Effect of *Thiobacillus* and superabsorbent on essential oil components in *Thyme* species

Pouneh Pouramini^{1,4}, Mohammad Hossein Fotokian², Hossein Dehghan³, Goetz Hensel^{4*}

¹Horticulture Department, Faculty of Agriculture Sciences, Karaj University, Karaj, Iran

²Agricultural College, Shahed University, Tehran, Iran.

³Medicinal Plants Research Center, Shahed University, Tehran, Iran.

⁴Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany.

*Corresponding author: Dr. Goetz Hensel

E-mail: hensel@ipk-gatersleben.de

Tel: +49 39482-5543

Fax: +49 39482-5515

Abstract 20

Optimal nutrition along with non-stress conditions has a significant impact on the quantity 21
and quality of essential oil in medicinal plants. The objective of this research was to examine 22
the possibility of improving the quantity and quality of essential oil in *thyme* through 23
nutrition of the seedlings using *Thiobacillus* bio-fertilizer and treatment by Superabsorbent. 24
For this purpose, seedlings of two *thyme* species (*Thymus vulgaris* and *Thymus daenensis*) 25
were sown in pots and exposed to different levels of *Thiobacillus* and superabsorbent. Results 26
of Gas Chromatography Mass Spectrometry (GC/MS) revealed that the main compounds 27
detected for *T. vulgaris* were thymol (31.5%), *p*-cymene (23.4%), γ -terpinene (13.9%), 28
linalool (38.3%) and carvacrol (2.7%) while the main compounds of *T. daenensis* were 29
thymol (51.2%), *o*-cymene (12.9%), γ -terpinene (4.5%), linalool (1.7%) and borneol (3.1%). 30
Furthermore, the application of *Thiobacillus* had a significant effect on α -pinene content 31
($p=0.05$). Moreover, the interaction between superabsorbent and *Thiobacillus* significantly 32
changed the percentage of thymol, borneol, and caryophyllene. In conclusion, the mean of 33
essence components in *T. vulgaris* was more than *T. daenensis* with the exception of thymol 34
and caryophyllene. 35

Keywords: borneol, carvacrol, caryophyllene, thymol, thyme 37

1. Introduction 40

The genus *Thymus*, with about 215 species, is one of the eight most important genera 41
of the Lamiacea family. *Thyme*, a plant native to the Mediterranean region (Spain, Italy, 42
France, Greece, etc.), has long been used as a source of essential oil and other constituents 43
(e.g. thymol, flavanoid, caffeic acid and labiatic acid) derived from the different parts of the 44
plant. In addition to their numerous traditional uses, the plant (herb) and its essential oil have 45
found diverse applications in pharmacy and medicine [1]. *Thymus vulgaris* is a perennial 46
medicinal plant, cultivated worldwide for a lot of uses like culinary, cosmetic and medical 47
purposes. This species has special activities such as antispasmodic, expectorant, antiseptic, 48
antimicrobial and antioxidant [2,3]. *Thymus daenensis* is native to Iran [4]. 49

Depending on the growing conditions (different environments, climates, soil, 50
geographical location) *Thyme* can provide various therapeutic properties [5]. Plant growth, 51
quality, and quantity of active ingredients mainly depends on genetic manipulation while 52
environmental factors play a major role by leading to changes in the growth, quality and 53
quantity of active substances such as alkaloids, glycosides, essential oils [6]. 54

Thyme contains 0.8 to 2.6% (generally 1%) of essential oils which is primarily 55
comprised (20 to 80%) of *phenols*, *monoterpenes* (e.g. *p-cymene* and *γ-terpinen*), and 56
alcohols (e.g. *linalool*, *α-terpinene* and *is thujan-4-ol*). In general, *thymol* constitutes the 57
highest content of phenolic compounds while *carvacrol* is a minor part of the essential oil of 58
Thyme [7]. In a study by Barazandeh and Bagherzadeh [8] investigating the essential oil of 59
the aerial parts of *Thymus daenensis*, GS/MS results showed that among the 43 indicated 60
components, *thymol* (73.9%), *carvacrol* (6.7%), *para-cymene* (4.6%), *β-bisabolene* (1.5%) 61
and *terpinene 4-L* (1.4%) were the main constituents. 62

Bio-fertilizers are of great importance and play the largest role in increasing the 63
productivity and maintaining soil fertility in sustainable agriculture [9]. Bio-fertilizers are 64

microbial inoculants consisting of living cells of microorganism like bacteria, algae and fungi 65
alone or combination which can help in increasing crop productivity. Organic manures can 66
serve as alternative to mineral fertilizers for improving soil structure, soil nutrients, soil 67
physical and chemical characteristics, increase soil fertility and plant plants resistance to 68
diseases and salt stress, decrease plant diseases, improving crop growth, development, yield 69
and quality through directly synthesizing hormones, antibodies and secondary metabolites 70
[10,11]. through organic and biological methods plays a key role in increasing qualitative and 71
quantitative herbal and essential oil yield [12]. 72

In many regions of Iran, soil type is calcareous with high pH. Therefore, some 73
nutrients such as phosphorus, iron and zinc are stabilized and non-absorbable for plants. In 74
such situations, *Thiobacillus* bacteria can reduce the pH of the soil in the presence of sulfur 75
resulting in the solubility of nutrients around the root area [13]. 76

These previously mentioned issues have increased along with limited water resources 77
in the arid and semi-arid ecosystems of Iran [14]. Water stress has many adverse effects on 78
the plant's processes such as reduction in the leaf area index, the number of leaves and shoots 79
dry weight [15,16]. Medicinal plants are not accepted and water stress reduces the quantity 80
and quality of active ingredients while these herbs need to perfect vegetative and 81
reproductive growth in order to combine the ingredients [16]. Superabsorbents are able to 82
reduce the need to be irrigated by up to 33% with no side effects on the soil and the 83
environment [14]. Superabsorbent is natural or synthetic contain over 99% water. Hydrogels 84
have been defined as polymeric materials which exhibit the ability of swelling in water and 85
retaining a significant fraction (>20%) of water within their structure, without dissolving in 86
water [17]. Superabsorbent hydrogels are increase soil capacity to hold water, nutrients and 87
improve the plant's growth and yield [18]. Although the application of superabsorbent and 88
Thiobacillus have been separately studied, to our knowledge, their combined effects have not 89

yet been examined on medicinal plants. Therefore, this research was conducted to investigate the possibility of improving the quantity and quality of essential oil in *Thyme* through nutrition of the seedlings by *Thiobacillus* bio-fertilizer and increase of available water through the application of a superabsorbent.

2. Materials and methods

2.1 Experimental condition

The experiment was conducted in a greenhouse on a research farm of an Agricultural Engineering Park affiliated with the Ministry of Agriculture in Jihad, Karaj, Iran (56.5 ° East longitude and 35.47 ° N latitude) during 2012. Plant growth was carried out in a greenhouse at 24/18°C day/night mean temperature and 65% air relative humidity. Properties of used soil are presented in Table 1.

2.2 Treatments

A factorial experiment using a completely randomized design with two replications was performed. The experiment consists of three factors of *Thiobacillus* (two levels of present and absent), superabsorbent (three levels of 0, 0.5 and 1 g k⁻¹ soil) and *Thyme* (*Thymus vulgaris* and *Thymus daenensis*).

2.3 Herb processing and essential oil isolation

The seeds were planted on 5 April 2011 and germinated after 17 days. The first flower was visible on 13 July, while the plants were harvested at a full flowering phase on 30 August, air-dried at 25°C in the shade and subsequently well crushed. The essential oil was extracted by hydro distillation for 120 min using a Clevenger-type system [19]. Collected essential oils were stored in dark flasks and kept at -18°C for GC/MS analysis.

2.4 Statistical analysis 115

Statistical analyses were conducted using SPSS version 16.0. A normality test of 116
collected data residuals was conducted. Analysis of variance (ANOVA) was performed 117
followed by Duncan's multiple range test to compare the effects of main and/or sub-main 118
factor levels combination. Pearson's correlation coefficients were used to detect associations 119
between dependent measures. Statistical significance was concluded with a *P*-value less than 120
0.05. 121

3. Results and discussion 123

3.1 Comparison of *Thymus vulgaris* and *Thymus daenensis* essential oil composition 124

The *Thymus vulgaris* essential oil included 31 constituents which represented 96.7% 125
of the total analyzed oil. The main compounds included thymol (31.5%), *p*-cymene (23.4%), 126
 γ -terpinene (13.9%), linalool (3.4%), thymol methyl ether (3.6%) and carvacrol (2.7%). 127
Further compounds and their constituents are presented in Table 2. In contrast, the *Thymus* 128
daenensis species contained 33 compounds in the essential oil which represented 96.19% of 129
the total analyzed oil. The main compounds included thymol (51.18%), *O*-cymene (12.87%), 130
 γ -Terpinene (4.48%), linalool (1.68%) and borneol (3.09%). The major differences are in the 131
thymol content as well as monoterpenes (Table 2, grey highlighted). While some compounds 132
are present only in one species, some other compounds show only minor differences between 133
both species. 134

The components identified in *Thymus vulgaris* are in accordance with a study 135
analysing material from Saudi Arabian markets [20]. Since *Thymus daenensis* had a higher 136
thymol content compared to *Thymus vulgaris* previous studies focused on this species. Our 137
results find thymol still the main component of the *Thymus* oil but the quantities are below 138

previous published data. The reason for that might be the different soil characteristics because 139
the studies have been performed in different parts of Iran [4,8]. 140

Extractable oil composition strongly depends on the method used. In a study by 141
Charles and Simon [21] comparison of different extraction methods showed that hydro 142
distillation on basil lead to the greatest number of constituents with the highest number of 143
volatile constituents in the organic solvent extract. Therefore, hydro distillation appears to be 144
an excellent method for extracting the essential oil of basil as it results in good yield, good 145
recovery of essential oil constituents, is less labor-intensive, and is simpler and faster than 146
steam distillation. 147

3.2 Treatment with superabsorbent and *Thiobacillus* on essential oils content 148 149

Analysis of variance of some components of thyme essential oil is presented in Table 150
3. The difference between two thyme species was significant ($P \leq 0.01$) for all of the 151
quantities of the compounds (Table 4) while the superabsorbent effect was not significant for 152
any compound. The *Thiobacillus* effect was only significant for α -Cymene ($P \leq 0.05$). In the 153
case of treatment interactions, it was found that the superabsorbent $\times$ *Thiobacillus* interaction 154
had a significant effect on borneol and caryophyllene compounds. Furthermore, the species $\times$ 155
Thiobacillus interaction was significant for thymol. The triple interaction among species $\times$ 156
superabsorbent $\times$ *Thiobacillus* had a significant effect on borneol and caryophyllene. 157

A review of the literature regarding the application of *Thiobacillus* showed that there 158
was little information about the use on medicinal plants. In a recent study, the effect of Bio- 159
fertilizer with superabsorbent on the yield and essential oil content of lemon verbena (*Lippia* 160
citriodora Kunth.) were analysed. The authors could show that sole application of treatments 161
was useful, but their combined use was more effective to increase dry mass and oil yield of 162
lemon verbena [18]. In another study in dragonhead, the effect of chemical fertilizer and 163

microbial inoculum application on the yield and nutrients content of *Dracocephalum* 164
moldavica L. seeds was evaluated. In a field experiment, the highest yield was obtained by 165
chemical fertilizers, while seed inoculation with sulfur oxidizing bacteria (*Thiobacillus* 166
strains) improves the iron nutrient content of the seeds [21]. Also, in the presence of sulfur, 167
Thiobacillus increased nutrient uptake, growth and production of *Melissa officinalis* oil 168
compared to the control [12]. 169

Razban and Pirzad [16] investigated the effect of superabsorbent on *Matricaria* 170
chamomilla growth under different irrigation regimes. They reported that superabsorbent was 171
able to compensate for the reduction of biomass yield due to lack of water. 172

3.3 Relations between the main compounds of essential oil 174

Pearson's correlation coefficients between traits showed a significant positive 175
association between the numbers of traits ($P \leq 0.01$) (Table 5) suggesting these may be due to 176
pleiotropic effects of genes controlling the same pathways, close linkage, and/or epistasis 177
effects. 178

3.4 Interaction between factors 180

3.4.1 Superabsorbent $\times$ *Thiobacillus* interaction 181

Combined levels of the superabsorbent $\times$ *Thiobacillus* interaction were significant for borneol 182
and caryophyllene (Table 4 and Figure 1; $P \leq 0.05$). The maximum amount of borneol was 183
observed in S_0T_0 treatment, although there was no significant difference in the amount of 184
borneol in the treatment $S_1T_{0.5}$. Although the maximum amount of caryophyllene was 185
observed in $S_{0.5}T_{0.5}$, the difference of this treatment with $S_0T_{0.5}$ and S_1T_0 treatments was not 186
statistically significant (Figure 1). The differences among superabsorbent levels were not 187
statistically significant for essential components. One reason for this may be the low 188

difference among levels of superabsorbent. With the increase of the content in superabsorbent treatment levels, the differences among these levels may be significant for essence components.

3.4.2 Species $\times$ *Thiobacillus* interaction

The interaction between species $\times$ *Thiobacillus* was significant for thymol ($P \leq 0.05$; Table 4). Using *Thiobacillus* in *Thymus daenensis* led to a significant reduction in the amount of thymol and an increase in the amount of thymol in *Thymus vulgaris* (Figure 2).

3.4.3 The triple interaction among species $\times$ superabsorbent $\times$ *Thiobacillus*

Significant differences among combinations of this interaction were observed only for Borneol and caryophyllene ($P \leq 0.05$) (Table 6). The maximum amount of caryophyllene was obtained with *Thymus daenensis* in the $S_{0.5}T_{0.5}$ treatment, with no statistically significant differences of this treatment with S_1T_0 . Application of superabsorbent and *Thiobacillus* did not improve the amount of borneol. The amount of borneol in *Thymus vulgaris* was significantly higher than *Thymus daenensis*.

The maximum amount of borneol was observed in the S_0T_0 treatment, although there was no significant difference in the amounts found in the $S_1T_{0.5}$ treatment. Although the maximum amount of caryophyllene was observed in $S_{0.5}T_{0.5}$, but the difference of this treatment with $S_0T_{0.5}$ and S_1T_0 treatments was not statistically significant (Figure 1).

The interaction between species $\times$ superabsorbent was not significant for all traits, while for species main factor it was significant at $P \leq 0.01$. Referring to an acceptable amount of coefficient of variation (CV %), it is concluded that non-significance of some parts of variation for essence components sources is not due to the amount of experimental error.

The correlation between thymol with others was negatively significant, with the exception of
a positive correlation with caryophyllene. In conclusion, thymol is the most important part of
essence component in *Thymus*; any trying to increase thymol content will decrease other
essence components.

4. Conclusion

The interaction between *Thiobacillus* and superabsorbent on oil and essential oil yield was
significantly greater for borneol and caryophyllene. Furthermore, *Thymus vulgaris* oil yield
was higher than *Thymus daenensis*. Despite the fact that *Thymus vulgaris* is not native to Iran,
its high value in the pharmaceutical, cosmetic, health and culture, food and export will
encourage its cultivation. In addition, the variance analysis of our data showed the amount of
all components in *T. vulgaris* are much higher than *T. daenensis*, with the exception of
thymol and caryophyllene. Hence, in the cultivation of *Thymus* for the production of essence
components, cropping of *T. vulgaris* is recommended.

Tables

Table 1. Selected physical and chemical properties of soil used in this study.

Tissue	P [ppm]	K [ppm]	Total Nitrogen [%]	Organic matter [%]	Calcium carbonate equivalent [%]	pH	EC [ds/m]	Percent saturation [%]
Loamy	38	344.7	0.22	2.19	7.5	8.26	1.32	45

Table 2. Compounds identified in *Thymus vulgaris* and *Thymus daenensis*

No.	Compound	<i>Thymus vulgaris</i>		<i>Thymus daenensis</i>		Class
		RT [min]	[%]	RT [min]	[%]	
1	α -Thujene	11:12	1.51	11:05	0.90	MH
2	α -Pinene	11:44	1.17	11:35	0.68	MH
3	Camphene	12:10	0.78	12:05	0.52	MH
4	β -Pinene	13:49	0.36	13:44	0.30	MH
5	Octen-3-ol	-	-	13:86	0.23	Others
6	β -Myrcene	14:39	3.32	14:32	0.88	MH
7	Phellandrene	-	-	14:91	0.18	MH
8	α -Phellandrene	15:23	0.44	-	-	MH
9	<i>p</i> -Cymene	16:90	23.40	-	-	MH
10	α -Terpinene	-	-	15:60	1.14	MH
11	<i>o</i> -Cymene	-	-	16:31	12.87	MH
12	1,8-Cineole	-	-	16:52	1.94	MO
13	γ -Terpinene	18:63	13.94	17:95	4.48	MH
14	β -Terpineol	18:80	0.87	18:31	0.84	MO
15	α -Terpinolene	19:50	0.25	19:24	0.15	MH
16	Linalool	20:47	3.38	20:06	1.68	MO
17	Borneol	23:34	1.02	23:73	3.09	MO
18	Terpinen-4-ol	23:85	0.84	-	-	MO
19	Borneol	24:49	1.61	-	-	MO
20	α -Terpineol	-	-	25:76	3.30	MO
21	Dihydro carvone	-	-	26:16	0.13	MO
22	Thymol methyl ether	26:72	3.58	26:47	1.19	MO
23	Carvacrol methyl ether	27:10	1.65	26:89	0.58	MO
24	Thymoquinone	-	-	28:59	3.02	MO
25	Thymol	30:62	31.50	30:64	51.18	MO
26	Carvacrol	31:13	2.66	-	-	MO
27	Caryophyllene	35:06	1.55	34:96	2.41	SH
28	Aromadenderene	-	-	35:67	0.17	SH
29	α -Humulene	-	-	36:28	0.28	SH
30	Geranyl propionate	-	-	37:00	0.12	Others
31	Geranyl propionate	37:13	0.46	-	-	Others
32	α -Amorphene	37:26	0.12	-	-	SH
33	Germacrene D	37:46	0.13	-	-	SH
34	Viridiflorene	-	-	37:95	0.15	SH
35	Cubebol	37:99	0.10	-	-	SH

36	β -Bisabolene	-	-	38:47	0.42	SH
37	γ -Cadinene	38:77	0.18	39:05	0.16	SH
38	δ -cadinene	39:13	0.43	-	-	SO
39	Thymohydro quinone	-	-	40:82	1.18	SO
40	Caryophelen oxide	41:54	0.75	41:52	2.13	SO
41	Eudesmol	42:87	0.09	-	-	SO
42	α -epi-Cadinol	43:68	0.21	-	-	SO
43	α -Cadinol	44:17	0.10	-	-	SO
44	(E)-14-Hydroxy-9-epi-caryophyllene	44:81	0.13	-	-	SO
45	α -epi-Bisabolol	-	-	45:20	0.12	Others
46	Hexadecanoic acid	54:87	0.13	54:84	0.13	Others
47	Abiatatriene	-	-	60:20	3.22	MH
Total Identified			96.67		96.19	

MH – monoterpene hydrocarbons; MO – oxygenated monoterpenes; SH – sesquiterpene hydrocarbons; SO – oxygenated sesquiterpenes

233

234

235

Table 3. Analysis of variance of the effects of superabsorbent and *Thiobacillus* on the essential oil of two species of thyme

S.O.V	df	Mean squares									
		α -Thujene	α -Pinene	Camphene	β -Myrcene	<i>p</i> -Cymene	γ -Terpinene	Linalool	Borneol	Thymol	Caryophyllene
S	1	0.562 ^{**}	1.321 ^{**}	0.702 ^{**}	11.323 ^{**}	2110.034 ^{**}	441.438 ^{**}	19.706 ^{**}	3.683 ^{**}	2545.542 ^{**}	28.423 ^{**}
SA	2	0.024 ^{ns}	0.058 ^{ns}	0.038 ^{ns}	0.061 ^{ns}	7.561 ^{ns}	11.952 ^{ns}	0.005 ^{ns}	0.232 ^{ns}	74.919 ^{ns}	0.251 ^{ns}
T	1	0.022 ^{ns}	0.003 ^{ns}	0.001 ^{ns}	0.018 ^{ns}	40.755 [*]	0.832 ^{ns}	0.438 ^{ns}	0.061 ^{ns}	1.322 ^{ns}	0.295 ^{ns}
T×SA	2	0.026 ^{ns}	0.054 ^{ns}	0.034 ^{ns}	0.001 ^{ns}	10.054 ^{ns}	4.751 ^{ns}	0.238 ^{ns}	0.481 [*]	11.094 ^{ns}	0.559 [*]
S×SA	2	0.021 ^{ns}	0.030 ^{ns}	0.010 ^{ns}	0.075 ^{ns}	0.319 ^{ns}	11.882 ^{ns}	0.338 ^{ns}	0.001 ^{ns}	41.655 ^{ns}	0.263 ^{ns}
S×T	1	0.055 ^{ns}	0.107 ^{ns}	0.018 ^{ns}	0.002 ^{ns}	25.058 ^{ns}	0.695 ^{ns}	0.096 ^{ns}	0.034 ^{ns}	433.359 [*]	0.112 ^{ns}
S×T×SA	2	0.009 ^{ns}	0.026 ^{ns}	0.036 ^{ns}	0.008 ^{ns}	22.564 ^{ns}	5.611 ^{ns}	0.183 ^{ns}	0.384 [*]	61.169 ^{ns}	0.469 [*]
Error	11	0.013	0.027	0.028	0.022	8.132	3.251	0.317	0.089	43.097	0.108
CV %	-	26.4	25.1	29.2	17.5	16.9	29.2	29.4	24.6	11.6	16

Symbols: S, Species; SA, Superabsorbent; T, *Thiobacillus*.

*, ** significant differences at $P \leq 0.05$ and $P \leq 0.01$, respectively.

Table 4. Mean comparisons between two species for essence components by Duncan's multiple range test

Species	α -Thujene	α -Pinene	Camphene	β -Myrcene	<i>p</i> -Cymene	γ -Terpinene	Linalool	Borneol	Thymol	Caryophyllene
<i>T. vulgaris</i>	0.583	0.887	0.743	1.529	26.181	9.192	2.823	1.603	46.297	0.964
<i>T. daenensis</i>	0.277	0.418	0.401	0.156	7.428	0.615	1.011	0.8197	66.895	3.141

-Refer to variance analysis (Table 3), the differences between two *Thymus* species is statistically significant at $P \leq 0.01$.

Table 5. Pearson's correlation coefficients between some essential components of two species of *Thyme*

Compound	α -Thujene	α -Pinene	Camphene	β -Myrcene	<i>p</i> -Cymene	γ -Terpinene	Linalool	Borneol	Thymol
α -Pinene	0.91 ^{**}								
Camphene	0.782 ^{**}	0.848 ^{**}							
β -Myrcene	0.748 ^{**}	0.781 ^{**}	0.701 ^{**}						
<i>p</i> -Cymene	0.789 ^{**}	0.798 ^{**}	0.733 ^{**}	0.932 ^{**}					
γ -Terpinene	0.76 ^{**}	0.696 ^{**}	0.655 ^{**}	0.881 ^{**}	0.874 ^{**}				
Linalool	0.635 ^{**}	0.654 ^{**}	0.7 ^{**}	0.836 ^{**}	0.812 ^{**}	0.732 ^{**}			
Borneol	0.619 ^{**}	0.691 ^{**}	0.764 ^{**}	0.634 ^{**}	0.635 ^{**}	0.546 ^{**}	0.831 ^{**}		
Thymol	-0.606 ^{**}	-0.641 ^{**}	-0.591 ^{**}	-0.790 ^{**}	-0.887 ^{**}	-0.816 ^{**}	-0.674 ^{**}	-0.437 [*]	
Caryophyllene	-0.761 ^{**}	-0.786 ^{**}	-0.669 ^{**}	-0.893 ^{**}	-0.892 ^{**}	-0.837 ^{**}	-0.859 ^{**}	-0.745 ^{**}	0.733^{**}

*, ** significant differences at $P \leq 0.05$ and $P \leq 0.01$ probability level, respectively

239

Table 6. Comparison interaction of species, superabsorbent and *Thiobacillus* on borneol and caryophyllene on *Thymus vulgaris* and *Thymus daenensis*

Interactions	Caryophyllene [%]	Borneol [%]
V × S0 × T0	0.9934 ^d	2.289 ^a
V × S0 × T0.5	0.9528 ^d	1.301 ^{bcd}
V × S0.5 × T0	0.8876 ^d	1.281 ^{bcd}
V × S0.5 × T0.5	1.124 ^d	1.621 ^{abc}
V × S1 × T0	0.8859 ^d	1.278 ^{bcd}
V × S1 × T0.5	0.9458 ^d	1.849 ^{ab}
D × S0 × T0	2.351 ^c	1.081 ^{cde}
D × S0 × T0.5	3.112 ^{bc}	0.9007 ^{cde}
D × S0.5 × T0	2.82 ^c	0.9435 ^{cde}
D × S0.5 × T0.5	3.92 ^a	0.3738 ^e
D × S1 × T0	3.715 ^{ab}	0.6984 ^{de}
D × S1 × T0.5	2.93 ^c	0.9215 ^{cde}

-Treatment symbols: T₀, 0.0 g *Thiobacillus*; T_{0.5}, 0.5 g *Thiobacillus*; S₀, 0.0 g superabsorbent; S_{0.5}, 0.5 g superabsorbent; S₁, 1.0 g superabsorbent; V, *Thymus vulgaris*; D, *Thymus daenensis*.

-Values with different letters in a column are significantly different according to Duncan's Multiple Range Test (P ≤ 0.05)

240
241
242
243
244

245

Figure legends

246

Figure 1. Caryophyllene and borneol change trend across superabsorbent and *Thiobacillus*

247

interaction levels over the *Thymus* species. Values with different letters in each graph are

248

significantly different according to Duncan's Multiple Range Test (P ≤ 0.05).

249

250

Figure 2. Mean comparisons of Thymol under the interaction of species × *Thiobacillus*. V

251

and D present *Thymus vulgaris* and *Thymus daenensis*, respectively. Values with different

252

letters are significantly different according to Duncan's Multiple Range Test (P ≤ 0.05).

253

254

255

256

5. References

1. Leung AY, Foster S (1996) Encyclopedia of common natural ingredients used in food, drugs, and cosmetics. John Wiley & Sons, Inc DOI 10.1021/np960461r
2. Abdulkarimi R, Daneshyar M, Aghazadeh A (2011) Thyme (*Thymus vulgaris*) extract consumption darkens liver, lowers blood cholesterol, proportional liver and abdominal fat weights in broiler chickens, volume 10, Issue 2, Ital J Animal Sci DOI.org/10.40801/ijas.2011.e20
3. Satyal P, Murray BL, Mcfeeters RL, Setzer WN (2016) Essential Oil Characterization of *Thymus vulgaris* from Various Geographical Locations. Food J 5(4), 70, DOI 10.3390/foods5040070
4. Nickavar B, Mojab F, Dolat-Abadi R (2005) Composition of the Volatile Oil of *Thymus daenensis* Celak. subsp. *daenensis*. J Med Plants 1:45-49
5. Intodia SK, Sahu MP (2005) Effect of sulphur fertilization on growth of opium poppy in calcareous soils of south Rajasthan. Indian J Plant Physiol 10:90-93
6. Esmaeeli A, Amiri H (2006) The study of quantitative and qualitative changes of essential oil from *Smyrniun cordifolium* boiss. In lorestan province. J Med. Plants 5:36-41
7. Naghdi badi H, Makizadeh tafti M. (2002) Review of common thyme. J Med. Plants 2:1-12
8. Barazandeh M, Bagherzadeh K (2007) Investigation on the Chemical Composition of the Essential of *Thymus daenensis* Celak from Four Different Regions of Isfahan Province. J Med. Plants 3:15-19
9. Mehnaz S, Lazarovits G (2006) Inoculation effects of *Pseudomonas putida*, *Gluconacetobacter azotocaptans*, and *Azospirillum lipoferum* on corn plant growth under greenhouse conditions. Microb Ecol 51:326-335 DOI 10.1007/s00248-006-9039-7
10. Sharafzadeh Sh and Ordookhani, K (2011) Organic and Bio Fertilizers as a Good Substitute for Inorganic Fertilizers in Medicinal Plants Farming. Aust J Basic Appl Sci 5(12); 1330-1333
11. Kumari B, Hora A, Mallick MA (2017) Stimulatory effect of PGPR (Plant Growth Promoting Rhizospheric Bacteria) on Medicinal and Growth properties of a potential medicinal herb *Chlorophytum borivillianum*: A review. J Plant Sci Res 33(2): 151-156
12. Yadegari M, Barzegar R (2010) The effect of sulphur and *Thiobacillus* on nutrient absorption, growth and essential oil in lemon balm (*Melissa officinalis* L.). J Herbal Drugs 1:28-32
13. Besharati H, Saleh rastin N, Malakouti MJ (2004) Study of thiobacilli survival on different carriers. Iranian J Soil Waters Sci 18:170-181
14. Banj Shafiei A, Eshaghi Rad J, Alijanpour A, Pato M (2012) Effects of super-absorbent application and irrigation period on the growth of pistachio seedlings (*Pistacia atlantica*), (Case study: Dr. Javanshir nursery, Piranshahr). Iranian J Forest 4(2): 101-112
15. Lazcano-Ferrat I, Lovatt CJ (1999) Relationship between Relative Water Content, Nitrogen Pools, and Growth of *Phaseolus vulgaris* L. and *P. acutifolius* A. Gray during Water Deficit. Crop Sci DOI 10.2135/cropsci1999.0011183X0039000200028x
16. Razban M, Pirzad AR (2012) Evaluate the effect of varying amounts of super absorbent under different irrigation regimes on growth and water deficit tolerance of german chamomile (*Matricaria chamomilla*), as a second crop. J Sustain Agricult Prod Sci 21(2): 123-137
17. Zohuriaan-Mehr MJ and Kabiri K (2008) Superabsorbent Polymer Materials: A Review. Iranian Polymer J 17(6): 451-477

18. Shahhoseini R, Saeidi K, Babaahmadi H, Ebadi MT (2018) Effect of Fertilizers and Superabsorbent Hydrogel on the Yield, Essential Oil Content and Composition of Lemon verbena (*Lippia citriodora* Kunth.) Cultivated in Iran. J Essential Oil Bearing plants. 21:1, 230-236, DOI: 10.1080/0972060X.2017.1422808
19. Brown DE & Walton NJ (1999) Chemicals from plants: perspectives on plant secondary products. Imperial College Press, London
20. Al-Asmari AK, Athar MdT, Al-Faraidy AA, Almuhaiza MS (2017) Chemical composition of essential oil of *Thymus vulgaris* collected from Saudi Arabian market. Asian Pac J Tropic Biomed 7(2): 147–150
21. Charles DJ and Simon JE (1990) Comparison of Extraction Methods for the Rapid Determination of Essential Oil Content and Composition of Basil. J American Soc Horticult Sci 115(3): 458-462
22. Rahimzadeh S, Sohrabi Y, Pirzad A, Sheykhbaglou R (2017) Effect of Biological and Chemical Fertilization on the Yield and Nutrients of Moldavian Balm (*Dracocephalum moldavica* L.) Seeds. J Siberian Fed Univ 10(3): 323-332

Figure 1. Caryophyllene and borneol change trend across superabsorbent and *Thiobacillus* interaction levels over the *Thymus* species. Values with different letters in each graph are significantly different according to Duncan's Multiple Range Test ($P \leq 0.05$).

Figure 2. Mean comparisons of thymol under the interaction of species $\times$ *Thiobacillus*. V and D present *Thymus vulgaris* and *Thymus daenensis*, respectively. Values with different letters are significantly different according to Duncan's Multiple Range Test ($P \leq 0.05$).