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Application of pesticide combinations on watermelon affects pollen viability, germination, and storage

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ABSTRACT

To increase watermelon (*Citrullus lanatus*) production, pesticides are now being used in higher quantities. Many pesticide combinations are harmful to seed production. This study was carried out to assess the effects of pesticide combinations (i) α-cypermethrin + mancozeb (α-CpMa) and (ii) λ-cyhalothrin + acetamiprid + metalaxyl + copper oxide (λ-ChAMeC) on *in vitro* germination, viability, and storage of watermelon pollens. Pesticides were applied on field, on three varieties of watermelon plants (kaolack, F1-koloss, and F1-sugar dragon), before and during blooming. The pollens were subjected to viability and germination tests directly after harvesting, or stored at +10°C or −20°C before testing. *In vitro* germination and viability of pollen were carried out on modified Brewbaker and Kwack medium. A CpMa and λ-ChAMeC inhibited pollen germination in all the three varieties of watermelon. α-CpMa was the most harmful pesticide when applied during blooming, with up to 26.5% decrease in pollen germination. A decrease of pollen germination and viability was also observed after 4 and 7 days of storage. Pollen from the variety kaolack showed a higher germination rate and, freezing at −20°C was the better storage condition. These results could help to scale up pollen sharing and seed production in watermelon breeding programs.

1. INTRODUCTION

Watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai] is popular annual fruit crops of the gourd family Cucurbitaceae [1]. Being monoecious [2], *C. lanatus* originates from northeastern Africa [3]. According to Food and Agriculture Organization Corporate Statistical Database, the cultivated area of watermelon in 2016 was approximately 3.51 million hectares worldwide, and the annual global production of watermelon is about 117.20 million tons, making it among the top five most consumed fresh fruits [4]. Watermelon fruit is an important source of carotenoids and a precursor of vitamin A [5].

Watermelon production is subjected to many pests (whiteflies, aphids, etc.) and diseases, such as downy mildew, powdery mildew, anthracnose, and Gummy stem blight. [6] in the tropics. Moreover, disease incidences are highly conducive during raining seasons, leading to a huge use of different insecticides, fungicides, and combinations by farmers. In sub-Saharan Africa, pesticide application always starts after the appearance of disease symptoms on leaves during watermelon plant development and continues till fruit harvest. However, many commercial pesticide formulations significantly inhibit pollen viability and germination [7,8]. Preserving the germinative capacity of pollen, and especially its viability using efficient means of conservation and storage is of great importance in plant breeding programs [9]. Seedlings production involves controlled pollination between parents with interesting complementary traits [10], using high-quality pollens. Many studies have provided full knowledge on pollen harvesting, conditioning, storage, and viability [11,12]. Few previous works have been done on the detrimental effects of fungicides on pollen germination [13]. In this way, captan has been reported to reduce pollen viability in many apple cultures [14]. Combinations of pesticide formulations containing different active principles can be seriously detrimental to pollen quality. In Cameroon, rice and vegetable farmers are gradually using higher rates of pesticide combinations, such as α-cypermethrin + mancozeb or λ-cyhalothrin + acetamiprid + metalaxyl + copper oxide [15], to reduce loss from pests and diseases and increase yield.

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The aim of this study was to assess the risk posed the in-field temporally application of commonly used combinations of pesticides on the viability and germination of fresh and stored watermelon pollen grains.

2. MATERIALS AND METHODS

2.1. Field experiment and pollen collection

All the three varieties of *C. lanatus* (kaolack, F1-koloss, and F1-Sugar Dragon) produced and distributed by the seed company TECHNISEM were cultivated in the field at Nsimeyong II (Latitude 3°50′0 N, Longitude 11°29′0 E, Altitude 700 m a.s.l.) Yaoundé Cameroon. Field experiment was carried out during the dry season (from September 2015 to February 2016). Plants were watered at 10 l/m^2/day. Pesticides were applied to the plants every week, starts from the fifth week after direct seeding. Systemic pesticides complex (λ-cyhalothrin at 15 g/l + acetamiprid at 20 g/l in liquid formulation and metalaxyl 120 g/kg + copper oxide 600 g/kg in solid formulation) or contact pesticides complex (α-cypermethrin 50 g/l in liquid formulation and mancozeb 800 g/kg in solid formulation) were applied ether before blooming or during blooming. Pesticides were applied at the following doses: λ-cyhalothrin + acetamiprid at 2.7 ml/l, metalaxyl + copper oxide at 3.33 g/l, α-cypermethrin at 2.5 ml/l and mancozeb at 8.5 g/l. Male flowers were hand collected in the morning (6–7 AM) as previously described [16]. Briefly, the harvested flowers were dried at room temperature (29 ± 1°C) and humidity (30%–40%) for 1 hour. The pollen was then put in special plastic bags, locked up in a 2-l container half filled with ice blocks and transported to the Laboratory of Biotechnology and Environment of the University of Yaounde I for *in vitro* germination and storage.

2.2. *In vitro* pollen germination, viability, and storage

Brewbaker and Kwack culture medium [17] was modified by adding 20 mg/l H_3BO_3, 41.6 mg/l $Ca(NO_3)_2$, 10 mg/l KNO_3, 21.7 mg/l $MgSO_4$, 7 H_2O, and 2 g/l sucrose. The solution was heated at 120°C and 1% agar was added to the medium with continuous slow stirring. The pollen grains were spread on slides containing the cooled solid medium. The slides were then placed in Petri dishes under a saturated atmosphere. The pollen were incubated at 27°C for 24 hours and stained as previously described [18]. A pollen grain was considered as germinated when the pollen tube reached a length greater than twice the pollen diameter [19]. The number of germinated pollens was evaluated using an ×40 optical microscope.

Pollen viability was examined using malachite green approach [20]. Thus, the aborted or dead pollen stains green while the viable pollen has its protoplasm stained pink. The enumeration of viable pollens was done under an optical microscope at magnification of ×40.

The freshly harvested pollens were filled in vials in triplicates and, respectively, stored at 10°C and −20°C. *In vitro* germination assessment of pollen was conducted after 4 and 7 days.

2.3. Statistical analysis

The experimental design used was a split plot with pesticide treatment as main factor and the watermelon variety as a secondary factor in three replicates. The results were subjected to an analysis of variance using the software R, version 3.3.3. To determine significant differences between pesticide treatments, varieties, and their interactions, the Duncan post-test was used at 5% confidence level.

3. RESULTS AND DISCUSSION

3.1. Results

3.1.1. In vitro viability and germination conditions of watermelon pollen

Preliminary *in vitro* viability and germination tests carried out on fresh pollen showed that pollen from the three varieties of watermelon were viable (Fig. 1A) and germinated (Fig. 1B) in the modified Brewbaker and Kwack culture medium. Viable pollen grains were stained in red (Fig. 1Aa), while non-viable pollen were stained in green (Fig. 1Ab). A pollen grain was considered to have germinated (Fig. 1Bb), when the length of the pollen tube was twice the diameter of the pollen grain [21].

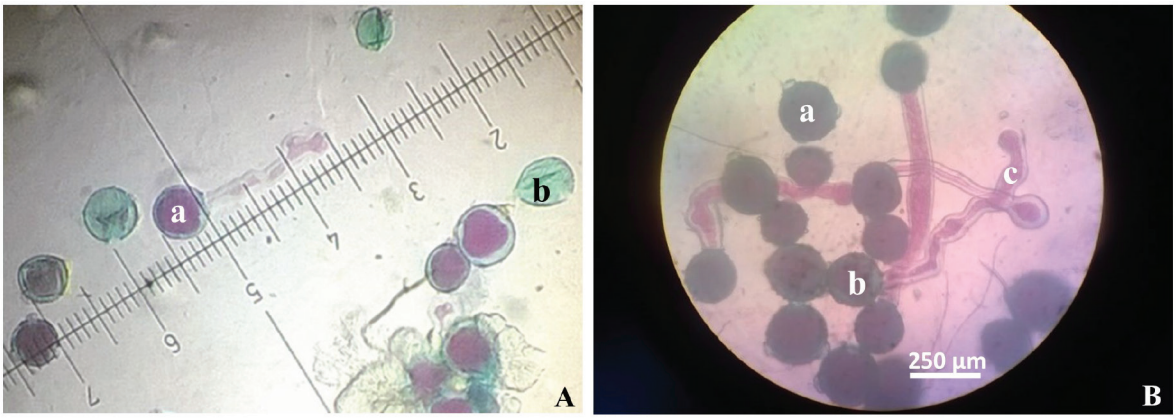

Figure 1: Watermelon pollen viability (A) and germination (B) showing viable pollen (Aa), non-viable pollen (Ab), non-germinated pollen (Ba), germinated pollen (Bb) with pollen tube (Bc). Photographs of pollen were taken directly from the Olympus Optical Co., Ltd microscope.

3.1.2. Effects of pesticides and application time on viability of fresh and stored watermelon pollen grains

As per the effect of pesticide applications, the viability of fresh pollen collected on the flowers of watermelon treated with α-CpMa and λ-ChAMeC before and during blooming was not significantly affected (Table 1). But, pollen collected from plants treated with α-CpMa showed a viability decrease of 2.3%, while pollen from untreated plants showed a loss of viability of 21.21% after 7 days of storage at 10°C. Pollen viability was most affected when plants were treated with α-CpMa during blooming and pollen were stored during 7 days at 10°C; having a significant loss of 40.27% when compared with untreated fresh pollen.

3.1.3. Storage and germination potentials of the three varieties of watermelon pollen grains

The rates of germination of fresh pollen grains of *C. lanatus* varieties kaolack, F1-koloss, and F1-sugar dragon were 72.4 ± 11.8, 69.15 ± 11.12, and 64.59 ± 9.79%, respectively, before storage. Germination of pollen grains from the three varieties significantly decreased ($p < 0.05$) after 4 days of storage at 10°C. After 7 days of storage, the rate of germination significantly decreased ($p < 0.05$) by 77.97%, 82.13%, and 86.9% for varieties kaolack, F1-koloss, and F1-sugar dragon, respectively. About 56.74% and 22.03% of it germination capacities after, respectively, 4 days (41.08 ± 9.83%) and 7 days (15.95 ± 7.48%) of storage at 10°C (Fig. 2).

3.1.4. Effects of pesticides and time of application on in vitro germination of fresh and stored watermelon pollen grain

In general, the mean germination capacities of non-treated and pesticide treated *C. lunatus* pollen grains stored in freezer (−20°C) and in refrigerator (10°C) significantly ($p < 0.01$) decreased after 4 and 7 days of storage (Fig. 3). Germination rates of fresh pollen grains collected from plants treated with α-CpMa (56.8%) and λ-ChAMeC (65.64%) during blooming significantly decreased ($p < 0.01$) when compared with non-treated fresh pollen grains (77.28%). However, germination rates of pollen grains collected from the same plants treated before blooming were similar to that of non-treated pollen grains. Fresh pollen grain from watermelon plants treated with α-CpMa during blooming showed the highest loss (26.5%) of fresh pollen' germination potentials. After treatment with λ-ChAMeC and α-CpMa before blooming, the pollen grains of *C. lunatus* stored for 7 days at −20°C germinated at 23.13 ± 7.01% and 14.9 ± 9.64%, respectively, conserving 30% and 19% of their germination potential (Fig. 3A). When watermelon plants were treated during blooming, the pollen grains collected and stored for 7 days at −20°C germinated at 18.85 ± 5.34 % and 11.4 ± 4.74%, respectively, conserving only 24.4% and 14.8%% of their germination capacity. After 7 days of storage in the refrigerator (10°C), pollen grains from *C. lunatus* plants treated with λ-ChAMeC and α-CpMa before and during blooming, less conserved their germination capacity (Fig. 3B). Pollen from plants treated with α-CpMa during blooming germinated at 4.56 ± 2.21%, loosing up to 94.1% of their germination capacity.

3.2. Discussion

With respect to pollen viability, our results showed a weak loss of viability due to pesticide treatments or storage. These results are in agreement with those of Youmbi et al. [12], which found a viability rate greater than 80% for *Picea glauca* pollen after long period of storage. Alexander's stain may overestimate the viability of pollen grain [22]. The results obtained in our study show that the viability rate was usually lower in the pollen of watermelon plants treated during flowering than in the pollen of plants treated before flowering. These results are in agreement with those of Cali and Candan [13], who showed that some pesticides have negative effects on pollen viability.

Untreated watermelon fresh pollens germinated at approximately 77% on modified Brewbaker and Kwack medium. This result corroborates those of Kwon et al. [23] who obtained up to 87% germination rate of untreated watermelon pollen. Also, it has been

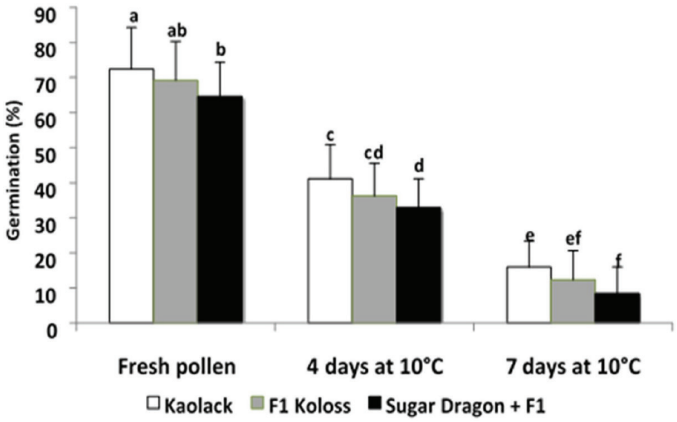

Figure 2: Germination rate of the three varieties of fresh and stored watermelon pollen. Bars bearing the same letters are not statistically different according to Duncan's test at $p = 0.05$.

Table 1: Effect of α-CpMa and λ-ChAMeC on viability of fresh and stored watermelon pollens. The averages followed by the same letter in the same row are not statistically different according to Duncan's test at $p = 0.05$.

Pesticide treatments		Fresh pollen	Pollen stored at 10 °C		Pollen stored at -20 °C	
			4 days	6 days	4 days	6 days
No pesticide		95.27 ± 2.65	91.33 ± 1.6 a	75.06 ± 0.56 a	96.34 ± 1.63 a	83.64 ± 2.34 a
λ-ChAMeC	Before blooming	95.19 ± 2.84	84.84 ± 1.52 b	68.28 ± 1.58 b	87.78 ± 1.87 b	77.67 ± 1.36 b
	During blooming	95.39 ± 2.65	81.7 ± 1.81 c	64.95 ± 1.28 c	84.07 ± 2.15 c	73.08 ± 1.14 c
α-CpMa	Before blooming	94.91 ± 3.29	77.63 ± 2.21 d	61.43 ± 1.83 d	81.28 ± 3.01 d	69.48 ± 1.86 d
	During blooming	93.11 ± 3.13	73.43 ± 2.21 d	56.39 ± 1.81 e	78.05 ± 1.75 d	66.99 ± 1.4 e

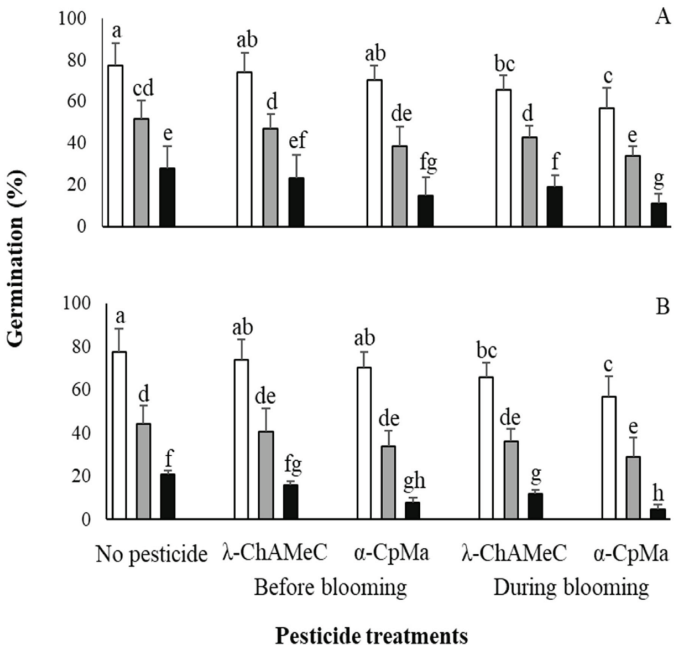

Figure 3: Effect of α-CpMa and λ-ChAMeC on *in vitro* germination of fresh and stored watermelon pollens. A, stored at -20 °C; B, stored at +10 °C; white bars, fresh pollen; grey bars, 4 days storage; black bars, 7 days storage.

shown that *Dacryodes edulis* pollen requires up to 24 hours of incubation to germinate at 96% on Brewbaker and Kwack medium supplemented with sucrose [12]. However, Baker and Baker [24] stated that the environment required for *in vitro* germination of pollen remains directly related to the genetics of the species, as well as the quality and quantity of nutrient reserves. It has been established that micronutrients, also called germination stimulating substances, play a specific role in triggering and promoting *in vitro* germination of pollen [25].

The results obtained in this study showed that pesticides inhibited *in vitro* germination of pollen. Control pollens had the best germination rates *in vitro*, followed by pollen from plants treated with systemic (λ-ChAMeC) or contact (α-CpMa) pesticides before flowering, and the last were pollens from watermelon plants treated during flowering. These results are consistent with those of authors who showed that the fungicides applied to pollens lead to a decrease in their *in vitro* germination, deformation and cracking of the pollen tubes [26,27]. Zarrabi and Imani [28] demonstrated that high pesticide application during flowering, induced modification of pollen' morphological characters and significantly reduce pollen germination and pollen tube elongation of peach and nectarine. Therefore, the negative effects of fungicides on the *in vitro* germination of pollens are variable and depend on the type of pesticides used.

A decrease in germination rate and pollen viability rate was observed at the fourth and seventh day of storage. These results are consistent with those of Daher et al. [29] who showed that the *in vitro* germination rate of pollen decreases with increasing storage time. During storage, the germination rate was usually higher in pollen treated with pesticides before blooming than in those treated during blooming. Regarding the temperature regime for pollen storage of the three varieties of watermelon, the results obtained in this study showed that storage at −20 °C is better. This is consistent with the results of Kwon et al. [23] who found that watermelon pollens stored at −10°C germinated better than those kept at +10 °C. Pesticides inhibited the fertility of watermelon pollens stored at −20°C and +10 °C. Thus, pollens from non-treated plants recorded the best germination rates, followed by pollens from plants treated with systemic (λ-ChAMeC) and those treated with contact (α-CpMa) pesticides. However, a loss of germination capacity of watermelon pollens stored at −20°C and 10°C was noted and may be induced by regulatory enzymes and metabolites essential for pollen germination [30].

4. CONCLUSION

The aim of this study was to evaluate the effects of pesticide combinations (i) α-cypermethrin+mancozeb (α-CpMa) and (ii) λ-cyhalothrin + acetamiprid + metalaxyl + copper oxide (λ-ChAMeC) on *in vitro* germination, viability and storage of watermelon pollens. The results showed that α-CpMa and λ-ChAMeC inhibited the *in vitro* germination of stored and fresh watermelon pollens. The decrease in germination rate was more pronounced in pollens from plants treated with contact pesticide (α-CpMa) during blooming. *Citrullus lanatu*s pollens were better preserved at −20°C. Systemic pesticide (λ-ChAMeC) used in this study seems to be less harmful to watermelon pollens. Therefore, in case of absolute necessity, watermelon farmers and breeders may apply systemic pesticide before flowering. These results could help to scale up pollen sharing, seed production, and quality in watermelon breeding programs.

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