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REGULAR ARTICLE

Evaluating the biocontrol potential of insects auxiliary and bio- insecticide extracted from *Citrullus colocynthis* against *Parlatoria blanchardi* in date palm in Saoura Oases, Algeria

Benlarbi Larbi*, Boulanouar Ali, Akermi Moulay M'hammed

Laboratory for the enhancement of plant resources and food security in semi-arid zones in southwest Algeria - Bechar. Bechar 08000, Algeria

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**Corresponding Author:*
Email:
Benlarbi.larbi@univ-bechar.dz
Telephone: 00213 667101012
FAX: 0021349 23 89 74

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ABSTRACT

Saoura, is an Algerian desert region, characterized by a height density of date palm trees (more than 300000).Despite this high density, the production of dates has seen a continuous decrease due to multiple reasons including increasing water salinity, the spread of many fungal diseases and insect pests where the most serious is *Parlatoria blanchardi*. The random use of Malathion during the invasions of desert locusts caused an ecological imbalance, where the majority of the natural enemies of date palm scale (DPS) disappeared, as consequence the population of DPS expanded increasingly. This study aims to find alternative methods to control pests taking into consideration the ecological balance. For this purpose, three entomofauna inventories were carried out to study the dynamics of insect populations and for introduction and acclimatization of new auxiliary insects to biocontrol the large infestation of DPS. we also looked to find a biodegradable insecticide extracted from *Citrullus colocynthis* (desert plant pariah by insects due to its toxicity). By comparing the results we noticed that effectiveness of the colocynth aquatic extracts on the target insect was as high as 65.99 % however it's side effects on ecosystem were negligible unlike Malathion which exterminated over than 301 non-target insects where 68% are useful such as bees, butterflies, some entomophagous insects and even some vertebrate such as *Oenanthe leucopyga*. Finally, a phytochemical screening and toxicological studies are recommended to determine exactly which component in colocynth is more effective on DPS and its undesirable effect on human health.

1. Introduction

Date palm is one of the main wealths of Algeria after oil and that through its favorable climate, which allows the palm tree to grow and complete the ripening of its fruits. The exploitation of date palm represents a major asset for the development of the Algerian Sahara especially at the current time where Algeria allocates much importance to natural resources. As a result, development, conservation and the rational exploitation of palm groves have become an urgent necessity. Thus, to achieve these main aims, the research in this field become very necessary. Indeed, a well-maintained palm can produce up to 100 kg of dates per year.

However, its growth, flowering and fruiting closely linked to ecological factors.

Various constraints hamper date palm cultivation, such as (i) abiotic stresses, including water scarceness, prolonged dryness, drawdown of groundwater, climate change, and soil salinization (Idder ,2011; Manickavasagan et al., 2012) (ii) biotic interactions, such as insect pests, cryptogamic and phytoplasmic diseases (El Shafie, 2012) and (iii) anthropogenic perturbations including all inappropriate human activities like the excessive use of pesticides and fertilizers, abandonment of phœniciculture and palm plantations, exodus and expansion of urbanization (Idder, 2011; Manickavasagan et al., 2012) which have all led to reduce yields of production and problems of marketing (El-Juhany, 2010; Manickavasagan et al., 2012).

The Date Palm Scale (DPS) *P. blanchardi* Targioni-Tozzetti 1892 (Homoptera: Diaspididae) is the most serious pest of date palm in most areas producing dates, because it settles throughout the green parts of the tree, preventing and affecting the vital functions of the tree like photosynthesis, respiration, and transpiration (Bénassy 1990; Abivardi 2001). The phenomenon of waxy crust usually results in death of premature palm trees and weakening of older individuals (Smirnoff, 1957). In addition, the DPS is widely distributed in the most date palm-growing areas of the world except in the USA and some countries of the southern hemisphere such as South Africa and Namibia (Zaid et al., 1999). Thus, heavy damage caused by this pest is considerable and leads to considerable economic losses at both regional and national scale (Smirnoff, 1957).

The population of DPS is limited by certain number of parasites and predators such as *Cybocephalus palmarum* (Coleoptera: Nitidulidae) as established several year ago (Smirnoff, 1957). Nevertheless, the disappearance of some natural enemies of DPS using pesticides caused an ecological imbalance. Then, in the absence of bio-control, the population of DPS intensified the reproduction up to four generations per year (Munier, 1973); the situation becomes worse particularly after the renewing of attacks and the invasion widen more and more.

In view of the above, the purpose of this study was to provide the most accurate and up-to-date overview to the oasis ecosystem and find an eventual environmentally benevolent alternative to fight against this pest using auxiliary and botanical insecticides. Using different equipment and collecting methods, we realized an entomofauna inventory in Saoura region (Boulanouar, 2009). Among the numerous insects inventoried, some have been a subject of a bio-ecological study; these species were selected according to their abundance, frequency and their harmful effect on palms.

For these reasons, the list of inventoried species were compared to that made by Peyerimhoff in the same region since 1928. This allowed us to identify species newly emerged or completely disappeared.

Bioinsecticides are used to kill or repel pests that results from dried, ground plant material, crude plant extracts, or chemicals isolated from plants. The recorded use of plant material or plant extracts for pest control dates back at least 200 years (Capinera, 2008).

In the present study, we chose a plant of desert, which not easily affected by insects and a cultivated plant of same species. *Citrullus colocynthis* (L.) (Cucurbitaceae), commonly known as wild gourd, colocynth or bitter apple. *C. colocynthis* has gained increasing interest as a natural insecticide and its activity has been evaluated against many economically important pest species. *C. colocynthis* has deterrent, antifeedant, growth-regulating and fertility – reducing properties on insects (Seenivasan et al., 2004)..

2. Materials and Methods

2.1. The study area

Saoura constitutes the western part of the Algerian Sahara, of approximately 789,000 km^2, deriving its name from the valley of Saoura, trees palm groves were the subject of the study: Taghit (30°51'43.72" N 02°00'20.66"W), Igli (30°26'44.22" N02° 16'36.76"W) and Beni Abbes (30°07'29.14" N02° 10'17.85"W).

2.2. Comparative study between chemicals and biocides effects

Treatment with chemicals

We have selected twelve-(12) palm date tree with a height of 3 to 4 m, then we divided them into three groups of four trees, group A, B and C. Each palm of the 'group A'(Fig.1) had undergone a particular type of treatment with a given concentration of Malathion 1%, 2% or 3%, but the last one remained as witness (spray distilled water only). The same treatment repeated for the palms of groups B and C (Fig.1). After one day of treatment, we took three

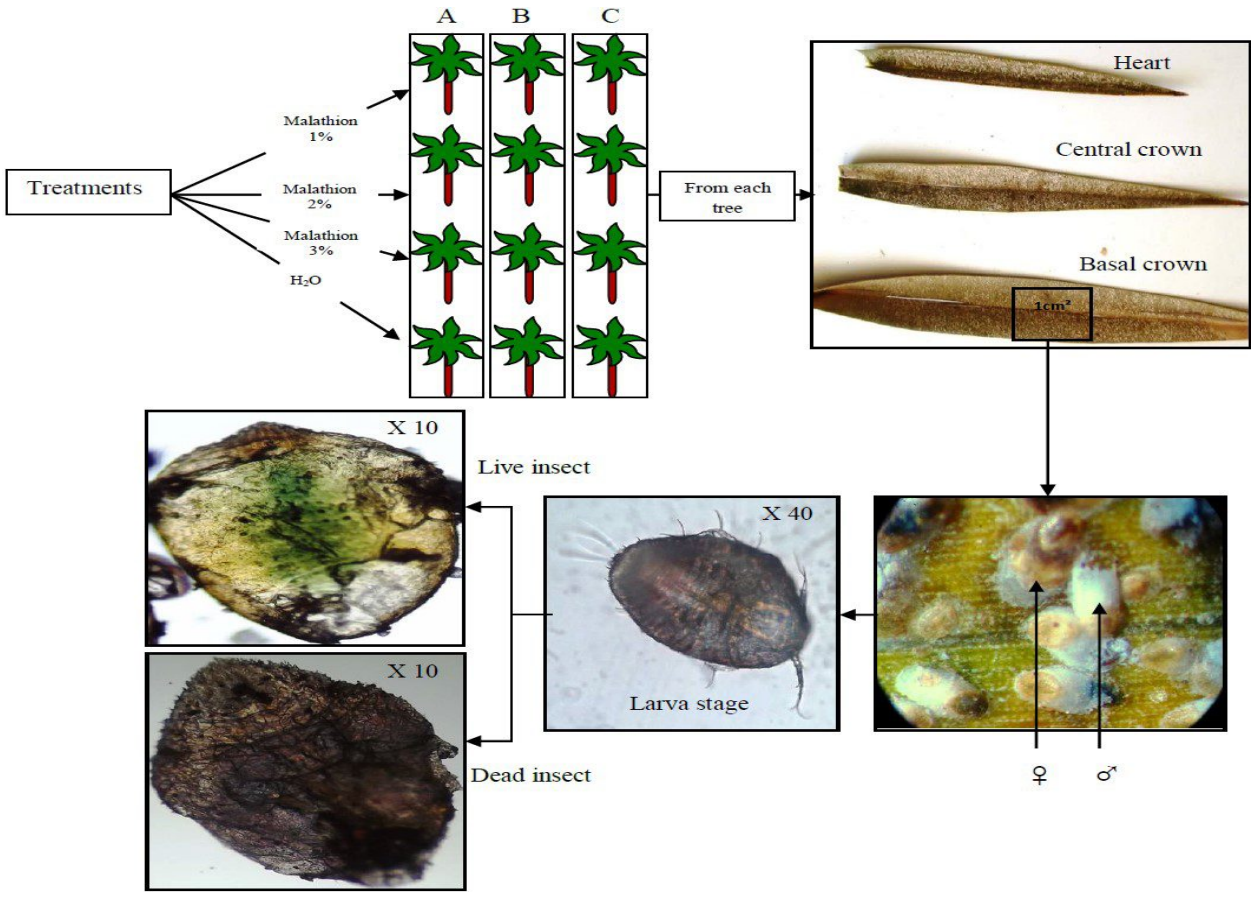

Figure 1: Summary of methodology steps of treatments using Malathion.

leaflets from each treated palm tree, one from the basal crown, and the others from central crown and heart. Leaflets collection process was repeated every five days after treatment for a month, thus 18 leaflets from each palm in total were collected. Later on, we cut one square of one cm^2 from the center of each leaflet where the infestation was very high. Dead insects counting process was based on several morphological and physiological criteria; it was by the use of a needle to remove the waxy shield of the insect, where the microscopic observations allowed us to identify the living or the dead insects.

It has been used a commonly mathematical technique (Abbott 1925) to assess mortality in insecticide trials when there is need to correct for a change (decrease) in the background population density (in the check or control plots):

% Corrected control =

$$\frac{\%\ alive\ in\ the\ check - \%\ alive\ in\ the\ treatment}{\%\ alive\ in\ the\ treatment} \times 100$$

During the treatment period, we also studied the undesirable effect of the insecticide on the other taxa by covering of the ground under the date palm by a sheet of greenhouse plastic and that enables us to count the number of the non-target species dead.

Treatment with biocides by spraying

We used the Colocynth fruits (*Citrullus colocynthis*). The plant harvesting took place in the region of Igli province of Saoura situated in the south west of Algeria. The harvested fruits were washed and then dried in the shade. The aqueous extract was obtained by melting 20g of dried and ground bark,. The whole was brought to boiling for approximately 3 h in reflux mounting. Then we filtered it using a filter paper.

The basic solution was prepared by diluting the initial extract to obtain concentrations of 1%, 2% and 3% followed by addition of 2 ml of paraffin liquid as an adhesive for each 200 ml of concentration. In the field, we repeated the same steps of the previous treatment, except for the replacement of Malathion by the colocynth extracts however the counting process was the same.

This type of treatment consisted in the irrigation of four infested male palm trees (2 m) with an aqueous extract of colocynth; the concentrations were in the order 1%, 2% and 3% however, the third one

was irrigated with water. Then we followed the same precedent steps for accounting.

2.3. Statistical Analysis

The data collected were expressed as mean ± standard deviation (SD). One-way ANOVA was carried out to test for any significant difference by using EXCEL 2010.

Differences among means at P ≤ 0.05 level considered significant and the results were interpreted.

To know the side effects of Malathion, the biocide and the systemic treatment, we focused in our comparative study between chemicals and biocides effects of each one.

The side effects of Malathion was more active and it showed results were statistically significant (*df* 3,8; F= 21,981; *P*= 3.222E-04) (Fig 4-b). .

The biocide was less active then the chemicals effects and the results ware statistically significant (*df* 3,8; F= 22,229; P= 3.097E-04)(Fig 5-b).

The systemic treatment showing promising results and which was comparatively greater than positive control and the result was not statistically significant (df 3,8; F= 2,753; P= 0,112)(fig 6-b).

3. Results and discussions

3.1. Diagnosis of the ecological situation in Saoura's oases

Studies began with a diagnosis of the situation of the entomofauna in the oasis ecosystem, by the realization of inventories. we compared our inventory with previously published inventories to identify species newly emerged or completely disappeared, in order to know the consequences of the disappearance or the emergence of each one.

3.2. Evolution of Saoura's entomofauna

In 1928, Peyerimhoff identified in these areas a total 164 species. We collected 161 species among 5032 individuals in 2009, 163 species among 6128 individuals in 2013 (Fig.2a). Under the impact of multiple fluctuations either abiotic such as climate conditions, or biotic (human, plants…), the entomofauna of Saoura reported several evolutions concerning the appearance of new taxa or the disappearance of others. A simple comparison be-

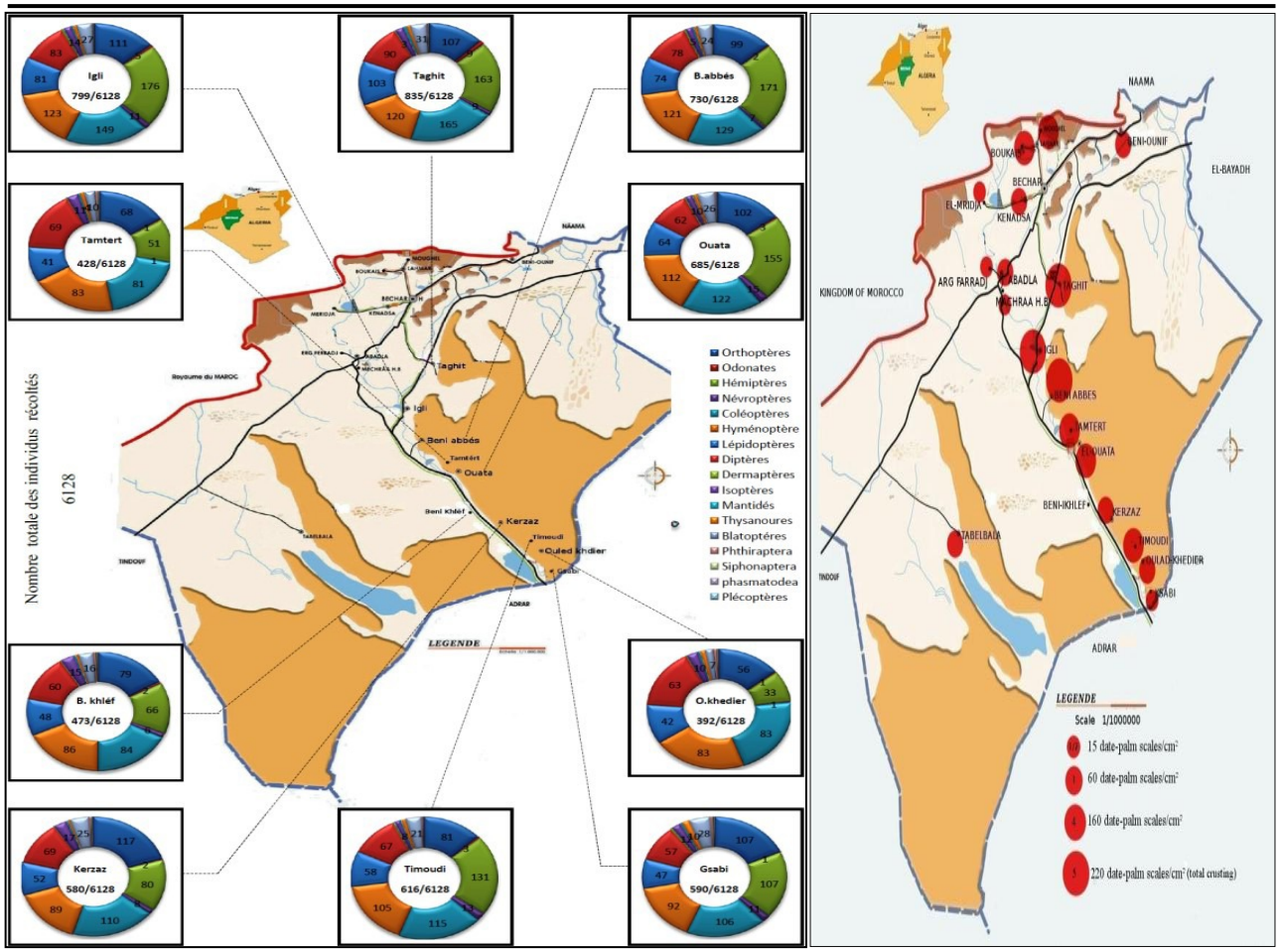

Figure 2: (a) Spatial distribution of collected insects 2013; (b) Density of the DPS in different oases of Saoura.

Comparison of both inventories Peyerimhoff 1928 and our first inventory 2009		comparison of both inventories first inventory 2009 second inventory 2013	
Missing species	New species	Missing species	New species
Scarites occidentalis *Scarites Eurytus* *Plocaederus caroli* *Derolus mauritanicus* *Cebocephalus sp*	*Xylocopa pubescens* *Dysgonia algira* *Danaus chrysippus* *Oryctes rhinocéros*	*Chilochorus bipustulatus*	*Bactrocera oleae* *Ceratitis capitata* *Cydia pomonella*

Table 1: Different inventories comparisons.

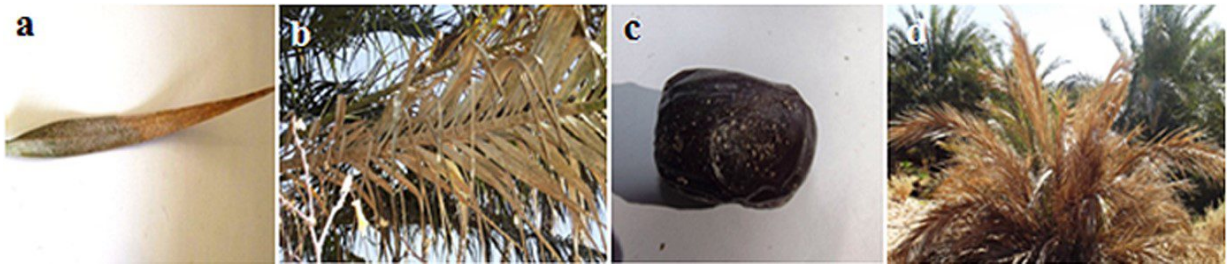

Figure 3: Symptoms and steps of DPS infestation: (a) leaflets yellowing (b) leaves drying (c) infested date (d) palms death.

tween inventories realized during different periods allowed us to detect these species. Where we took in consideration date palms and fruit trees pests in oasis ecosystem as well their natural enemies (Table 1).

On the other hand, the excessive use of insecticides, Malathion particularly, appeared to cause the disappearance of the many natural predators of DPS such as *Chilochorus bipustulatus* (Coleoptera: Coccinellidae) and *Cybocephalus sp* (Lepidoptera: Nitidulidae). As result of this, the population of DPS widens much more than it was expected, exceeding four generations in a year; according to Brun (1990), the first one (spring generation) is the most injurious in palms. All green parts of the trees were damaged; especially palms of the basal crown, even fruits were not spared from injury. The damages caused by this scale insect were of several kinds: (i) during insect feeding (aspiration) the DPS injects a certain quantity of a toxin that alters chlorophyll and (ii) the insect crust prevents the correct respiration and photosynthesis. These actions conduce to reduction of production, deterioration of fruit quality, a rapid aging and the premature death of palms (Fig.3). Moreover, insects that feed on plants' vascular tissues (xylem and phloem) appear to be the most common vectors of plant viruses (Gray, 1999).The excessive density of DPS in some oases is represented in the Figure 2b.

As a result, the ecological balance in the palm groves is extremely sensitive, each species plays a well-defined role in the trophic chain; its disappearance may lead to a collapse of the whole system.

3.3. Chemical treatment with Malathion

Malathion spraying with different concentrations had rapid and a very significant effect on the population of Semm (local appellation of the DPS); particularly on males during the period of the flight, there was a very acute population decline of up to 60% after 5 days; to reach 82.85% after one month, when we used the concentration 3% (Fig.4a). Our results corroborate with that of Ghazwan et al. (2008), where they reported that some chemicals (Actara 25WG and Confidor 200S L) can reduce the population of Semm until 78.77%. For another insect *Sitophilus oryzae*, Malathion can reduce the population as high as 87.8% for the concentration 1.2 ppm (Aly S. D et al., 2011).

During the treatment period, we identified over 301 non-target insects dead, of which 60% were useful, such as bees, butterflies and some auxiliary enemies of cochineal and even vertebrae such as insectivorous birds (Fig.4b). Malathion mainly used in agriculture as a pesticide (insecticide and acaricide) with broad spectrum, especially to control sucking pests. Although its human toxicity is relatively low, it is a product with a little persistent in soil, but it is very ecotoxic particularly to aquatic ecosystem, and its active product of decomposition, Malaoxon is very toxic. It is for these reasons prohibited from use in the European Union, since 1 December 2008 (Uzun et al., 2009). Malathion as a foliar residue is highly toxic to honeybees for as much as 48 h post application. Malathion's average half-life ranges from 1-10 days on foliage. This latent residue is suspected to cause acute losses in

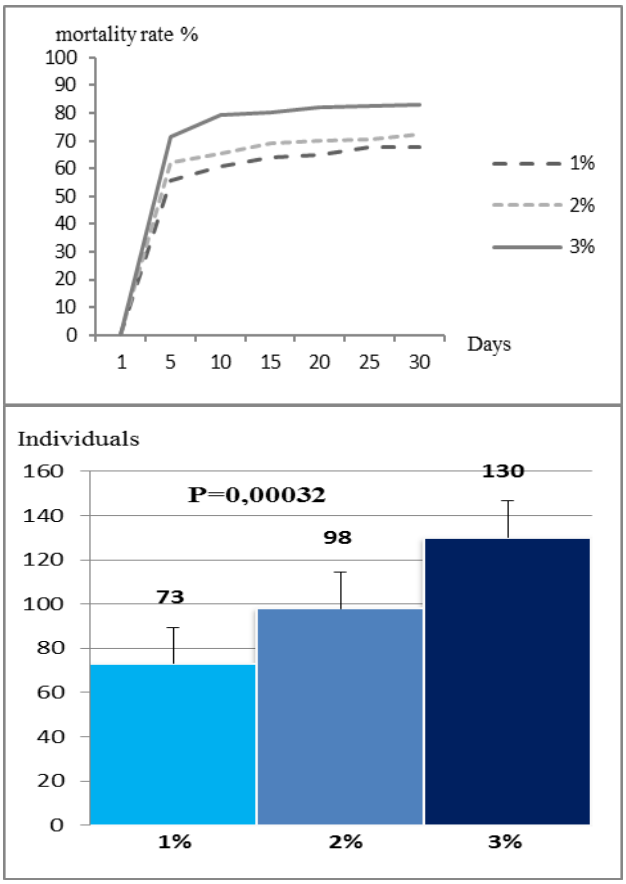

Figure 4: (a) DPS's evolution during the Malathion treatment. (b) The side effects of Malathion.

other beneficial insect populations (Gary et al,. 1984). In addition to killing the targeted pests, a range in mortality has been observed for non-target insects and spiders. In tests conducted in Pleasanton and Woodside, California, mortality of dipterids, Lepidoptera larvae, spiders, cynipoidea, hemiptera, and psocoptera observed in a treated area with Malathion bait spray (Dahlsten, 1985). Sublethal effects also occur to mammals and reptiles due to their susceptibility to the inhibition of acetylcholinestease (Giles et al.,1970).

This insect (DPS) improved defenses against any fluctuation of the ecological factors, whether biotic or abiotic.They are distinctive for their sluggish or sessile habits, reduced morphology and the waxy secretions they produce. These secretions, which are mixtures of true waxes plus lipids, resins and other substances; are produced by integumentary wax glands, (Howard 2001). This insect can resist Malathion (molecules contain two extra carboxylic acid ethyl ester parts). Malathion-resistant insects always show increased carboxylesterase activity (Resh, 2003). Esterases of these types are not broad-spectrum enzymes, and therefore Malathion resistance is usually specific (Resh, 2003) usually the insects resistant to Malathion are not resistant to other insecticides. Our results also corroborate these data.

As conclusion, the oasis ecosystem is a very fragile and a closed ecosystem therefore human intervention must be precautionary and vigilant, and particularly concerning the use of synthetic insecticides.

3.4. Treatment with aqueous extract of Colocynth

The colocynth (*C. colocynthis*) belongs to Cucurbitaceae family. It is a desert plant that grows in sandy arid soils. It is native to the Mediterranean region and Asia. The fruit is spherical with a 5–10 cm diameter and extremely bitter at taste. We chose this plant because the overwhelming majority of insects avoid the consumption of its fruits which characterized by a high degree of bitterness and toxicity. It is very available with large quantities in the study area. February is the appropriate period for treatment; it precedes the fruiting and the ripening stage, so it is the ideal time for treatment in order to avoid poisoning of dates, because they are among the fruits consumed without washing. On the other hand, palm date achieves a very important vegetative stage where plant biomass increases by the appearance of new palm leaves. This growth accompanied by an evolution of the population of the DPS that intensifies its attacks to colonize the new palms. The aquatic extract of colocynth with concentrations of 1%, 2% and 3% had a clear impact on the insect but it was relatively slow comparing to chemicals, where the mortality rate exceeding 58% after 15 days of treatment. These results (Fig.5a) are close to the results obtained by El Dossary *et al*., (2008), where he used other extracts such as glory bower extract 3% *(Clerodendron inerme)* 62.30%,Oleander extract 3% *(Nerium oleander)* 66.93%. Wild rue 3% *(Pegnanum harmala)* 69.20% Castor oil plant 3% *(Ricinus communis)* 70.80% after 14 days.

DPS use piercing-sucking mouthparts to remove plant sap, during treatment the insect absorb the aqueous extract of colocynth which leads to their death. The Cucurbitaceae members are well protected with exceedingly bitter large steroid type molecules named cucurbitacins. Most insects will not feed on wild species of the Cucurbitaceae because of the bitter cucurbitacins (Bell 1995). The biological activities of triterpenoids have an antinutritional effect. They inhibit food intake of some phytophagous insects and cause death and malformations in future generations (Carpinella et al., 2003). Cucurbitacins and particularly 2-O- fl -D-

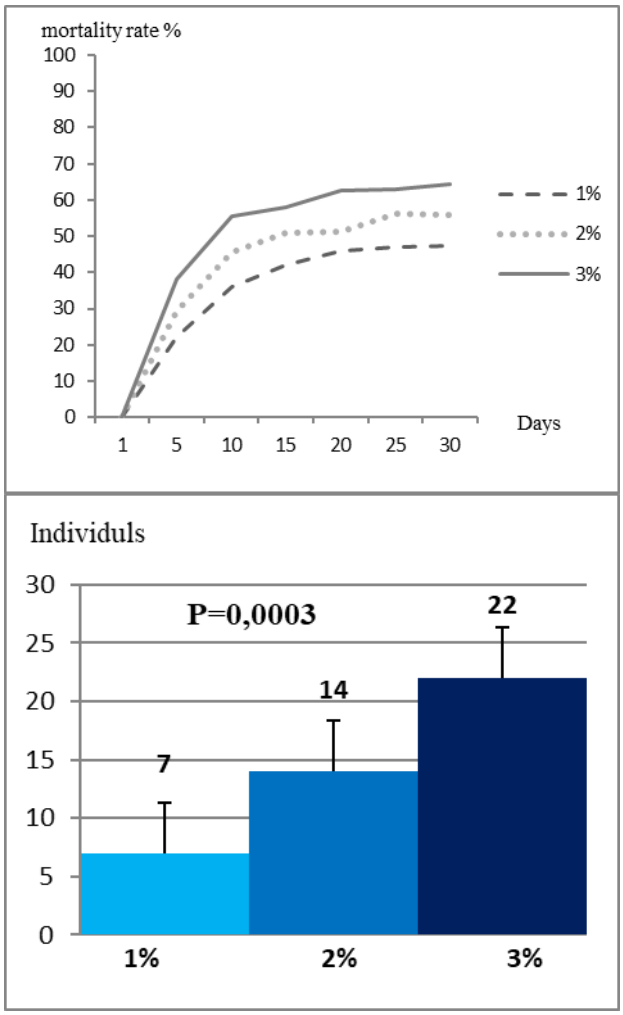

Figure 5: (a) DPS's evolution during the biocide treatment. (b) The side effects of the biocide.

Metabolites	Party	Compounds	References
Flavonoids	Fruits	Isovitexin, iso-orientin3'-methyl ether iso-orientin	Maatooq and *al.*,1997
Saponins	Fruits	2-O-B-D glucopyranosyl cucurbitacin I, J, K and L	Seger and *al.*, 2005
Glycosides	Fruits	Three flavone glycosides: isosaponarine, iso-vitexinisoorientin and 3'-O-methylether; Two glycosides cucurbitacins: 2-O-β-D-glucopyranosyl-cucurbitacin I 2-O-β-D-glucopyranosyl-L cucurbitacin	Delazar and *al.*, 2006
		Two new triterpene glycosides cucurbitacins: colocynthosides A and B	Yoshikawa and *al.*,2007
Alkaloids	Fruits	Choline- Pyridine derivatives: $C_{10}H_{15}NO_3$ and $C_{20}H_{32}NO$ - The derivative of pyridine or quinoline: $C_{16}H_{24}NO_7$	Darwish-Sayed and *al.*, 1973

Table 2: Different components of the colocynth's fruits.

glucopyranosyl cucurbitacin E represented a deterrent, antifeedant, growth-regulating and fertility reducing properties on insects (Sachdev-Gupta et al., 1993)., Table 2 represents the results of the phytochemical screening of colocynths fruit where Saponins and Glycosides contains a large quantity of this molecule.

Numerous researches have been done on *C. colocynthis* against a number of pests such as cockroaches, adult honeybee, housefly, cotton leaf worm, bed bug and mosquito (El-Naggar et al., 1989). A study by Seenivasan et al. (2004) showed that the petroleum ether and ethyl acetate seed extracts showed anti-oviposition, adult emergence, and ovicidal and repellent activity against the pulse beetle *Callosobruchus maculates.*

Concerning the side effects, biocides are biodegradable and volatile vegetable products for these reasons the effect this latter remains proportionally slow and very limited where undesirable effects on the non-target insects are very slightly marked.

During the treatment period we identified more than 43 insects (non-target) is dead, whether half are opophagous insects such as Hemiptera (Fig.5b).

A systemic insecticide is an insecticide, which penetrates the tissues of the plant and is transported by the sap, which is very effective against sucking insects, biting or phytophagous. This kind of products usually has no effect on the insects which does not consume fragments or fluids from the treated plant, colocynth extracts caused the dead approximately of about 65.99% of the total population (Fig.6a).

The more shares of insects (non-target) death are defoliator such as orthoptera, which feed by foliage and tissues rich in extract of colocynth where the total number of insects is 17(Fig.6b).

Finally, the experience of plants on earth estimated at billions of years, and it is sure that it's more than that of the human being, during this period the plants evolve several strategies and techniques to fight against the pests. Repulsive are phyto-products target only pests; they have no effect on pollinators and beneficial insects, unlike those synthetized by humans it's really time bomb with countdown delay.

4. Conclusion

It has been shown in this study that extracts of Colocynth fruits, are effective against DPS (*Parlatoria*

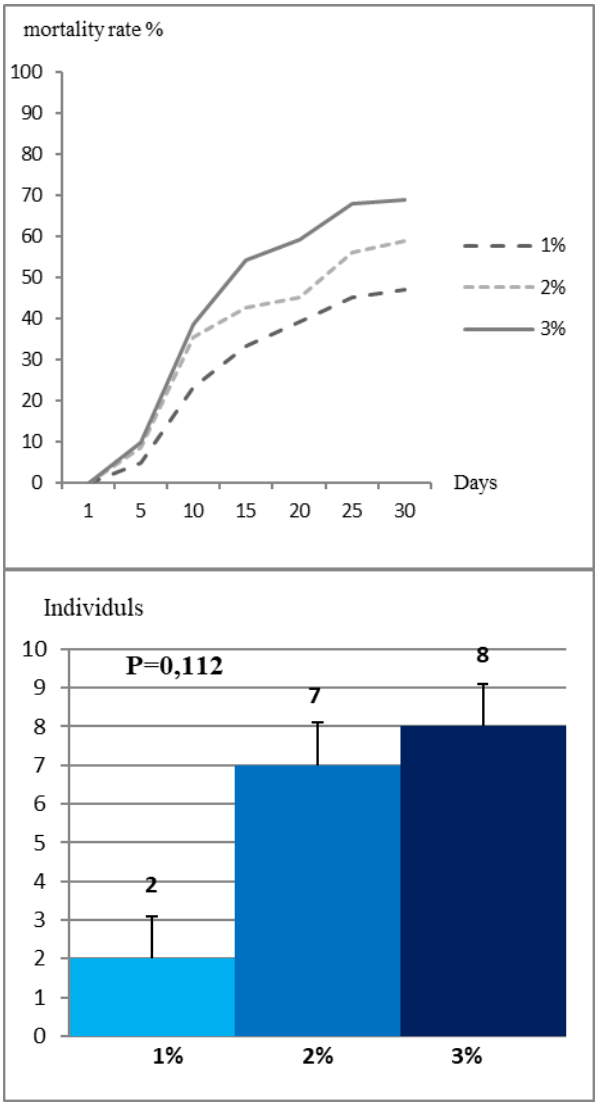

Figure 6: (a) DPS's evolution during the systemic treatment (b) The side effects of systemic treatment.

blanchardi) but, it should not be considered absolutely safe. The active components in certain botanical preparations used as insecticides are relatively toxic for animals and humans also. However, most used at very low concentrations of active ingredients, minimizing the risk to humans and wildlife from exposure. In summary, extracts of Colocynth fruits can be used with prevention to control DPS in palms. The results are similar to other artificial or natural extracts but it is less dangerous for the human health and the oases ecosystem, this last one is a closed ecosystem where the reparation of this imbalance is very expensive it requires a lot of money and time.

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