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TOXICITY EFFECTS OF GLYPHOSATE AND METRIBUZIN ON FIVE SPECIES OF SOIL-DWELLING PREDATORY MITES

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The toxicity of glyphosate and metribuzin 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) was evaluated under laboratory conditions. Three different concentrations (half of the recommended dose (HRD), recommended dose (RD) and double the recommended dose (DRD)) of both herbicides were tested. All soil-dwelling predatory mites under study showed high susceptibility to both herbicides, demonstrating that they can significantly reduce the population of all five species of soil-dwelling predatory mites. HRD of glyphosate can reduce the population of all five species of soil-dwelling predatory mites by > 50%, except *C. setirostris,* as its mortality was 30%, whereas HRD of metribuzin can only reduce the population of *L. dentatus* and *M. muscaedomesticae* by > 50%. Obviously, glyphosate seems to be more toxic to all five species of soil-dwelling predatory mites than metribuzin. *L. dentatus* was found to be the most sensitive soil-dwelling predatory mite, whereas *C. setirostris* was found to be the least sensitive to both herbicides, as well as a control (well water), compared with all the tested soil-dwelling predatory mites.
Finally, the highest mortality rates (100%) were obtained for DRD of both herbicides. Therefore, extra care and serious caution must be exercised when diluting and applying these herbicides to ensure their RD is never exceeded as they can kill all soil-dwelling predatory mites studied here in significant numbers, which leads to a sharp reduction in their populations as well as a reduction in their role in the ecological balance.
Keywords: Toxicity, glyphosate, metribuzin, soil-dwelling 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 these chemicals which once efficaciously suppressed or controlled them. 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, can cause less biodiversity. A decrease in biodiversity can lead to increases in the growth of pathogens, harmful insects, or 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, earth worms, and other organisms.
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 mediums, especially Collembolans and Oribatid mites (Santos-Roch *et al.,* 2011). Research findings indicate that the effects of the application of various herbicides differ on the diversity and number of micro arthropods (Mohammed *et al.,* 2017). Herbicide 2, 4-D sodium is reported to have no significant effect on mite populations according to studies (Edwards, 1972), whereas Simazine treatment causes 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, including 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 they improve the soil aggregate structure, all of which improves soil quality and thus its productivity (Abbott, 1989; Palacious-Vargas, 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 combination which, due to their creating new surface areas for microbial colonization, have very important ramifications on the processes of decomposition and mineralization (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 and mites, including those known as aerial predators, which attack leaves and buds (Abbasipour *et al.,* 2006; Wekesa *et al.,* 2007; Ukabi *et al.,* 2009). Many countries use various species of predatory mites in biological control programs (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 genus *Geolaelaps* and *Stratiolaelaps* (Walter and Campbell, 2003). Species of the genus *Stratiolaelaps* are frequently assigned to the refuse 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 herbicides, can directly affect the physiochemical properties of soil (Robidoux *et al.,* 1999). This includes harmful effects on non-target species such as beneficial soil organism such as earthworms. Selective (e.g. metribuzin) and non-selective (e.g. glyphosate) herbicides were specifically selected in the present study due to their extensive use and potential for pre-emergence treatment by farmers worldwide.

The non-selective herbicide glyphosate (N-(phosphonomethyl) glycine) is widely used in agricultural and non-agricultural settings in many countries around the world (Uren-Webster *et al.,*2014). In 1974, glyphosate was formulated by John E. Frazn and in 1993 it was registered foruse in agricultural and residential settings as a non-selective herbicide. Its greatest use is in the absolute weed control, both annual and perennial plants as well as others like grasses, sedges, broad-leaved weeds and woody plants. Glyphosate is the principle ingredient in several products sold under various trade names, including Touchdown® (TD: Syngenta) and the most popular, Roundup® (Mosanto) (Howe *et al.,* 2004). Several previous studies have proved that glyphosate can induce neurotoxicity in Zebrafish, *Xenopus* and *Caenorhabditis elegans* (Richard *et al.,* 2005; Paganelli *et al.,* 2010; Negga *et al.,* 2012; McVey *et al.,* 2016; Roy *et al.,* 2016; Alhewairini, 2017). Moreover, glyphosate can directly affect fish and earthworms as well as birds and small mammals, as glyphosate kills plants that are used for food, nest support and shelter (Santillo *et al.,* 1989; Cox, 1995).

Metribuzin is widely used in agriculture as a selective herbicide in both pre- and post-emergence situations to control grasses and broad leaved weeds in various crops, such as sugar cane, tomatoes, potatoes and soya beans (Landgraf *et al.,* 1998). Furthermore, it has been found that metribuzin can contaminate groundwater (Undabeytia *et al.,* 2011). It has been reported that metribuzin can significantly affect the growth and survival of earthworms (Travlos *et al.,* 2017).

The aim of this study was to evaluate the toxicity of glyphosate and metribuzin against five species of soil-dwelling predatory mites, *L. dentatus*, *A. casalis*, *R. roseus, M. muscaedomesticae* and *C. setirostris*.

MATERIALS AND METHODS

***Herbicides*:** The commercial formulation of glypho-48 (glypho-48, 48% w/v, Glyphosate) was obtained from Montajat Pharmaceuticals company and metribuzin (Metrozin,70% w/w, Metribuzin) was obtained from Delta Saudi Company. The recommended doses were 1.1L/100L for glypho-48 and 0.5kg/hectare for Metrozin.

Experimental protocol:

Collection of soil samples: Predatory mites living in the debris of date palm trees, citrus trees and organic manure were collected during April 2018 at certain localities in the Qassim region, Saudi Arabia. Moist soil samples at 0–10 cm depth were 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. A large sample of mites with different body sizes was collected so that immature and mature specimens (male and female) will be represented in the sample. This is especially important for mites because males are required for identification at the species level for many species. Soil samples were sifted through a 2-mm sieve while still moist. Thereafter, samples were extracted by using a Berlese-Tullgren funnel for one day as it is the most useful tool for extracting large quantities of mites living in soil and the debris of date palm trees, citrus trees and organic manure (Upton, 1991; McSorely and Walter, 1991).

Preserving the mites for study: The mites were preserved in small vials with 80% alcohol. The addition of 5% glycerol is recommended to prevent mites from drying out if the alcohol evaporates. The vials used for storage were small so that the mites could be easily found later.

Preparing the 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 the mites: Individual mites were singly mounted on glass slides in Hoyers fluid, covered with a glass cover and gently heated for one minute to stretch the mite bodies (Evans 1992; Krantz1978). 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.* (1996) and El-Benhawy *et al.* (2006). The taxonomy of the collected mites was carried out by using an Olympus Camera DP25 attached to an Olympus Microscope BX51.

Effect of herbicides on soil-dwelling predatory mites: Three different concentrations (half the recommended dose (HRD), recommended dose (RD) and double the recommended dose (DRD)) of glyphosate and metribuzin were tested. Five separate stock colonies of *L. dentatus*, *A. casalis*, *R. roseus*, *M. muscaedomesticae* and *C. setirostris* were maintained in the experimental laboratory. Each stock colony was divided into four treatment groups with 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 glyphosate and metribuzin, and a control (well water), were sprayed directly onto the Petri-dishes by using a small knapsack sprayer (1L). Dead mites were then counted three days after the application to determine the percentage of mortality.

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

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

Where: n = Number of soil-dwelling predatory mite, T = Treated, Co = Control.

The mortality percentages of the date palm mite were calculated manually by direct observation under a binocular. Thereafter, a Microsoft Excel Program was used to calculate the average obtained data. Statistically, all variables of the obtained data were analyzed using one-way analysis of variance (ANOVA).

RESULTS

The toxicity of glyphosate and metribuzin on soil-dwelling predatory mites, *L. dentatus*, *A. casalis*, *R. roseus*, *M. muscaedomesticae* and *C. setirostris* was evaluated under laboratory conditions. All five species of soil-dwelling predatory mite were found to be susceptible to both herbicides (glyphosate and metribuzin) as both herbicides can kill significant numbers of these mites at all concentrations tested (HRD, RD and DRD) compared with the control (well water).

Table 1. Effect of three concentrations of glyphosate on the mortality of five different adult predatory soil mite species *L. dentatus*, *A. casalis*, *R. roseus*, *M. muscaedomesticae* and *C. setirostris* under laboratory conditions.

Predatory species	Concentrations of glyphosate	No. of predatory mites(Adults)		
		Average pre-spray count	Average post-spray count *	Reduction % **
L. dentatus	Control	20.00	20.00	0.00 a
	HRD	20.00	8.00	60.00 b
	RD	20.00	3.00	85.00 c
	DRD	20.00	0.00	100.00 d
A. casalis	Control	20.00	18.00	0.00 a
	HRD	20.00	9.00	50.00 b
	RD	20.00	3.00	83.34 c
	DRD	20.00	0.00	100.00 d
R. roseus	Control	20.00	18.00	0.00 a
	HRD	20.00	8.00	55.60 b
	RD	20.00	3.00	83.34 c
	DRD	20.00	1.00	94.45 d
M. muscaedomesticae	Control	20.00	19.00	0.00 a
	HRD	20.00	9.00	52.70 b
	RD	20.00	3.00	84.30 c
	DRD	20.00	0.00	100.00 d
C.setirostris	Control	20.00	20.00	0.00 a
	HRD	20.00	14.00	30.00 b
	RD	20.00	2.00	90.00 c
	DRD	20.00	1.00	95.00 c

* Average counts made three days post treatment. ** Mortality values calculated with the Henderson-Tilton equation. Values indicated by a different letter in the column are significantly different from each other at $P < 0.05$. RD recommended dose of glyphosate; HRD half recommended dose and DRD double recommended dose.

Table 1. Effect of three concentrations of metribuzin on the mortality of five different adult predatory soil mite species *L. dentatus*, *A. casalis*, *R. roseus*, *M. muscaedomesticae* and *C. setirostris* under laboratory conditions.

Predatory species	Concentrations of metribuzin	No. of predatory mites(Adults) Average pre-spray count	Average post-spray count *	Reduction % **
L. dentatus	Control	20.00	20.00	0.00 a
	HRD	20.00	8.00	60.00 b
	RD	20.00	2.00	90.00 c
	DRD	20.00	0.00	100.00 d
A. casalis	Control	20.00	19.00	0.00 a
	HRD	20.00	15.00	21.10 b
	RD	20.00	3.00	83.34 c
	DRD	20.00	0.00	100.00 d
R. roseus	Control	20.00	20.00	0.00 a
	HRD	20.00	12.00	40.00 b
	RD	20.00	4.00	80.00 c
	DRD	20.00	1.00	95.00 d
M. muscaedomesticae	Control	20.00	17.00	0.00 a
	HRD	20.00	6.00	64.80 b
	RD	20.00	2.00	88.00 c
	DRD	20.00	0.00	100.00 d
C. setirostris	Control	20.00	20.00	0.00 a
	HRD	20.00	11.00	45.00 b
	RD	20.00	4.00	80.00 c
	DRD	20.00	1.00	95.00 d

*Average counts made three days post treatment. ** Mortality values calculated with the Henderson-Tilton equation. Values indicated by a different letter in the column are significantly different from each other at $P < 0.05$. RD recommended dose of metribuzin; HRD half recommended dose and DRD double recommended dose.

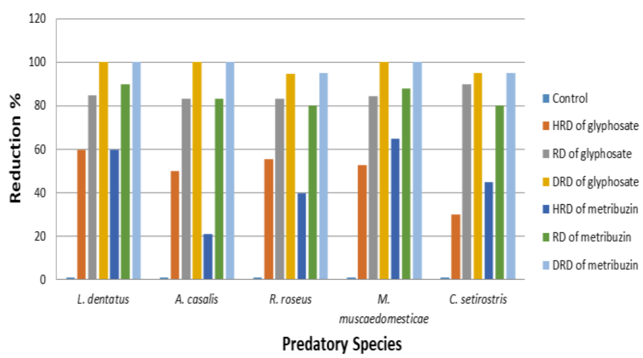

Figure 1. Comparison of the effects of three different concentrations of glyphosate and metribuzin (HRD, RD and DRD) and the control (well water) on the mortality of five different adult predatory soil mite species, *L. dentatus, A. casalis, R. roseus, M. muscaedomesticae* and *C. setirostris* under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

In addition, glyphosate and metribuzin can significantly decrease their populations by >80% and 90% at RD and DRD, respectively (Table 1, 2). Clearly, the RD and DRD of glyphosate and metribuzin showed a slight difference on the mortality of all five species of soil-dwelling predatory mites, whereas both herbicides differ from each other on the mortality percentages at HRD. For example, HRD of glyphosate can reduce the population of all five species of soil-dwelling predatory mites by > 50% except *C. setirostris*, as its mortality was 30%, whereas HRD of metribuzin only reduces the population of *L. dentatus* and *M. muscaedomesticae* by > 50% (Figure 1). Furthermore, there was a significant decline in the population of soil-dwelling predatory mites at each concentration compared with the control of each species. Obviously, glyphosate seems to be more toxic to all five species of soil-dwelling predatory mites. Moreover, *L. dentatus* was found to be the most sensitive soil-dwelling predatory mite, whereas *C. setirostris* was found to be the most insensitive to both herbicides compared with all the other tested soil-dwelling predatory mites (Figure 1).

DISCUSSION

Unfortunately, several beneficial and predatory insects and mites have become targets of many chemical compounds, including pesticides, due to the extensive use of these chemical compounds forvarious industrial, agricultural and residential purposes. This includes the misuse of these chemicals, such as exceeding the recommended dose or mixing more than one agent together to achieve higher mortality. It seems unknown that such use can directly cause a sharp reduction in the population of several predatory insects and mites. Therefore, the main purpose of this study was to evaluate the toxicity of glyphosate and metribuzin on five species of soil-dwelling predatory mites, *L.dentatus*, *A. casalis*, *R. roseus*, *M. muscaedomesticae* and *C. setirostris*. The application of the two herbicides had a direct effect on the mortality of all five species of soil-dwelling predatory mites compared with the control (well water). The application of both herbicides produced a sharp reduction in the population of all soil-dwelling predatory mites tested in this study, especially at DRD.

Mohammed *et al.* (2017) documented that soil plots treated with glyphosate or multrazine were found to significantly reduce the population of soil microarthropods. They also highlighted that three Collembolla species (*Cryptophagus sp*, *Dicyrtoma sp* and *Tomocerus sp*) were completely eliminated from all treated plots with low, standard and high concentrations of both herbicides. Travlos *et al.* (2017) reported that HRD of metribuzin can cause a reduction in the weight of earthworms by approximately 22% compared with untreated ones. They also found a 70% survival rate of earthworms five days after exposure to DRD of metribuzin.The results obtained here were consistent with their findings as DRD of both herbicides (glyphosate and metribuzin) can cause a sharp reduction in the population of soil-dwelling predatory mites, *L.dentatus*, *A. casalis*, *R. roseus*, *M. muscaedomesticae* and *C. setirostris* (>90%). The results of this study showed that different herbicides have different effects on the population of soil-dwelling predatory mites, as glyphosate was found to have greater effects on the mortality of all five species of soil-dwelling predatory mite than metribuzin (Figure 1). Thus, the rank of sensitivity was *L. dentatus*, *M. muscaedomesticae*, *A. casalis*, *R. roseus* and *C. setirostris*for glyphosate and *L. dentatus*, *M. muscaedomesticae*, *C. setirostris*, *R. roseus* and *A. casalis* for metribuzin. Moreover, *L. dentatus* was found to be the most sensitive soil-dwelling predatory mite, whereas *C. setirostris* was found to be the least sensitive to both herbicides compared with all the tested soil-dwelling predatory mites.

In contrast with the obtained results, glyphosate has often been found to be less toxic than other herbicides, especially when assessed under field conditions. Therefore, it can be claimed that the effects of the herbicides might be influenced by many environmetal factors such as rainfall, soil type and climate condition.

Sandy soil is known to have a low content of organic matter, which influences the community structure as well as the abundance and diversity of soil microorganisms. As a result of the high demand for both herbicides, itis very necessary to highlight how dangerous they are, not only tothe target species but also to many beneficial mites which live in the soil. As sandy soil already has a low number and little diversity of soil microorganisms, it is especially susceptible to herbicide use. Therefore, unnecessary application must be avoided and the recommended dose of herbicides and pesticides must not be exceeded in order to keep beneficial insects and mites at a suitable population level. Further investigations are required to assess the possible risks for humans from such herbicides, especially those who apply them or live near a treated area.

***Conclusion*:** This study has demonstrated the effect of two herbicides (glyphosate and metribuzin) on the populations of soil-dwelling predatory mite, *L.dentatus*, *A. casalis*, *R. roseus*, *M. muscaedomesticae* and *C. setirostris*. It indicated that all three concentrations (HRD, RD and DRD) of both herbicides can significantly reduce the population of these soil-dwelling predatory mites (>90%) compared with the control. Glyphosate was found to be more toxic than metribuzin. Therefore, extra care and serious caution must be taken when diluting and applying these herbicides to never exceed their RDs as they can significantly reduce the populations of all soil-dwelling predatory mites studied here, which will lead in turn to a sharp reduction in their numbers as well as a suppression of their role in the ecological balance.

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