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In vitro and *in vivo* Evaluation of 3 Insecticides and Bio-Insecticide Effects on Olive Pollen Germination and Tube Growth

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Abstract: In order to determine if insecticide treatment during bloom had an effect on olive productivity, three insecticides: Dimethoate (Dimate), Deltamethrine (Decis) and Oleoparathion (Oleokriss) and a bioinsecticide (Bactospeine) were studied over Coratina olive cultivar. These compounds, were applied, at variable concentrations of 100, 10 and 1% RFR, to pollen culture medium or sprayed to field-grown trees during green-cluster stage. Their effectiveness was evaluated on the germination capacity (% germination and tube growth). When added to pollen culture medium, increased germination percentage of Coratina pollen and *in vitro* enhanced tube elongation were obtained when Bactospeine at 100, 10 and 1% RFR. Germination and tube length were reduced when Dimethoate, Deltamethrine and Oleoparathion were applied to Coratina pollen germination media at 100% RFR. Oleoparathion at 100% and 10% RFR inhibited both pollen germination and tube growth. In contrast, at 1% RFR, Oleoparathion was less inhibitory. Deltamethrine at 100% RFR inhibited pollen germination and tube growth. An intermediate inhibitory effect on pollen germination and tube elongation were observed when Dimethoate was used at 100% RFR. When the insecticides were sprayed on the shoots of Coratina olive cultivar just before blooming, treatments showed similar trends with low pollen germination and tube growth in presence of Oleoparathion and increasing to high levels in presence of Bactopeine. Germination and tube length were reduced when Dimethoate, Deltamethrine and Oleoparathion at 100% RFR were applied to pollen insecticide-free media. In contrast, at 10 and 1% RFR, these insecticides were less inhibitory and showed no difference from the control. Applications of Oleoparathion most severely reduced pollen germination, retarded germination, ruptured pollen tubes and damaged stigmatic surfaces *in vivo*.

Key words: *Olea europaea* L., pollen germination, pollen tube morphology, bio-insecticide

INTRODUCTION

Olive is one of the most important crops in Tunisia and many insecticides are used and targeted specially into the open bloom. Current pest control recommendations for olive tree include applications of insecticides to control olive fruit fly (*Bactrocera oleae* Gmel.) (Laccone *et al*., 2000; Parlati *et al*., 2000; Calvitti *et al*., 2002), olive tree psyllid (*Euphyllura olivine*) (Zouiten and Hadrami, 2001) and to prevent *Prays oleae* Bern. (Jardak and Ksantini, 1986; Ramos *et al*., 1999). These plant insects occur frequently causing serious crop damage in olive crops because they infect various flower parts during bloom. Chemical treatments to manage these pests have proved to be effective but they are shown to be highly polluting to the environment and their use is not always justified (Iannotta *et al*., 1998). Many of beneficial insects present in the olive orchards are seriously affected by conventional treatments (Campos and Civantos, 2000) and their increased require increased pathogen virulence (Lacey *et al*., 2001) and have favored the development of resistance in many species (Baker, 1982).

In contrast, microbial control agents such as Bactospeine are far more environmentally friendly than pesticides and serve as alternatives to broadspectrum chemical insecticide (Bae *et al*., 2004; Zouari *et al*., 2002).

Scientific literature contains numerous reports describing insecticide efficacy but only a few reports documented the effect of insecticides on tree physiology and productivity (Rosenberger *et al*., 2003). Effects of insecticides and particularly bio-insecticide applied during bloom period on pollen capacity (viability, germination and tube elongation) are little known particularly with *Bacillus thuringiensis* Berliner (commonly referred to as Bt) which is well known as a biological insecticide and was used against olive moth *Prays olaea* to reduce the first generation larvae (Jardak *et al*., 1993; Ianotta *et al*., 1998; Mazomenos *et al*., 1999). The recent one report was

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of Mehri *et al*. (2006) on olive pollen using *in vitro* culture of pollen collected from three olive cultivars Gerboui, Coratina and Meski. Pollen germination, tube growth and tube morphology were inhibited by insecticides when incorporated onto germination media even at levels well below recommended field application rates.

The objective of this study was to examine the effectiveness of three insecticides and a bio-insecticide sprayed during olive bloom period of Coratina olive cultivar on the pollen germination capacity (% germination and tube growth and morphology), by *in vitro* and *in vivo* assays. The effects of Dimethoate (Dimate), Deltamethrine (Decis) and Oleoparathion (Oleokriss) and Bactospeine applied at three concentrations 100, 10 and 1% RFR (recommended field rate) were assessed by applying in germination medium (*in vitro*) or by spraying to shoots grown in the field (*in vivo*).

MATERIALS AND METHODS

Our collection of olive trees was preserved in the field at High Institute of agronomy, Chott-Meriem (central part of Tunisia). This study was conducted over three years since: 2005 for *in vitro* assays and 2005-2006 for *in vivo* assays.

Coratina olive cultivar was used as a pollen source and three trees were used for these essays. Three insecticides included in comparisons were Dimethoate, Deltamethrine, Oleoparathion and a microbial control agent (Bactospeine). The class, trade name, formulation, active ingredient and recommended field rate for the four compounds are shown in Table 1. Three concentrations of the compounds: the recommended field rate (100% RFR), 10 and 1% RFR were used.

Pollen germination and tube growth: Three assays were conducted on pollen grains of Coratina olive cultivar

- **Control:** The shoots were sprayed with distilled water at green cluster stage. At white-button stage, pollen grains were collected and cultured on insecticide-free medium.
- ***In vitro* assays:** At white-button stage, pollen grains were collected and cultured on medium (Mehri *et al*., 2003) containing one of the three insecticides and the bio-insecticide at 100, 10 and 1% RFR concentrations. The pollen was collected from flowers soon after dehiscence of anthers and dried in the laboratory over night. Shoots sprayed with distilled water and germination medium without insecticide served as control.
- **Spray of insecticides *in vivo* assays:** The shoots of Coratina cultivar were sprayed at green cluster stage (about 15 days before blooming) with three insecticides and bio-insecticide at 100, 10 and 1% RFR concentrations. At white-button stage, pollen grains were collected and cultured on insecticide-free medium. Pollen capacity (germination and tube growth) was assessed at three concentrations of the compounds: 100% the recommended field rate RFR, 10 and 1% RFR.

The experimental design was a randomized complete block with 4 treatments using three-tree plots. All test plots were located in a single row through the center of the orchard. All sprays were applied at 3 concentrations using a conventional sprayer delivering 400 L ha^{-1} about 15-18 L $tree^{-1}$. Individual shoots of uniform diameter and length containing inflorescence were sprayed once in the end of April (green cluster stage). Insecticide and bio-insecticide sprays and control were with three shoots in treatment. The controls, the shoots were sprayed with distilled water.

The plants were at the green cluster stage at the time of spray applications. Three replicates per treatment were used. The three plants of Coratina cultivar were used as three experimental blocks, selected for their uniform size and vigour with high flowering load. All trees received identical management practices (fertilizing, watering, etc.).

Inflorescence of treated shoots and control were collected at white-button stage. Anthers were taken off and allowed to dry in the laboratory, they were dehisced overnight at room temperature and pollen collected. Pollen grains were cultured on free insecticide medium with three replications per treatment. Pollen from shoots sprayed with distilled water and germinated in free-insecticide medium served as control.

The culture medium for pollen germination used was prepared as described by Mehri *et al*. (2003), containing 0.7% agar, 20% sucrose, 100 ppm H_3BO_3 at pH = 5. Cultures were kept in dark at 25°C. Counted pollen grains

Table 1: Selected insecticides and their Recommended Field Rates (RFR)

Active ingredients	Trade name	Formulation	Active ingredient (%)	RFR
Bacillus thuringieusis	Bactospeine 16000	WP	16000 UI mg^{-1}	100 g hL^{-1}
Deltamethrine	Decis EC 25	EC	25 g L^{-1}	200 cc hL^{-1}
Dimethoate	Dimate	EC	40%	100 cc hL^{-1}
Oleoparathion	Oleokriss	EC	Parathion Ethyl 3%	750 cc hL^{-1}

EC: Emulsifiable Concentrate, WP: Wetable Powder

were at least 100 per Petri dish. The experiments were conducted as randomized designs with 3 Petri dishes as replicates.

Statistical analysis: A factorial treatment was used in this study. Each treatment consists of (3 insecticides + 1 bio-pesticide x 3 concentrations with control) on Coratina olive cultivar. The effect of insecticides on germination and pollen tube length for each assay was analyzed by analysis of variance.

RESULTS AND DISCUSSION

Germination and tube growth: The treatment interaction was significant indicating that Coratina olive cultivar differed in its response to the insecticide treatment and to insecticidal concentrations when the compounds were added to the pollen germination medium (Table 2-4) or when sprayed on the field-grown shoots (Table 5).

Control: Pollen germination was achieved in the presence of 20% sucrose, 0.7% agar, 100 ppm boric acid. Germination rate and pollen-tube length were determined after 3, 10, 20 and 28 h incubation.

In Coratina cultivar, the mean germination percentage in the control (when pollen exposed to the water spray was incubated in insecticide-free media) was 34.7% after 3h incubation. After 28 h incubation, the percent germination average 62.4% and pollen tube length was 553 μm (Table 2).

When shoots were sprayed with distilled water and insecticides and bio-insecticide were applied to pollen germination medium: Bactospeine at 100% RFR increased significantly germination and tube growth (Table 2). The optimal concentration of Bactospeine for Coratina germination pollen and tube elongation was 100% RFR which was 90.5% and 647 μm, respectively, after 28 h incubation. Significative effect was signaled between control and Bactospeine treatment but no significative differences were found between pollen grains germination from the Bactospeine concentrations of 100, 10 and 1% RFR. Bactospeine remained stimulatory with germination rate of 82.4% at 10% RFR and 85.09% at 1% RFR.

Table 2: Evolution of germination rate and tube growth of Coratina pollen grains cultured in medium containing Bactospeine at 3 concentrations 100, 10 and 1% RFR

	Incubation			
Treatments (%)	3 h	10 h	20 h	28 h
Pollen germination				
Control	34.72	54.60	59.05	62.40±2.90
Bactospeine 100	48.00	74.70	88.59	90.50±3.07
Bactospeine 10	22.95	55.95	80.00	82.48±1.40
Bactospeine 1	23.47	56.77	83.00	85.09±1.90
Tube length (μm)				
Control	268.50	480.00	523.20	553.00±5.11
Bactospeine 100	280.00	478.00	625.00	647.00±5.90
Bactospeine 10	139.60	271.00	420.00	432.00±4.59
Bactospeine 1	110.50	217.00	365.00	396.00±3.40

The media contained 0.7% agar, 20% sucrose, 100 ppm H_3BO_3 (pH = 5). The values represent means from replicates

Table 3: Evolution of germination rate and tube growth of Coratina pollen grains cultured in medium containing 3 insecticides and a bio-insecticide at 100% RFR

	Incubation			
Treatment at 100% RFR	3 h	10 h	20 h	28 h
Pollen germination (%)				
Bactospeine	48.00	74.70	88.59	90.50±3.07
Dimethoate	13.20	33.50	47.88	49.50±0.92
Deltamethrine	17.00	21.50	24.02	26.03±1.60
Oleoparathion	3.22	4.57	5.38	5.68±0.90
Control	34.72	54.60	59.05	62.40±2.90
Tube length (μm)				
Bactospeine	280.00	478.00	625.00	647.00±5.90
Dimethoate	215.30	382.00	426.20	460.00±5.60
Deltamethrine	48.30	62.00	65.00	68.00±1.30
Oleoparathion	26.20	34.00	36.60	37.50±1.50
Control	268.50	480.00	523.20	553.00±5.11

The media contained 0.7% agar, 20% sucrose, 100 ppm H_3BO_3 (pH = 5). The values represent means from replicates

Table 4: *In vitro* germination (germination rate and tube length) of Coratina pollen grains after 28 h incubation in medium containing insecticides at 3 concentrations 100, 10 and 1% RFR at 25°C and in darkness

Treatments (%)	Pollen germination (%)	Tube length (μm)
Control	62.40±2.90	553.0±5.11
Bactospeine 100	90.50±3.07	647.0±5.90
Bactospeine 10	82.48±1.40	551.1±4.59
Bactospeine 1	85.09±1.90	549.4±3.40
Dimethoate 100	49.50±0.92	460.0±5.60
Dimethoate 10	51.39±1.10	501.3±3.90
Dimethoate 1	55.33±1.30	492.4±4.70
Deltamethrine 100	26.03±1.60	68.0±1.30
Deltamethrine 10	31.22±1.05	142.8±1.03
Deltamethrine 1	59.48±2.67	493.8±3.60
Oleoparathion 100	5.66±0.90	37.5±1.50
Oleoparathion 10	10.94±1.40	40.1±0.90
Oleoparathion 1	34.32±1.34	491.3±4.10

The values represent means from replicates

Table 5: Germination rate and tube length of Coratina pollen grains when insecticides were sprayed on shoots before bloom at 3 concentrations of 100, 10 and 1% RFR

Treatments (%)	Germination (%)	Tube length (μm)
Oleoparathion		
100	33.10	469
10	38.40	521
1	55.02	596
Bactospeine		
100	78.90	856
10	64.30	707
1	55.50	667
Deltamethrine		
100	44.20	568
10	52.64	615
1	53.66	650
Dimethoate		
100	42.58	598
10	50.19	652
1	52.95	679
Control	53.95	668

The values represent means from replicates

Data indicate also that when Bactospeine was added to the medium, germination and tube growth of Coratina variety were enhanced especially during the first hours of incubation. At 3 h incubation and at 100% RFR of Bactospeine, the pollen germination rate was 48% and tube growth 280 μm. After 28 h on culture medium, the relative germination reached 90.5% and tube elongation of 647 μm.

Deltamethrine and Oleoparathion were less effective than Bactospeine in stimulating germination for Coratina pollen (Table 3). Coratina pollen cultivated in presence of Bactospeine started to germinate earlier and had a faster apparent pollen tube growth rate than in presence of Dimethoate, Deltamethrine and Oleoparathion at 100% RFR indicating that Bactospeine had no toxic effects on pollen germination and tube growth.

These results depend on the concentration of the insecticides tested. If the concentration of Bactospeine decreased from 100 to 1% RFR, a limited decline of germination and tube growth rates were observed. On the opposite, decreasing Dimethoate, Deltamethrine and Oleoparathion from 100 to 1% RFR appeared to promote the germination rate and pollen tube length of Coratina (Table 4). Limited germination of Coratina pollen was obtained in any of the media containing Deltamethrine and Oleoparathion. Pollen germination and tube growth were severely inhibited at 100% RFR Oleoparathion with 5.6 and 26.03% in presence of Deltamethrine, compared to the control with 62.4%.

As shown in Table 4, Deltamethrine and Oleoparathion at 100% RFR, not only prevented of Coratina pollen germination when contained in the germination media but also inhibited further development of germinating pollen and arrested the elongation of pollen tubes. Decreasing Deltamethrine and Oleoparathion concentration from 100 to 1% stimulated both germination and tube elongation of Coratina pollen. In presence of Deltamethrine, the tube length passes from 68 μm at 100% to 493.8 μm at 1% and in presence of Oleoparathion, germination percentage increases from 37 μm at 100% RFR to 491.3 μm at 1% RFR. Assays conducted in presence of Oleoparathion and Deltamethrine at 1% RFR were effective to show the olive pollen sensitivity to different concentrations of insecticides.

In contrast, germination did not differ significantly from the control when Dimethoate was incorporated into the medium. Pollen germination and tube length in the presence of Dimethoate at 100% RFR exhibited slight but significant inhibition (49.5% and 460 μm) compared to control (62.4% and 553 μm tube elongation). Decreasing concentration from 100 to 1%, Dimethoate had a little stimulatory effect on pollen germination and significant effect on pollen tube growth, the limited germination rate from 49.5 to 55.3% and tube length growth from 460 to 492 μm (Table 4). These data were consistent with the results obtained by Laccone *et al.* (2000). Studying the efficacy, persistence and phytotoxicity of Dimethoate in controlling *Bactrocera oleae* on Coratina oil olives, they showed that Dimethoate at 40 mL hL^{-1} was effective and higher persistency and no toxic effects. According to Rodriguez *et al.* (2003), Dimethoate is an organophosphorus insecticide which is easily degraded.

In vitro, when Coratina pollen grains were germinated in the presence of Deltamethrine (26%) and Oleoparathion (5.6%) at 100% RFR, tube length was only 68 and 37.5 μm, respectively. Pollen grains that germinated in presence of Bactospeine at 100% RFR presented higher tube length (647 μm comparing to the control 553 μm) (Table 4). The decreasing of Bactospeine concentration (from 100 to 1% RFR) enhanced pollen germination but had an inhibitory effects on pollen tube growth with mean tube length ranging from 647 μm at 100% RFR to 549 μm at 1% RFR. Tube length of Coratina pollen germinated in presence of Deltamethrine and Oleoparathion at 1% RFR were significantly different from those of 100 and 10% RFR with mean tube elongation 493 and 491 μm. Intermediate inhibitory effects on tube length were observed with Dimethoate (Table 4) where pollen exhibited 460, 501 and 492.3 μm of tube length, respectively at 100, 10 and 1% RFR. Also pollen tube growth was less affected by the presence of Dimethoate than was by Deltamethrine and Oleoparathion.

When shoots were sprayed with insecticides and bio-insecticide and pollen was cultured on insecticide-free medium: Sprayed on Coratina shoots just before blooming, Dimethoate, Deltamethrine and Oleoparathion at 100% RFR significantly reduced pollen germination and tube elongation compared with water controls. In contrast Bactospeine at 100 and 10% increased germination and enhanced pollen tube growth for Coratina olive cultivar tested (Table 5). There were no significant differences in mean pollen germination percentage and tube growth among control (water sprayed) and the three insecticide flower sprayed at 10 and 1% RFR (Table 5). In control (pollen from inflorescence sprayed with water), the mean germination percentage was significantly greater than in Oleoparathion, Deltamethrine and Dimethoate at 100% RFR. While the treatments with Bactospeine sprayed at 100, 10 and 1% were higher than control.

In Coratina, there were no significant differences in mean per cent pollen germination among control (water spray) and 1 and 10% RFR Deltamethrine and Dimethoate

sprayed inflorescence. Oleoparathion sprays at 100% RFR significantly decreased germination in comparison with the control and resulted in a further decrease in comparison to Deltamethrine and Dimethoate at 100% RFR.

Oleoparathion at 100% RFR that had the greatest inhibition on pollen germination also had the greatest inhibitory effects on tube growth. Among the insecticide treatments, Oleoparathion at 100% RFR had the greatest inhibitory effects on pollen germination (33.1%). Pollen collected from shoots which received Oleoparathion sprays made less extensive tube growth that controls (469 μm). Oleoparathion (Table 5) sprayed at 100% RFR resulted in the least germination after 48 h incubation in Coratina cultivar (33.1%) compared to the control. At 1% Oleoparathion, no significant effect on mean percent pollen germination was found with control (55%).

Sprayed at green cluster stage, Dimethoate and Deltamethrine at 100% RFR reduced germination and tube growth but less severely decreased than with Oleoparathion. At 10 and 1% RFR, germination percentage and tube elongation were not significantly different from those of the control. Tube elongation was no different from controls.

Field application of these insecticides on olive have inhibited pollen germination and tube growth both *in vitro* and *in vivo* assays but not equally suggesting that the contrasts between various insecticide groups showed different results. Bactospeine is a *Bacillus thuringiensis* (Bt) used for controlling pests, is a most widely used microbial control agent and serve as alternatives to broadspectrum chemical insecticide (Lacey *et al*., 2001). It is a delta-endoxin which exhibit larvicidal toxicity upon ingestion lepidopterean and dipterian larvae (Zouari *et al*., 2002). Civantos and Sanchez (1993) used Bt as integrated control in Spanish olive groves and concluded that the results were similar to those with organophosphate insecticides. Bt is a prevalent organism on wine grapes where it has the potential to control insects and also to control spoilage and mycotoxigenic fungi on grapes and to influence the phyllospheric microflora (Bae *et al*., 2004). The efficacy of Bt has been evaluated against olive pyralid (*Palpita unionalis* Hubner), it reduced the population if *P. larvae* on shoots and was more effective if applied twice and sprayings are spaced at 7-10 days on olive trees (Albanese *et al*., 2000).

Dimethoate is an organophosphate and a very toxic systemic insecticide which kills insects by interfering with the action of important enzymes in the nervous system has been used as larvicide treatment against olive fly (*Bactrocera oleae* Gmel). It resulted in successful control of the pest and it is no detected in olive and oil samples tested after analyzing the active substances (Parlati *et al*., 2000).

Deltamethrine is a pyrethoid insecticide used to control the bark beetle, *Phloeotribus scarabaeoides* Bern. in olive orchards. It has a negative effect on different parasitoid families and especially parasitoids of *Prays oleae*, the major pest of olive trees (Rodriguez *et al*., 2003). It was found to be toxic for the Heteroptera populations.

In vivo, Coratina olive cultivars showed similar trends with low pollen germination and tube growth in presence of Oleoparathion and increasing to high levels with Bactopeine treatment. Oleoparathion treatments retarded germination, ruptured pollen tubes and damaged stigmatic surfaces *in vivo*. Germination and tube length of pollen were reduced when Dimethoate, Deltamethrine and Oleoparathion at 100% RFR were sprayed in the field. In contrast, at 10 and 1% RFR, these insecticides were less inhibitory.

The nefast activity of insecticides treatment *in vivo* (sprayed on shoots during green-cluster stage and incubated on insecticide-free medium) reported in this investigation was confirmed by the same investigation under *in vitro* conditions (insecticides were applied in germination medium) but rates *in vivo* were lower. It suggested that the undehisced anther wall may protect pollen from contact with insecticide sprayed at green-cluster stage. The results suggest that *in vitro*, the toxic effect of insecticides appear during germination, resulting in the pollen is arrested in its development by direct contact with insecticide (Mehri *et al*., 2006).

Tube morphology: In addition to inhibiting pollen germination and tube elongation, Deltamethrine and Oleoparathion applied to culture medium or sprayed on shoots at green cluster stage, also influenced tube morphology. There were highly significant differences in the mean elongation of pollen tubes among treatments. The mean length of pollen tube in Bactospeine medium were significantly greater than the three insecticides used but no significant differences were found among the 3 concentrations used (100, 10 and 1%).

In control and in presence of Bactospeine on culture medium, pollen had tubes that were straight, long and smooth with tapering ends (Fig. 1a). Pollen tubes grown in the presence of Deltamethrine and Oleoparathion were characterized by swelling and rupture in the tip (apical) region (Fig. 1b and c). Pollen tubes exhibited abnormal growth, with a sinuous and wavy configuration. Jaycox and Owen (1965) suggested the possibility of physical action of sprays damaging pollen grains.

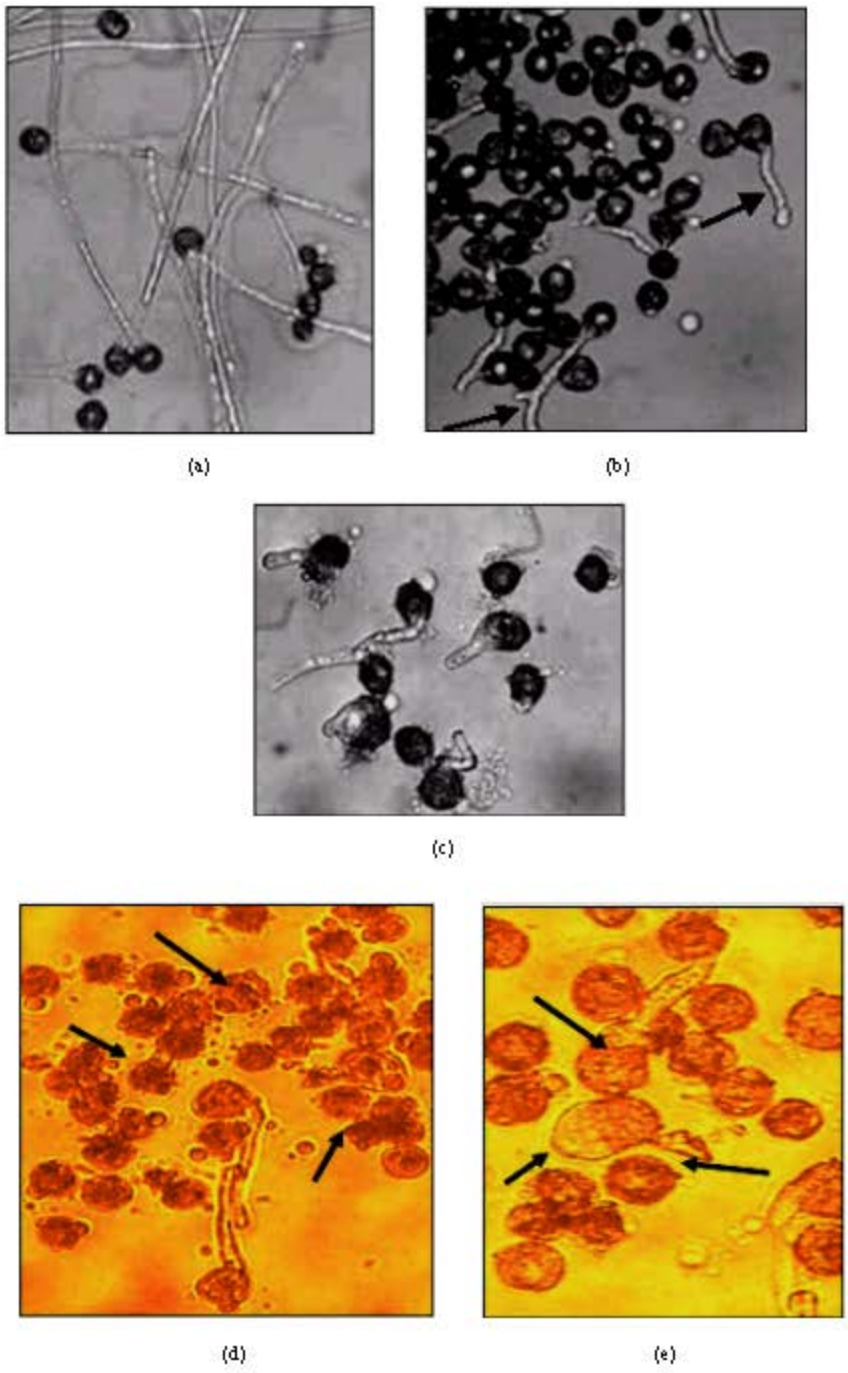

Fig. 1: Tube morphology of coratina olive pollen after incubation in germination medium containing insecticides (a-c) or exposed to insecticide sprays and incubated in insecticide-free media (d-c). (a): Pollen showing straight and long tubes in presence of Bactospeine, (b and c): swelling pollen tube and rupture in the tip region with abnormal tube growth with a sinuous and wavy configuration in presence of insecticides and (d and e) shoots exposed to insecticide sprays and pollen cultured in insecticide-free medium showing a high proportion of abnormal pollen: small and aggregated grains into clumbs and collapsed at polar ends and irregularly shaped and abnormal tube growth

Compared with controls, Oleoparathion and Deltamethrine sprayed on the trees resulted in pollen with more abnormal morphology. A higher proportion of abnormal grains were observed in 100% RFR of the 2 insecticides. There were from 6% at 100% RFR Deltamethrine to 24% at 100% Oleoparathion. These abnormal pollen grains were often smaller and aggregated into clumps and collapsed at polar ends. They are irregularly shaped and were empty as lacking cytoplasmic components (Fig. 1d-c).

In addition to decreased germination rates, pollen collected from shoots which received insecticide sprays produced shorter tubes than control. Patterns of pollen tube growth differed among the type and concentration of insecticides. The decrease in pollen capacity associated with insecticidal sprays can affect fertilization and subsequent fruit set, it significantly altered pollen morphology. Further investigations to consider must include effects on pollination.

The carry-over effects of the three insecticides and the bio-insecticide on vegetative and reproductive behaviour of olive cultivar the season following application need further investigation. It includes effects on plant growth, pollen development and pollination to select the less damaging pesticides for use at or near pollination in olive trees.

CONCLUSIONS

Chemical treatments remain one of the most important management activities in olive production and many insecticides are currently registered in Tunisia for controlling many olive pests. In the current study, three insecticides and a bio-insecticide at three concentrations are applied to pollen culture medium or sprayed at green-cluster stage of Coratina olive cultivar. They were evaluated to compare their effectiveness on pollen capacity (germination and tube growth) in both *in vitro* and *in vivo* conditions.

The results of these experiments indicate a high correlation between germination capacity (germination and tube growth) and type and concentration of insecticides when added to culture medium or sprayed on shoots. One of the most important findings of this investigation was the increase in germination percentage and tube elongation induced by Bactospeine; a microbial control agent. This work shows for the first time that application of Bt bio-insecticide sprayed on shoots or added to culture medium, causes stimulation of olive pollen germination and tube growth and this promoting effect of Bactospeine is associated with the concentrations used. In contrast Deltamethrine and Oleoparathion at 100% RFR applied during bloom period, were the most inhibitory on pollen germination and tube growth *in vitro* and *in vivo*. Intermediate inhibitory effects on pollen germination and tube elongation were observed when Dimethoate was added in germination medium or sprayed on shoots. The insecticide products may have toxic effects while the biopesticide had stimulation.

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