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# Laboratory and Field Evaluation of the Toxicity of Oxamyl against the Red Palm Weevil, *Rhynchophorus ferrugineus* (Olivier)

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## ABSTRACT

The widespread as well as serious damage produced by the red palm weevil (RPW), *Rhynchophorus ferrugineus* Olivier, have forced emphasis of various research teams on finding appropriate methods for managing and controlling it. A huge fund was given for managing and controlling RPW in several countries. In this study, the potential toxicity of oxamyl to RPW was evaluated under both laboratory and field conditions. In the laboratory, oxamyl had significant effects on the mortality of RPW. After exposure to direct spray of oxamyl, adult mortality was 62, 82 and 100% whereas larvae mortality was 72, 77 and 100% after 1, 24 and 48 h, respectively. Alive adult and larvae were completely paralyzed after 24 hours. There was significant difference between 2 treatments, 1 and 24 h, in both adult and larvae. The mortality of both adult and larvae was 100% after 48 hours of exposure to oxamyl. In the field, however, Oxamyl was applied in two different applications. A foliar application by spraying and watering application by adding the to the irrigation water. After 2 days of applications, daily monitoring was conducted for 10 days to record any dead or paralyzed adults and/or larvae. Dead and paralyzed adults or larvae were found particularly in foliar application compared with the control. Furthermore, females egg-laying defect with serious reduction in their movement was recorded especially in foliar application. Egg hatching was not affected with minimal activity in both applications compared with control. Moreover, no mortality of adults emerging from pupae was recorded and they only showed detectable reduction on their movement in both field treatments. Finally, this study would recommend the use of oxamyl in managing and controlling the infestation of RPW.

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## INTRODUCTION

In Saudi Arabia, the number of date palm tree was over 18 million in 2004 (Anonymous, 2004) whereas it was more than 28 million date trees in 2015 (General Authority for Statistics, 2015). About 24% (~7 million) of these palm trees are located in Qassim region (central region). Furthermore, a sharp increase in the number of planted date palm trees between 2004 and 2015 by about 10 million shows their economic importance. Like any other plants, the date palm tree has many pests which can directly or indirectly affect their viability as well as their production. The most important date palm tree pest is the red palm weevil (RPW), *Rhynchophorus ferrugineus* Olivier.

The RPW infestation has rapidly spread since 1980 (Gomez and Ferry, 1999). Its infestation was recorded in many countries such as Saudi Arabia in 1987 (Abraham *et al*., 1998), Egypt in 1992 (Cox, 1993), Jordan and Palestine in 1999 (Kehat, 1999), Spain in 1994 (Barranco *et al*., 1996), United Arab Emirates 1985 (Zaid *et al*., 2002) and Iran in 1996 (Faghih, 1996).

RPW can be considered as the most highly destructive pest for date palm tree. It can play a critical role in the viability of palm trees as it can create a serious damage affecting normal growing conditions of the trees. Damage by RPW widely weakens the palm trunk which results in reducing their production and growing or making them less tolerance to the environmental conditions (*e.g.* strong winds). Therefore, this has forced several countries to generate a new program with a huge fund for management and controlling RPW. In California for example, approximately 70 million US dollar were spent annually on ornamental palms (Hussain *et al*., 2013) whereas about 450 million US dollar was made available for controlling RPW on date palm trees for 5 years program in Saudi Arabia. Moreover, 30% estimated loss of the global date production is due to the infestation by RPW in the Arabian Peninsula (El-Sabea *et al*., 2009).

The control and management of RPW is very difficult as the infestation cannot be easily detected and diagnosed.

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RPW can also continue feeding and damage the trunk without any symptoms in the early stage. In Saudi Arabia, it has also reported that up to three complete generations were found in a single palm within a year (Hussain *et al*., 2013). Despite the difficulty of detecting the infestation by RPW, successfully diagnosed and detected infestation at the early stage can save the palm whereas neglected or delayed detection until the late stage infestation might lead to serious damage or loss to palm tree. At the end of the infestation, damage symptoms are very detectable as tunnels in the trunk, oozing of thick yellow fluid from the palm, empty pupal cases, external entrance and toppling of the crown (Hussain *et al*., 2013; Abo-El-Saad *et al*., 2013). Appearance of these symptoms indicates serious damage to the palm tree.

Since 1998 several tactics based on the integrated pest management (IPM) have been extensively used by researchers such as the use of pheromone trap, surveillance and inspection, chemical control and cultural control (Abraham *et al*., 1998; Shawir *et al*., 2014). Nevertheless, insecticide applications are largely used in different ways as the main tactic for controlling RPW. Insecticides were used as soil treatment, frond axil filling, trunk injections, tree fumigation, crown drenching, wound dressing and dipping offshoots (Hussain *et al*., 2013; Al-Shawaf *et al*., 2013). The wide-spread of RPW to different geographical areas has encouraged researchers to focus their work on management of RPW. Effect of three insecticides carbosulfan, pirimiphos-ethyl and rogodial on all stages of RPW was tested in the UAE laboratory (El-Ezaby, 1997). The UAE laboratory results showed that these three insecticides had high mortality on all stages of RPW. In Saudi Arabia and during last two decades, a huge number of laboratory experiments were conducted to evaluate and test the sensitivity of RPW to a wide range of insecticides. These include chlorpyriphos, endosulfan, pirimophos-methyl, fipronil, deltamethrin, dimethoate, methomyl, methidathion, fenitrothion, Salut, carbaryl, beta-cyfluthrin, acephate, emamectin benzoate and imidacloprid (Abraham *et al*., 1998; Ajlan *et al*., 2000; Abo-El-Saad *et al*., 2001, 2011; Al-Rajhy *et al*., 2005; Al-Shawaf *et al*., 2010; Al-Jabr *et al*., 2013). Some insecticides were tested in other laboratory and/or field experiments worldwide including azadirachtin, spirotetramat, methidathion, aluminum phosphide and oxamyl against all stages of RPW (Cabello *et al*., 1997; Kaakeh, 2006; Llacer and Jacas, 2010; Shar *et al*., 2012; Shawir *et al*., 2014).

Oxamyl is a member of the carbamate group. It has several trade names such as Thioxamyl, Vydate and DPX 1410 (USEPA, 2004). Oxamyl has been extensively used in many countries including developed countries such as the USA as a broad spectrum pesticide to control insects, mites, roundworms and ticks and can be considered as an insecticide, acaricide, and nematicide. Oxamyl has been used to protect a wide range of crops including vegetables, fruit, wheat and ornamental plants and has widely used on cotton. It is not persistent in the environment and does not accumulate in fat. According to the literature review, toxicity of oxamyl to RPW has not been tested since 1997 when Cabello *et al*. (1997) evaluated its toxicity against the larval stage of RPW in the laboratory. However, toxicity evaluation of oxamyl to RPW adults in laboratory and field has never been tested and verified.

Therefore, the aims of this study were to evaluate the toxicity of oxamyl to adults and larvae of RPW as well as its effectiveness to control all stages of RPW in the field.

## MATERIALS AND METHODS

*Insects*

All stages of RPW including adults (males and females), larvae and pupae were collected from a date palm farm which is recently infected (has not been treated with any insecticides) by RPW in the north of Al-Qassim region.

*Chemicals and solutions*

Oxamyl (Fymate 24% oxamyl) was obtained from Astra Company. Recommended application rates 4 ml/1L for direct spray and 15 ml/tree for mixing with irrigation water were used in laboratory and field study.

*Treatment*

The recently RPW-infected farm was selected to do all required aspects of this study. In laboratory, collected RPW were maintained at 25 ± 2°C and used for bioassays immediately after collection. Ten adult and 10 larvae with three replicates (60 RPW in total) were placed in a plastic container with palm pieces as a source of food. Thereafter, they were exposed directly to oxamyl or distilled water (without oxamyl) as a control by using conventional hand sprayer.

Peeled palm pieces (to make them more attractive) were soaked for 1 min in spray solution of oxamyl or distilled water (without oxamyl) as a control and left to dry on clean tissue paper. Dried peeled palm tissues were then placed in the plastic containers. Ten adults and 10 larvae in each container replicated three times (60 RPW in total) were put in with treated tissues to get them in contact with/ or ingestion of oxamyl with feeding. Mortality rate was recorded after 1, 24 and 48 h by counting dead RPW and expressed as percentage mortality.

In the field, 30 infected palm trees of the same age (7-8 years old) (Sukari cultivar) were determined and divided into 3 treatment groups (A, B and C). In group (A), oxamyl solution was applied directly to 10 trees (a foliar application) (~ 10 L directly sprayed to a single date palm tree). In group (B), oxamyl was added by irrigation water to 10 trees. In group (C), 10 trees were sprayed by only well water (without oxamyl) as a control. Agricultural power sprayer 1000 L was used to spray the solution. After 48 h of application, a daily investigation was started by selecting a single date palm tree in all treatment groups (A, B and C) up to 10 days. All direct observations and mortality was recorded upon investigation. Each palm tree was opened up to expose the infected area and the effect on RPW was recorded.

*Statistical analysis*

The mortality of RWP was also calculated manually by direct observation. Collected data were calculated by using Microsoft Excel. The collected data for all variables were statistically analyzed using the MSTATC microcomputer program (MSTATC, 1990). The least significant difference (LSD) test was used to compare the mean mortality percentages. Only differences significant at $P \leq 0.05$ are considered in the text. Curves for the mortality assays were plotted using Graph Pad Prism version 7. Each point indicated the mean mortality percentages ± SEM of each treatment with oxamyl and the graphs were fitted using a nonlinear regression with a four parameter logistic equation where the upper plateau was set to 100% and the lower plateau was set to 0. The results were expressed as mean mortality percentage ± SEM for each treatment.

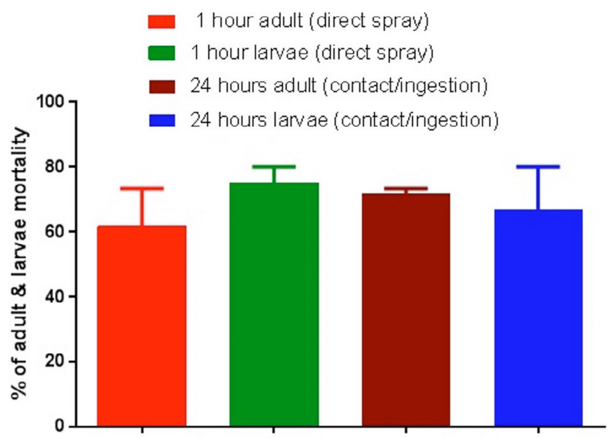


Fig. 1. Comparison of the effects of oxamyl on the mortality of adults and larvae after 1 and 24 h of exposure to direct spray or contact/ingestion (under laboratory conditions) expressed as percentage of the control mortality of adults and larvae after exposure to distilled water (Control). Each point is the mean of mortality percentage ± SEM for 3 replicates.

**Table I.- The mortality percentage of RPW (adults and larvae) after 1 and 24 h of exposure to oxamyl (direct spray or soaked palm pieces).**

| Treatment | Direct spray | | Contact/ingestion | |
|---|---|---|---|---|
| | **Adults** | **Larvae** | **Adults** | **Larvae** |
| 1 hour | 62.2 ± 4.69*b* | 72.23 ± 6.93*b* | 0.0 ± 0.0*b* | 0.0 ± 0.0*b* |
| 24 hours | 82.2 ± 8.4*a* | 77.8 ± 6.36*a* | 68.9 ± 1.9*a* | 62.23 ± 7.73*a* |

LSD 0.05= 13.67, h X methods; *a-b* means within time or method followed by the same letter are not significantly different (P = 0.05).

Fig. 2. RPW treated in the laboratory by direct spray with oxamyl, 1 h after treatment (top) and 24 h after treatment (bottom).

## RESULTS

The toxic effects of oxamyl on RPW (larvae, adult and pupae) were tested under both laboratory and field conditions. In both treatments, mortality as well as detectable observations were recorded daily. Under laboratory conditions, oxamyl had significant toxic effects based on the mortality of RPW. Adults (males, females), larvae and pupae of RPW were exposed to oxamyl by direct spray. Adults' mortality was 62, 82 and 100%, whereas larvae mortality was 72, 77 and 100% after 1, 24 and 48 h, respectively (Fig. 1). Males showed more sensitivity to oxamyl than females. Alive adult and larvae showed a significant reduction in their movement after 1 hour and were completely paralyzed after 24 h. Statistically, there was significant difference between 1 and 24 h in both adult and larvae (Table I). In addition, younger larvae were more sensitive to oxamyl than older ones but their mortality was 100% after 48 h of exposure either to direct spray or to contact/ingestion (Fig. 2). Pupae did not show any effects after exposure as adults were found to be alive (Fig. 3). However, mortality of adults and larvae exposed to soaked palm pieces in oxamyl (contact/ingestion) was 69.0 and 100% and 62.0 and 100% after 24, and 48 h, respectively. After 1 h of exposure to soaked palm pieces adult and larvae did not show mortality and no change on their movement, whereas alive adults and larvae were completely paralyzed after 24 h. The mortality difference between adults and larvae was statically significant.

Fig. 3. Adult emerging from pupae after treatment with oxamyl in the field (a foliar treatment).

In both treatments (direct spray or soaked palm tissue), the differences were significant after 1 and 24 h, whereas after 48 ho of exposure there was no significant difference as there was 100% mortality in both adults and larvae.

In the field, oxamyl was applied in two different ways; a foliar application or by adding with irrigation water. After 3-10 days of application, dead and paralyzed adults and larvae were recorded daily especially with foliar application (Fig. 4). Recently hatched larvae were observed alive with minimal activity in both applications compared with the control. Furthermore, an egg-laying defect in females was observed with serious reduction in their movement especially with foliar application (Fig. 5). In all days of treated irrigation water treatments, larvae were found alive with acceptable activity inside palm trunk during the 10 days of investigation whereas all adult and larvae found dead or paralyzed were in the peripheral region of tree trunk. Moreover, no mortality of adults emerging from pupae was recorded and they only showed detectable reduction on their movement in both field treatments (a foliar application and adding with irrigation water).

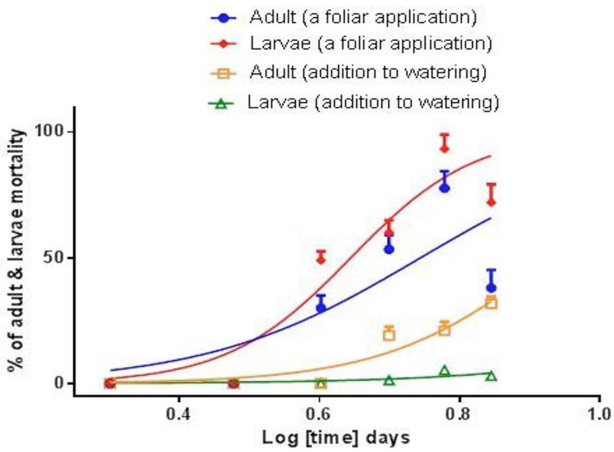


Fig. 4. Comparison of the effects of oxamyl on the mortality of adult and larvae during 7 days of investigation after a foliar application and adding with irrigation water (under field conditions) expressed as percentage mortality after exposure to well water (Control). Each point is the mean of mortality percentage ± SEM but in most cases the error bars are smaller than the symbols used. The lines were fitted using a non-linear regression in Graph Pad Prism 7 with the maximum plateau being 100% and the minimum being 0%. Each curve indicates the mortality of adult and larvae after exposure to oxamyl by a foliar application and adding with irrigation water (under field conditions) compared with control.

Fig. 5. RPW treated with oxamyl on the date palm tree. The top left, top right, bottom left and bottom right pictures were taken after 3, 4, 5 or 6 days, respectively, after direct spray treatment.

## DISCUSSION

All currently available insecticides have been extensively used for controlling RPW. Toxicity of many traditional insecticides to all stages of RPW has already been evaluated in the laboratory and/or field. Moreover, a large number of these insecticides have been banned or restricted such as chlorpyriphos. Many other insecticides such as permethrin, deltamethrin and fenvalerate were found to be ineffective against RPW (Abo-El-Saad *et al*., 2001) but they still in use for the control of RPW. In Saudi Arabia for example, farmers recently complained about the ineffectiveness of most available insecticides. This might be because RPW has developed resistance due to the extensive use or misuse of insecticides. The mechanism of RPW resistance to insecticides is still poorly understood (Al-Ayedh *et al*., 2015). Farmers might exceed the recommended application rate or make a mixture of various insecticides to control RPW without any concern of environmental consequences, residues and toxicity to non-target species with the buildup of RPW resistance makes it very hard and close to impossible to control RPW. Therefore, this study was designed to evaluate the toxicity of oxamyl to RPW in both laboratory and field. Stokes and Laughlin (1970) were first to report that oxamyl had systemic nematicidal properties if used as a foliar spray. Therefore, it can be applied in two ways: as a foliar spray or by adding to irrigation water and taken up into the plant (Kennedy, 1986). Under laboratory conditions, oxamyl can produce significant lethal toxic effects on RPW. Oxamyl showed high effectiveness against RPW as it can cause more than 60% mortality in adults and larvae after 1 hour of exposure. A noticeable reduction in the movement of both adults and larvae was recorded immediately after direct spray of oxamyl, which lead to complete paralysis. Oxamyl can also produce 100% mortality in RPW after 48 h of exposure by either direct spray or contact/ingestion. Moreover, males were found to be more sensitive to oxamyl than females. This finding was consistent with previous studies that males are more sensitive to many insecticides such as fipronil (Al-Shawaf *et al*., 2010). Oxamyl however, did have effects on adult emergence from pupae but these adults did not show any effects from subsequent exposure, as adults were found alive with minimal reduction of movement compared to controls. On the other hand, no mortality of adult and larvae exposed to oxamyl soaked palm pieces (by contact/ingestion) after 1 h of exposure whereas the mortality was about 69 and 100% for adults and about 62 and 100% for larvae after 24 and 48 h of exposure to oxamyl, respectively. Alive adults and larvae were completely paralyzed after 24 h. The mortality differences between adults and larvae were statically insignificant. These findings were consistent with previous studies that recorded 7-days old larvae fed with oxamyl had 100% mortality (Cabello *et al*., 1997). They also indicated that oxamyl could rapidly cause larval mortality. They also found that the mortality of 30-days old larvae fed with oxamyl was 71.8%. Obtained results showed that oxamyl did not only can cause a rapid death in larvae but also in adults (males and females).

Under field conditions, a single recently RPW infected farm was recently used in this study to ensure that no other factors might directly influence the toxicity of oxamyl such as traces of previous insecticide applications. Therefore, it would not be possible to find a large number of different stages of RPW in the treated trees as the selected farm has recently infested by RPW. Oxamyl was applied in two applications (a foliar application or adding to irrigation water). Dead and paralyzed adults and larvae were found daily up to 10 days post-spray especially with foliar application. Eggs were successfully hatched with minimal activity in both applications compared with control. Furthermore, female egg-laying defect was only recorded with serious reduction in their movement especially with a foliar application (Fig. 5). On the other hand, the mobility of larvae was very low at all 10 days of investigation in oxamyl added in irrigation water treatment with acceptable activity of alive larvae inside the trunk whereas all adult and larvae were found dead or paralyzed in the peripheral region. To understand and explain these findings a deep assessment of the anatomy of date palm stem was taken into consideration. Zimmermann and Tomlinson (1965) and (1966) investigated the course of vascular bundles in the stem of palms. According to these studies only relatively few bundles extended to the center of the stem. All bundles in parts of their course were found in the peripheral region, this region is crowded with bundles. In the central, uncrowded part of the stem, all bundles when traced up words rotate uniformly and describe a helix in the direction of the phyllotactic spiral. Both Fan (1982) and Metacalfe (1982) confirmed these findings. Therefore, the obtained result would be reasonable as treatments had no effects on larval stages especially when located in the center of the palm trunk. This would confirm that a foliar application of oxamyl was found to be more effective to control all stages of RPW except pupal stage at any location of the trunk compared with oxamyl in irrigation water treatment. A foliar application would be also recommended to avoid environmental impacts of oxamyl as it can be readily leached from soil by rain to reach the ground water (Wagenet, 1985; USEPA, 1987). The high mobility of oxamyl has the potential to result in contamination of underground water especially with sandy soil (low organic content) (USEPA, 1987; USDA, 1990). Its mobility in soil depends on the amount of organic content in the soil (Harvey and Han, 1978; Bromilow *et al*., 1980) and under aerobic conditions the half-life is 2-4 weeks whereas under anaerobic conditions it is less than 2 weeks. Therefore, much careful consideration of the safety regulations must be taken especially when adding or mixing oxamyl with irrigation water to treat infested trees as it might leach into surrounding soil which results in increase of contamination of underground water.

Higher concentrations of oxamyl might be more effective against all stages of RPW in the laboratory or in the field but beyond this approach, its environmental impacts and residues must be justified. Dates are directly consumed and/or exported after picking, so high level of residues might result in direct effect in human health or rejection of shipped dates.

In fact, there are many agricultural malpractices that directly enhance the RPW infestation and make date palm trees more attractive to RPW. However, such practices are beyond the scope of this study.

## CONCLUSION

This study has provided evidence emphasizing the effectiveness of oxamyl against adults and larvae of RPW. Laboratory experiment showed that oxamyl could produce high mortality on adults and larvae of RPW with direct spray or feeding (ingestion). In field experiments, foliar treatment of oxamyl showed high effectiveness against adults (males and females) and larvae whereas oxamyl added irrigation water treatment showed low effectiveness compared with foliar treatment. Both treatments were found less effective against pupae and eggs hatching. Therefore, it would be very valuable to investigate the potential effectiveness of oxamyl for use in farms that can minimize the infection as well as spread of RPW as trunk injection and offshoots dipping might be successful. Moreover, it is possible to recommend oxamyl for treating date palm offshoots as this is one of the most important elements of integrated pest management to regulating insecticide based quarantine protocols.

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*Statement of conflict of interest*

Authors have declared no conflict of interest.

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