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Effect of mixing some microbial control agents with pollen grain suspension to control two early date palm pests and fruit setting

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Abstract:

Date palm crop (*Phoenix dactylifera*) is the backbone of the agricultural economy in the New Valley Governorate, which cultivated more than two and half million date palm trees especially the semi-dry varieties. Field trials were conducted to investigate the efficacy of mixing some microbial control agents, *Bacillus thuringiensis* sub sp. *Kurstaki*, *Metarhizium anisopliae* (Sorok) and *Beauveria bassiana* (Bals.) with pollen grain suspension to combat the greater date moth, *Arenipses sabella* (Hampson) (Lepidoptera: Pyralidae) and the lesser date moth, *Batrachedra amydraula* (Meyrick) (Lepidoptera: Batrachedridae) which considered as an early date palm pests as well as the fruit set. The highest reduction in the fruit infestation with no significant differences (94.26 and 96.04 and 94.14 and 96.45%) was obtained with *M. anisopliae* and *B. thuringiensis* during 2020 and 2021 seasons, respectively. Meanwhile, the low effects against *A. sabella* (51.48 and 53.55%) were recorded in the case of the treatment with *B. bassiana*. The maximum reduction against *B. amydraula* infestations (88.36 and 87.88%) followed by (80.39 and 80.43%) and (72.78 and 71.89%) was obtained when pollen was mixed with, *B. thuringiensis*, *B. bassiana* and *M. anisopliae* during the two successive seasons, respectively. Regarding the effect of mixing the microbial control agents with the pollen suspension on the initial fruit set and fruit retention ratios, no side effect was observed, and a good result was obtained if compared to the control. This means that, the materials carrying microbial agents did not affect the vitality of pollen grains and stigma receptivity. These results encourage the mixing of biological compounds with pollen grain suspension during mechanical pollination of date palms in order to maximize the economic return.

Introduction

Recently, the Arab Republic of Egypt has tended to expand the cultivation of date palms, especially in the New Valley Governorate. This expansion requires conducting all agricultural operations using all modern methods in order to save time, effort

and money. The greater date moth and the lesser date moth are considered the early date palm pests under this region conditions (Gameel and Sayed, 2009; Gameel *et al.*, 2014; Sayed *et al.*, 2014 and Mansour *et al.*, 2019).

Regarding the greater date moth, spathes, bunches, fruit stalks and fruits were attacked in March and April. When the larvae infested at the later stage of growth, bunch bases broken and caused extreme damage to fruits and affected its quality. This usually happens during August such bunches are heavy enough and then these infested bunches were unable to bear their weight (Saleh, 1974; Ali *et al*., 1988 and Abdel-Rahman *et al*., 2007).

Gameel (2017) recorded that, the greater date moth, *Arenipses sabella* (Hampson) (Lepidoptera: Pyralidae) is considered a key insect pest on the date palm under El-Kharga Oasis conditions. The main date palm cultivar (Saidy) suffered from the greater date moth, *A. sabella* attacks, over 80% of the inspected date palm were found infested with the pest. The general average of the cut bunch bases was 9.58% (This value expresses the economic loss in the crop for the Saidy variety in the El-Kharga Oasis). Different larval instars of *A. sabella* were observed in full activity status in the frond bases during mid January. Unopened spadix of the date palm males recorded the earliest infestation during the last week of January. Also, Gameel *et al.* (2017) found that, the direct date fruit losses ranged between (20.87 and 26.23 %) on Saidy and (47.21 and 44.62%) on Tamr (Dray date) in El-Dakhla Oasis.

The lesser date moth *Batrachedra amydraula* (Meyrick) (Lepidoptera: Batrachedridae) is an early pest on date fruit in the Oases of the New Valley (Saleh, 1974; Temerak *et al.*, 2007; Gameel *et al.*, 2014 and Sayed *et al.*, 2014).The lesser date moth, is a pest of date fruits. The larvae of the first generation feed on the small fruits after fruit set. The larvae enter from the top between the three carpels inside the young fruit. Each larva has its independent entry pore in the fruit and may attack from three to four fruits during its lifetime. Usually, each larva eats more than a third of the fruit and it may sometimes feed on the entire content and consume seed in varieties in which this is tender, leaving only the outer fruit skin. These infested fruits wither and are suspended from the stalks by the threads secreted by the larvae or they fall onto the ground. The larvae of the second and third generations enter inside the fruits near the calyx or through the calyx and they feed on the placenta, fruit flesh and the fruit kernel. After some time, these fruits become reddish in color. Hence, the name Homera is given to the insect, which means red in Arabic. The damaged fruits begin to drop from bunches. The infestation of the dropped fruits can be differentiated by the pore present on each infested fruit filled with the faeces of the larvae and by the presence of the silk weaving secreted by the larvae (Venezian and Blumberg, 1982 and Kinawy, 2012).

Microbial control agents such as *B. thuringiensis*, *B. bassiana* and *M. anisopliae* and other species were evaluated against many date palm pests. Hussein *et al.* (2020) found that the mortality percent of adult females of the dust mite, *Oligonychus afrasiaticus* (McGregor) were 73.3, 88, 92 and 92%, respectively, after seven days of treatment with *B. bassiana*, *Metarhizium acridum*, *Lecanicillium muscarium* and *Isaria fumosorosea*, respectively. Under El-Kharga Oasis conditions, Temerak *et al.* (2007) recorded that, the infestation reduction ratios with the lesser date moth were 89.3, 88.6 and 87.2% after the treatment with *Paecilomyces farcinus*, *M.*

anisopliae (Bioranza product) and *Bacillus thuringiensis* subsp *aegypti* (Agerin product), respectively.

Aziz *et al.* (2014) evaluated the pathogenicity of *B. bassiana* on *B. amydraula* under laboratory conditions and found that, a significant and high mortality rate on the eggs, first and fifth instars of the Homera larvae which reaches 100%, 96.19% and 91.20% after 7 days of treatment. However, mortality rates were found to be decreased to 88.97% for fifth instars after 10 days of treatment, while results showed parasitism potentially reaches 94.50% in pupae and 90.22% in adults.

Artificial pollination is a necessary method for commercial date production (Nixon, 1951 and Ream and Furr, 1970). Pollen grain germination is closely related with both the environmental conditions and stigma receptivity. Pollination of 60-80% of the female flowers is considered satisfactory and will usually lead to a good fruit set. The pollination efficiency is affected by several factors and consequently fruit set is highly dependent on these factors.

The pollination time, flowering period of male palm, the type of pollen, its viability and amount and the female flower receptivity are the main factors to take into account (Brown and Perkins, 1969 and El-Salhy *et al.*, 1997). Several studies have been conducted to increase the efficiency of the pollination process in date palm by mixing the pollen suspension with different materials. Moreover, using of pollen grains mixed with pure water as a carrier was successful in the pollination of the Fard date palm cultivar and it was recommended to use 0.5g/liter of water in the pollination of it (Al-Sabahi *et al.*, 2006).

Also, it was recommended to use 0.5 g of pollen grains/Liter of water concentration to pollinate Jabree and 0.1 g/liter for Helaly Oman (Alabri *et al.*, 2006). Pollination by spraying pollens water suspension mixed with different carriers had an announced effect on yield and fruit quality in various date palm cvs rather than pollination with the traditional method (El-Salhy *et al.*, 2010 and Ahmed, 2014).

In order to face the increasing expansion of date palm cultivation in that province to save some agricultural operations, this study was conducted to evaluate the effect of mixing some biocides with pollen grain suspension to control two early date palm pests (*A. sabella* and *B. amydraula*) and fruit set.

Materials and methods

This study was conducted in a date palm orchard situated at El-Kharga Oasis, New Valley Governorate, Egypt, during 2020 and 2021seasons, on 18 years old Saidy date palm cultivar (As a semi dry date palm). Sixteen date palms that are uniform in vigor and in good physical condition were selected. The number of spathes per palm were adjusted to ten by removing excess earliest, latest and smallest clusters for achieving this work.

The biocides products were:

1. Protecto 9.4%WP, *Bacillus thuringiensis* subsp *Kurstaki* (32000 Inter. Units) at the rate of 125 gm / 100 L.
2. Bioranza (0.29%) (A natural metabolite of the Deuteromycete, *Metarhizium anisopliae* Sorok) at the rate of 200 ml/100 L.
3. Biovar 10 % (A natural metabolite of the Deuteromycete, *Beauveria bassiana* (Bals.) at the rate of 200gm / 100 L.

All compounds locally produced by Insect Pathogen Unit, Plant Protection Research Institute, ARC, MOA, Egypt. Pollination was uniformed in respect of source and method to avoid residues of metaxenia. Each bioproduct was mixed individually with 5 gm pollen grains/L

water and applied one time/season during the second half of March. Pollination treatment sprays were applied at the third day of spathe cracking. The combination was applied by using small hand sprayer (1/2 liter capacity) at the amount of 50 ml/bunch. The comparison palm was treated with pollen suspension only. The experiment was set up in a complete randomized block design with four replications. One date palm tree was considered as one replicate. Samples size was 10 strands / one date palm taken at random from each replicate.

Inspection times were conducted at two weeks interval from the mid of April until beginning of July during the two successive seasons (2020, 2021). In each strand, the infested fruits with the greater and lesser date moths, the naturally fallen fruits, initial fruit sit, and fruit retention were examined (El-Salhy *et al.*, 2010; Ahmed, 2014 and Sayed *et al.*, 2014).The initial fruit set (I.F.S.) was counted using 10 strands per spathe after 4 weeks from pollination and the percentage these values was calculated using the following equation:

$$\text{Initial fruit set}(\%) = \frac{\text{Av. number of set fruit per strand}}{\text{Av. number of set fruit} + \text{Av. number of flower scars}} \times 100$$

Fruit retention: In the first week of July, the previous 10 strands were inspected and counted then the percentage of retention fruits was calculated.

Data were statistically analyzed by F-test and the means were compared according to Duncan's Multiple Range Test (Snedecor and Cochran, 1971).

Results and discussion

Data in Tables (1 and 2) indicated that, all tested microbial control agents (*B. thuringiensis*, *M. anisopliae* and *B. bassiana*) when mixed with pollen grain suspension induced a remarkable reduction on the infestation rates with *A. sabella* and *B. amydraula* with different levels during 2020 and 2021 seasons.

Concerning to the greater date moth, data in Table (1) indicated that, the period of infestation with the pest extended from the second half of April until the first of May. After then, the infestation was decline to zero until the end of inspection time (Beginning of July) of the two successive seasons.

Table (1) : Reduction% of *A. sabella* infestation after the treatment with a mixture of microbial control agents and the pollen suspension, during 2020 and 2021 seasons.

Inspection date and treatments	16/4		1/5		Mean*	
	Infestation %	Reduction %	Infestation %	Reduction %	Infestation %	Reduction %
2020						
Beauveria bassiana	5.86	50.43	6.56	52.53	6.21 b	51.48b
Metarhizium anisopliae	0.68	94.25	0.79	94.26	0.74c	94.26 a
Bacillus thuringiensis	0.50	95.77	0.51	96.31	0.51 c	96.04a
Control	11.82		13.82		12.82 a	
2021						
Beauveria bassiana	6.04	50.25	6.27	56.87	6.16 b	53.55 b
Metarhizium anisopliae	0.72	94.07	0.84	94.22	0.78 c	94.14a
Bacillus thuringiensis	0.56	95.38	0.36	97.52	0.46c	96.45a
Control	12.14		14.54		13.34 a	

***Means followed by the same letter in each column are not significantly different at 0.05 level of probability**

The results indicate that the low effects against *A. sabella* 51.48 and 53.55 % were recorded in case of the treatment with *B. bassiana* during. 2020 and 2021 seasons, respectively. The highest reduction in the fruit infestation with no significant differences (94.26 and 96.04 and 94.14 and 96.45 %) were obtained with *M. anisopliae* and *B. thuringiensis* during 2020 and 2021 seasons, respectively.

Regarding the infestation with the lesser date moth, as shown in Table (2), the infestation time extended from the second half of April to the beginning of June. After then, the infestation was decline to zero until the end of inspection time (Beginning of July) of the two successive seasons.

In 2020, the maximum average level of infestation reduction (88.36%) was recorded when the pollen grain mixed with *B. thuringiensis* followed by (80.39%) in case of *B. bassiana*. Meanwhile, the low average effect (72.78%) was obtained when the bunches treatment with *M. anisopliae*.

The same trend of results was obtained during 2021 season, where the combination of *B. thuringiensis* with the pollen grain suspension induced the high average rate of the infestation reduction (87.88%) followed by (80.43 and 71.89%) in the case of *B. bassiana* and *M. anisopliae*, respectively.

Table (2) : Reduction% of *B. amydraula* infestation after the treatment with a mixture of microbial control agents and the pollen suspension during 2020 and 2021 seasons.

Inspection date and treatments	2020							
	16/4		1/5		1/6		Mean*	
	Infestation %	Reduction %	Infestation %	Reduction %	Infestation %	Reduction %	Infestation %	Reduction %
Beauveria bassiana	1.38	83.82	1.57	83.24	0.78	43.88	1.24 bc	80.39 b
Metarhizium anisopliae	2.22	73.97	2.73	70.86	0.30	78.41	1.75 b	72.78 c
Bacillus thuringiensis	0.84	90.51	0.99	89.43	0.43	69.06	0.75 c	88.36 a
Control	8.53		9.37		1.39		6.43 a	
	2021							
Beauveria bassiana	1.48	82.94	1.48	84.13	0.83	37.12	1.26 bc	80.43 b
Metarhizium anisopliae	2.35	72.92	2.83	69.66	0.25	81.06	1.81 b	71.89 c
Bacillus thuringiensis	0.87	89.98	1.01	89.17	0.46	65.15	0.78 c	87.88 a
Control	8.68		9.33		3.33		6.44 a	

***Means followed by the same letter in each column are not significantly different at 0.05 level of probability**

When the pollen grain suspension was mixed with the biological control agents such as *B. thuringiensis*, *B. bassiana* and *M. anisopliae* do not induce any negative effect on the initial fruit set and fruit set ratios if compared to the control as shown in Table (3)

In 2020, higher initial fruit set (84.37 and 82.89 %) was obtained after the treatment with *M. anisopliae* and *B. thuringiensis*, respectively. The low initial fruit set(78.23 %) was obtained after the treatment with *B. bassiana*. During 2021season, the maximum initial fruit set 87.46 %, followed by 83.19 and 78.80% was recorded in case of *B. thuringiensis*, *M. anisopliae* and *B. bassiana*, respectively. Regarding to, the effect of mixing some microbial control agents with pollen grain suspension on the fruit retention ratio, all tested compounds induced a positive significant effect if compared with

control which recorded for 66.79 and 62.45% in 2020 and 2021 respectively. The percentage of fruit set was (80.87 and 84.55%), (82.91 and 81.20%) and (74.31 and 76.55%) when treated with *B. thuringiensis*, *M.anisopliae* and *B. bassiana* in 2020 and 2021, respectively.

Table (3) : Percentage of the fruit set after the treatment with a mixture of microbial control agents and the pollen suspension during 2020 and 2021 seasons.

Season	2020		2021	
	Initial fruit set	Fruit retention	Initial fruit set	Fruit retention
Beauveria bassiana	78.23 b	74.31b	78.80 c	76.55c
Metarhizium anisopliae	84.37 a	82.91a	83.19 b	81.20b
Bacillus thuringiensis	82.89 a	80.87a	87.46 a	84.55a
Control	66.79 c	66.79c	65.56 d	62.45d

***Means followed by the same letter in each column are not significantly different at 0.05 level of probability.**

Finally, it can be observed that, the infestation rate with *A. sabella* is twice the rate of infestation with *B. amydraula*. This is consistent with what Gameel *et al.* (2014) and Gameel (2017) emphasized in previous studies in the same province that the greater date moth is the main pest in the New Valley. It is clear that the process of mixing pollen grains with the tested biological compounds has achieved several successes such as: **(1)** *B. thuringiensis* (Protecto product), *M. anisopliae* (Bioranza product) and *B. bassiana* (Biovar product) when mixed with pollen grain suspension induced a significant reduction on the infestation rates with *A. sabella* and *B. amydraula*. More than 90% reduction in the infestation with *A. sabella* was obtained when date palm bunches treated with a mixing pollen grains *and B. thuringiensis* or *M. anisopliae*. Poor results were recorded in case of *B. bassiana*. Regarding the lesser date moth, all treatments gave satisfactory results, ranging between70 and 88%. It can be arranged in descending order as follows: *B. thuringiensis*, *B. bassiana* and *M. anisopliae*. Nearly the same trend of results was obtained by Temerak *et al.* (2007) who found that, the infestation reduction ratios with the lesser date moth were 88.6 and 87.2% after the treatment with *M. anisopliae* (Bioranza product) and *B. thuringiensis* subsp *aegypti* (Agerin product), respectively. Aziz *et al.* (2014) evaluated the pathogenicity of *B. bassiana* to *B. amydraula* under laboratory conditions and found that, high mortality rates in the eggs, first and fifth instars of the *B. amydraula* larvae reached to 100%, 96.19% and 91.20% after 7 days of treatment. **(2)** No side effect was observed on the initial fruit set and fruit set ratios and a good result were obtained if compared to the control. This means that, the materials carrying microbial agents did not affect the vitality of pollen grain and stigma receptivity. **(3)** Mixing pollen grains with various carriers and nutrient minerals were beneficial in establishing mechanical pollination and obtaining an economical yield with good fruit quality. Also, it is responsible for enhancing pollination efficiency (Furr and Hewitt, 1964; Khalil and Al-Shawaan, 1982; El-Mardi *et al*., 1995; Hussein and Hassan, 2001; Ragab *et al*., 2004; Ashour *et al*., 2004 and El-Salhy *et al*., 2007). Previous studies showed that pollination by spraying pollens water suspension mixed with different carriers had an announced effect on yield and fruit quality in various date palm cvs rather than pollination with the traditional method (El-Salhy *et al*, 2010 and Ahmed, 2014). In conclusion, it can be recommended that, during the date palm pollination process that used a mixing pollen grains (5 gm/L water) and the recommended dose of *B. thuringiensis* or

M. anisopliae to combat *A. sabella* and *B. thuringiensis* or *B. bassiana* to control *B. amydraula* during the second half of March

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