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Research Article

Preliminary study on non-chemical management factors for bulb mite *Rhizoglyphus robini* (Acari: Acaridae) control in the saffron crop

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Abstract: Saffron is the most expensive agricultural product and like other products, is affected by some limiting factors, including soil pests such as bulb mite *Rhizoglyphus robini* Claparede (Acari: Acaridae). Due to its polyphagous habit, it remains in the soil for a long time and is one of the most important pests of saffron. To determine the effect of non-chemical management factors including soil solarization days, fertilization and year on the population of bulb mite and yield of saffron, a split-plot experiment based on a randomized complete block design in three replications was conducted between 2007 and 2011 in Gonabad, Iran. Fertilization (Cow manure) of zero, 20 and 40 t/ha as the main plots and soil solarization of zero, 10, 20, 30 and 45 days as sub-plots were studied for four years. The results showed that simple and interaction effects of the three factors including year, fertilization and soil solarization days on the population of bulb mite and saffron yield were significant. Simple linear regression was fitted between the population of bulb mite and saffron yield as well as multiple linear regression for population of bulb mite and saffron yield separately. The population of bulb mite increased during the experiment by increasing the two factors of the year and fertilization separately. Whereas, with increasing soil solarization days, population of bulb mite decreased except for 10 days solarization treatment and, unexpectedly, in 45 days of solarization treatment, the population increased in the fourth year.

Keywords: Fertilization, Soil solarization, Bulb mite, Saffron, Cow manure

Introduction

Saffron is one of the most important crops that provides a significant portion of Iran's non-oil exports. In arid and semi-arid regions such as Iran, water as the most limiting factor in the share of production determines the priority of cultivation. Accordingly, since ancient times Iranian farmers, especially farmers in Khorasan Province have been cultivating this crop with low water need. The saffron plant grows with the onset of autumn rainfall, and when spring rainfall ends, its growth stops. This character has caused the crop to survive in areas with low rainfall and provide more income than other crops. Mites of the *Rhizoglyphus* genus are a major pest of many agricultural products and warehouses causing economic damage to some crops such as saffron. Manson (1971) mentioned

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that the hosts of this mite are: garlic, carrot, gladiolus, iris, narcissus, onion, potato, dahlia and amaryllis, and other tuberous plants. Rahimi and Kamali (1993) reported *Rhizoglyphus robini* Claparede for the first time on saffron corms from Gonabad and Qaeen cities in Iran. They examined the growth period of the mite in laboratory conditions and reported that this species is a multi-generation pest and its life cycle at 25 °C and %75 ± 5 relative humidity on saffron corms, onion and potato tubers was 13.75 and 15.12 and 17.57 days, respectively. They also stated that this species attacks saffron corms and, in addition to feeding, it creates routes for penetration of saprophytic agents into saffron corms, which would cause decay of the corms. In addition, in a study by Fashing and Hefel (1990) the growth period of *R. robini* on artificial medium of Bot and Meyer at temperatures of 16, 27 and 35 °C with the period of 12/12 (light/dark) was estimated to be 11.25, 13 and 35.82 days, respectively. Rahimi *et al*. (2008) reported that summer irrigation increases the population of *R. robini* in saffron fields; however, the effect of sowing depth on the population of mite was different according to the season. Moreover, they stated that after several years with the aging of the farm, the population of mite will increase; therefore this pest is the most important limiting factor for saffron cultivation. Rahimi and Arbabi (2006) floated saffron corms for 30 seconds in a solution 3 mg/l Omite (Propargite), and used this chemical to disinfect the soil at a rate of 30 liters per hectare. According to their results, seed disinfection was more effective than soil disinfection for controlling the population of the mite ($P < 0.05$). Wang and Lin (1986) introduced *R. robini* as one of the most dangerous pests of gladiolus in Taiwan and recommended some pesticides for the disinfection of gladiolus corms such as bromopropylate, benzoximate, demeton-methyl, and aluminum phosphide tablets. Other methods such as sterilization and solarization of the soil which cause physical, chemical and biological changes in the soil can improve plant growth and have been recommended by many researchers (Ahmadian Yazdi, 2002; Moosavi and Teymori, 1992; Nasr Esfehani, 2000; Katan, 1992; Ono and Morita, 1993). Gerson and Smiley (1990) studied the solarization method using a transparent plastic layer to reduce the population of *R. Robini* in gladiola and garlic fields, and stated that after 10 days, the mites up to the depth of 20 cm and after 20 days, the mites up to the depth of 30 cm were destroyed. They also recommended complete sterilization of the soil for one month. Ono and Morita (1993) recommended the same approach against *R. robini* in onion fields in Japan. Katan (1992) reported that the soil solarization with transparent plastics reduced the population of pathogens in soil. Moosavi and Teimouri (1992), showed by covering the dry soil with transparent plastic sheets in the warm season for 20 to 40 days, the population of Pratylenchus nematodes in the treatment of 40 days in some of the repetitions reached zero. Ahmadian Yazdi (2002) reported that the effect of solarization on the reduction of the population of root-knot nematodes (*Meloidogyne* spp.) in greenhouse and field conditions was 88%. Extensive research has been done especially on the effect of solarization on successful control of soil pathogenic agents, plant-parasitic nematodes and weeds in Iran and in the world. To investigate the effect of solarization on *R. Robini* mites in saffron farms, this study was carried out in Gonabad city of Razavi Khorasan province for four consecutive years.

Materials and Methods

This experiment was carried out at the Agriculture and Natural Resources Research Station of Gonabad city (34°22’N, 58°45’E), Iran from 2007 to 2010. A field with a long history of saffron cultivation in which the *R.*

robini population was known to be high was selected for the trial. The project was implemented as follows.

Implementation of the project

The project was based on a randomized complete block design with split plot design including three main plots (cow manure of zero, 20 and 40 tons per hectare) and five subplots (soil solarization of zero, 10, 20, 30 and 45 days covered with transparent plastics) in three replications. Day-Zero treatment under plastic for each block (main plot) was considered as control. The dimensions of the plots were 12 m^2 and the distance between the plots, were selected at 0.5 m. The manure was uniformly spread and irrigated. Once the soil was at the field capacity, the manure and soil were uniformly mixed with rototiller, and on all plots, a transparent plastic with a thickness of 30 micrometers was placed. Then, after zero, 10, 20, 30 and 45 days of solarization, the plastic was torn by a razor.

Planting the saffron corms

At the end of soil solarization and after removing plastic covers, the plots were plowed by the worker for ventilation of the soil. Then in mid-September, saffron corms were planted uniformly with 5 cm spacing along the lines and 25 cm between the lines and at a depth of 15 cm.

Sampling the mite population

From each experimental unit, five soil samples were taken from a depth of 15 to 20 cm by a shovel. After mixing them together, a one-kilogram compound sample was selected as a sample of each unit and transferred to the lab in a nylon bag. After 72 hours in the laboratory, the mites in the soil sample were extracted by Berlese funnel and their numbers counted by a binocular and recorded in tables. The percentage of mortality in the first year was obtained by using the Henderson-Tilton formula (Henderson and Tilton, 1955). Sampling of the mite population was carried out in the first year before and after soil solarization and in subsequent years in April of each year.

Sampling the saffron yield

In November of each year, harvesting of the flowers was done uniformly several times until the end of flowering. The number of flowers per plot (saffron yield) was recorded after removing the marginal effects (two lateral rows and half a meter at the beginning and end of each plot).

Determination of the corm contamination

In the summer of last year (physiological sleep time of the saffron corms), from each experimental unit, one kilogram of corm was randomly taken from different parts of soil. After removing tufts around the corms, the percentage of healthy and contaminated corms was determined.

Statistical analysis

The variance residues were normalized at a 5% level by transforming the data to $\sqrt{(x + 1)}$. Analysis of variance was performed for all data. Comparison of the means was done based on Tukey's test at a 5% level. Also, Simple and multiple linear regression models were fitted for bulb mite population, saffron yield, fertilization, solarization and year factors using SPSS version 16 software (SPSS, 2007).

Result and Discussion

In the first year, the relationship between the saffron mite population and the saffron yield was not significant at 5% level. This result can be explained by the low population of bulb mites and saffron yield on the first year. But on the second, third and fourth years, this relationship was significant ($P < 0.05$), and the increase in the population of bulb mite reduced the saffron yield. Based on simple linear model, 33.8%, 28.3% and 23.28% of the variations in the yield reduction of saffron

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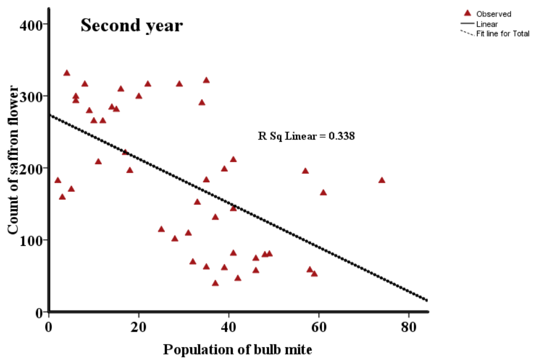
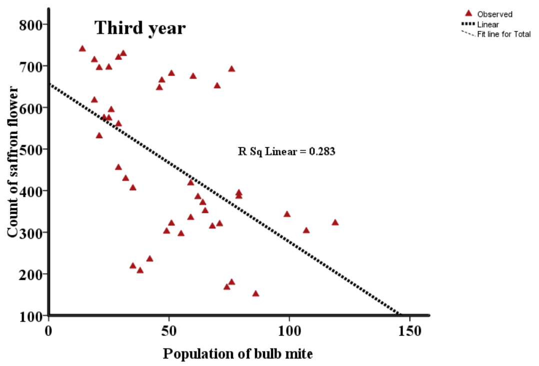
were related to the increase in the mite population of the second, third and fourth years respectively (Fig. 1a, b, c). In addition, multiple linear regression was fitted for non-chemical management factors and bulb mite population and the coefficient of determination (r^2) was calculated to be 64.9%. Based on regression relation, most influential independent factors on the population of bulb mite was the year with a Beta coefficient of 0.649, That is, one unit of change in the standard deviation of the year creates 0.649 units of variation in the standard deviation of the bulb mite population. Other factors were also important in influencing independent factors. The solarization factor with a Beta coefficient of -0.375 and fertilization factor with a Beta coefficient of 0.295, participated in the regression model (Table 1). The direct effect of increasing fertilizer on the increase in the population of bulb mite can be due to saprophytic habit of *R. robini* and the suitability of the environment for growth and development. Regarding saffron yield, the most influential independent factor was the year with a Beta coefficient of 0.918. The solarization and fertilization factors were also considerable with a Beta coefficient of 0.233 and -0.077, respectively (Table 1). One of the reasons for the small indirect effect of increasing fertilizer on saffron yield can be due to the increase in the population of bulb mites. During the 4 years of this experiment, the bulb mite population, which was affected by different levels of solarization, showed a steady trend. Accordingly, with increased solarization exposure for up to 10 days, the mite population increased, but as the solarization exposure continued beyond 10 days, the population of bulb mite decreased (Fig. 2).





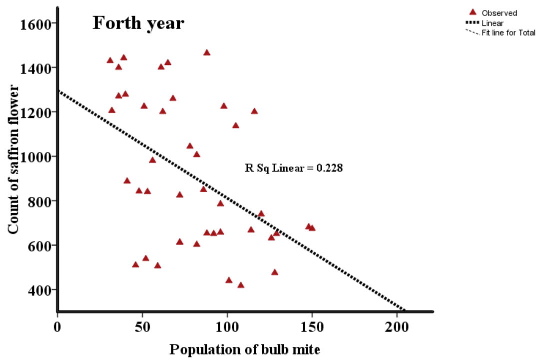


Figure 1 Relationship between bulb mite population and count of saffron flower for the second, third and fourth years.

Table 1 Relationship between Bulb mite population and saffron yield with pest management factors in multiple regression.

Dependent variables	r^2	ρ	Coefficients	β_0	β_{year}	$\beta_{Fertilization}$	$\beta_{Solarization}$
Bulb mite population [1]	0.649	< 0.001	multiple reg.	2.392	1.858	0.580	-0.077
			Beta		0.649	0.295	-0.375
Saffron yield [2]	0.903	< 0.001	multiple reg.	-5.536	8.166	-0.047	0.149
			Beta		0.918	-0.077	0.233

[1, 2] Data was transformed into $\sqrt{(x + 1)}$.

In the first year, the effect of fertilization on the bulb mite population was significant at zero, 10, and 20 days of solarization treatments (P < 0.05). The population of mites was more in the treatment of 40 t/h of cow manure than non-fertilized treatment. The highest mite population was observed in 10 days of solarization treatment with fertilizer levels of 20 and 40 t/h. This can be due to the positive effect of the heat of solarization. The lowest population of mites was observed in 45 days of solarization, which could be due to the negative effects of increasing the temperature above the biological tolerance of the bulb mite (Fig. 2a). In the second year of the experiment, the main trend of the chart did not change compared to the first year (Fig. 2b). In the third year, while continuing the trend of previous years, an enormous increase in the population of the bulb mite was observed in the treatment of 40 t/h of cow manure and solarization for 10 days compared to other treatments, which continued in the fourth year. The reason for this increase seems to be due to the positive interaction of the cow manure 40 t/h and solarization for 10 days. On the fourth year of the experiment, the increase in the population of mite was observed in 45 days of solarization treatment compared to 30 days of solarization treatment, which was not expected. It is noteworthy that the first indication of this new trend was observed in the treatment of 20 t/h of cow manure and solarization for 45 days in the third year. The reason for this population growth in 45 days of solarization in the fourth year may be related to the loss and depletion of the ecological niches caused by the 45 days of solarization at the beginning of the experiment (causing a high temperature in the soil). This has changed the situation in favor of the bulb mites that are transported by corms to the field after solarization, and it has emerged as an upward trend on the fourth year (Fig. 2c, d).

The results of the analysis of combined experiments of year, fertilization and solarization on mite population and saffron yield were significant (P < 0.05, Table 2). Accordingly, the effect of all independent factors of the experiment on bulb mite population and saffron yield was significant at level of 0.05. The year factor with an effect size of 0.427 and 0.822 had the highest and fertilization factor with an effect size of 0.087 and 0.006 had the least effect on bulb mite population and saffron yield, respectively. The low effect size of the fertilization factor on the saffron yield (0.6%) could be due to the direct effect of this factor on the population of bulb mite which reduced the positive effect of the fertilization on saffron growth (Table 2). Overall, with increasing amount of fertilizer, the saffron yield decreased and the population of bulb mite increased (Table 3).

Regarding the interaction effects, the greatest effect on bulb mite population and saffron yield were observed on the interactions of solarization × fertilization ($\omega2 = 0.057$) and solarization × year ($\omega2 = 0.036$), respectively (Table 2). In the case of the bulb mite population, it seems that the growth rate of bulb mite at different levels of fertilization, increased significantly with increasing temperature (caused by solarization up to 10 days) (Fig. 2). This trend was even more pronounced on the third and fourth year (Fig. 2c, d). Regarding the saffron yield, it seems that increasing the temperature for disinfection of the soil by solarization (from 10 to 45 days) before planting, decreases the population of bulb

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mites and other pathological agents in the soil, which has a positive effect on plant growth and number of flowers over a period of four years (Table 3).

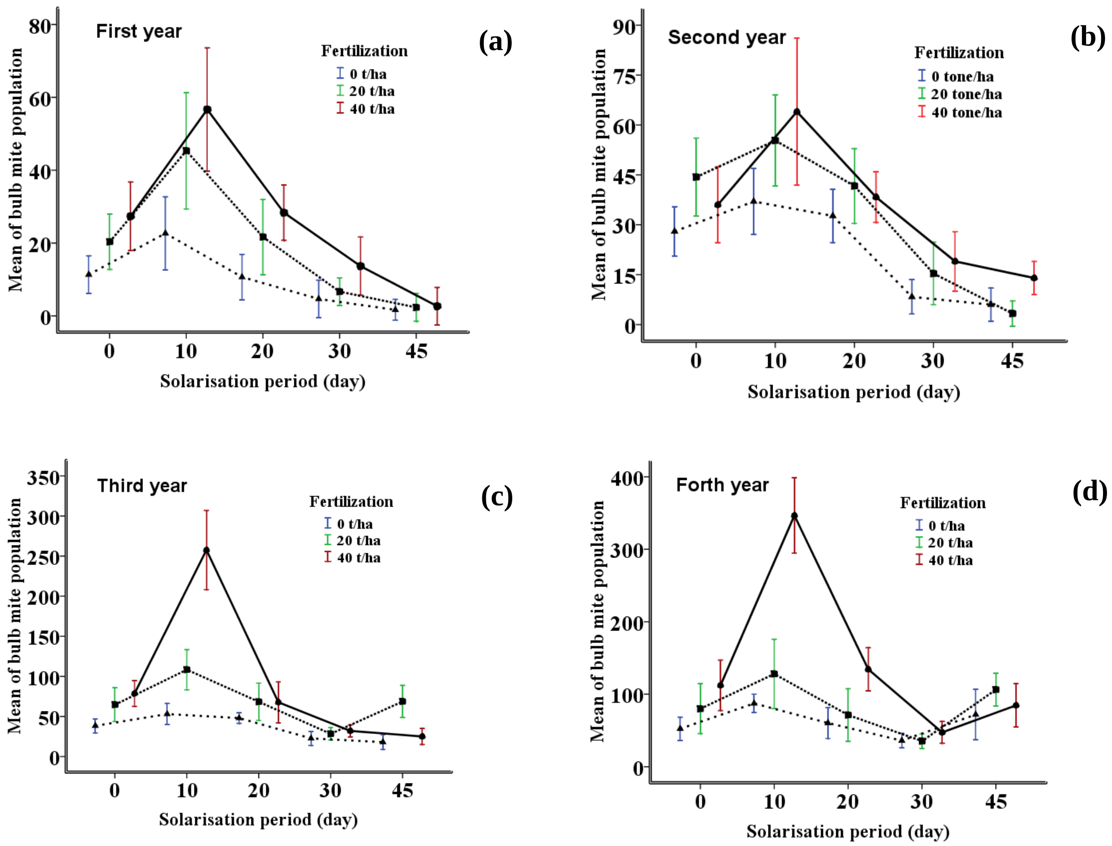

Figure 2 Relationship between solarization and fertilization in the bulb mite population for a four-year period.

Table 2 *Analysis* of combined experiments for the population of the bulb mite and saffron yield.

Source of variance	df	Number of mites per 100 grams of saffron corm[1]		Number of saffron flowers per plot [2]	
		Mean square	Effect size (ω^2)	Mean square	Effect size (ω^2)
Replication	2	0.101 ns		0.197 ns	
year	3	262.685 **	0.427	5001.129 **	0.842
Error1	6	0.499		0.214	
Fertilization (Fer.)	2	80.537 **	0.087	55.119 **	0.006
Fer. × year	6	6.144 **	0.019	76.462 **	0.026
Error2	16	0.376		0.429	
Solarization (Sol.)	4	136.992 **	0.297	299.286 **	0.067
Sol.× year	12	7.319 **	0.047	53.041 **	0.036
Sol. × Fer.	8	13.021**	0.057	21.155 **	0.009
Sol. × year × Fer.	24	3.880 **	0.048	8.903 **	0.012
Error3	96	0.164		0.195	
C. V		6.2		2.5	

[1, 2] Data was transformed into $\sqrt{(x + 1)}$.
** and ns: significant at 0.01 and non-significant.

Table 3 Grouping untransformed mean of bulb mite population and saffron yield for three factors.

Treatments		Measured variables	
		Bulb mite population	Saffron yield
Year	First	18.40 [d]	22.98 [d]
	Second	29.56 [c]	183.16 [c]
	Third	65.29 [b]	459.42 [b]
	Forth	96.96 [a]	890.56 [a]
S. E.		1.091	3.005
Fertilization (t/ha)	0	32.55 [c]	420.98 [a]
	20	50.78 [b]	422.50 [a]
	40	74.32 [a]	323.60 [b]
S. E.		0.945	2.062
Solarization (day)	0	49.47 [b]	218.36 [e]
	10	105.17 [a]	286.67 [d]
	20	51.94 [b]	447.33 [c]
	30	22.44 [d]	481.22 [b]
	45	33.72 [c]	511.56 [a]
S. E.		1.220	3.359

Comparison of means based on Tukey's test at a significant level of 5%.

Conclusion

Data analysis showed that the population of the bulb mite increased with year (Tables 2, 3). Therefore, in the integrated management of bulb mite, saffron fields should not be aged. This is in line with the method of the cultivation of saffron in Spain (Dar *et al*., 2017). It is recommended to transfer the saffron corms to a new farm after a five-year period to obtain economic yield. If it is not possible to transfer, at first the corms are removed from the soil and after 30 days of soil solarization on the hottest days (mid-June to mid-July), they are transferred back to the farm in September. According to the results of this study, the population of bulb mite increased with the increase of cow manure, because it corresponds to the pest's saprophytic behavior. Therefore, composted manure should be used to prevent bulb mite farm contamination. The highest saffron yield was obtained at the level of 20 t/h of cow manure. This is in line with the results of Mohammadzadeh and Pasban (2007), who stated that the consumption of more than 35 t/h of cow manure reduced the yield of saffron flowers. Therefore, solarization along with fertilization at the beginning of summer before the saffron replanting in the infected fields increases the saffron yield and reduces the bulb mite population. As a result, if the crop health is maintained after solarization until the end of the year, the effects of this method will probably last up to a five-year period. Results showed that 30 days of solarization had the best reduction effect on the bulb mite population. Gerson and Smiley (1990) also found that one month of solarization is enough to completely disinfect the soil. Therefore, the highest yield of saffron and the lowest population of mites was found in the treatment of 20 t/h of cow manure and 30 days of soil solarization which is recommended as the best treatment of this experiment. This treatment is more effective in a long history of saffron cultivation and to be impossible to re-cultivate saffron. Also, it seems that planting saffron immediately (in September of the year) after solarization has detrimental effects on the saffron plant, therefore more studies should be conducted on the appropriate time lapse between solarization and corm planting.

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مطالعه مقدماتی بر روی عوامل مدیریت غیرشیمیایی کنه پیاز گل *Rhizoglyphus robini* (Acari: Acaridae) در محصول زعفران

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چکیده: زعفران یکی از گران‌ترین محصولات کشاورزی است و مانند سایر گیاهان تحت تأثیر برخی از عوامل محدودکننده از جمله آفات خاکزی مانند کنه پیاز گل *Rhizoglyphus robini* Claparede (Acari: Acaridae) قرار دارد. به‌دلیل چندخوارگی، این آفت مدت طولانی در خاک باقی می‌ماند و یکی از مهم‌ترین آفات زعفران است. به‌منظور تعیین اثر سه عامل مدیریت غیرشیمیایی آفت شامل: سال، آفتاب‌دهی و کوددهی خاک بر جمعیت کنه و عملکرد زعفران، آزمایشی در قالب کرت‌های خرد شده بر پایه طرح بلوک‌های کامل تصادفی در سه تکرار در سال‌های ۱۳۸۶-۱۳۸۹ در شهرستان گناباد انجام شد. عامل کوددهی (کود گاوی) در سه سطح صفر، ۲۰ و ۴۰ تن در هکتار در کرت‌های اصلی و عامل آفتاب‌دهی خاک در پنج سطح صفر، ۱۰، ۲۰، ۳۰ و ۴۵ روز در کرت‌های فرعی طی چهار سال مورد مطالعه قرار گرفت. نتایج نشان داد که اثرات ساده و متقابل سال، کود دهی و آفتاب‌دهی خاک بر روی جمعیت کنه و عملکرد زعفران معنی‌دار می‌باشد. رگرسیون خطی ساده بین جمعیت کنه و عملکرد زعفران در سال‌های مختلف آزمایش به‌دست آمد. هم‌چنین رگرسیون خطی چندگانه برای متغیرهای مستقل جمعیت کنه و عملکرد زعفران به‌طور جداگانه برازش شد. با افزایش مقدار دو عامل سال و کود دامی به‌طور جداگانه، جمعیت کنه در طول آزمایش افزایش یافت. هم‌چنین با افزایش مدت آفتاب‌دهی، جمعیت کنه به‌جز در تیمار ۱۰ روز آفتاب‌دهی کاهش، ولی برخلاف انتظار در تیمار ۴۵ روز آفتاب‌دهی در سال چهارم افزایش نشان داد.

واژگان کلیدی: کوددهی، آفتاب‌دهی خاک، کنه، زعفران، کود دامی

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