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# EVALUATION OF THE SIDE EFFECTS OF OXAMYL AND HYMEXAZOL ON FIVE SPECIES OF SOIL-DWELLING PREDATORY MITES

**Saleh S. Alhewairini[1,*] and Mahmoud M. Al-Azzazy[1,2]**

**[1]Department of Plant Production and Protection, College of Agriculture and Veterinary Medicine, Qassim University, P.O. Box 6622, Buraidah 51452, Qassim, Saudi Arabia. [2]Agricultural Zoology and Nematology Department, Faculty of Agriculture, Al Azhar University, Cairo, Egypt.**
**[*]Corresponding author's e-mail: hoierieny@qu.edu.sa**

Side effects of oxamyl and hymexazol on five species of soil-dwelling predatory mites, *Lasioseius dentatus* (Fox) (Acari: Ascidae), *Androlaelaps casalis* (Berlese) (Acari: Laelapidae), *Rhodacarus roseus* (Oudemans) (Acari: Rhodacaridae), *Macrocheles muscaedomesticae* (Scopoli) (Acari: Macrochelidae) and *Cunaxa setirostris* (Hermann) (Acari: Cunaxidae) were tested under stringent laboratory conditions. Both oxamyl and hymexazol were found to drastically kill all five predatory soil mites listed above at three different concentrations (half of the recommended dose (HRD), recommended dose (RD) and double the recommended dose (DRD). Oxamyl was found to be more toxic than hymexazol to all five predatory soil mites as it could reduce their population by > 40% at the HRD, except *R. roseus*, as its population was reduced to 27.80%. In addition, the application of oxamyl can decrease the population of five predatory soil mites by >83% at RD while at DRD a total mortality rate could be seen. In conclusion, this research study has provided evidence which highlights the reduction of population of five predatory soil mites even after the exposure to half of the recommended dose of oxamyl and hymexazol. Therefore, precautionary measures should be taken to maintain the sustainability and to avoid disturbing the natural balance of the ecosystem.
**Keywords:** Toxicity, Oxamyl, Hymexazol, soil predatory mites.

## INTRODUCTION

Agricultural production has come to rely more heavily on pesticide use during the past two decades. Often, however, diseases, weeds and insects have developed resistance to those chemicals which once efficaciously suppressed or controlled them (Aktar *et al*., 2009). Broad spectrum pesticides kill the target pest or disease, but they are also likely to have effects on beneficial non-target organisms and predators in spite of the chemical producers' attempts to make their products very specific. Because of this, pesticide use, be it broad-based or more specific ones, can cause less biodiversity (Marer, 2000). A decrease in biodiversity can lead to increases in the growth of pathogens, harmful insects, or other weeds. There is also a justifiable worry that various soil organisms may be accidentally harmed by the use of pesticides, and this will undermine soil porosity or aggregation through the suppression of fungi, earthworms, and other soil microorganisms (Aktar *et al.,* 2009).

Arthropods, performing key tasks as herbivores, detritivores, predator and prey, are an essential component of a functional ecosystem. Micro arthropods are the most diverse and numerous arthropods in litter and soil media, especially Collembolans and Oribatid mites (Vargas *et al*.,2007). Research findings indicate that the effects of the application of various herbicides differ on the diversity and number of micro arthropods. Herbicide 2, D-4 sodium is reported to have no significant effect on mite populations according to studies (Edwards, 1972), whereas Simazine treatment caused a significant decrease in Oribatid populations (Prasse, 1973). Research also indicates that long-term use of herbicides causes a significant reduction in collembolan populations (Mitra *et al*., 1983). Other factors which influence the diversity, number, and community structure of micro arthropods are the quantities of macro and micro nutrients, the biodiversity and age of the rehabilitating habitat, the accessibility of organic matter, and substrate quality (Loranger *et al*., 1988).

Soil micro arthropods are a key part of all ecosystems, even agricultural ecosystems, due to the essential tasks they perform in the soil. They decrease the bulk density of soil and increase soil horizon mixing, soil pore space, aeration and drainage, litter decomposition and water holding capacity, and improve the soil aggregate structure, all of which improve soil quality and thus its productivity (Abbott, 1989; Vargas *et al*., 2007). As they are an integral part of ecosystem processes and are very responsive to land management practices, they are useful as bio-indicators quantifying environmental degradation (Larson and Pierce, 1994; Costello *et al*., 2013). Moreover, soil micro arthropods are essential in fragmentation and in combination which, due to them creating new surface areas for microbial colonization, have very important ramifications on the processes of decomposition and mineralization (Webb, 1977; Elkins and Whitford, 1982). It is crucial to preserve beneficial aerial and soil mites, especially predatory species, to encourage a more stabilized pest population and natural balance although there is still little knowledge about the habits of many micro arthropods, such as mites, which together form the food chain below ground (Fouly, 1997; Walter and Campbell, 2003; Heckmann *et al*., 2007). A large number of the group Gamasida (Mesostigmata) can serve a role as efficient biological control agents against insect pests, plant-parasitic nematodesand mites, including those known as aerial predators, which attack leaves and buds (Hyvonen and Persson, 1996; Abbasipour *et al*., 2006; Wekesa *et al*., 2007; Ukabi *et al*., 2009). Many countries use various species of predatory mites in biological control programs (Fouly, 1997; Takano-Lee and Hoddle, 2002; Cuthbertson *et al*., 2007). Among the predatory mites, the most important group of aggressive soil predators is thought to be the family Laelapidae Berlese, particularly members of the subfamily Hypoaspidinea, which includes the genera *Geolaelaps* and *Stratiolaelaps* (Walter and Campbell, 2003). Species of the genus *Stratiolaelaps* are frequently assigned to the refuse of genus *Hypoaspis* Canestrini. Some species of this genus are currently in commercial use in greenhouses, particularly in the Northern Hemisphere, as a means of biological control against agricultural pests.

Many chemical compounds including nematicides, fungicides and miticides can directly affect the community of soil as they are added directly to the soil. This includes harmful effects on non-target species as well as beneficial soil organism such as earthworms.

Oxamyl is a crystalline, systemic and contact pesticide. It is used for controlling insects, plant parasitic nematodes, and mites on various crops. Though not considered as a persistent pesticide with a half-life period of a few days or weeks, its over application may lead to unwanted conditions especially in loamy sand and silt loam soils. It has been detected in ground water in many parts the US (U.S. EPA, 1992, 2000). It inhibits the enzyme acetylcholinesterase (AChE), which hydrolyzes the excitatory neurotransmitter acetylcholine at the neuromuscular synapse (Ahmed *et al*., 2013).

Hymexazol is a heteroaryl hydroxy compound and is used as a systemic fungicide for controlling fungal diseases of soil and seed. Payne and Williams (1990) recommended seed treatment with hymexazol at 10.5–14g $kg^{-1}$ seed. At the same time, they also warned the risk of phytotoxic effects. embryotoxicity tests indicated that the 96h $LC_{50}$ value of hymexazol for embryo D. Rerio was 648.55mg/L and decreased the heart rate and increased the voluntary swing, inhibition of normal development, induced cardiac edema and yolk sac edema. Fan *et al.* (2018) found the $LC_{50}$ of hymexazol against 96h embryo of zebrafish as 648.55 mg/L. They also reported decreased heart rate, increased voluntary swing, inhibition of normal development, induced cardiac edema and yolk sac edema.

The side effects of both oxamyl and hymexazol on five predatory soil mites have never been tested and documented. Therefore, this study was aimed to assess the side effects of oxamyl and hymexazol on predatory soil mites *Lasioseius dentatus* (Fox), *Androlaelaps casalis* (Berlese), *Rhodacarus roseus* (Oudemans), *Macrocheles muscaedomesticae* (Scopoli) and *Cunaxa setirostris* (Hermann).

## MATERIALS AND METHODS

***Solutions*:** Commercial formulation of oxamyl (Fymate 24% w/v, oxamyl) was obtained from Astra Company and hymexazol (Gizazol 30% SL, hymexazol) was obtained from Aljammaz Agriculture Company. The recommended application rates (4ml/1L for Fymate and 50ml/100L for Gizazol) for direct spray mixture were only used in this study.

***Experimental Protocol Collection of soil samples*:** Predatory mites living in debris of date palm trees, citrus trees and organic manure were collected during April 2018 from certain localities in Qassim region, Saudi Arabia. Moist soil samples at 0–10 cm depth was collected and were immediately transferred to the Acarology Laboratory at the Department of Plant Production and Protection, College of Agriculture and Veterinary Medicine, Qassim University. Large samples of mites were collected with different body sizes so that immatures and adults (males and females) could be represented in the sample. This is especially important for mites because females are required for identification at the species level for many species. Soil samples were sieved while still moist through a 2-mm sieve. Thereafter, samples were extracted by using Berlese-Tullgren funnel for one day as it is the most useful tool for extracting large quantities of mites living in soil, debris of date palm trees, citrus trees and organic manure (Upton, 1991; McSorely and Walter, 1991).

2.2.2. Preserving mites for study

Mites were preserved in small vials with 80% alcohol. Addition of 5% glycerol is recommended to prevent mites from drying out if the alcohol evaporates. Vials used for storing were small so that mites can be easily found later.

***Preparing Mites For Microscopic Study Clearing of specimens***: The collected mites were placed in an aqueous solution of 85% lactic acid as a clearing agent for two days at room temperature.

***Preserving mites***: Mite individuals were singly mounted on glass slides in Hoyers fluid, covered with a glass cover and gently heated for one minute to stretch mite bodies (Evans, 1992; Krantz, 1978). Identification and classification of the collected predacious mites were based on the terminology of Chant (1963), Lindquist and Evanz (1965), Evans and Till (1979), Krantz (1978), Zaher *et al*. (1986) Smiley *et al.* (1986) and El-Benhawy *et al*. (2006). Taxonomical of collected mites was carried out by using an Olympus Camera DP25 attached to an Olympus Microscope BX51.

***Effect of herbicides on soil predatory mites*:** Three different concentrations (the half-recommended dose (HRD), recommended dose (RD) and double recommended dose (DRD)) of oxamyl and hymexazol were tested. Five separate stock colonies of *L. dentatus*, *A. casalis*, *R. roseus*, *M. muscaedomesticae* and *C. setirostris* were maintained in the experimental laboratory. Every stock colony was divided into four treatments which divided into four replicates, which included twenty adult predators with twenty grams of soil as a similar environment. Thereafter, three different concentrations (HRD, RD and DRD) of both oxamyl and hymexazol including a control (well water) with four treatments were sprayed directly on the Petri-dishes by using a small knapsack sprayer (1L). Dead mites were then counted after three days of the application to determine the percentage mortality.

***Statistical analysis*:** The percentage reduction in the average populations of *L. dentatus, A. casalis, R. roseus, M. muscaedomesticae* and *C. setirostris* was calculated using the equation of Henderson and Tilton (1955).

$$\text{Corrected}(\%) = (1 - \frac{\text{n in Co before treatment} \times \text{n in T after treatment}}{\text{n in Co after treatment} \times \text{n in T before treatment}}) * 100$$

Where,

n = Number of predatory soil mites,

T = Treated, Co = Control.

The mortality percentages of predatory soil mites were calculated manually by direct observation. Thereafter, Microsoft Excel Program was used to calculate the average obtained. Statistically, all variables of the obtained data were analyzed using one-way analysis of variance (ANOVA). The results are expressed as mean mortality percentage ± SEM for each treatment.

## RESULTS

Lethality is defined as the capacity of a chemical or compound to cause death. Lethality of different concentrations (HRD, RD and DRD) of two commonly used compounds *viz.*, oxamyl and hymexazol on the populations of five predatory soil mites (*L. dentatus*, *A. casalis*, *R. roseus*, *M. muscaedomesticae* and *C. setirostris*) were tested under stringent laboratory conditions. All the species of predatory soil mites were susceptible to both oxamyl and hymexazol, where a significant number of deaths could be observed in all the tested concentrations (HRD, RD and DRD) compared with the control (well water). Application of oxamyl decreased the population by >83% at RD while at DRD, a clear change in the mortality rate could be seen and 100% mortality was observed in *L. dentatus*, *A. casalis* and *M. muscaedomesticae* while *R. roseus* and *C. setirostris* had 94.45% and 95% mortality rates (Tables 1, 2).

At the RD, oxamyl had a more or less uniform rate of lethality against the species tested; whereas a considerable difference could be seen in the mortality of the species tested against hymexazol. *L. dentatus* showed highest mortality (89.5%) followed by *C. setirostris* and *M. muscaedomesticae* with mortality rates of 73.0% and 65.0% respectively while *A. casalis* and *R. roseus* had the same mortality rate (63.0%).

In case of HRD, *M. muscaedomesticae* had the maximum mortality (61.20%) against oxamyl and *L. dentatus* had the maximum mortality (63.20%) against hymexazol. *R. roseus* had the least mortality rate against both oxamyl (27.80%) and hymexazol (16.0%). Of all the species tested, *R. roseus* undoubtedly was the most insensitive one against both oxamyl and hymexazol at all concentrations.

**Table 1. Effects of three concentrations of oxamyl on five different adult predatory mite species *L. dentatus*, *A. casalis*, *R. roseus*, *M. muscaedomesticae* and *C. setirostris* under stringent laboratory conditions.**

| Predatory species | Concentrations of oxamyl | No. of predatory mite(Adults) | | |
|---|---|---|---|---|
| | | Average pre-spray count | Average post-spray count * | Reduction % ** |
| *L. dentatus* | Control | 20.00 | 18.00 | 0.00 a |
| | HRD | 20.00 | 9.00 | 50.00 b |
| | RD | 20.00 | 3.00 | 83.40 c |
| | DRD | 20.00 | 0.00 | 100.00 d |
| *A. casalis* | Control | 20.00 | 19.00 | 0.00 a |
| | HRD | 20.00 | 11.00 | 42.20 b |
| | RD | 20.00 | 3.00 | 84.00 c |
| | DRD | 20.00 | 0.00 | 100.00 d |
| *R. roseus* | Control | 20.00 | 18.00 | 0.00 a |
| | HRD | 20.00 | 13.00 | 27.80 b |
| | RD | 20.00 | 3.00 | 83.84 c |
| | DRD | 20.00 | 1.00 | 94.45 d |
| *M. muscaedome-sticae* | Control | 20.00 | 18.00 | 0.00 a |
| | HRD | 20.00 | 7.00 | 61.20 b |
| | RD | 20.00 | 3.00 | 83.00 c |
| | DRD | 20.00 | 0.00 | 100.00 d |
| *C. setirostris* | Control | 20.00 | 20.00 | 0.00 a |
| | HRD | 20.00 | 12.00 | 40.00 b |
| | RD | 20.00 | 3.00 | 85.00 c |
| | DRD | 20.00 | 1.00 | 95.00 d |

*Average counts made one-week post treatment.

** Mortality values calculated with the Henderson-Tilton equation. Mean followed by the different letter in a column are significantly different from each other at $P < 0.05$.

RD - recommended dose of oxamyl; HRD - half recommended dose and QRD - quarter recommended dose.

**Table 2. Effect of three concentrations of hymexazol on five different adult predatory mite species *L. dentatus*, *A. casalis*, *R. roseus*, *M. muscaedomesticae* and *C. setirostris* under stringent laboratory conditions.**

| Predatory species | Concen-trations of hymexazol | No. of predatory mite (Adults) | | |
|---|---|---|---|---|
| | | Average pre-spray count | Average post-spray count * | Reduction % ** |
| *L. dentatus* | Control | 20.00 | 19.00 | 0.00 a |
| | HRD | 20.00 | 7.00 | 63.20 b |
| | RD | 20.00 | 2.00 | 89.50 c |
| | DRD | 20.00 | 0.00 | 100.00 d |
| *A. casalis* | Control | 20.00 | 19.00 | 0.00 a |
| | HRD | 20.00 | 15.00 | 22.00 b |
| | RD | 20.00 | 7.00 | 63.00 c |
| | DRD | 20.00 | 3.00 | 84.30 d |
| *R. roseus* | Control | 20.00 | 18.00 | 0.00 a |
| | HRD | 20.00 | 15.00 | 16.00 b |
| | RD | 20.00 | 7.00 | 63.00 c |
| | DRD | 20.00 | 3.00 | 84.30 d |
| *M. muscaedo-mesticae* | Control | 20.00 | 20.00 | 0.00 a |
| | HRD | 20.00 | 15.00 | 25.00 b |
| | RD | 20.00 | 7.00 | 65.00 c |
| | DRD | 20.00 | 2.00 | 90.00 d |
| *C. setirostris* | Control | 20.00 | 18.00 | 0.00 a |
| | HRD | 20.00 | 10.00 | 44.50 b |
| | RD | 20.00 | 5.00 | 73.00 c |
| | DRD | 20.00 | 0.00 | 100.00 d |

*Average counts made one-week post treatment.
**Mortality values calculated with the Henderson-Tilton equation.
Mean followed by the different letter in a column are significantly different from each other at $P < 0.05$.
RD - recommended dose of hymexazol; HRD - half recommended dose and DRD - quarter recommended dose.

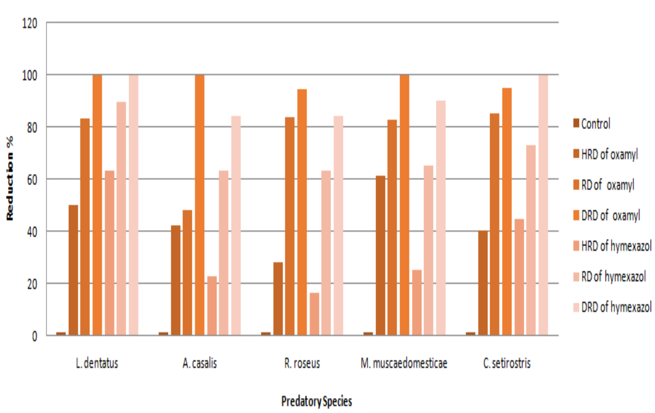


**Figure 1. Comparison of the effects of three different concentrations of oxamyl and hymexazol (HRD, RD and DRD) and the control (well water) on the mortality of five different adult predatory soil mite species *L. dentatus*, *A. casalis*, *R. roseus*, *M. muscaedomesticae* and *C. setirostris* under stringent laboratory conditions.**

## DISCUSSION

Extensive use of chemical compounds which are generally known as insecticides, nematicides, fungicides and others for agricultural and residential purposes has led to the destruction of the populations of several beneficial and predatory insects, nematodes and mites. Oxamyl is also one such chemical. It is an active ingredient in broad-spectrum insecticide/ nematicide. It is widely used for managing unwanted populations of insects, mites and nematodes. Exceeding the recommended dose or mixing two or multiple agents together for achieving higher mortality is often seen among the less educated farmers. This practice causes a sharp decline in the population size of beneficial organisms. It was therefore, considered worthwhile to evaluate the lethality of different concentrations of oxamyl and hymexazol on the predatory soil mites *A. casalis*, *C. setirostris*, *L. dentatus*, *M. muscaedomesticae* and *R. roseus*. According to literature, there is no information available regarding the side effects of oxamyl and hymexazol on predatory soil mites. However, oxamyl was found to be toxic to birds, fish and other wildlife (Dupont, 1984), although it does not accumulate in fat (U.S. EPA, 1987) whereas in high concentration hymexazol was found to inhibit *Saccharomyces Pseudomonas* (Kamimura *et al.*, 1976), red clover rhizobia (Heinonen-Tanski *et al.*, 1982) and various phytopathogenic bacteria (TomiTa *et al.*, 1975).

As has been shown (Figure 1), both the RD & DRD of oxamyl could not obtain 100% mortality to the populations of *R. roseus* and *C. setirostris*. In such a case, there is a probability of the growers to make additional applications resulting into the development of resistance against oxamyl by the very population of *R. casalis*. However, the RD of oxamyl caused ≥83% mortality to all the species, a figure good enough to be further managed by natural enemies. Besides, a rich biodiversity boosts the productivity of ecosystem. Therefore, it is important to preserve beneficial aboveground-belowground mites, especially the predatory ones, to bring about a more stabilized pest population and maintain a natural balance. Unfortunately, the knowledge on the role of many micro arthropods, including mites, in nutrients cycling below ground is still fragmentary (Fouly, 1997; Walter and Campbell, 2003; Heckmann *et al*., 2007). On the other hand, the direct application of both oxamyl and hymexazol to the soil can increase the risk potential of exposing non-target organism such as predatory soil mites to them. Under certain conditions, sandy soil possesses low amountsof organic matter. This has a direct influence on the diversity and abundance of microorganisms in the subterranean ecosystem. Therefore, highlighting the effects of different doses of such chemicals which are being used at a large scale especially in the area where the present study was conducted becomes a necessity. Due to uncontrolled overuse of such chemical compounds may wipe out the already less diverse soil microorganisms in sandy soil. Other alternatives like biocontrol methods may further be tested and the likelihood of risks on humans and livestock in and around such areas may also be evaluated.

***Conclusion*:** High application rates lead to the selection of resistance as this practice removes the susceptible individuals from a population of varying tolerance and thereby reducing the chance of interbreeding which can minimize the population of the resistant strain. This research study has provided information which emphasizes that a huge decline in the population of five predatory soil mites were seen even after the exposure to half of the recommended dose of oxamyl and hymexazol. Therefore, extra care should be taken to avoid the practice of extensive application of many chemical compounds including pesticides for various industrial, agricultural and residential purposes, as they can kill several beneficial insects and mites and disturb the natural balance of the ecosystem.

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