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The Effect of different irrigation intervals and anti-transpiration compounds on yield and yield components of Black Cumin (*Nigella sativa*)

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Abstract

Water deficit stress, permanent or temporary, limits the growth and the distribution of natural vegetation and the performance of cultivated plants more than any other environmental factor. Mechanism involved is still not clear. In order to investigate the effect of different irrigation intervals and anti-transpiration compounds on yield and yield components of black cumin (*Nigella sativa*), an experiment was conducted at research station, Faculty of Agriculture, Ferdowsi University of Mashhad, in 2012 - 2013. A split-plot layout based on randomized complete block design with three replications was used. Irrigation intervals (8, and 16 days) were allocated to main plots and anti-transpiration compounds including chitosan (0.25, 0.5, 1 percent) and *Plantago psyllium* mucilage (0.5, 1, 1.5 percent) and arabic gum (0.25, 0.5, 0.75 percent) were allocated to sub plots. Results showed that irrigation intervals had significant effects on number of capsule per plant, number of seeds per capsule, number of seeds per plant, seed weight per plant, seed yield, biological yield and harvest index (HI), but there was no significant effect on weight of 1000 seeds. The 8 days irrigation interval produced more grain yield compared to 16 days irrigation intervals (621.56kg/he, 484.23kg/he). The greatest seed yield (760 kg/he) and lowest (419 kg/he) respectively at treatment anti-transpiration compound chitosan 1% with 8 days irrigation interval and arabic gam 0.25% was obtained with 16 days irrigation interval. It seems that due to the lack of water in the area, anti-transpiration compound chitosan 1% with 8 days irrigation interval is the best combination for *nigella sativa* grain production in Mashhad.

Keywords: Black cumin, Irrigation intervals, Anti-transpiration compounds, Yield

Introduction

The ever-increasing tendency to the use of medicinal plants in the world has grown concerns about their cultivation and production processes. As medicinal plants are more compatible with the nature, special interest and attention has recently been given to herb therapy, and use of

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medicinal plants, being limited by the rise of pharmaceutical drugs, has become again common and widespread due to a number of reasons (Omidbaigi., 2000). *Nigella sativa* is one of the herbs that has a variety of uses and has been being used in Iran's traditional medicine since old times. Today this plant is considered as one of the most important kinds of medicine. Therefore, it is of great importance to conduct some researches on the herbs around the country due to different ecological requirements. *Nigella sativa* belonging to buttercup family, with the scientific name of *Ranunculaceae,* is an annual, dicotyledonous, herbaceous plant. This plant is widespread within North Africa, southern Europe, the Mediterranean regions to India and Southeast and West Asia, and Australia, but researchers believe its origin as being in the Middle East and West Asia. Its geographical distribution in Iran is typically extended in the center, Northeast in Tabriz, South in Fars and East in Khorasan (Davazdaemami and Majnonhoseni., 2006؛Das et al., 1992). In several studies, *nigella sativa* has been reported to have anti-oxidative, anti-inflammatory, strengthening of immune system, and anti-histamine and oil extract properties. Furthermore, several effects such as lowering blood sugar, lipids, and hypertension, excretion of bile and uric acid, protection of liver, kidney and cardiovascular tissues as well as anti-seizure, anti-cancer, anti-microbial and anti-parasitic effects related to this plant have been reported (Khoram Del et al., 2008). Thus, considering the importance of *nigella sativa* in pharmaceuticals, food and cosmetics industries, it is strongly needed to conduct comprehensive studies on the cultivation and development of the plant**.** Previous studies have indicated that the construction of active ingredients in medicinal plants is influenced by genotype and environmental factors. Water, not only ecologically but also physiologically, is of great importance, because it contributes to most of the internal processes in plants and almost all the metabolic activities in plant cells, including construction of active ingredients in medicinal plants, depend on water (Norozpoor and RezvaniMoghaddam., 2002؛Riaz et al., 1996؛Safikhani et al., 2007). Shortage of absorbed water by plants can cause a series of morphological, physiological and biochemical changes as following: reduced swelling and cell growth, thereby, reducing the leaf area and plant height, closure of stomata and photosynthesis limitation, increased soluble compounds for adjusting osmotic pressure, reduction in nutrients uptake and ultimately decrease in the plant productivity, and these reactions create different effects, depending on the plant's growth phase, and severity and duration of the stress (Lebaschi et al., 2003). With an average rainfall of 240 mL, Iran is classified as one of the dry regions in the world. High rates of evapotranspiration, water resources constraints and other factors have made researchers to pay more attention to the studies on the effects of drought stress (Hassani et al., 2005). In dry regions, most of water loss occurs as a result of plant transpiration. Reducing the rate of water loss through evapotranspiration, in addition to reduced water consumption, while preserving the existing water resources and their optimum use, in large parts of the country, soil salinity can also be partly avoided. Mean while, the use of anti-transpiration materials is a promising tool for the regulation of transpiration to maintain the plant's water in an optimum level. In this respect, the strategies such as the use of anti-transpiration materials have the potential for regulation of transpiration. As a results of some mechanisms like stomatal closure, reflecting more sunlight and reduced shoot growth, the anti-transpiration material can play a role in increasing plant resistance to water shortage conditions (Ibrahim et al., 2010؛Iiriti et al., 2009؛Kattlewell et al., 2010). Today in agriculture, different materials are used as anti-transpiration, including chitosan and natural compounds in medicinal plants. Stimulating the biosynthesis of abscisic acid, chitosan influences on stomatal opening and the rate of water loss in the plant. In an experiment carried out by Del Amora et al (2010) the effect of anti-transpiration pinole compounds, treatments of carbon dioxide concentrations and irrigation intervals on the pepper-plant under drought stress was studied. Anti-transpiration compound with 2.5%, carbon dioxide with two concentrations of 380 and 200 mM and irrigation at intervals of 4 and 8 days were applied to the plant. Results showed that water potential of the plant treated with anti-transpiration compounds was more than that of control plant. Also, the plants treated with this compound showed more resistance than those under tension conditions. Iiriti et al (2009) examined the effect of the anti-transpiration compound of chitosan on bean plant. Results showed that chitosan stimulating abscisic acid synthesis in the treated plant would result in stomatal closure, reduction of stomatal conductance, transpiration rate and water content. They also pointed out that the anti-transpiration effect of chitosan was because of its stimulatory effect in increasing abscisic acid concentration in the treated leaves of bean plant. Ibrahim et al (2010) conducted an investigation over the effect of irrigation intervals and kaolin anti-transpiration compoundon pumpkin. The experiment was done over three irrigation intervals (8, 12, 16 days) and kaolin at 0, 3, 6 percent levels. Results showed that with irrigation every 8 days, bush dry weight, leaf weight per plant, average fruit weight and yield increased. Furthermore, the 6% kaolin in the 12 day irrigation interval increased water use efficiency. Present study aims to investigate the possibility of improving the yield and yield components of *nigella sativa* through anti-transpiration compounds, under drought stress.

Materials and Methods

The experiment was carried out in the 2012-2013 crop year at the Agriculture Faculty of Ferdowsi University, Mashhad, Iran. Before testing, a farm was selected to determine the chemical properties of soil and then, soil sampling was conducted. Physicochemical properties of the soil of the experiment site are shown in table 1. The study was conducted using the split plots in the completely random blocks with three replications, so that the main plots would include irrigation intervals (8 and 16 days) and the subplots would include chitosan anti-transpiration compounds (0.25, 0.5, 1 percent) and *Plantago psyllium* mucilage (0.5, 1, 1.5 percent) and arabic gam (0.25, 0.5, 0.75 percent) with three replicates. Each subplot was considered to be 150 cm long and 100 cm wide and the distance between subplots was 50 cm and distance between rows was 30 cm. Also in one block, the distance of main plots, 100 cm and the distance between two blocks was considered 200 cm, so that the moisture of adjacent plots doesn't affect each other. The planting date was April 16 and planting was performed manually in the furrows with a depth of 0.5 cm. Thinning operations were performed in the four and eight leaf stages. In order to keep uniformity in emergence of seeds, after planting, continuous irrigation was done and irrigation treatments began at the six leaf stage after full deployment of seedlings and continued until physiological maturity. Anti-transpiration compounds were sprayed coincidently with applying drought stress till the flowering stage once a week at sunset. When the plants were yellow, but capsule had not cleaved yet, harvesting operation was performed in July 5 (16 days irrigation interval) and July 25 (8 days irrigation interval). At first, ten plants per plot were randomly selected for measurement of yield components. After removal of margins, to determine the yield level, the remaining area was harvested. To analyze the variance of the experimental data and drawing of diagrams, JMP 8 and Excel software was used. All the averages were compared according to LSD test ($p<0.05$).

Table 1- Physicochemical properties of soil experiment

Soil texture	K (mg/kg)	P (mg/kg)	Total N (%)	Electrical conductivity (ds/m)	Soil pH
Clay loam	334	19.7	0.3	8.35	7.9

Result and Discussion

Number of Capsule per Plant

The results obtained from variance analysis (Table 2) indicated that the effect of irrigation intervals and anti-transpiration compounds on the number of capsules per plant was significant ($p<0.01$). Maximum number of capsules per plant was observed in 8 days irrigation intervals showing a significant difference with 16 days irrigation intervals (Table 3). The mean comparison (Table 3) showed that there are significant differences amongst the anti-transpiration compounds treated with chitosan and control and other treatments. 1 percentof chitosan treatment had the highest number of capsules per plant. The lowest number of capsules per plant was observed in 0.25 percent of arabic gum treatment. Interaction of irrigation intervals and anti-transpiration compounds on the number of capsules per plant was significant ($p<0.01$), so that the highest and lowest number of capsules were observed at 8 days irrigation intervals with 1 percent chitosan and 16 days irrigation intervals and control treatment, respectively (Table 4). Results are consistent with the results by Norozpoor and RezvaniMoghaddam (2002). Considering the fact that *nigella Sativa* is a plant with terminal flowers and limited growth, in which the flower and fruit form at the end of each branch, the number of capsules per plant follows number of flowering branches. Bagheri et al (2010) reported that application of atrazine anti-transpiration underrain-fed conditions increased the number of bolls in safflower plant. It seems that under optimum conditions of irrigation, it is possible for the plant to create a close relationship between the number of capsules per plant and genetic potential. When the plant is provided with optimum irrigation, it will experience greater vegetative growth, produce more branches and thus increase the number of capsules (Bannayan et al., 2008).

Number of Seeds per Capsule

The effect of irrigation intervals and anti-transpiration compounds on the number of seeds per capsule was significant ($p<0.01$) (Table 2).The results obtained from the mean comparison (Table 3) showed that maximum number of seeds per capsule was observed in 8 days irrigation intervals representing a significant difference with 16 days irrigation intervals. There was a significant difference between the various levels of anti-transpiration compounds and control sample. 1 percent of chitosan treatment had the highest number of seeds per capsule. The lowest number of seeds per capsule was observed in 0.25 percent of arabic gum treatment. Interaction of irrigation intervals and anti-transpiration compounds on the number of seeds per capsule was significant ($p<0.01$)(Table 2), so that the highest and lowest number of seeds per capsule were observed at 8 days irrigation intervals with 1% chitosan and 16 days irrigation intervals and 0.25% arabic gum treatment, respectively (Table 4). Bannayan et al (2008) reported that increase in the seeds in *nigella Sativa* and reduction in irrigation intervals coincide. In many crop plants, the occurrence of water stress during the flowering period is critical and in this case, the number of flowers that turns into seeds become significantly reduced. Kazempourand Tagbakhsh (2002) reported an increase in the number of grains of corn, as a result of use of anti-transpiration corn and under limited irrigation. Being in appropriate environmental conditions provided by anti-transpiration material at the seed formation stage, the plant would make use of its maximum power at the flowering stage to increase then umber of seeds per capsule (Bagheri et al., 2010).

Table 2- Analysis of variance for number of capsules per plant, number of seeds per capsule, seed number per plant, seed weight per plant, seed weight, 1000 seed weight, seed yield, biological yield, and harvest index in *Nigella Sativa*

		mean squares							
S.O.V	df	number of capsules per plant	number of seeds per capsule	seed number per plant	seed weight per plant	1000 seed weight	seed yield	biological yield	harvest index
Replication	2	1.5*	8.49**	38140.19*	0.011*	1.25 ns	291.56 ns	144.4**	0.87*
Irrigation interval	1	2.71**	112.99**	22847.7*	0.15**	0.18ns	286305.25**	181060.3**	16.06**
Error $_a$	2	1.26	0.12	7854.51	0.007	0.15	2201.46	0.13	0.35
Anti-transpiration compounds	9	42.91**	963.44**	399691.6**	1.43**	2.12ns	260928.82**	1138949.4**	127.19**
Anti-transpiration compounds× irrigation	9	5.89**	62.81**	24080.21 ns	0.11**	0.90 ns	39031.93**	25241.4**	20.58**
Error $_b$	36	3.08	5.39	168636.39	0.043	7.48	27076.4	4.67	3.1

ns, * and ** means non- significant and significant at the 5% and 1% probability levels, respectively

Number of Seeds per Plant

The results obtained from variance analysis (Table 2) indicated that the effect of irrigation intervals on the number of seeds per plant was significant (p<0.05). The effect of anti-transpiration compounds on the number of seeds per plant was significant (p<0.01). The results of mean comparison (Table 3) indicated that the number of seeds per plant increased with decreasing irrigation intervals. In addition, there was a significant difference between different levels of anti-transpiration compounds and the control. The highest number of seeds per plant was achieved in 1 and 0.5 percent chitosan treatments and the lowest number of seeds per plant was observed in the control treatment. Also, there was no significant difference between irrigation intervals and anti-transpiration compounds on the number of seeds per plant (Table 2). Akbarinia et al (2005) reported that with the increasing of irrigation intervals, number of seeds in *nigella sativa* increased as well. It sounds like that presence of moisture increased number of capsules per plant, which this, in turn, increases the number of seeds per plant. Slatyer and Bierhuizen (1964) reported that anti-transpiration agents have increased the number of grains in the case of corn. It appears that an increase in the number of seeds under lower drought stresses might lead to the more and larger capsules, as well as better growth of plants (Akbarinia et al., 2004).

Table 3- Mean comparisons of yield and yield components of *Nigella sativa* under different levels of anti -transpiration compounds and irrigation intervals

Treatments	number of capsules per plant	number of seeds per capsule	seed number per plant	seed weight per plant (g)	seed yield (Kg/he)	biological yield (Kg/he)	harvest index (%)
Irrigation interval							
8 days	6.1a	61.48a	353.25a	0.78a	621.56a	1981.53a	31.36a
16 days	5.6b	58.73b	314.22b	0.68b	484.23b	1871.666b	30.32b
Anti-transpiration compounds							
Control	4.7g	56.79h	180.73e	0.57f	454.31e	1658j	29.16e
Chitosan 1%	7.24a	66.98a	471.76a	1a	659.5a	2127a	33.31a
Chitosan 0.5%	7.32a	65.73b	442.12ab	0.94b	640.66ab	2079ab	33.16a
Chitosan 0.25%	6.39b	64.37c	389.24bc	0.94b	618.5ab	2052c	32.47b
Psyllium mucilage 1.5%	5.91bc	61.38d	342.29cd	0.67c	540.66c	1999d	30.37c
Psyllium mucilage 1%	5.89cd	57.93f	334.7cd	0.66cd	547c	1992f	30.18c
Psyllium mucilage 0.5%	6c	57.39g	335.24cd	0.62ed	530.16c	1867g	30.17c
Arabic gum 0.75%	5.17ed	57.93f	262.06de	0.61ef	561.16c	1947e	30.26c
Arabic gum 0.5%	4.88gf	56.95hg	281.83d	0.62ed	493d	1822h	29.73d
Arabic gum 0.25%	5.46ef	54.94i	296.85d	0.64cde	484ed	1792i	29.60d

Means, in each column and for each treatment, followed by at least one similar letter are not significantly different at 5% probability level

1000 Seed Weight

The results obtained from variance analysis (Table 2) indicated that the effect of irrigation intervals and anti-transpiration compounds, and the interaction of these two on the seed weight were not significant. Norozpoor and RezvaniMoghaddam (2002) reported that 1000 seed weight per capsule plant was not affected by irrigation intervals. Overall, 1000 seed weight is considered a factor which is mostly under genetic control, has high heritability and is less affected by environmental factors.

Seed Weight per Plant

The effect of irrigation intervals and anti-transpiration compounds on the seed weight per plant was significant ($p<0.01$)(Table 2). The results obtained from of mean comparison (Table 3) indicated that with increasing of irrigation intervals, the seed weight per plant was reduced. Also, the highest weight of seed was obtained in 1 percent chitosan treatment and the lowest weight of seed was observed in the control treatment. There was a significant difference in different levels of chitosan treatments. Interaction of irrigation intervals and anti-transpiration compounds on the seed weight per plant was significant ($p<0.01$)(Table 2), so that the highest and lowest seed weight were observed at 8 days irrigation intervals with 1 percent chitosan and 16 days irrigation intervals and control treatment, respectively (Table 4). The increase in seed (or grain) weight in the absence of sufficient water in plant is associated with duration and rate of grain filling; and the longer the period and the faster the rate is, the highest seed weight is gained. Also, with increase in the photosynthetic rate as a result of spraying anti-transpiration compounds, in the seed formation time, the plant allocates more assimilates to the seed, thereby, increasing the weight of seeds (Bagheri et al.,2010).

Seed Yield and Biological Yield

The effect of irrigation intervals and anti-transpiration compounds on the seed yield and biological yield was significant ($p<0.01$) (Table 2). The results obtained from mean comparison (Table 3) indicated that with increasing of irrigation intervals, the seed yield increased. At different levels, the anti-transpiration compounds showed significantly greater seed yield and biological yield compared to the control. Also, the highest weight of seed was achieved in 1 percent chitosan treatment and the lowest seed yield and biological yield were observed in the control treatment (Table 3). Interaction of irrigation intervals and anti-transpiration compounds on the seed yield and biological yield were significant at 1% level (Table 2), so that the highest seed yield and biological yield were observed at the optimum irrigation conditions (8 days intervals) and anti-transpiration agent of 1 percent chitosan (Figure 1). These results are consistent with those reported by Koutroubas et al (2002). The increase in seed yield can be at tributed to better vegetative growth, canopy development and, therefore, better use of solar radiation and higher photosynthesis in optimum irrigation conditions (Filippo et al., 2002). Thakuria et al (2004) reported that anti-transpiration compounds increased the seed yield in sunflower. Considering the number of capsules per plant, number of seeds per capsule and seed weight, the three of which are considered as the yield components of *nigella sativa*, spraying of anti-transpiration compounds in drought stress conditions leads to reaching of the maximum seed yield. Due to exposure to the favorable conditions, the plant tends to apply the favorable environmental conditions, created by spraying of anti-transpiration compounds, to complete the generative phase. Perhaps it can be stated that the increase in grain yield in optimum irrigation conditions is largely due to its effect on the number of capsules per plant directly and increase in number of seeds per plant indirectly (Akbarinia et al., 2004 ؛Iiriti et al., 2009**)**.

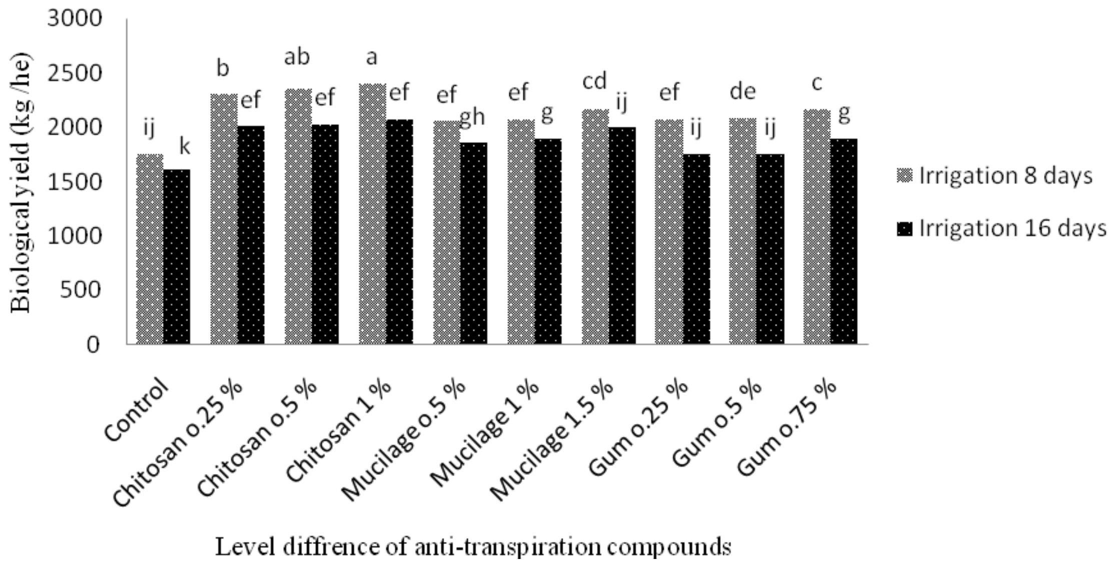

Fig1-Interaction irrigation intervals and anti-transpiration compounds on biological yield

Harvest Index (HI)

The results obtained from variance analysis (Table 2) indicated that the effect of irrigation intervals and anti-transpiration compounds on HI became significant ($p<0.01$). The mean comparison (Table3) suggests that there are significant differences between 8 and 16 days irrigation intervals. Also, there was a significant difference between anti-transpiration compounds and the control. The highest HI was achieved in 1 and 0.5 percent chitosan treatments and the lowest HI was observed in the control treatment and the interaction of irrigation intervals and anti-transpiration compounds on HI was significant ($p<0.01$)(Table 2). Also, the highest HI was observed at the optimum irrigation conditions (8 days irrigation intervals) and different levels of chitosan anti-transpiration agent and the lowest HI was observed at 16 days irrigation intervals and control treatment (Table 4). It sounds like that the reduced HI at 16 days irrigation intervals treatment and control treatment has resulted from decrease in vegetative growth and competition of chlorophyll organs with reproductive organs, which therefore, less assimilates will be allocated to the seed. The plants treated with 8 days irrigation intervals and chitosan anti-transpiration agent took the best advantage of water and, with a favorable vegetative growth supporting thoroughly the reproductive organs, reached a high HI. After pollination, the materials are transmitted to seeds and the water plays an important role in transmission process of materials. Therefore, it seems that due to lack of water, in the 16 days irrigation interval treatment and control treatment, transmission process of water decreased, resulting in the reduced HI. In drought stress conditions, the effect of anti-transpiration compounds on HI increase is associated with the improvement of metabolic and enzymatic activities, protein synthesis and osmotic regulation of plant under anti-transpiration compounds consumption. The results are consistent with those reported by (Mozzafari et al., 2000؛Mousa-Zadeh et al., 2009)

Table 4- Mean comparisons of irrigation intervals and anti-transpiration compounds interaction on yield and yield anti-transpiration components of *Nigella sativa*

treatments	number of capsules per plant	number of seeds per capsule	seed weight per plant	harvest index
I_1K_1	6.45c	65.88c	1.02b	34.15a
I_1K_2	7.36b	66.63b	1.06b	34.35a
I_1K_3	8.2a	70.93a	1.14a	34.51a
I_1M_1	6.29cd	57.86i	0.68d	30.22defg
I_1M_2	6cde	59.25h	0.68d	30.27def
I_1M	6cde	61.84f	0.69d	30.59cd
I_1G_1	5.34fg	56.87kj	0.64ed	29.73ghi
I_1G_2	5.21fg	58.93h	0.64ed	29.81efghi
I_1G_3	4.97gh	59.35h	0.64ed	30.21defg
I_1C	5.18fg	57.27ji	0.64ed	29.77fghi
I_2K_1	6.33c	62.87e	0.83c	30.80c
I_2K_2	7.28b	64.83d	0.86c	31.98b
I_2K_3	6.28cd	63.02e	0.86c	32.11b
I_2M_1	5.7ef	56.92kj	0.57f	30.11defgh
I_2M_2	5.7ef	56.87kj	0.64ed	30.10defgh
I_2M_3	5.74def	60.92g	0.64ed	30.16defgh
I_2G_1	5.58ef	53.01m	0.64ed	29.46i
I_2G_2	4.55hi	54.97l	0.60ef	29.65hi
I_2G_3	5.38fg	57.97i	0.58f	30.31cde
I_2C	4.32i	57.92i	0.55f	28.55j

Means, in each column and for each treatment, followed by at least one similar letter are not significantly different
I1=8 days irrigation interval, I2=16 days irrigation interval, K1=chitosan 0.25 percent, K2=chitosan 0.5 percent, K3=chitosan 1 percent, M1=mucilage 0.5 percent M2=mucilage 1 percent, M3= mucilage 1.5 percent, G1=gum 0.25 percent, G2=gum 0.5 percent, G3=0.75percent

Conclusion

According to the results obtained in the present study, the use of anti-transpiration compounds under drought stress conditions showed a significant impact on the yield and yield components of *nigella sativa*. Spraying of chitosan anti-transpiration compounds compared to the control reduced the effect of drought stress and improved the negative effects of drought stress. Considering the mean comparisons and the relationships among the traits, it was turned out that choosing the appropriate amount of spray greatly influences on enhancing each of the traits. Providing the appropriate conditions, 1% chitosan treatment can enhance the yield under drought stress. Spraying by arabic gum did not improve the growth conditions. According to this experiment, 1% chitosan treatment and 1.5% *Plantago psyllium* mucilage is considered the most appropriate strategy to enhance the yield of *nigella Sativa* under drought stress.

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