



Original article

Alfalfa (*Medicago sativa* L.) seed yield in relation to phosphorus fertilization and honeybee pollinationSaad Naser Al-Kahtani^a, El-Kazafy Abdou Taha^{a,b,*}, Mohammed Al-Abdulsalam^a^a Department of Arid Land Agriculture, College of Agriculture and Food Sciences, King Faisal University, Al-Ahsa, Saudi Arabia^b Department of Economic Entomology, College of Agriculture, Kafrelsheikh University, Kafrelsheikh, Egypt

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ABSTRACT

This investigation was conducted at the Agricultural and Veterinary Training and Research Station, King Faisal University, Al-Ahsa, Saudi Arabia, during the alfalfa growing season in 2014. The study aimed to evaluate the impact of phosphorus fertilization and honeybee pollination on alfalfa seed production. The experiment was divided into 9 treatments of open pollination, honeybee pollination, and non-pollination with three different levels (0, 300 or 600 kg P₂O₅/ha/year) of triple super phosphate. All vegetative growth attributes of Hassawi alfalfa were significantly higher in the non-insect pollination plots, while the yield and yield component traits were significantly higher with either open pollination or honeybee pollination in parallel with the increasing level of phosphorus fertilizer up to 600 kg P₂O₅/ha/year in light salt-affected loamy sand soils. There was no seed yield in Hassawi alfalfa without insect pollination. Therefore, placing honeybee colonies near the fields of Hassawi alfalfa and adding 600 kg P₂O₅/ha/year can increase seed production.

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1. Introduction

Alfalfa (*Medicago sativa* L.) is a Fabaceae perennial herb and is an important legume used for forage worldwide. Additionally, it is an important source of nectar and pollen for honeybees in several locations around the world, including Al-Ahsa, Saudi Arabia (Taha, 2015a,b). It is a cross-pollinated plant, and pollination occurs with the help of insects, wind, and other external elements. The honeybees *Apis mellifera* L. and *A. florea* F. were found to be the most active pollinators in alfalfa flowers (Taha et al., 2016). A number of wild pollinators also visit the flowers and participate in pollination (Cane and Schiffhauer, 2003; Hayter and Cresswell, 2006; Cecen et al., 2008; Taha et al., 2016). Different insect pollinators have been shown to vary in how effectively they deposit and remove pollen from individual flowers (Thomson and Goodell, 2001). For example, the tripping rate varies between bee species

visiting alfalfa racemes (Cane, 2002), and distinct species deposit different quantities of pollen on cranberry flowers during a single visit to a flower (Cane and Schiffhauer, 2003). Such differences in tripping rates and pollen deposition can be influenced by whether a pollinator forages for pollen or for nectar (Breazeale et al., 2008), and these differences have been shown to influence fruit and seed set (Thomson and Goodell, 2001; Pinheiro et al., 2014).

An insufficient number of suitable pollinators causes a reduction in fruit and seed production (Taha and Bayoumi, 2009; Maiti and Maiti, 2011). Of the total pollination activities, insects represent more than 80% and honeybees represent nearly 80% of the total insect pollinators (Robinson and Morse, 1989; Taha and Bayoumi, 2009; Taha et al., 2016). Alfalfa's floral structure facilitates cross-pollination. The rate of cross-pollination depends on insect activity, environmental conditions and the availability of other vegetation (Kumar and Lenin, 2000; Breazeale et al., 2008).

Improvements in artificial pollination increase the number of fruits in each branch. In cases where this plant cannot be pollinated, seed production is decreased substantially (Bolanos et al., 2000; Taha and Bayoumi, 2009; Bomfim et al., 2015). In Saudi Arabia, seed production can be achieved using the honeybee (*A. mellifera* L.) as managed pollinators (Taha et al., 2016). The flowers require bee visits for pollination, and when a bee opens the keel petals, the enclosed stamen and pistil snap forward, forcefully striking the bee (Cecen et al., 2008).

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Alfalfa removes large quantities of nutrients from the soil. Phosphorus has been the nutrient needed in the largest quantities for alfalfa production. Low phosphorous (P) availability reduces the yield and persistence of this perennial plant (Berg et al., 2005). Phosphate nutrition interacts with the carbohydrate supply and influences carbohydrate partitioning in plants including alfalfa (Li et al., 1998). The availability of P also influences protein accumulation and utilization in leaves (Rufty et al., 1993) and roots (Li et al., 1998). Phosphorus also enhances the symbiotic nitrogen (N) fixation process in legume crops (Cihacek, 1993). Phosphorus is an essential ingredient for *Rhizobium* bacteria to convert atmospheric N (N₂) into an ammonium (NH₄) form useable by plants. *Rhizobium* are able to synthesize the enzyme nitrogenase that catalyzes the conversion of N₂ to two molecules of ammonia (NH₃) (James et al., 1995). Phosphorus becomes involved as an energy source when 16 molecules of adenosine tri-phosphate (ATP) are converted to adenosine diphosphate (ADP) as each molecule of N₂ is reduced to NH₃ (Carroll, 2001; Erdal et al., 2008). The ATP is generated during the process of photosynthesis, when light energy is transformed and stored in the form of ATP for later use by the plant. Phosphorus influences nodule development through its basic functions in plants as an energy source. Inadequate P restricts root growth, the process of photosynthesis, translocation of sugars, and other such functions that directly or indirectly influence N fixation by legume plants (Berg et al., 2005).

Generally, legumes require more P than grasses for root development and energy-driven processes. Traditionally, these nutrients have been provided to plants through applications of manure or chemical fertilizers to the soil (Beegle, 1995). The deep roots of alfalfa are also able to access nutrients that cannot be reached by shallow-rooted crops (Gossen et al., 2004).

Phosphorus functions in alfalfa plants include energy storage and transfer (such as ADP and ATP), structural biochemical components, seed formation, calcium and magnesium phytate, maintenance and transfer of genetic code, root growth, rapid crop establishment, early maturity, and quicker recovery (Berg et al., 2005). Other studies reveal that P applied to low P soils can increase the percent N in legumes and result in greater dry matter yields (Reid et al., 2004; Madani et al., 2014; Zhang et al., 2014). This is believed to be one of the reasons why legumes are dependent on symbiotic N and have a higher P requirement than grasses that depend on fertilizer N.

The present investigation aimed to study the effect of honeybee pollination and phosphorus fertilization on alfalfa growth and seed production.

2. Material and methods

The experiment was carried out at the Agricultural and Veterinary Training and Research Station, King Faisal University, Al-Ahsa, east Saudi Arabia, during the 2014 growing season. The experimental soil is light salt-affected loamy sand. Alfalfa cv. Hassawi was established by direct seeding at the rate of 50 kg/ha. The seeds were inoculated with *Rhizobium meliloti* before seeding. Local cultural practices were applied throughout the experimental period, except for the studied treatments.

2.1. Soil characteristics

Soil traits such as salinity, pH, calcium, magnesium, potassium, sulfate ion, bicarbonate, chlorine, and the percentage of sand, silt and clay of the study area were determined and presented in Table 1.

Table 1

Soil physicochemical properties of the experimental site in Al-Ahsa, east of Saudi Arabia.

Soil properties	
EC ds/m	6.85
pH	7.74
Cations (meg/l)	
Ca ²⁺	12.6
Mg ²⁺	2.4
Na ⁺	29.3
K ⁺	24.1
Anions (meg/l)	
HCO ₃ ⁻	0.3
SO ₄ ⁻²	26.2
Cl ⁻	17.0
Textural class (%)	
Sand	84.50
Silt	12.50
Clay	3.00

2.2. Pollination and phosphorus fertilization treatments

To study the effects of bee pollination and phosphorus fertilization on alfalfa seed yield, the experiment was laid out as a complete block randomized design (CBRD) with three replicates. The treatment details are as follows: open pollination (T₁), closed pollination by honeybee only (T₂), and without pollination (T₃). Each pollination treatment was treated with three levels of phosphorus fertilization [0 kg P₂O₅/ha/year (P₁), 300 kg P₂O₅/ha/year (P₂), and 600 kg P₂O₅/ha/year (P₃)]. The phosphorus was applied before planting and after each cut as triple super phosphate. In T₁ plots, plants were left to open pollination, and 3 m² sections were randomly selected to take measurements. In T₂ plots, an insectarium (10 × 6 × 2 m) was fixed, and the plants inside the insectarium were sprayed with a suitable insecticide. At the commencement of flowering, one Carniolan honeybee (*Apis mellifera carnica* Pollmann) colony of seven combs was placed inside the insectarium. In T₃ plots, 3 m² sections were isolated from all insect pollinators by using wooden cages (3 × 1 × 2 m) covered with wire screen, which was fixed two weeks before anthesis.

2.3. Vegetative traits and seed yield component

The plant height (cm), number of racemes/stem, number of flowers/raceme and number of pods/raceme were measured on 25 randomly selected plants from each treatment. The harvest index (HI) was calculated as the percentage ratio of seeds to the total above-ground biomass of 10 stems taken at random from each plot. The number of seeds per pod was counted. The weight (g) of 1000 seeds was estimated by weighing 1000 dried seeds drawn randomly from the seed yield of each treatment using an electronic balance. Seed yield/m² at harvest was determined by removing the capsules of all plants per m² in each treatment, and the seeds were separated and weighted using an electronic balance. The successful fruiting index was calculated using the following equation:

$$\text{Successful fruiting index} = \frac{\text{Total number of pods/raceme}}{\text{Total number of flowers/raceme}} \times 100$$

3. Statistical analysis

Data were statistically analyzed by ANOVA using the SAS[®] software program (SAS Institute, 2003). The means of the treatments

Table 2
Influence of honeybee pollination and phosphorus fertilization on the growth parameters of Hassawi alfalfa in Al-Ahsa, east of Saudi Arabia.

Treatment	Plant height Cm	No. racemes/stem	No. flowers/raceme
T ₁ P ₁	59.83 ^c	7.73 ^b	8.32 ^d
T ₁ P ₂	61.83 ^c	8.00 ^b	8.87 ^{cd}
T ₁ P ₃	65.33 ^c	8.60 ^b	9.52 ^{bc}
T ₂ P ₁	79.80 ^{ab}	8.73 ^b	8.54 ^d
T ₂ P ₂	80.87 ^{ab}	9.33 ^{ab}	9.18 ^c
T ₂ P ₃	79.89 ^b	10.17 ^a	9.53 ^{bc}
T ₃ P ₁	85.00 ^a	8.84 ^b	9.19 ^c
T ₃ P ₂	85.33 ^a	9.72 ^b	10.18 ^b
T ₃ P ₃	83.33 ^{ab}	10.57 ^{ab}	11.44 ^a
Significant	**	*	*

T₁ = open pollination, T₂ = closed pollination by honeybee, T₃ = without pollination, P₁ = 0 kg P₂O₅/year, P₂ = 300 kg P₂O₅/year, and P₃ = 600 kg P₂O₅/year. Means of each column followed by the different letters are significantly different. ** indicate $p < 0.01$ and * indicate $p < 0.05$.

were compared using Duncan's Multiple Range Test (Duncan, 1955).

4. Results

The data presented in Table 2 show significant differences among the treatments in all studied vegetative traits of Hassawi alfalfa. The plant height, number of racemes/stem, and number of flowers/raceme of Hassawi alfalfa were significantly higher in non-insect pollination plots compared to the pollinated ones. In all treatments, the number of racemes/stem and the number of flowers/raceme of Hassawi alfalfa increased in parallel with increasing phosphorus levels.

The data presented in Table 3 clearly show that the yield and yield components traits of Hassawi alfalfa significantly increased with pollination and with the increase of P fertilizer level up to 600 kg P₂O₅/ha/year in a light salt-affected loamy sand soil. The P level of 600 kg P₂O₅/ha/year in open pollination plots (T₁P₃) resulted in the highest values for the studied parameters, with 8.10 pods/raceme, 4.15 g/1000 seeds, 39.40 g seed yield/m², an 85.05% successful fruiting index, and a 9.40% harvest index. The highest number of seeds/pod (2.78 seeds) was obtained from plots fertilized with 600 kg P₂O₅/ha/year and pollinated by honeybees (T₂P₃). The highest abortion percentage (100%) was obtained from non-pollinated plants without any effects from phosphorus fertilization.

5. Discussion

Phosphorus fertilization and insect pollination significantly affected all of the studied vegetative traits of Hassawi alfalfa. Non-pollinated plants (T₃) were exceeded open pollinated (T₁) and honeybee pollination (T₂) by 42.07% and 6.52% for plant height, 14.36% and 1.26% for the number of racemes/stem, and 10.46% and 7.61% for the number of flowers/raceme, respectively. Adding P at a level of 300 kg P₂O₅/ha/year reduced the increases to 38.01% and 5.52% for plant height, while it raised the increases to 21.50% and 4.18% for the number of racemes/stem and 14.77% and 10.89% for the number of flowers/raceme, respectively. Phosphorus fertilization at a level of 600 kg P₂O₅/ha/year reduced the increases in plant height to 27.55% and 4.31%, while it raised the increases to 22.91% and 3.93% for the number of racemes/stem and 20.17% and 20.04% for the number of flowers/raceme, respectively. These results confirm the findings of Berg et al. (2005) and Madani et al. (2014), who found that in P poor soil, alfalfa responded significantly to phosphorus fertilization.

Seed yield and yield components, which included the number of pods/stem, number of seeds/pod, abortion percentage, weight of 1000 seeds, and seed yield/m², were significantly ($p < 0.01$) influenced by pollination and phosphorus fertilization levels. The highest number of pods/stem, weight of 1000 seeds, and seed yield/m² were obtained from plants fertilized with 600 kg P₂O₅/ha/year in an open pollination treatment. The non-pollinated plants failed to form any pods on any plants, which reflects the important role of insects, especially honeybees, in the pollination and seed production of Hassawi alfalfa. Alfalfa seed production is enhanced by cross-pollination, which is largely achieved by the honeybee (Taha et al., 2016). If a flower is not tripped, pollination cannot occur. Tripping occurs when the action of a flower-visiting bee releases pressure on interlocking keel petals and the enclosed stamens and pistil abruptly snap upward from the keel. Thus, there were significant differences between the means of pollination for all investigated plant traits.

Although some crop plants can produce seeds through other means of pollination, the presence of honeybees as alfalfa pollinators (Taha et al., 2016) is important to increase productivity (Cecen et al., 2008). Our findings demonstrated the great importance of insect pollinators, especially honeybees, for the seed set in alfalfa, since the results indicated 100% abortion for all pistillate flowers that did not receive any entomophilous visitation. Our results were consistent with the findings obtained by Taha and Bayoumi (2009) and Adamson (2011), who reported that insect pollination plays a vital role in producing high yields due to their help in the pollination of crop plants. The importance of insects visits to flowers and their pollination activity has been recognized in squash (Skinner and Lovett, 1992), watermelon (Stanghellini et al., 1997; Bomfim et al., 2015; Giannini et al., 2015; Kiill et al., 2016), radish (Chandrashekar, 2005), cucumber (Stanghellini et al., 1997), Egyptian riverhemp (Sajjad et al., 2009), summer seed watermelon (Taha and Bayoumi, 2009), sesame (Kumar and Lenin, 2000; Mahfouz et al., 2012), apple tree, avocado, and pear (Giannini et al., 2015). Adding 300 kg P₂O₅/ha/year resulted in non-significant differences between open pollination and honeybee pollination for the number of pods/raceme, the number of seeds/pod, and the weight of 1000 seeds, while significant differences ($p < 0.01$) were observed for the successful fruiting index, abortion percentage, and seed yield/m². Except for the number of seeds/pod, all values of the seed yield components in open pollination plots significantly ($p < 0.01$) exceeded the values of honeybee pollination plots when phosphorus fertilization was applied at a level of 600 kg P₂O₅/ha/year. The superiority of the open pollination treatment might be attributed to pollination by dwarf honeybee that become widely distributed in Al-Ahsa area (AL-Kahtani and Taha, 2014). The dwarf honeybee was active on the flowers in the day-time; this is in contrast with the Carniolan honeybee, which was active only at limited times (Taha et al., 2016). On the other hand, the numbers of seeds/pod in the honeybee pollination plots were better than those in the open pollination. Indeed, there is no fruit set without the deposition of the minimum amount of pollen needed for fruit development (Delaplane and Mayer, 2013), but fruit quality is affected by the number of pollen grains deposited on the stigma of a flower (Serrano et al., 2006; Pinheiro et al., 2014), and that might be correlated with the body size of the insect pollinator.

Adding phosphorus to open pollination and honeybee pollination plots at a level of 300 kg P₂O₅/ha/year caused significant increases in the number of pods/raceme (14.84% and 12.50%), the weight of 1000 seeds (67.06% and 67.06%) and seed yield/m² (25.08% and 15.11%) compared with the results for the non-fertilized and non-pollinated plants, respectively. In comparison to the non-fertilized and non-pollination treatments, adding 600 kg P₂O₅/ha/year resulted in an increasing number of pods/

Table 3
Influence of honeybee pollination and phosphorus fertilization on seed yield of Hassawi alfalfa in Al-Ahsa, east of Saudi Arabia.

Treatments	No. pods/raceme	Successful fruiting index	Abortion %	No. seeds/pod	Weight (g)		Harvest index (%)
					1000 seeds	Seed yield /m ²	
T ₁ P ₁	6.40 ^d	76.92 ^e	23.08 ^c	1.49 ^c	1.70 ^d	24.68 ^d	7.10 ^{bc}
T ₁ P ₂	7.35 ^c	82.86 ^c	17.14 ^e	1.95 ^b	2.84 ^c	30.87 ^c	7.50 ^b
T ₁ P ₃	8.10 ^a	85.05 ^a	14.95 ^f	2.76 ^a	4.15 ^a	39.40 ^a	9.40 ^a
T ₂ P ₁	6.30 ^d	73.77 ^f	26.33 ^b	1.42 ^c	1.70 ^d	23.16 ^e	6.50 ^c
T ₂ P ₂	7.20 ^c	78.43 ^d	21.57 ^d	1.87 ^b	2.84 ^c	28.41 ^d	7.70 ^b
T ₂ P ₃	7.96 ^b	83.53 ^b	16.47 ^e	2.78 ^a	3.96 ^b	35.01 ^b	8.60 ^a
T ₃ P ₁	0.00 ^e	0.00 ^g	100.00 ^a	0.00 ^d	0.00 ^e	0.00 ^f	0.00 ^d
T ₃ P ₂	0.00 ^e	0.00 ^g	100.00 ^a	0.00 ^d	0.00 ^e	0.00 ^f	0.00 ^d
T ₃ P ₃	0.00 ^e	0.00 ^g	100.00 ^a	0.00 ^d	0.00 ^e	0.00 ^f	0.00 ^d
Significant	**	**	**	**	**	**	*

T₁ = open pollination, T₂ = closed pollination by honeybee, T₃ = without pollination, P₁ = 0 kg P₂O₅/year, P₂ = 300 kg P₂O₅/year, and P₃ = 600 kg P₂O₅/year. Means of each column followed by the different letters are significantly different. * indicate $p < 0.01$ and * indicate $p < 0.05$.

raceme (26.56% and 26.34%), weight of 1000 seeds (44.12% and 32.94%), and seed yield/m² (59.64% and 41.86%) for the open pollination and honeybee pollination treatments, respectively. The increase in seed yield and yield components due to the application of different levels of phosphorus in Hassawi alfalfa was in accordance with the results reported by Mullen et al. (2000), Gossen et al. (2004), and Berg et al. (2005). The production of significantly higher seed yields due to an application of P₂O₅ over the control could be related to the differences in the number of pods per stem, the number of seeds per pod and seed weight. Higher seed yield could be obtained through integrated management, especially if this rate is applied along with other cultural practices that increase the number of stems/m², the number of racemes/stem and the number of flowers/raceme.

6. Conclusion

Pollination by honeybee and other insects is a critical input in Hassawi alfalfa seed production. The findings of this study suggest that even though insects, especially honeybees, are available for pollination, phosphorus levels up to 600 kg P₂O₅/ha are required for the loamy sand soils in Al-Ahsa, KSA, to increase seed yield.

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