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Biological impact of insect growth regulators (IGRs) on the development of the oribatid mite *Scheloribates laevigatus* (Acari: Oribatida: Scheloribatidae) in the laboratory

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Abstract

The biological impact of insect growth regulators (IGRs) pesticides on ecologically important soil oribatid mites' fauna was evaluated using life history parameters in *Scheloribates laevigatus* (Koch) (Acari : Oribatida: Scheloribatidae) , under microcosm conditions. Four novel IGR pesticides namely, Easo 30% WG (Indoxacarb), Kafrozed 5% EC (Chlorfluazuron), Alsystin 48% SC (Triflumuron) and Klegron 10% EC (Flufenoxuron) were tested. Laboratory fresh adults were reared on plaster of Paris (9:1) supplied with fungal food treated with agricultural doses of tested IGR pesticides. There was negligible mortality of individuals in control vessels 3.23%, Klegron 10% EC caused the highest mortality of 35.16 %, and significantly decreased the longevity of adults to 17.26. The growth rate of *S. laevigatus* decreased in IGRs reared vessels with moderate influence of Kafrozed 5% EC and Alsystin 48% SC, while a significantly toxic effect of Klegron 10% EC on all instars was recorded ($P<0.05$). Furthermore, fecundity declined to 1.8 /day in Easo 30% WG and 1.1 in Klegron 10% EC than the control vessels. Chitin formation in adults was decreased and the hard brown exoskeleton looks pale in all reared vessels treated with IGR pesticides.

Introduction

Oribatid mites are usually among the most abundant and species richness arthropods in soils (Behan, 1978 and Danks, 1981). *Scheloribates laevigatus* (Koch) (Acari: Oribatida: Scheloribatidae) is a cosmopolitan species of oribatid mites. They are characterized by hard exoskeletons and long life cycles. The activity of oribatid mites and other detritivores animals enhances soil fertility, formation of humus and nutrient recycling. A little information about their demography and life histories population biology under stressed condition is known. Suppression of oribatid mites can result in soil compaction and thatch accumulation (Franzluebbers, 2004 and Potter, 1993).

Insect growth regulators (IGR) are new approach compounds that adversely affect insects by regulating or inhibiting specific biochemical pathways or processes essential for insect growth and development, disrupting the process of

growth, development, and metamorphosis (Ishaaya and Horowitz, 1997). The insects exposed to such compounds may die due to abnormal regulation of hormone-mediated cells on certain physiological regulatory processes essential to the normal development of insects or their progeny (Tunaz and Uygun, 2004). Therefore, does not necessarily be toxic to its target, but may lead to various abnormalities in the insect that impair survival and reduce their reproductive potential (Siddall, 1976). The IGR pesticides can inhibit the production of chitin, and the affected insects become unable to synthesize new cuticles, and to successfully molt into the next stage (Ijumba *et al.*, 2010).

Although generally effective of IGR on pest control, it may adversely disturb the development of non-target invertebrates that have useful ecological functions. All pesticides that are applied to crops reach a considerable portion of the soil. The impact of such treatments of IGR on Non-target invertebrates is poorly developed.

Therefore, the present experiments were conducted to evaluate the toxicity effect of four IGRs, on reproduction and growth parameters in *S. laevigatus.* This species is a relevant bioassay model, is an ecologically important soil micro-arthropod, occurs abundantly in moist and fertile topsoil, and can be reared in the laboratory throughout the year.

Materials and methods

Four novel IGR pesticides namely, Easo 30% WG (Indoxacarb), Kafrozed 5% EC (Chlorfluazuron), Alsystin 48% SC (Triflumuron) and Klegron 10% EC (Flufenoxuron) were selected for the study, they are recommended for fruit trees and cotton crops.

1. Mite extraction and identification:

Samples were collected from grapes orchard soil. Micro-arthropods were extracted using a Berlese funnel into water cups. The extracted animals were poured into a Petri-dish where the oribatid mites were sorted from the rest of the micro-arthropods. Collected mites were preserved in 70% ethyl alcohol; the proper clearing technique involves placing the mites into lactic acid for two weeks for further identification. Temporary mounts of specimens were made and examined under a binocular stereomicroscope, using keys and fine taxonomic details provided in Balogh and Balogh (1990) and Krantz and Walter (2009).

2. Toxicity test on life history parameters:

The treatments were conducted in plastic vessels (3 cm diameter and 3.5 cm height) filled to half with a mixture of plaster of Paris and charcoal (calcium hydroxide-charcoal" mix in a ratio 9:1). Charcoal substrate was made into a smooth paste with distilled water. Laboratory fresh adults were reared on (5 mm Ø) agar discs of *Rhizoctonia solani* fungal diet treated previously with one ml of the recommended agricultural doses of each pesticide solution in pre-equipped (9 cm) culture plates of PDA medium. long-term microcosm studies were conducted to compare the sub-lethal effects on reproduction and growth in *Scheloribates laevigatus* using the recommended agricultural doses of Easo 30% WG at 150 ppm (15 g /100 L) in Grapes, Kafrozed 5% EC at 500 ppm (100 g / ha), Alsystin 48% at 262.5 ppm (26.25 cm^3/ 100L) in grabs, and Klegron 10% at 1000 ppm (200 cm^3/ acre). Five replicates rearing vessels for each pesticide solution were carried, untreated control vessels were used for comparison. The rates of fecundity, juvenile success and mortality were recorded at regular 3 days intervals. The vessels were kept at room temperature,

fungal diets were renewed at 3 days intervals. For each test, data were subjected to analysis of variance (ANOVA), followed by Tuckey's HSD test for comparison of treatments versus a control.

Results and discussion

Results of studies on the impact of IGR pesticides on *S. laevigatus* are presented in Table (1). The lethal effect of the pesticides on emerged instars was evident in treated vessels. There was negligible mortality of adults in control rearing vessels 3.23% during the experimental period (Figure1). Klegron 10% EC was the highly toxic substance causing the highest mortality of 35.16 % among emerged instars. Longevity in adults recorded 17.26 while in control vessels was 102.55. A significant decrease in life span ($P<0.05$) was also recorded than the control ones. Furthermore, a noticed pale exoskeleton formed in adults emerged through the experiment.

Alsystin 48% SC and Easo 30% WG treatments showed moderate toxicity to mites' offspring. However, survived immature recorded 24.59 for Easo 30% WG and 23.18 for Alsystin 48% SC. The IGR pesticide Kafrozed 5% EC. was the least toxic effect substance on individuals.

Similarly, IGR pesticides affected the rate of egg production in *S. laevigatus* and fecundity. In control specimens adults laid (3.8 per day) declined in all treated vessels; a significant decrease was found for the lowest fecundity 1.1 eggs per day in Klegron 10% EC treatment ($P<0.05$). The long-term sub-lethal toxicity of IGRs on *S. laevigatus* was evident from a low survival of juveniles. The juvenile success in the control set was 58.41 but decreased significantly to 12.79% ($P<0.05$) in Klegron 10% EC treated vessels. Among the chemicals tested, Kafrozed 5% EC had minimum inhibited major life history parameters than Alsystin 48% SC and Easo 30% WG, whereas Klegron 10% EC had a strong lethal effect on immature and adults ejected. The IGR pesticides also affected chitin synthesis in the dorsal shell of *S. laevigatus* by decreasing the formation of heavily sclerotized shell in adults. However, there were no rates of low chitin synthesis in control vessels (Figure 2). The findings of the present study showed that agricultural doses of the IGRs are harmful to the well growth and reproduction of *S. laevigatus*.

Soil invertebrate populations are influenced by soil factors; several studies have demonstrated a relationship between soil factors and micro-arthropod community composition (Badejo and Akinwole, 2006). Four novel IGR pesticides namely, Easo 30% WG (Indoxacarb), Kafrozed 5% EC (Chlorfluazuron), Alsystin 48% SC (Triflumuron) and Klegron 10% EC (Flufenoxuron) were selected for the study. These chitin synthesis inhibitors can disrupt the development of immature stages of insects and are very effective against several groups of insect pests (Deng *et al.*, 2008; Praveena *et al.*, 2011 and Rajasekar and Jebanesan, 2012).

Indoxacarb has a wide range of controlling insect pests, such as tomato fruit worms, potato tuber moth, tunnel makers' cotton leaf worms and grape worms. It is registered in many countries of the world and used on many field crops, vegetables, and fruits. Indoxacarb is a modern group of chemicals that has some side effects on mites, it closes sodium channels inside nerve cells, so the larvae stop feeding and moving then paralyzed and die quickly (Mann, 2003–2004).

Chlorfluazuron is used globally under the Atabron brand to control Lepidoptera on cotton, strawberries and

fruit trees and vegetables. It inhibits chitin synthesis in insects. In addition to Triflumuron chitin synthesis inhibitor pesticide acting as inhibition of moulting, it is used to control Lepidoptera, Diptera and Coleoptera on soya beans, fruit and vegetable trees (Mann, 2003–2004). Flufenoxuron is a type of acaricide for controlling mites and other insect pests, is a chitin synthesis inhibitor, it moderately persists in soil. It applies to fruit including apples, cherry grape, citrus, strawberry, cotton, and some vegetables (Lewis *et al*., 2016).

Preliminary screening studies showed negligible short-term lethal effect of the IGRs chemicals on Oribatid mites at agriculturally relevant concentrations; LC_{50} values were not estimated in the present study. Therefore, long-term microcosm studies were conducted to compare the sub-lethal effects on reproduction and growth in *S. laevigatus*, especially since it is a long-life cycle species, a model of beneficial agriculture animals living in the top layer of fresh unpolluted soil. They have heavily sclerotized dorsal surfaces formed of chitin. Therefore, the rates of growth and fecundity in *S. laevigatus* are considered potential indices of the impact of xenobiotics in soil and to evaluate ecotoxicity of chemical pollutants. Lebrun (1980) studied the applicability of non-target micro-arthropod species as bioindicators of pesticide residues in soil and showed that some species of Collembola are suitable for bioassay studies. However, microcosm studies under controlled laboratory conditions are essential to validate the impact of toxicants in field conditions.

Present findings suggest that sub-lethal toxic effects of IGRs on life history parameters in the oribatid mite *S. laevigatus* are indications of ecological imbalances in the soil. Wardle *et al.* (2000) exhibited that, physical and chemical stresses can reduce the taxonomic and functional diversity of beneficial soil organisms like Oribatida and Collembola and affect the ecological ecosystem.

Although many reports supposed the high specificity of IGR insecticides for pest control, and low toxicity to non-target organisms, because they act as chitin synthesis inhibitors, ecdysone agonists or juvenile hormone analogues (Tunaz and Uygun, 2004). But the present study recorded some impact of IGRs on non-target animals. Some authors have demonstrated biological ill effects of IGRs on non-target organisms, which suggest ecological consequences. Many studies reported ill effects of several IGRs on growth and reproduction in worker bumblebees *Bombus terrestris* (Mommaerts *et al*., 2006). Our present study is also compatible with Wang *et al*. (2012) in earthworm and Campiche *et al.* (2006) who showed that IGRs namely methoprene, fenoxycarb, precocene II, tebufenozide, hexaflumuron, and teflubenzuron produced significant ill effects like chitin synthesis inhibition, mortality of adults and juveniles, decreased fecundity, etc. on *Folsomia candida* at environmentally relevant concentrations. Tripathi and Sharma (2005) showed that cypermethrin significantly reduced the population of Acari, Coleoptera, and Collembola, but increased the population of other soil arthropods. Therefore, the negative implications of xenobiotics on non-target detritivores like oribatida would cause imbalances in the structure and function of the soil ecosystem.

Table (1): Duration (In days) of developmental stages of *Scheloribates laevigatus* exposed to IGR pesticides in laboratory rearing vessels.

Control		
Developmental stages	**Number**	**Development time (days)**
Egg	3.8±0.01	2.5±0.13
Larva	4.6±0.19	5.21±0.07
Protonymph	8.3±0.12	8.61±0.21
Deutonymph	17.01±0.18	18.02±0.14
Tritonymph	28.5±0.03	33.11±0.06
Sum of Juveniles	58.41±0.52	
Life cycle		67.45±0.61
Longevity		102.55± 0.58
Life span		169.45±0.03

Data are the mean of five Replicates vessels ±standard deviation.

Easo 150 ppm		
Developmental stages	**Number**	**Development time (Days)**
Egg	1.8±0.05	2.1±0.16
Larva	1.3±0.14	6.31±0.01
Protonymph	3.2±0.05	10.01±0.11
Deutonymph	6.05±0.03	22.06±0.04
Tritonymph	14.04±0.13	35.01±0.03
Sum of Juveniles	24.59±0.35	
Life cycle		69.49.±0.35
Longevity		70.71± 0.05
Life span		140.2±0.4
Kafrozed 500 ppm		
Developmental stages	**Number**	**Development time (Days)**
Egg	2.3±0.01	3.4±0.13
Larva	2.3±0.06	6.28±0.01
Protonymph	5.2±0.07	9.81±0.01
Deutonymph	8.11±0.06	22.12±0.06
Tritonymph	17.04±0.05	36.16±0.7
Sum of Juveniles	32.65±0.24	
Life cycle		73.77±0.09
Longevity		81.94±0.13
Life span		155.71±0.22
Alsystin 262.5 ppm		
Developmental stages	**Number**	**Development time (Days)**
Egg	2.6±0.03	2.8±0.16
Larva	2.9±0.11	5.21±0.07
Protonymph	2.2±0.18	13.41±0.11
Deutonymph	6.04±0.04	23.08±0.12
Tritonymph	12.04±0.15	37.11±0.09
Sum of Juveniles	23.18±0.48	
Life cycle		80.61±0.55
Longevity		58.14±0.23
Life span		138.75±0.78
Klegron 1000 ppm		
Developmental stages	**Number**	**Development time (Days)**
Egg	1.1±0.03	3.6±0.04
Larva	1.5±0.08	8.01±0.03
Protonymph	1.2±0.11	12.21±0.01
Deutonymph	2.05±0.14	24.02±0.06
Tritonymph	8.04±0.07	38.15±0.07
Sum of Juveniles	12.79±0.4	
Life cycle		85.99±0.21
Longevity		17.26±0.17
Life span		103.25±0.38

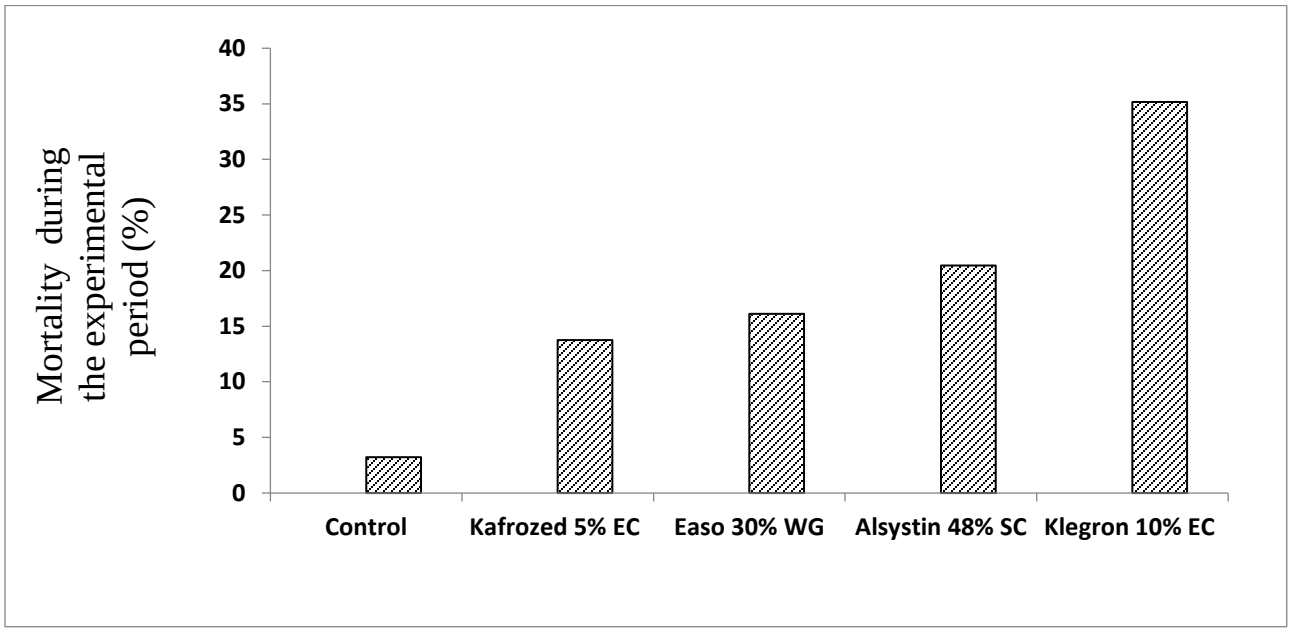

Figure (1): Impact of tested IGR insecticides on survival of *Scheloribates laevigatus* individuals.

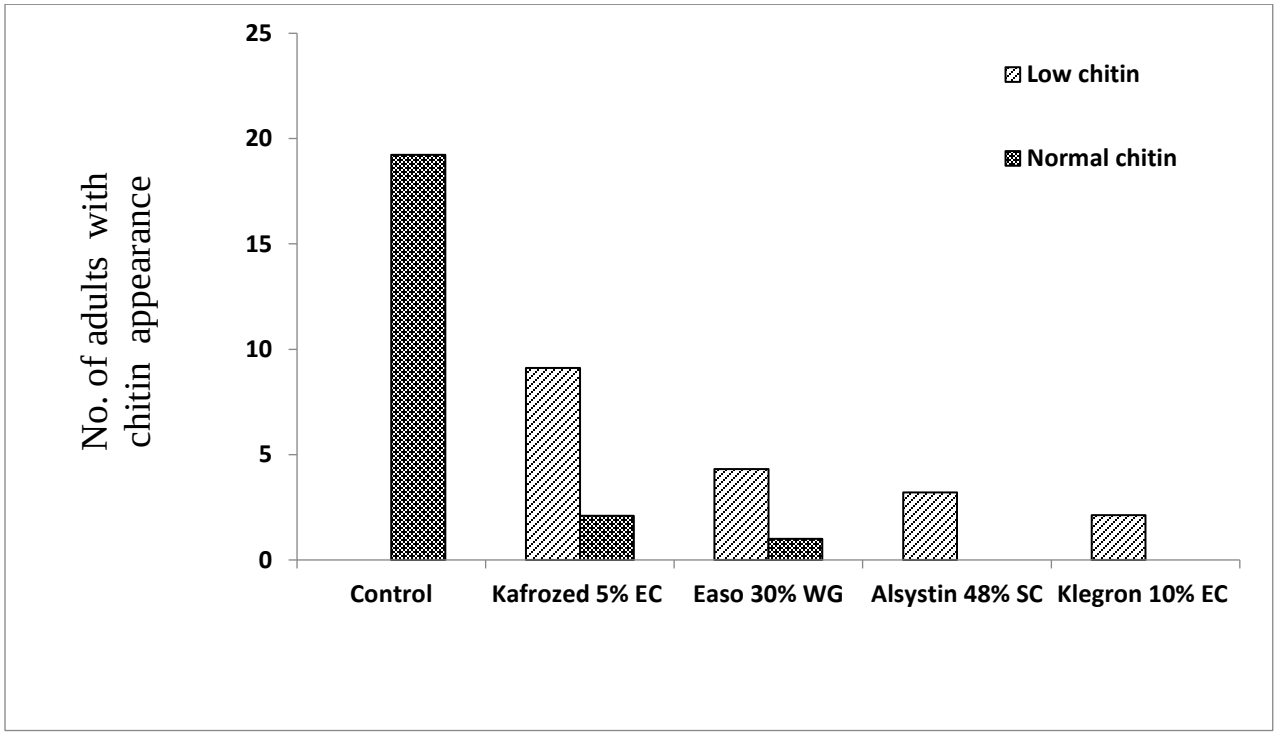

Figure (2): number of adult *Scheloribates laevigatus* emerged with low chitin appearance.

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