INTERNATIONAL JOURNAL OF PLANT, ANIMAL AND ENVIRONMENTAL SCIENCES

IJPAES

Volume-5, Issue-2, April-June-2015 Coden: IJPAJX-USA, Copyrights@2015 ISSN-2231-4490

Received: 11th Jan-2015 Revised: 15th Mar-2015 Accepted: 16th Mar-2015

Research article

COMPARATIVE STUDY OF THE EFFECT OF ABSENCE OF ENTOMOPHILOUS POLLINATION ON THE PRODUCTIVITY OF RAPESEED AND FABA BEAN

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ABSTRACT: Several crops receive benefits from the presence of pollinating insects and therefore a decline or disappearance of these could compromise agricultural production, essentially that of cross-pollinated and partially cross-pollinated crops. A comparative study of the effect of the absence of entomophilous pollination on some parameters of rapeseed and faba bean, two partially cross-pollinated crops, was performed through a field experiment carried out on plots placed under insect-proof cage to simulate the absence of pollinating bees (self-pollination) and other plots placed outside the cage to ensure the presence of bees (open-pollination). Field trial was conducted in 2013/14 at the experimental station of Douyet of INRA-Morocco according to a completely randomized blocks design, with two replications. Effect of the absence of entomophilous pollination was evaluated on morphological, phenological and agronomic parameters, such as plant height, flowering duration, number of fruits per plant, number of seeds per fruit and seed yield. In rapeseed, absence of entomophilous pollination reduced drastically the number of fruits per plant by 15.54% and seed yield per plant by 33.45%, while in faba bean, these parameters were reduced, respectively, by 28.20% and 20.83%. Also, under bee's absence conditions, average number of rapeseed seeds per silique decreased by 20.92%. However, for faba bean, no significant difference was observed between bees absence and bees presence for this parameter, with an average seeds number per pod of 2.94 and 2.74, respectively. In absence of pollinating bees, seed yield of rapeseed was more affected than that of faba bean. Thus, in particular environments with decline of bees, it would be wiser to integrate faba bean rather than rapeseed in the traditional cereal-based cropping system. Protection of pollinating bee populations is a necessity, not only for the maintenance of plant and animal biodiversity, but also for preserving the economic activities related to agriculture and beekeeping.

Key words: Pollinating insects, Partially cross-pollinated crops, Faba bean, Rapeseed, Agronomic parameters, Yield reduction.

INTRODUCTION

Entomophilous pollination is a key factor in sexual reproduction of many plant species. The symbiosis between insects and plants allows the maintenance of biodiversity through cross-pollination and sustainability of some ecosystems. Bees, bumblebees and other pollinating insects are foraging to collect pollen and nectar. By this foraging activity, they will ensure "unconsciously" cross-pollination and thus cross-breeding of angiosperms. Pollination by insects is a widespread ecological process: more than 80% of wild plant species [1] and almost 75% of the cultivated plant species [2] rely on insects, especially wild bees, for the production of seeds and fruits. As a facet of global changes, the recent decline in insect pollinator communities is currently a major concern because of its impact on ecological and agricultural systems [3]. Climate change, characterized by increased trend of drought and heat, as well as changes in human land use and agricultural intensification is considered the main cause of pollinating insects decline. Since the 1950's, there has been a reduction of bee species richness and populations density [4].

The loss of some pollinating species could affect crop yields if those crops are dependent on these species for their pollination. Therefore, the decline of pollinators is likely to cause an agricultural crisis for such crops. The pullback of pollinator diversity is accompanied, in fact, by lower pollination efficiency, lower crop yields and a tendency to substitute insect-pollinated by self-pollinated species [5]. Furthermore, there is a real threat of extinction of plant species dependent on pollinators to ensure their reproduction and life. This would have a negative impact on biodiversity and hence on the sustainability of the global environment and agriculture.

Faba bean and rapeseed are a two self-compatible species partially cross-pollinated, and many authors have reported that they are dependent on pollinating entomofauna for their seed production. The foraging activity of insect pollinators (bees and/or bumblebees) on the faba bean and rapeseed ensures cross-pollination that significantly improves the production of the plant more than self-pollination [6, 7, 8, 9, 10, 11]. Aouar-Sadli et al. [12] found that the abortion rate among faba bean was significantly higher in the absence of bees (37%) than in their presence (19%), the average number of seeds per pod and average number of seeds per plant were lower in the absence of entomophilous pollination than in its presence. Musallam and al. [13] concluded that honeybee pollination increased faba bean seed yield by as much as 49%. Sabbahi et al. [7] have shown that there is an increase in seed yield of increased of at least 44% and a decrease in flowering period of about 4 days in the presence of honeybees compared to their absence. Likewise, Shakeel and Inayatullah [14] found that, for rapeseed, average number of siliques per plant was 189±1.7 siliques in the pollinated plots (entomophilous pollination) and 142±2.4 siliques in the covered plots (self-pollination).

Because of the economic importance of faba bean and rapeseed crops in the world and Mediterranean area, including Morocco, and also because of decline of bees due to climate change and increasing use of insecticides, it is very interesting, in scientific (agronomic) and environmental terms, to evaluate the effect of insect pollinators on these crops. The main objective was to check whether the absence of pollinators has a negative effect on some parameters of agronomic interest of faba bean and rapeseed under Moroccan conditions. The second objective of this study is to compare for both crops the effect of the absence of insect pollinators on seed yield and other related agronomic parameters.

This study has been performed for the first time in the Mediterranean area. To our best knowledge, no previous study has focused on comparing the negative effect of the absence of bees on the productivity of two economically important partially cross-pollinated crops which are integrated in a cropping system with cereals (especially wheat), that are the most strategic crops in Morocco and around the world. Apart from the ecological requirements of both crops, the expected results of this research would recommend, for a given environment, the crop that would produce more, taking into consideration the availability and abundance of bees.

MATERIALS AND METHODS

Plant material

Faba bean plant material used in this study is the line encoded S8712-6-m-4. It is productive and moderately resistant to Botrytis. Rapeseed plant material is the variety ‘Moufida’, that is canola type and highly productive [15].

Experimental site and design

The field trial was installed in the INRA experimental station of Douyet which is located at 10 km from Fez city (34°04’ N, 5°07’ W) at an elevation of 416 m and receives an average of 425 mm of precipitation per year. The soil is a cracking clay with vertic properties, it is fertile and deep. Cumulative rainfall recorded during the 2013-14 crop year was 300 mm. The trial was conducted in a completely randomized blocks experimental design, where the factor studied was presence/absence of pollinator bees, i.e. (entomophilous vs self-fertilization). The study consists of two blocks, each consisting of four randomized plots. Two of them contained rapeseed and faba bean plants growing under an insect-proof cage to prevent the penetration of bees as well as other insects and thus to ensure only self-fertilization in absence of these pollinators. The other two plots were outside the cage to ensure entomophilous pollination.

Measurements and observations

The main observations focused on plant vigor, disease and pests that can affect the development of the latter, the presence and activity of bees in flowering times in the free plots and the absence of any insect within the cage. The measurements were performed on a random sample of 10 plants per plot and mainly concerned some morphological and phenological traits and major yield components of both crops. Other parameters, such as temperature of vegetation cover, leaves temperature and air temperature were also measured to characterize the experiment environment within and outside the plant isolation cage.

- **Temperature measurement**

Temperature measurements of vegetation cover and leaves were performed using an infrared thermometer in two different days (24 April and 29 April). In each plot, the temperature of vegetation cover was measured at four different places, while keeping the thermometer at the same tilt angle to obtain the average temperature of vegetation cover of the plot. The latter was measured by calculating the average of temperatures taken on 24 and 29 April.

For faba bean, temperature of five leaves per plant, one leaf per node, going from the node located at the apex downwards was measured. For rapeseed, temperature of five leaves per plant, one leaf per branch, ranging from the lowest branch upwards was measured. To determine air temperature, two thermometers were installed, one in the middle of plots outside the cage and the other in the middle of plots under the cage. These thermometers were installed at a height of 60 cm from the ground. They measure the minimum and maximum temperatures during a day and the daily fluctuations in temperature.

- **Plant height**

After maturation of plants, their height, expressed in m, was measured from the ground to the highest point of the plant.

- **Flowering duration**

The duration of flowering was measured for both species in all plots based on the date of beginning of flowering and the date of the end of flowering per plot.

- **Number of fruits per plant**

When both species have almost completed their fruiting stage, the number of siliques per rapeseed plant and number of pods per faba bean plant was determined on the sample of ten plants. By calculating the average of ten values per plot, it was possible to obtain the average number of fruits per plant for each plot.

- **Number of seeds per fruit**

At harvest the number of seeds per faba bean plant was counted and divided by the number of pods per plant to get the number of seeds per pod for each faba bean plant sampled. Whilst, for rapeseed, given the high number of seeds per plant, the seeds counting was limited to ten siliques taken randomly from each of the ten plants sampled. The resulting number was divided by ten to obtain the average number of seeds per pod. The ten pods per rapeseed plant sampled served afterwards to measure average silique size and average seed weight.

- **Seed weight**

Faba bean seeds of the ten plants sampled were separately weighed using an electronic balance with a precision of 0.01 g, and individual seed weight, for each plant, was determined by dividing the measured plant seeds weight by the number of seeds. The average weight of one seed (g) was calculated by dividing the total of individual seed weight per plant (g) by the number of plants (10). Average seed weight (mg) of rapeseed was estimated on the basis of the seeds obtained from the ten pods already used to estimate the number of seeds per pod. The weighing was done using an electronic balance having an accuracy of 0.1 mg.

- **Rapeseed silique size**

The average size was estimated by measuring the size of ten siliques per plant.

- **Rate of flower blasting**

For each faba bean plant, the number of nodes with flowers and the number of fruiting nodes were identified, respectively, during the period of full bloom and the fruiting. Thus, the rate of flower blasting (RFB) (%) is estimated on the basis of the flowered nodes as shown in the following formula:

$$\text{RFB (\%)} = \left(100 - \left[\frac{\text{Number of fruiting nodes}}{\text{Number of nodes in bloom}} \times 100\right]\right)$$

For each rapeseed plant, the rate of flower blasting is estimated on the basis of the flowers of the terminal inflorescence and those of primary branch located just below the latter.

$$\text{RFB (\%)} = \left(100 - \left[\frac{\text{Number of formed siliques}}{\text{Number of flowers}} \times 100\right]\right)$$

- **Seed yield per plant**

The seed yield per plant (g) is determined by weighing all the plant seeds.

- **Reduction rate in yield, number of fruits and average number of seeds**

To assess the level of seed yield loss due to the absence of pollinator bees, the yield reduction rate (YRR) (%) was calculated on the basis of the plant average seed yield (g) in plots under and outside cage, using the formula shown thereafter. By the same formula below, the reduction rate in number of fruits per plant and number of seeds per fruit was calculated.

$$\text{YRR (\%)} = \frac{\bar{X}_2 \text{ Seed yield per plant (Outside cage)} - \bar{X}_2 \text{ Seed yield per plant (under cage)}}{\bar{X}_2 \text{ Seed yield per plant (outside cage)}}$$

Statistical analyses

Student test on equality of means for paired samples or groups was done to compare means of air temperatures measured in plots under cage and those outside the cage, using 'IBM SPSS Statistics 20' software. One-way analysis of variance (treatment (presence/absence of the cage) was made for each crop in order to check if there is a cage effect on the vegetation cover and leaves temperatures. The same analysis was done to check if there is an effect of absence of entomophilous pollination on the parameters measured in faba bean and rapeseed. The ANOVA test was performed using the 'MINITAB 16' software.

RESULTS

Temperature inside and outside insect-proof cage

The averages of all measured air temperatures are different inside and outside the cage. The average (mean ± standard deviation) of minimum, maximum temperatures and those taken at 9h00, 11h00 and 13h00 were respectively 10.53±6.23°C, 35.22±5.76°C, 28.64±5.20°C, 29.86±5.20°C and 32.36±5.92°C, outside the cage, while they were 9.47±5.14°C, 33.56±6.02°C, 25.64±4.05°C, 27.29±5.06°C and 31.43±6.03°C inside the cage. Comparing averages of measured air temperatures (Student t-test); significant differences were revealed between inside and outside the cage, except for the minimum temperature. These differences are estimated to be between 1°C and 3°C. This implies that relative humidity of the cold air inside the cage is greater than that of the hot air outside the cage. Because of technical constraints we were not able to measure the wind speed and insolation, but from our observations, the cage has an obvious windbreak effect, which could be beneficial for the growing plants under cage.

The average temperatures of vegetation cover in faba bean and rapeseed are slightly higher in plots outside the cage that in plots under cage. The analysis of variance showed that the cage had no effect ($F = 0.13$; $p > 0.05$) on the temperature of rapeseed vegetation cover, but it had an effect on the temperature of faba bean vegetation cover ($F = 67.44$; $p < 0.05$). Likewise, the average temperatures of faba bean leaves are almost the same inside and outside the cage ($F = 0.35$; $p > 0.05$) while those of rapeseed are slightly elevated outside the cage ($F = 10.50$; $p < 0.05$).

Plant height

Plants growing under insect-proof cage were significantly higher than those growing outside the cage for both rapeseed ($F = 21.32$; $p < 0.05$) and faba bean ($F = 91.87$; $p < 0.05$). The average plant height of rapeseed was 1.60 m and 1.46 m, respectively inside and outside the cage. The average plant height of faba bean was 0.99 m and 0.78 m, respectively inside and outside the cage.

Flowering duration

For faba bean, flowering started on 06/03/2014 and was spread over 40 days in plots outside the insect-proof cage and over 42 days in plots under cage. For rapeseed, flowering started on 04/04/2014 and was spread over 31 days in plots outside the cage and over 35 days in plots under cage. Therefore, flowering period of both species was lower in plots of insect-pollinated plants than of plants not pollinated by insects. Along the flowering period, one could observe the presence of pollinating insects, mainly bees, foraging flowers in plots outside the cage.

Rate of flower blasting

The rate of faba bean flower blasting in plots outside the cage and under cage was respectively of 53.23% and 64.45%. Results of ANOVA showed that the cage factor, preventing the contact of plant flower with bees and likely other insects, had a significant effect on this parameter ($F = 4.16$; $p < 0.05$). The rate of rapeseed flower blasting in plots outside the cage and under cage was respectively of 53.21% and 44.21%. Similarly, results of ANOVA showed that the cage factor has a significant effect on this parameter ($F = 13.26$; $p < 0.05$). Paradoxically, in rapeseed, flower blasting rate was higher outside the cage than inside. This would be a result of the attack of rose chafer as well as other serious pest that destroy buds, flowers and young siliques. Figure 1 show s a rose chafer and other insect on a rapeseed flower.

a b

Fig. 1 Rose chafer (a) and other pest (b) on buds and flowers of rapeseed

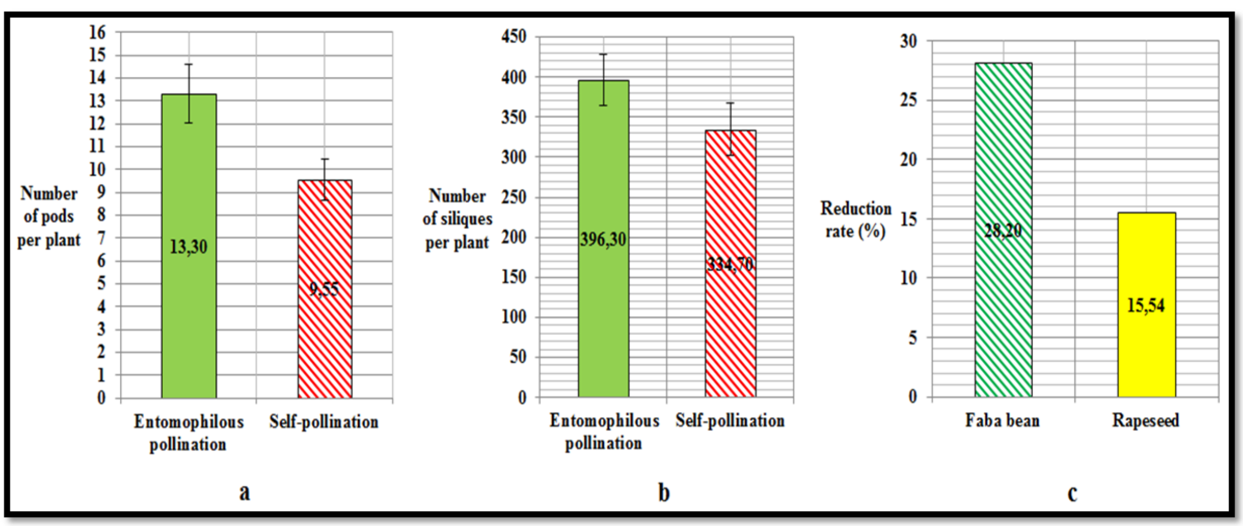

Fig. 2 Average number of pods per faba bean plant (a) and siliques per rapeseed plant (b) and reduction rate of this parameter in absence of entomophilous pollination (c)

Number of fruits per plant

The average number of pods per faba bean plant was 13.3 outside the cage where plants were open-pollinated in presence of bees and other insects. This value is statistically higher than the average number observed inside the cage in absence of bees and other any insects (9.22). Similarly, for rapeseed, the average number of siliques per plant was higher in plots outside the cage, with presence of bees and other insects (396.30) than plots inside the cage, in absence of any pollinating insect (334.70). Figure 2 shows the number of pods per faba bean plant and the number of siliques per rapeseed plant as well as the reduction rate observed in absence of pollinating insects. For the plants growing in the plots under cage, in absence of insects, the number of fruits fell by 28.20% for faba bean and 15.54% for rapeseed, compared to plants growing outside the cage, in presence of insects. There was a decrease in the number of fruits per plant for both species. However, the reduction rate was notably higher in faba bean than in rapeseed. These results indicated that the absence of bees or other pollinating insects could lead to an increased abortion rate, mainly reflected by a decrease of the number of fruits per plant. On the other hand, the average size of rapeseed silique is statistically higher ($F = 4.92$; $p < 0.05$) for plants open pollinated outside cage (6.27±0.46 cm) than those self-fertilized inside the cage (6.00±0.41 cm).

Number of seeds per fruit

Figure 3 shows the number of seeds per faba bean pod and the number of seeds per rapeseed silique as well as the reduction rate observed in absence of pollinating insects.

For faba bean, the absence of entomophilous pollination had no effect on the average number of seeds per pod ($F = 2.60$; $p > 0.05$). In fact, the average number of seeds per pod inside the cage (2.94±0.47) is statistically equal to that outside the cage (2.74±0.41). However, for rapeseed, this absence affected significantly and negatively the number of seeds per silique ($F = 13.19$; $p < 0.05$). In fact, the average number was 22.66±3.62 for plants outside the cage and 17.92±4.66 for plants inside the cage. This was the reason why the silique size was higher for plants outside the cage pollinated by bees and likely other insects. The average number of seeds per silique decreased for under cage plants by almost 21% compared to those growing outside the cage under entomophilous pollination conditions. On the other hand, average seed weight in plants pollinated by insects and plants self-fertilized was respectively, 2.15±0.71 mg and 2.33±0.55 mg for rapeseed and respectively 0.88±0.12 g and 0.90±0.13 g for faba bean. Absence/presence of pollinating insects had no effect on the average seed weight both for rapeseed ($F = 0.98$; $p > 0.05$) and faba bean ($F = 0.30$; $p > 0.05$).

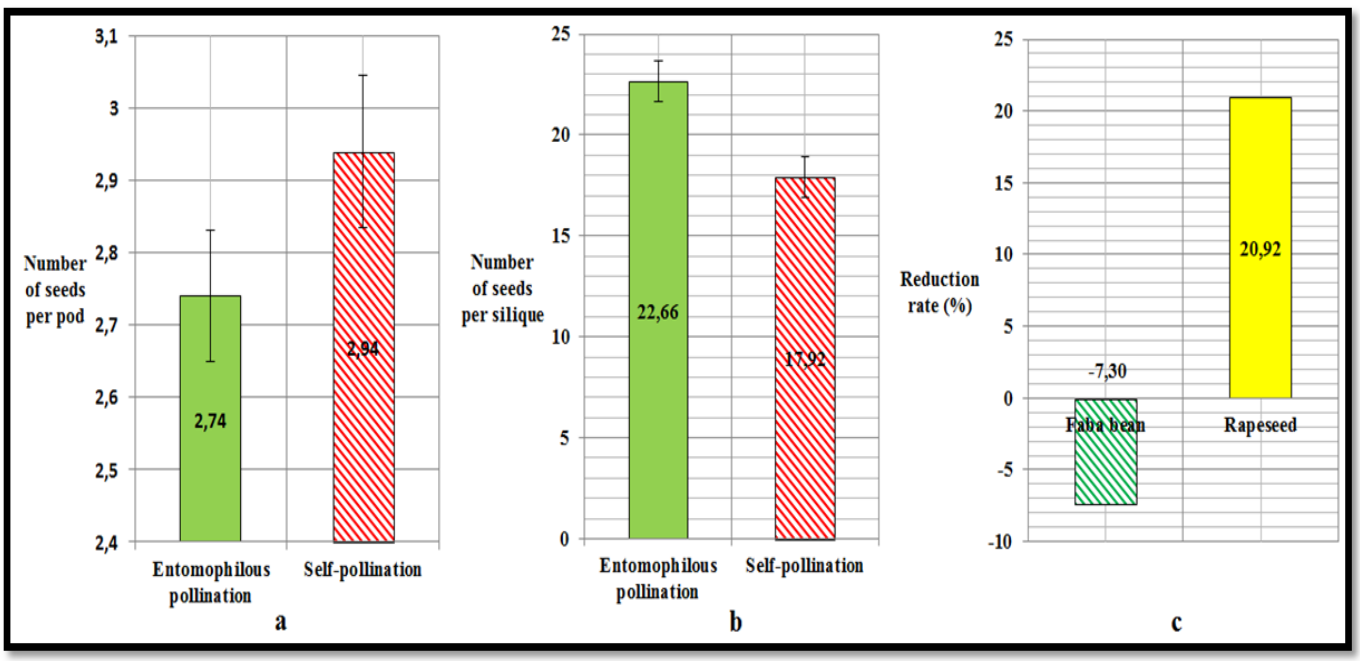

Fig. 3 Average number of seeds per faba bean pod (a) and seeds per rapeseed silique (b) and reduction rate of this parameter in absence of entomophilous pollination (c)

Seed yield per plant

Even though results of ANOVA showed that absence/presence of pollinator insects had no significant effect on seed yield per plant for faba bean (F = 3.59; $p > 0.05$), the average seed yield per plant in outside cage plots (30.78±11.55 g) was sufficiently higher than that observed in plots within the cage (24.37±9.35 g). There was a decrease of seed yield by almost 21% (Figure 4). For rapeseed, results of ANOVA showed that the presence of entomophilous pollination had a significant effect on the seed yield per plant (F = 6.92; $p < 0.05$). Average seed yield per plant in plots pollinated and non-pollinated by insects was, respectively, 20.12±10.44 g and 13.39±5.65 g. The seed yield reduction rate was about 33% (Figure 4).

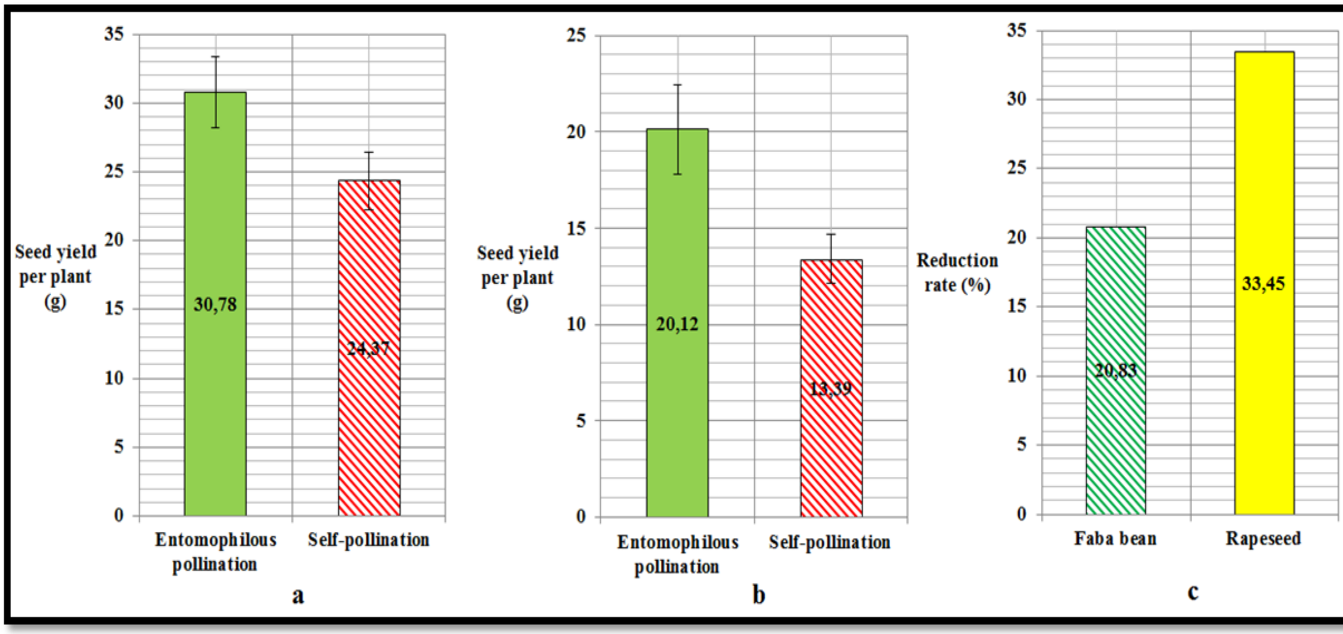

Fig. 4 Average seed yield per faba bean plant (a) and seed yield per rapeseed plant (b) and reduction rate of this parameter in absence of entomophilous pollination (c)

DISCUSSION

Data on air, vegetation cover and leaves temperatures showed that micro environmental conditions inside the cage were more favorable than outside the cage. This was confirmed by the general appearance of plants that was better under the cage. Also, in all growth stages, and particularly at maturity, plants of both crops were higher inside the cage than outside the cage due to the lower temperature and the higher relative humidity under the cage. One would expect that plants growing inside the cage yielded more than those growing outside the cage. The observations carried out on faba bean and rapeseed, during the flowering period, showed that the pollinating entomofauna of both crops was composed mostly of bees whose presence was very remarkable in the plots outside the cage. The flowering period of both crops was shorter in plots outside the cage compared to those inside the cage. Outside the cage, the plants were exposed to temperatures that are slightly higher than those inside the cage, and they tend to shorten their flowering period. The reduction of the latter was of two days for faba bean and four days for rapeseed. However, similar findings reported by previous works on rapeseed [16, 7], having shown that entomophilous pollination had an effect on the flowering period reduction, let suggest that flowering duration in the present study tended to increase because of absence of bees pollination rather than the slight lower temperature.

The rate of flowers blasting in faba bean was higher in self-pollinated plants than in plants pollinated by bees. This could be due to the higher number of flowers that have not been pollinated and therefore they did not give pods under self-fertilization conditions. Similarly, Varis and Brax [17] found that excluding insects from plots reduced the percentage of flowers producing pods in faba bean. However, the rate of rapeseed flowers blasting was lower in un pollinated plants by insects than in pollinated ones. This paradoxical finding is explained by the remarkable attack of rose chafers and other pest insects devouring floral buds, flowers and young siliques during the flowering period. For both crops, no insecticide was applied during flowering period in order not to kill bees as well as other likely pollinating insects. Fight against rose chafers was limited to trapping them by placing yellow bins, half filled with water.

In both crops, despite environmental conditions were more favorable for self-pollinated plants, seed yield of these was reduced by one-third for rapeseed and one-fifth for faba bean, compared to plants visited by pollinating bees. It is well known that total seed yield (t/ha) in rapeseed is strongly correlated to seed yield per plant. In Moroccan conditions, a significant and positive coefficient of correlation of 0.72 was found between both parameters in rapeseed [18]. Thus, the productivity or total seed yield of both crops was seriously affected in the absence of entomophilous pollination because some yield components were significantly affected. In fact, there was a drastic reduction in the number of pods per faba bean plant and siliques per rapeseed plant, as well as in the number of seeds per rapeseed silique. The reduction observed would be due to the high abortion rate occurred in the absence of pollinating insects. These results are in agreement with those obtained by Benachour [19], Mesquida et al. [9] and Varix and Brax [17] for faba bean and by Pordel and al. [20], Lerin [21] and Mesquida and Renard [10] for rapeseed. Regarding the slight increase in the number of seeds per pod in faba bean self-pollinated plants, it would be probably due to the compensation phenomenon caused by the reduced number of pods per plant under such conditions. The observed reduction in silique size for self-pollinated plants compared to those pollinated by bees was closely related to the reduced number of seeds per silique. In the same context, Singh and al. [22] showed that length of one silique was significantly higher in entomophilous pollination compared to self-pollination. Vaissière [23] reported that the fruit size is correlated with the number of seeds it contains.

CONCLUSION

The present study was carried out on faba bean and rapeseed, two partially cross-pollinated and economically important crops in the word and Mediterranean area, usually integrated in cereals-based cropping systems in rotation with those. It showed the negative effect of the absence of entomophilous pollination on the productivity of both crops. This effect was mainly exhibited in rapeseed by a drastic reduction in seed yield per plant, number of siliques per plant and number of seeds per silique. In faba bean, there was a significant reduction in seed yield per plant and number of pods per plant. Furthermore, it was showed that rapeseed was more affected than faba bean by the absence of bee's pollination. Therefore, in environments that suffer a bees decline, it would be wiser to recommend faba bean, instead of rapeseed, for rotation with cereal. Other additional experimental years will be needed to confirm these results and especially by trying to standardize the micro environmental conditions with and without bees. For that, it would be recommendable to conduct all the experimentation under cage, part of which without bees and the other part with bee hives. The findings of this research are in addition to previous reports sounding the alarm about the negative impact of the decline of bees on the agricultural production, especially of cross-pollinated and partially cross-pollinated crops.

It is therefore necessary to preserve pollinating bees to meet the pollination need of both crops, in particular, and other allogamous or partially allogamous crops, in general. This can only be done by protecting the habitat of this wildlife against heavy human pressures. The protection of pollinating bee populations is a necessity, not only for the maintenance of plant and animal biodiversity, but also to preserve the economic activities related to a sustainable agriculture and beekeeping.

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ISSN 2231-4490

International Journal of Plant, Animal and Environmental Sciences