowest score at the end of the survival period was zero and the highest was 4, which shows the effect of nano-silver on this variable. The difference between the minimum and maximum values for the most noticeable traits, which can occur due to the effects of nano-silver was investigated. The standard deviation of the variables under study was relatively low (Table 1).

Comparing mean levels of different amount of nano-silver in Duncan test showed surfaces 60 and 20 ppm nano-silver and control have the highest score of survival and 100 ppm has the lowest score survival at the end of the experiment.

Using different levels of nano-silver has significant effect on the height of thyme plants. Plants have the lowest height at 100 ppm nano-silver comparing to other treatments (Table 2). The largest diameter and canopy area belongs to 60 ppm nano-silver treatment. The lowest variability is observed at 100 nano-silver and control treatments. There is no fixed and specific process among treatments in terms of days to flowering and days to flowering were 50%. The highest yield is observed consequently in 40, 20 and 60 ppm nano-silver level. The lowest yield is observed in 100 ppm nano-silver. Moderate levels of using nano-silver have the highest essential oil content among treatments. Nano-silver levels of 40 and 20 ppm have the highest essential oils and the level 100 ppm has the lowest (Table 2).

Comparing the compositions of essential oils revealed that minor components do not show any particular trend with treatment by nano-silver .However there are significant differences between major components of essential oils (Table 3). The highest variation in major components can be seen with 60 ppm of nano-silver treatment, so the amount of α-terpinyl acetate is 14.2% in this essential oil while it is about 0.1% in other treatments. The other significant variation is in 100 ppm treatment because the amount of thymol in this treatment is less than carvacrol while in other treatments thymol is more than twice of carvacrol (Table 3).

According to this study and other papers, it is necessary

Table 1. Descriptive characteristics of the variables.

Traits	Max	Min	Mean
Survival rate in the start of the experiment	4	3.5	3.8±0.32
Survival rate at the end of the experiment	4	0	3.2 ±0.44
Plant height (cm)	35	11	19±6.8
Large diameter cover (cm)	78	18	50±12
small diameter cover (cm)	69	12	40±10
Canopy area (cm^2)	3342	580	1600±290
Days to flowering	66	38	48±7
Days to 50% flowering	72	45	59±9
Days to end of flowering	93	67	80±12
Yield (Kg/ha)	1185	800	1000±105
Essential oil amount (Kg/ha)	14.7	7.7	10±2
Essential oil (%)	1.52	0.83	1.2±0.1

Table 2. Comparing the traits mean in different nano-silver levels.

Traits	Blank	20	40	60	80	100
Survival rate in the start of the experiment	4^{a}	4^{a}	3.8^{a}	4^{a}	3.9^{a}	3.8^{a}
Survival rate at the end of the experiment	3.3^{ab}	3.4^{a}	3.1^{ab}	3.8^{a}	2.8^{c}	3.0^{c}
Plant height	19.8^{bc}	18.2^{bc}	19.4^{bc}	30.6^{a}	25.2^{ab}	15^{c}
Large diameter cover	35^{c}	44^{bc}	45.6^{bc}	69.8^{a}	56^{b}	32^{c}
Small diameter cover	27^{c}	31^{bc}	38^{b}	55.8^{a}	39^{b}	24^{c}
Canopy area	795^{d}	1180^{c}	1430^{bc}	3170^{a}	1850^{b}	650^{d}
Days to flowering	44^{c}	46^{c}	52^{b}	58^{a}	47^{c}	60^{a}
Days to 50% flowering	54^{c}	58^{c}	69^{a}	62^{bc}	56^{c}	67^{ab}
Days to end of flowering	85^{a}	76^{a}	78^{a}	82^{a}	81^{a}	87^{a}
Yield (Kg/ha)	873^{b}	972^{a}	1060^{a}	1170^{a}	890^{b}	868^{b}
Essential oil amount (kg/ha)	9.8^{b}	13.4^{a}	13.6^{a}	12.8^{a}	9.9^{b}	8.9^{c}
Essential oil (%)	1.2^{ab}	1.38^{a}	1.28^{ab}	1.09^{b}	1.11^{b}	1.05^{bc}

Numbers in each row with the same letters are not significantly different at 5% level.

Table 3. Comparison of the components of the essential oils (%) in different nano-silver levels.

Compound name	Sample blank	Sample (20)	Sample (40)	Sample (60)	Sample (80)	Sample (100)
o-Cymen	1.22	1.74	2.11	0.38	3.90	2.04
1,8 Cineol	0.45	0.52	-	0.16	0.59	1.35
cis-Sabinene hydrate	0.43	0.10	0.81	0.07	0.19	1.46
Borneol	1.60	-	1.26	0.20	0.11	3.26
Terpinen-4-ol	0.71	-	0.68	1.20	0.34	0.90
Benzoic acid 2-Hydroxy-methyl ester	0.21	-	3.04	-	1.38	0.04
Carvacrol- methyl ether	0.07	0.73	0.45	-	2.78	1.26
Nerol	0.04	-	-	-	2.78	1.26
Thymol	83.30	59.53	61.31	52.02	57.07	31.0
Carvacrol	3.62	24.84	25.63	23.25	23.80	49.11
α –Terpinyl acetate	0.11	0.15	0.1	14.2	0.06	0.1
ß -Caryophyllene	0.84	0.06	0.09	0.06	0.06	1.18
Germacren-D	0.02	2.16	1.35	1.14	1.56	-
ß-Bisabolene	1.82	0.56	0.36	-	0.18	0.76
Nerolidol	1.12	0.18	0.11	0.33	0.67	-

The amount of compared components in this table is more than 1% at least in one of the treatments.

to mention that nano-silver can be used to change the stages of plant growth and the amount of secondary metabolisms (secondary metabolites). One of the study which led to this conclusion was the effect of nano-silver on the saffron plants that the researchers declared by spraying 100 ppm of nano-silver, the corm and root number, weight and height of the plant were increased (Seif Sahandi et al., 2011). In another study the researchers found that increasing the concentration of nano silver from 20 to 60 ppm has led to an improvement in the seed yield of borage. Additionally, with increasing level of silver nitrate, the polyphenols compounds content were raised (Rezvani et al., 2012). However, the effect of nano-silver on plants needs more researches.

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REFERENCES

Aberoomand-Azar P, Saber-Tehrani M, Aghaei-Meibodi Z, Soleimani M (2010). Composition of essential oils of leaves, stems, and roots of *Thymus kotschyanus* var. *seuderiophorus* growing wild in Iran. Chem. Nat. Comp. 46(2):310-312.

Adams RP (2001). Identification of essential oil Components by Gas Chromatography / Quadrupole Mass Spectroscopy. Allured Pubi.Corp.Carol Stream, Ill, USA.

Amin G (2005). Popular Medicinal Plants of Iran. Tehran University of Medical Sciences Press, Tehran, Iran.

Bagci E, Baser KHC (2005). Study of the essential oils of two *Thymus* (*Lamiaceae*) taxa from Eastern Anatolian Region in Turkey. Flavour Fragr. J. 20:199-202.

Basir Y, Zarei H, Mashayekhi K (2011). Effects of nano-silver treatments on vase life of cut flowers of carnation. J. Adv. Lab. Res. Bio. 1 (2):49-55.

Bauer KD, Garbe H, Surburg H (1997). Common Fragrance and Flavor Materials, Wiley-VCH, Weinheim, Germany.

British Pharmacopoeia (1988). HMSO, London, 2:A137– A138.

Habibi H, Fakhr-Tabatabaee M, Bigdeli M (2006). Effect of Altitude on Essential Oil and Components in Wild *Thyme* (*Thymus kotschyanus* Boiss) Taleghan Region, Pajouhesh & Sazandegi, 73: 2-10.

Meriçli F (1986). Volatile Oils of *Thymus kotschyanus var*. *glabrescens* and *Thymus fedtschenkoi* var. *handelii*, J. Nat. Prod. 49(5):942.

Semnani MK, Rostami B, Akbarzadeh. M (2006). Essential oil composition of *thymus kotschyanus* and *thymus pubescens* from Iran. J. Essent Oil Res. 18(3): 272-274.

Nezhadali A, Akbarpour M, Zarrabi Shirvan B ,Mousavi M (2010). Comparison of volatile organic compounds of *Thymus vulgaris* using hydrodistillation and headspace solid phase microextraction gas chromatography mass spectrometry, J. Chin. Chem. Soc. 57:40-43.

Nickavar B, Mojab F, Dolat-Abadi R (2005). Analysis of the essential oils of two *Thymus* species from Iran. Food Chem. 90(4):609-611.

Pirigharnaei M, Zare S, Heidary R, Khara J, EmamaliSabzi R (2012). Determination and comparing of the essential oil components in wild and cultivated populations of *Thymus kotschyanus* Boiss. & Hohen. Afr. J. Plant Sci. 6(2):89-95.

Rechinger KH (1982) Flora Iranica. 1st ed. Akademische Druck- und erlagsanstalt. Austria. p. 152.

Rezvani N, Sorooshzadeh A, Farhadi N (2012). Effect of Nano-Silver on Growth of Saffron in Flooding Stress, WASET 61:606-611.

Rustaiyan A, Lajevardi T, Rabbani M, Yari M, Masoudi S (1999). Chemical constituents of the essential oil of *Thymus kotschyanus* Boiss. & Hohen from Iran. Daru 7(4):27-28.

Samavi S, Hassanzadeh N, Faghihi M, Rezaee Danesh Y (2009). Effects of Thyme (Zaatar) Essential oil and some chemical compounds in the control of citrus bacterial canker in Iran. J. Plant Pathol. 91(3):691-696.

Sefidkon F , Dabiri M, Rahimi-Bidgoly A (2000). The effect of distillation methods and stage of plant growth on the essential oil content and composition of *Thymus kotschyanus* Boiss. &Hohen. Flavour Fragr. J. 14(6):405-408.

Seif Sahandi M, Sorooshzadeh AH, Rezazadeh S, Naghdibadi HA (2011). Effect of nano-silver and silver nitrate on seed yield of borage. J. Med. Plant. Res. 5(2):171-175

Shah V, Belozerova I (2008). Influence of metal nanoparticles on the soil microbial community and germination of Lettuce seeds. Water Air Soil Pollut., 197: 143-148.

Zargari A (1997). Medicinal Plants, Tehran University Publications, Tehran, Iran.