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Effects of insect pollinators on onion seed production quality and quantity

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Abstract: To evaluate the effects of presence of insect pollinators on quantity and quality of seeds of two common cultivars of onion (*Allium cepa* L.), namely Red Azar Shahr and Yellow Sweet Spanish, an experiment in a factorial randomized complete block design was carried out at two sites. Bulbs of equal size were planted with plant to plant 35 cm and row to row 50 cm spacing. At each site, experimental groups differed only in pollination method. About a week before flowering start, at each site 3 groups of 10 individual inflorescences as block replications of each treatment combination (pollination × cultivar) were selected randomly and marked. For each onion cultivar, half of the plants were left uncovered (free pollination), whereas the other ones were isolated from insect pollinators by using wooden cages covered with cloth net. When about 10 percent of black seeds were visibly exposed in the umbels, harvest was done. After drying, the number and weight of seeds produced per inflorescence as well as 1000 seed weight was measured for each treatment combination. Four weeks after the harvesting date the germination test of seeds produced in each treatment combination was evaluated. Non availability of insect pollinators during the flowering period of onion caused substantial reduction on seed number and seed weight per umbel. Also, seeds from free pollination flowers showed higher germination capacity than those isolated from insect visitors. However, varieties as well as experimental sites did not show significant influences on seed setting of onion.

Keywords: Onion, pollination, seed, Iran

Introduction

Animal pollinators are thought to contribute between 15% and 30% of global food Production (Rubik, 1995). These products include many fruits, nuts, vegetable and oils as well as meat and dairy product produced by animals raised on insect-pollinated forage. The total economic value of pollination worldwide amounted to 153 billion euros in 2005 (Abrol, 2012). Among different pollinating agents, insects make a considerable contribution to crop production. Up to 90% of all flowering plant species rely on pollination by insects such as bees (Buchmann and Nabhan, 1996). Several scientists have estimated the value of insect-pollinated crops that are dependent on honey bees (Morse and Calderone, 2000), or the financial loss to society that could be expected if managed honey bees were removed from cropping systems (Southwick and Southwick, 1992). Losey and Vaughan (2006) found that native pollinators-almost exclusively bees- may be responsible for almost 3.07 billion dollars of fruits and vegetables produced in the United States. In

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Europe, pollination by honey bees and other insect taxa have been estimated to be 4.25 and 0.75 billions Euros, respectively (Borneck and Merle, 1989). The dependency of fruit setting on insect pollination in many agricultural crops is well studied (Losey and Vaughan, 2006).

Onion is grown in almost every country of the World. To obtain optimum seed yield, growers face some problems. Onion flowers are not capable of self-pollinating. So, the out-crossing becomes more critical due to the protandrous nature of the onion plant (Muller, 1983). The amount of out-crossing may vary from 8 to 71% under different conditions (van Der Meer and van Bennken, 1972). Among the pollinator agents, insects are dominant. Rao and Lazar (1983) studying onion pollination, recorded 9.8% fruit setting without pollinators. Also, onion does not produce good quality seed in the absence of abundant pollinators (Chandel *et al*., 2004) and loss of bulb yield may be as high as 28% after three consecutive generations of inbreeding. In other words, the presence of pollinators increases quantity and quality of the seeds. Estimates of increased seed set due to pollinators have been made in different parts of the world. An increase of 3.5 to 98.8 times more than the control in the seed yield has been reported due to assured pollination by bees in onion crop (Singh, 1997). In Poland, Woyke (1981) in an onion open field experiment found that plots caged without bees, caged with bees and not caged produced 2, 210 and 669 seeds per inflorescence, respectively. In India, Kumar *et al*., (1989) found greater onion seed set, seed yield and better seed germination from plots caged with bees, than from plots caged without bees and open plots and estimated seed yield of 73, 275, 97 kg/ha, respectively on plots caged without bees, caged with bees and not caged. According to Rao and Sunyanarayana (1989), non-availability of pollinators during the flowering period of onion caused only 17% fruit setting and free availability of pollinators increased fruiting up to 73%. Wilkaniec *et al*., (2004) found that the red mason bee (*Osmia rufa*) is an effective onion pollinator. According to the latter, the number of seed per inflorescence, the weight of 1000 seeds and germination capacity were higher when onion flowers were pollinated by red mason bee compared with self pollinated flowers. In the other hand, acute deficit of natural pollinators is one of the main reasons of low onion seed yields (Witter and Blochtein, 2003) and explains why attempts to increase the number of pollinating insects on plantations of this plant are still continuing (Mayer and Lunden, 2001).

Onion as a popular vegetable is grown in almost all 31 provinces of Iran. Onion is grown in an area of 50,000 ha. Of these, approximately 300 ha throughout the country are allocated to onion seed production. Depending on a number of factors (e.g., cultivar, date and swing space, etc.) the average seed yield may vary from 828 to 1446 kg/ha (Aminpour and Mortzavi Bak, 2004). However, according to local onion growers the average onion seed yield is estimated to be approximately 500 kg/hectare (Zamani, Pers. communication). This difference may be due to reduction in crop pollination services provided by insects. It is believed that factors such as continuous use of pesticides and decline in natural habitats to some extent are responsible for decreasing the availability of the natural insect pollinators (Saeed *et al.,* 2008). So, to obtain optimum seed yield, seed producers not only should be aware of the effect of pollinating insects and their efficiency in any given area, but also, employ the efficient insect pollinators.

The primary objective of the present study was to throw the light on the pollination potential of naturally occurring insects on onion seed production and to determine the most frequent onion flower insect visitors in the study sites.

Materials and Methods

During the season in 2010, the effect of insect pollinators on seed production of two common

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cultivars (Red Azar Shahr and Yellow Sweet Spanish) of onion (*Allium cepa* L.) was evaluated at two sites in a factorial randomized complete block design. The distance between the two sites was 40 kilometers, one at 15 kilometers west of Mashhad (36° 27′ N & 59° 57′ E) and the other site at 5 kilometers east of Chenaran (36° 27′ N & 59° 07′ E). At each site, 6 groups of 10 bulbs were planted for each cultivar. Bulbs of equal size (about 200g) were planted on 7th April 2010 with 35 cm on rows and 50 cm between rows spacing. Experimental groups differed only in pollination method. About a week before the beginning of flowering, three groups of 10 individual inflorescences at each site were selected randomly and marked as block replications of each treatment combination (pollination × cultivar). For each onion cultivar, half of the plants were left uncovered (free pollination), whereas the other ones were isolated from insect pollinators using wooden cages (1 × 2 × 1m) covered with cloth net. When about 10 percent of black seeds were visibly exposed in the umbels, harvest was done. Five umbels with a 5- cm stalk were cut from each group, 15 umbels of each treatment combination, placed individually in plastic bags and transferred to the laboratory, and left in disposable dishes to dry. After drying, number and weight of seeds produced per inflorescence as well as 1000 seed weight was determined for each treatment combination. Four weeks after harvesting date, the germination of seeds produced in each treatment combination was tested. The germination capacity of seeds was evaluated according to the method described by Wilkaniec *et al.,* (2004). To do this, samples of 100 seeds (3 replicates) from different treatments were taken four weeks after harvesting date and incubated at 21 ± 1º C for 12 days. Sprouted seeds were then counted to determine their germination capacity.

To survey the insect pollinators in each experimental site, a sampling procedure was carried out in the morning (8.00-10.00) and afternoon (16.00-18.00) and five times during the flowering period, at intervals of 3-5 days. Using a hand net all insects visiting flowers were collected. The collected insects were pinned, labeled with date and site and stored for identification.

Data analyses

Differences in quantity and quality of onion seed production were analyzed by three-way full factorial ANOVA (site × cultivar × pollination treatment). When ANOVA indicated a significant effect ($P < 0.05$), Fisher's Protected LSD test was used to determine significance between mean values. Prior to ANOVA analyses, the assumptions of normality and homogeneity of variances were evaluated. SAS statistical software, version 9.1, was used for all statistical analyses.

Results

At both sites, the mean numbers of seeds per inflorescence of uncovered onions in two cultivars differed significantly from the covered ones (Table 1). However, the effects of cultivar and area on seed number per inflorescence were not significant. There were no significant interactive effects of pollination, cultivar and area on seed number per inflorescence (Table 1). For these variables, the effect of block was not significant ($F_{2, 14} = 0.34$, $P = 0.71$).

There was a significant difference between pollination treatments in terms of seed weight ($F_{1, 14} = 17.9$, $P < 0.01$), but the effects of site and cultivar on seeds weight were not significant ($F_{1, 14} = 0.94$, $P = 0.35$ & $F_{1, 14} = 1.55$, $P = 0.23$, respectively). In addition, the interactions between pollination and site ($F_{1, 14} = 0.08$, $P = 0.76$), pollination and cultivar ($F_{1, 14} = 0.57$, $P = 0.48$), site and cultivar ($F_{1, 14} = 1.47$, $P = 0.25$) and pollination and site and cultivar ($F_{1, 14} = 0.02$, $P = 0.93$) on seed weight were not significant. There was no significant effect of block on seed weight ($F_{2, 14} = 0.55$, $P = 0.58$).

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Table 1 Comparison of the effect of presence/absence of insects as pollinator on quantity and quality of onion seeds. The results of Analysis of Variance on three factors: pollination treatments (Uncovered, Covered), cultivar (Red Azarshahr, Yellow sweet Spanish) and site (Mashhad, Chenaran). Razavi Khorasan province, 2010.

Source of Variation	d. f.	Seed number		1000 seed weight		Seed germination	
Block	2	2	0.71	0.48	0.58	0.56	0.58
Area (A)	1	0.66	0.42	0.38	0.33	3.19	0.09
Variety (V)	1	1.97	0.18	0.18	0.23	1.24	0.27
Pollination (P)	1	15.87	0.007	16.12	0.008	4.2	0.07
A * V	1	0.15	0.7	1.72	0.97	0.28	0.6
A * T	1	0.61	0.44	1.24	0.48	0.28	0.6
V * T	1	1.42	0.25	2.1	0.24	0.16	0.69
A * V * T	1	0.02	0.95	0.09	0.87	1.58	0.22
Error							14

All comparisons were made using a factorial ANOVA followed by Fisher's Protected LSD, $P < 0.05$. Significant effects ($P < 0.05$) are presented in bold.

Open pollinated treatments produced the highest number and weight of seeds as compared with treatments without any insect visitors (Fig. 1a & b). The mean numbers of seeds and their weight per inflorescence of free pollinated plants were 636 ± 42 and 2.21 ± 0.15 g., respectively, while insect exclusive pollination plants produced 173 ± 19 seeds per inflorescence which their mean weight of seeds per inflorescence was 0.57 ± 0.04 g.

The weight of 1,000 seeds obtained from insect inclusive (3.68 ± 0.14) and exclusive (2.71 ± 0.17) flowers was significantly different (Table 1). However, there were no significant influences of either site (Mashhad vs. Chenaran (3.13 ± 0.14 vs. 3.27 ± 0.26)) or cultivar (Red vs. Yellow (3.24 ± 0.24 vs. 3.17 ± 0.16)) on weight of 1000 seeds (Table 1). The interactive effects of pollination, cultivar and area on weight of 1,000 seeds were not significant (Table 1). For this variable, the effect of block was not significant either ($F_{2, 14} = 0.48$, $P = 0.62$).

ANOVA on seed germination revealed that the seeds of free pollination flowers had higher germination capacity (79.4 ± 1.8%) compared with the seeds of flowers pollinated without insects (74 ± 2.4%), with no significant difference (Table 1). Moreover, the effects of area (Mashhad vs. Chenaran (75 ± 2% vs. 79 ± 3%) and cultivar (Red vs. Yellow (75.7 ± 2.7% vs. 78.7 ± 1.65) on seed germination capacity were not significant (Table 1). The interactions between treatments on seeds weight were not significant (Table 1). There was no significant effect of block on seed germination capacity ($F_{2, 14} = 0.56$, $P = 0.58$).

In the study sites a variety of insects were found to visit onion flowers. Among the community of pollinators, hymenopterans (*Andrena* sp. (Andrenidae), *Halictus patellatus* (Morawitz 1873), *Halictus quadricinctus* (Fabricius1777), (Halictidae), *Xylocopa valga* (Gerstaecker1872), *Amegilla* sp. (Apidae), and dipterans (*Episyrphus balteatus* deGeer, *Eupeodes corollae*, *Sphaerophoria scripta*, *Eristalinus aeneus* (Syrphidae), *Musca domestica* L. (Muscidae) were the most frequent visitors. Part of the community of pollinators of onion has already been published (Rasekh Adel *et al*., 2012).

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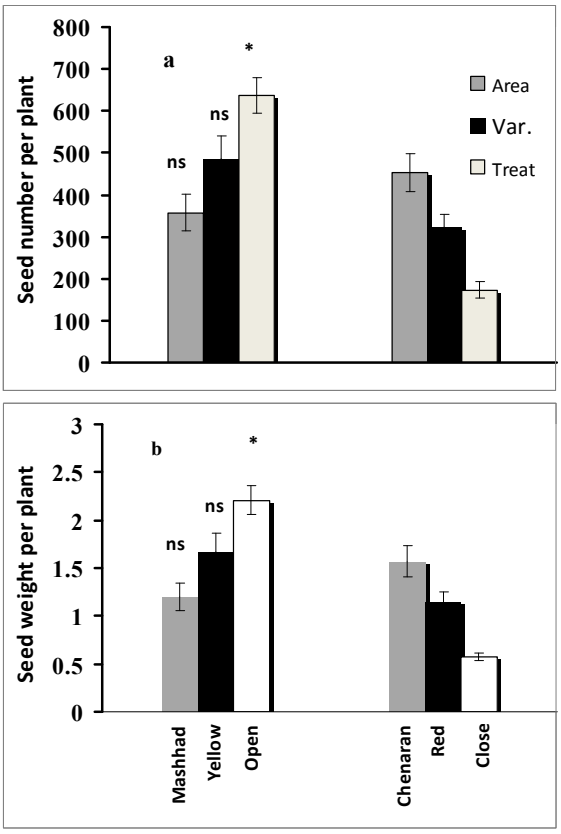

Figure 1 The effects of presence/ absence of insects on onion seed number (a) and weight (b) per inflorescence (Mean ± SE) in different treatment combinations. Two treatments (Uncovered and Covered); Two sites (Mashhad and Chenaran); two cultivars (Red Azarshar and Yellow sweet Spanish), in Razavi Khorasan province, 2010. Factorial ANOVA, followed by Fisher's Protected LSD, $P < 0.05$. * $P < 0.05$; ns, non-significant difference.

Discussion

This experiment highlighted the value of insects in seed production of onion in the study area. According to the obtained results, the average increased seed production in the open pollinated treatment was 74%. In other words, in the absence of insect pollinators, the expected onion seed yield per hectare would be around 130 kg as compared to 500 kg/ ha contributed by insects. The results of this study was in agreement with other works (e.g., Jablonski *et al.*, 1982; Tolon and Duman, 2003; Ahmad *et al.*, 2003; Kumar *et al.*, 1989; Rao and Lazar, 1983), and confirmed that the presence of insects during flowering period has substantial impact on onion seed setting. In this study, the number of seeds and weights of 1000 seeds in treatment without pollinating insect were significantly less than those of the free pollinated treatment. Similarly, Ahmad *et al.*, (2003) found that reduced or limited visiting of pollinators caused 50-61% reduction in seed production of onion. Also, Rao and Lazar (1983) observed only 9.8% seed setting in onion in cages. Moreover, Kumar *et al.*, (1989) recorded three times more yield in open pollinated treatment than the closed one.

Several factors have been suggested to influence onion pollination by insects. For example, as attractive flowering plants (including crops and weeds) in neighboring areas may pull away insect pollinators from plantations of flowering onion, it is suggested that part of the problem of the reduction in seed setting can be compensated by increasing the number of pollinators and decreasing the side effect of grown crops and number of sprays during flowering. Ahmad *et al.*, (2003) claim that proper visiting of pollinators could increase seed production up to 50% by minimizing the side grown attractive crops. Also, completing the spraying schedule before onset of flowering or application of insecticides in the evening could minimize the hazardous effect of insecticides on pollinators. In addition, spraying onion plants with some insects attractant may result in a higher rate of insect visiting flowers and consequently increase the percentage of flower set and lower number of aborted flowers. In an experiment, Al-Sahaf (2002) found that spraying of rose water on the inflorescence, vegetative part, or whole plant increased the number of visiting insects to the flowers and the insects spent longer periods foraging or grooming on the umbel, so the flower set percent was elevated.

In this study, free pollination flowers showed higher seed germination capacity than those isolated from insect visitors. This result is in agreement with earlier finding (Tolon and

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Duman, 2003), reporting that germination capacity and energy are higher when onion flowers are pollinated by honey bees. However, in our study difference of germination capacity between pollination treatments was not so much as those reported by other workers. For example, Chandel *et al*., (2004) reported that the difference in the onion seed germination capacity derived from plantations where pollinating insects were introduced in relation to plantations where they were absent reached over 20%.

In the present study, varieties as well as experimental sites did not show significant influence on seed setting of onion. Our results are supported by Ahmad *et al*., (2003) who found no significant difference in attraction of the four varieties of onion to pollinators. However, varieties possessing more concentration of nectar in flowers are more attractive to insect pollinators which in turn could result in a better seed setting.

Several studies have reported honeybees as the most frequent visitors of onion bloom (e.g., Bohart *et al*., 1970; Priti, 1998). However, in this study the abundance of honey bees was not high when compared with leaf cutting bees (Megachilidae) and yellow jacket wasps (Vespidae). This may be explained by the fact that onions are not favorites of honey bees because onion nectar is high in potassium, and the nectar tends to increase in viscosity as temperature increases, so bees prefer to forage on alternate nectar sources if available (Voss *et al*., 1999).

In conclusion, our results confirmed the positive role of insect visitors of onion flowers on seed production. Judging from our results, increased investment in the conservation of the insects providing such a service is justified. Moreover, detailed studies on the efficiency of each of the main insect pollinators of onion, and how to enhance the onion pollination are needed.

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تأثیر حشرات گرده‌افشان بر کمیت و کیفیت تولید بذر پیاز

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چکیده: به‌منظور ارزیابی تأثیر وجود حشرات گرده‌فشان بر کمیت و کیفیت بذر تولیدی دو رقم پیاز رایج به نام‌های قرمز آذرشهر و زرد شیرین اسپانیا، آزمایشی در قالب طرح بلوک‌های کامل تصادفی در دو مکان اجرا گردید. غده‌های پیاز هم‌وزن به‌فواصل ۳۵ سانتی‌متر بین بوته‌ها و ۵۰ سانتی‌متر بین ردیف‌ها کاشته شدند. تیمارهای این آزمایش در هر مکان فقط از لحاظ شیوه گرده‌افشانی متفاوت بودند. حدود یک هفته قبل از شروع گلدهی، در هر مکان ۳ گروه ۱۰تایی گل آذین به‌عنوان تکرارهای هر تیمار ترکیبی (گرده‌افشانی در رقم) به‌طور تصادفی انتخاب و علامت‌گذاری شدند. برای هر رقم پیاز، نصف بوته‌ها توسط پارچه توری ظریف و با استفاده از قفس‌های چوبی پوشیده شده و بقیه بدون پوشش و در معرض بازدید آزاد حشرات قرار داده شدند. هنگامی که در هر گل آذین حدود ۱۰ درصد بذور سیاه دیده شدند برداشت انجام گرفت. پس از خشک شدن بذور، تعداد و وزن بذر تولیدی به ازاء هر گل آذین تعیین و به‌علاوه وزن هزار دانه برای هر تیمار محاسبه شد. ۴ هفته پس از برداشت، آزمایش جوانه‌زنی اجرا گردید. عدم حضور حشرات گرده‌افشان در دوره گلدهی تأثیر چشمگیری در کاهش تعداد و وزن بذر تولیدی در هر گل آذین داشت. علاوه بر این، بذر حاصل از گل‌هایی که آزادانه مورد بازدید حشرات گرده‌افشان قرار گرفتند در مقایسه با گل‌هایی که از بازدید حشرات محروم بودند درصد جوانه‌زنی بالاتری داشتند. در این آزمایش مکان و واریته تأثیر معنی‌داری بر بذردهی پیاز نداشتند.

واژگان کلیدی: پیاز، گرده‌افشانی، بذر، ایران.

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