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POTENTIAL ILL EFFECTS OF IGR PESTICIDES ON LIFE-HISTORY PARAMETERS IN ECOLOGICALLY IMPORTANT SOIL COLLEMBOLA *CYPHODERUS JAVANUS* BORNER

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Abstract: The possible ill effects of insect growth regulator (IGR) pesticides on ecologically important soil microarthropod fauna was evaluated using life history parameters in *Cyphoderus javanus* Borner (Collembola: Insecta) under microcosm conditions. Laboratory reared fresh adults were kept for 16 days on sandy loam lateritic soil treated with agricultural doses of Applaud 25SC (Buprofezin), Takumi 20WG (Flubendiamide), and Rimon 10EC (Novaluron), and a pyrethroid Colt 25EC (Cypermethrin) was used as reference. There was negligible mortality of adults in control (3.1%), but the dose-related lethal effect of IGRs caused highest mortality (33.3%) and lowest survival success ($P<0.01$) in Applaud 2000 ppm treated soil. Growth rate of *C. Javanus* decreased in IGRs treated soil; rate of moulting decreased from 0.42 day^{-1} in control to 0.05 day^{-1} in Takumi 500 ppm treated soil ($P<0.05$). Similarly, fecundity declined to 1.8 day^{-1} in Applaud 2000, as against 4.2 day^{-1}in control ($P<0.01$). Survival rate of juveniles declined from 91.1% in control, to 67.6% in 500 ppm Applaud ($P<0.05$). Other pesticides affected juvenile success at higher doses, to 64.5% in Takumi 2000 ppm ($P<0.01$), and to 39.2% in Rimon 4000 ppm ($P<0.01$) treated soils. The above trends were comparable with the ill effects of Colt at 1000 ppm dose. Among the IGR chemicals, Rimon produced minimum ill effects, and Applaud and Takumi probably affected chitin synthesis. These life history parameters in *C. javanus* are suitable for ecotoxicological evaluations of xenobiotics in tropical soils.
Key words: IGR pesticides, Soil Collembola, Life history, Ecotoxicology.

Introduction

The indiscriminate use of synthetic organic pesticides and inorganic fertilizers has resulted in the deterioration of crop yield, soil quality, and ecological equilibrium of agricultural lands, mainly in the tropical regions. Therefore attempts are being made to develop methods that are economically viable, and ecologically safe to the system. An example is the third generation pesticides like insect growth regulators (IGR) that are recently being used for the control of pests and parasites in agriculture and public health. These hormone analogues or endocrine disrupting chemicals elicit their action on the insect metabolism, ultimately disrupting the process of growth, development and metamorphosis, particularly when applied during the sensitive period of insect development (Ishaaya and

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Horowitz, 1997). On the other hand, a considerable portion of all pesticides that are applied on crops reach the soil, and can disturb the non-target organisms and their ecological functions. The biological community in soil is comprised of a diverse range of microbes and invertebrates, and their synergistic interactions are crucial for ecological functions like decomposition, recycling of nutrients, breakdown of pollutants, etc. (Novak et al., 2010). Among the major groups of soil fauna, microarthropods surpass others in density and diversity, and a predominant group namely Collembola is very vulnerable to soil pollution (Fountain and Hopkin, 2005). A temperate Collembola, *Folsomia candida* Willem has the status of a standard biomarker species (ISO, 1999), and is employed for testing the ill effect of chemicals on soil invertebrates (Hopkin, 1997). However, this type of ecotoxicological studies on soil microarthropods is rare in India, and there is no ‘standard’ biomarkers established, even though many differences exist in the life history parameters of microarthropods between the temperate and tropical soils. The IGR pesticides can inhibit the production of chitin, and the affected insects become unable to synthesize new cuticle, and to successfully moult into the next stage (Ijumba et al., 2010). However, the ecological safety of these chemicals is not properly understood, and limited information is available on endocrine disruption in invertebrates (Lintelmann et al, 2003). Therefore, the present experiments were conducted to evaluate the sub-lethal toxicity of three IGRs, and a pyrethroid on reproduction and growth parameters in *Cyphoderus javanus* Borner (Collembola, Insecta). This species is ecologically relevant to the tropical soil conditions and is a potential bioassay model, because it is very sensitive to the edaphic changes, occurs abundantly in moist and fertile topsoil, and can be reared in the laboratory throughout year.

Materials and Methods

Rearing of Collembola and collection of samples

Adult specimens of *Cyphoderus javanus* were collected from field soils by floating on water (Choi et al., 2008), and reared in polythene vessels (6.5 cm diameter, 7.5 cm height), having 2 cm layer of moist soil, and few granules of Baker’s yeast as food source. The vessels were kept under controlled temperature (27±1 °C), moisture (50% WHC), and light dark cycle of 14: 10 h in a Biological Oxygen Demand incubator. Freshly emerged juveniles were reared separately by transferring the adults into new vessels, and the laboratory reared specimens of same age group were used for the experiments. The lateritic soil sample (0-15 cm topsoil, pH 6.2, EC 0.11 $mScm^{-1}$, WHC 24.2%, OC 0.74%) was collected from a local agricultural field with no history of chemical pollution. It was air-dried and passed through 1.0 mm mesh sieve to remove the fauna, stones and plant parts, and stored in labelled packets. Three novel IGR pesticides namely Applaud 25SC (Buprofezin), Takumi 20WG (Flubendiamide), and Rimon 10EC (Novaluron) 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; Rajasekar and Jebanesan, 2012). A common broad spectrum pyrethroid insecticide namely Colt 25EC (cypermethrin) was used for comparison of the toxicity on *C. javanus*.

Toxicity screening on life history parameters

Preliminary screening studies showed negligible short-term lethal effect of the IGR chemicals on *C. javanus* at agriculturally relevant concentrations; hence LC_{50} values were not estimated. Therefore, long-term microcosm studies were conducted to compare the sub-lethal effects on reproduction and growth in *C. javanus* using the recommended agricultural doses of Applaud 25SC at 500 and 2000 ppm (62.5 g and 250 g a.i. ha^{-1}), Takumi 20WG at 500 and 2000 ppm (50 g and 200 g a.i. ha^{-1}), Rimon 10EC at 2000 and 4000 ppm (100 g and 200 g a.i. ha^{-1}), and Colt 25EC at 100 and 1000 ppm (12.5 g and 125 g a.i. ha^{-1}) in distilled water. The treatments were made in polythene vessels (6.5 cm diameter, 7.5 cm height); each containing 10 g moist soil, few granules of yeast, one ml of pesticide solution, and approx. 10 specimens of average 15 days of age group. Untreated control vessels were used for comparison, and the sets were maintained as stated above for 16 days. The rates of survival, fecundity, juvenile success, and number of exuvia were recorded at regular 24 h intervals.

Results

Results of screening studies on the impact of IGR pesticides on *C. javanus* are presented in Table-1 and Figure-I. There was negligible mortality of adults in untreated control (3.1%) during the 16 days experimental period, but dose-related lethal effect of the pesticides was evident in treated sets. Applaud at 2000 ppm dose produced highest mortality of 33.3%, and significantly decreased the survival success of adults to 81.3% of test population ($P<0.01$). In contrast, all other treatments showed insignificant differences with control set for mortality and survival success of adults. However, Colt at 1000 ppm dose produced 19.4% mortality and 81.8% survival success of adults during the same period. Similarly, IGR pesticides affected the rate of egg production in *C. javanus*; fecundity in control specimens (4.2 day^{-1}) declined in all treated sets, except in Rimon 2000 ppm treated set. However, significant decrease was found only for lowest fecundity (1.8 day^{-1}) in Applaud 2000 ppm treated soil ($P<0.01$). In contrast, the pyrethroid inhibited fecundity at both doses, to 1.3 day^{-1}at 100 ppm.

Table 1: Comparison of sub-lethal ill effects of IGR pesticides on important life history parameters in *C. javanus* (values in parenthesis show significance level (t test) of differences between control and treated sets; * = P < 0.05, ** = P < 0.01, *** = P < 0.001)

Treat-ments	Repli-cations	Initial number	Average survival	Survival success %	Number of eggs	Number of juveniles	Hatching success %	Number of exuvia
Control	18	10.72 ± 0.44	10.54 ± 0.42	98.28	43.80 ± 5.93	39.91 ± 6.14	91.12	4.40 ± 1.01
Applaud 500 ppm	6	10.83 ± 0.75	9.11 ± 1.01	84.05	24.25 ± 6.15	16.40 ± 4.77(t = 2.12 *)	67.61	1.70 ± 0.37
Applaud 2000 ppm	8	10.13 ± 0.13	8.24 ± 0.46(t = 3.25 **)	81.33	15.14 ± 1.77(t = 3.16 **)	6.38 ± 2.23(t = 3.56 ***)	42.16	2.06 ± 0.44
Takumi 500 ppm	6	11.0 ± 0.52	9.86 ± 1.10	89.59	34.18 ± 8.95	26.66 ± 7.61	77.99	0.46 ± 0.17(t = 2.21 *)
Takumi 2000 ppm	8	10.75 ± 0.37	9.74 ± 0.56	90.56	24.33 ± 5.89	15.70 ± 2.85(t = 2.55 *)	64.54	1.07 ± 0.22(t = 2.21 *)
Rimon 2000 ppm	13	11.54 ± 0.39	11.04 ± 0.55	95.67	48.44 ± 7.28	28.13 ± 4.63	58.07	3.68 ± 0.87
Rimon 4000 ppm	10	12.0 ± 0.39	10.98 ± 0.43	91.46	33.11 ± 6.27	12.99 ± 3.02(t = 3.13 **)	39.25	4.04 ± 0.98
Colt 100 ppm	6	10.0 ± 0.89	9.32 ± 0.61	93.23	12.42 ± 3.89(t = 2.95 **)	11.14 ± 4.21(t = 2.60 *)	89.68	2.67 ± 0.84
Colt 1000 ppm	6	12.0 ± 0.00	9.81 ± 1.32	81.78	2.92 ± 1.14(t = 3.91***)	2.32 ± 1.40(t = 3.45 **)	79.64	1.82 ± 0.50

(P<0.01), and 0.3 day^{-1}at 1000 ppm (P<0.001). The long-term sub-lethal toxicity of IGRs on *C. javanus* was evident from low survival success of juveniles. The juvenile success in control set was 91.1%, but decreased significantly to 67.6% (P<0.05), and 42.2% (P<0.001) in Applaud 500, and 2000 ppm treated sets, respectively. In case of other IGR pesticides the juveniles were affected by higher doses, causing 64.5% (P<0.01) survival in Takumi 2000 ppm, and 39.2% (P<0.01) survival in Rimon 4000 ppm treated soil. However, Colt produced significant decrease in both doses: 89.7% (P<0.01) in 100 ppm, and 79.6% (P<0.01) in 1000 ppm. The IGR pesticides also affected growth rate of *C. javanus*, by decreasing the rate of moulting. Rate of moulting in control specimens was 0.42 day^{-1}, which decreased in treated sets. The ill effect was severe in Takumi, with only 0.05 moultsday^{-1}in 500 ppm (P<0.05), and 0.11 moults day^{-1} in 2000 ppm (P<0.05) treated specimens. However, lower rates of moulting in other treated sets including that of Colt were statistically insignificant. The above findings showed that agricultural doses of the IGRs are harmful to the growth and reproduction of *C. javanus*. Among the chemicals tested, Rimon had minimum ill effects, whereas Applaud and Takumi inhibited major life history parameters, as observed in the case of broad-spectrum pyrethroid.

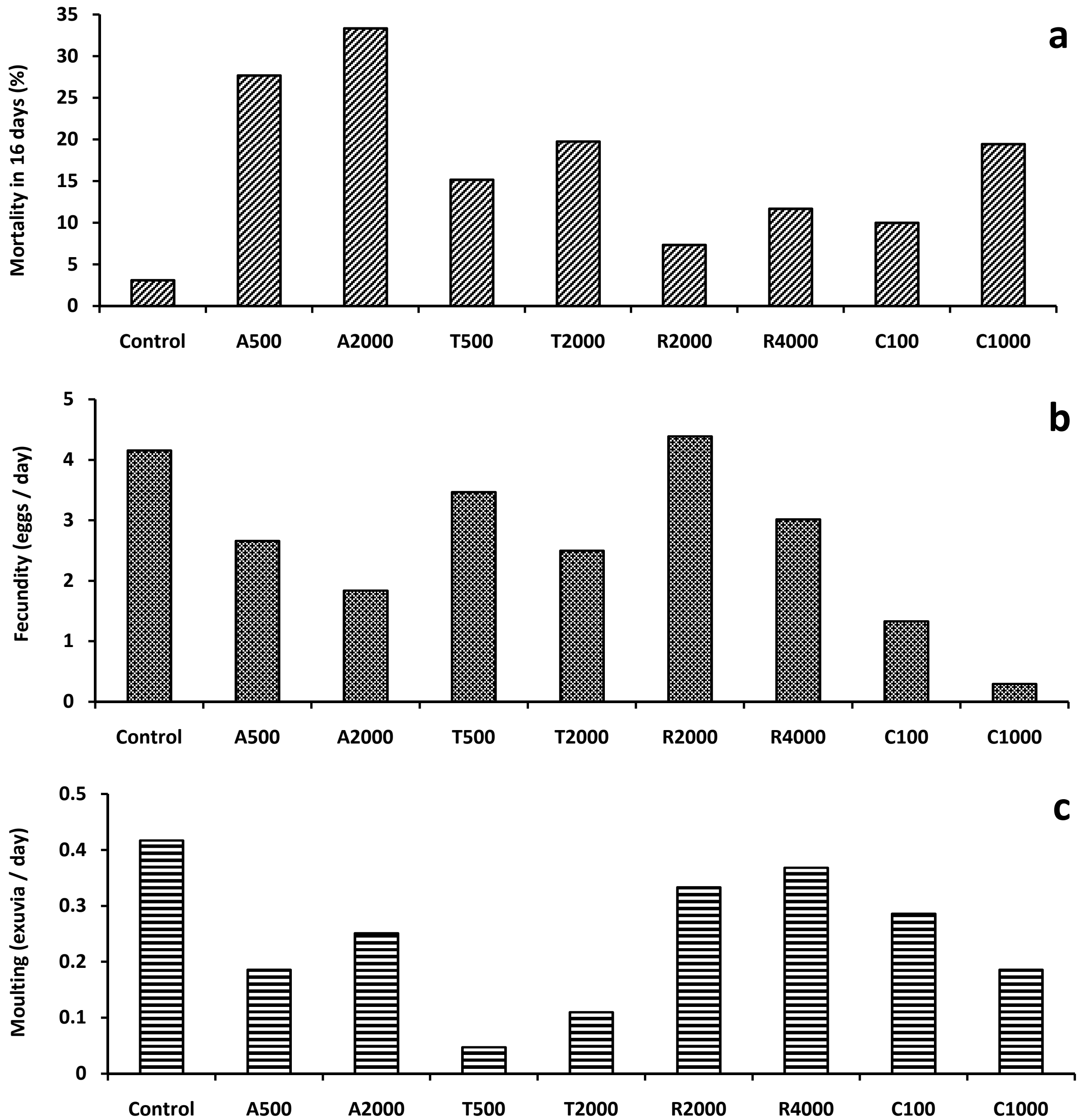

Figure I: Comparison of sub-lethal ill effects of IGR pesticides on adult survival (a), fecundity (b), and rate of moulting (c) in *C. javanus*. (values on X-axis show the doses of pesticides in ppm; A = Applaud, T = Takumi, R = Rimon, C = Colt).

Discussion

Collembola are primitive insects that grow in size and moult frequently throughout the adult life. Similarly, adults lay eggs for an indefinite period in fresh unpolluted soil. Therefore the rates of moulting and fecundity in *C. Javanus* are considered potential indices of the impact of xenobiotics in soil. The Collembola reproduction test was developed to evaluate ecotoxicity of chemical pollutants, and this assay is widely used because it is very sensitive, and breeding is also easy (Riepert, 1995). Lebrun (1980) advocated on the applicability of non-target microarthropod species as bioindicators of pesticide residues in soil, and showed that some species of Collembola are suitable for bioassay studies. However, microcosm setups under controlled laboratory conditions and standard protocols are essential to identify specific biomarker indices, and to validate the impact of toxicants in field condition. Gorney (1975) and Eijsackers (1980) reported that some species of Collembola are potential animals for laboratory screening. Similarly, Joy and Chakravorty (1991) showed that among different Collembola species *Cyphoderus sp*. was more sensitive than *Xenylla sp*. to short-term direct toxicity of several insecticides. Present findings suggest that sub-lethal toxic effects of IGRs on life history parameters in Collembola are indications of ecological imbalances in soil. According to Wardle et al. (2000) anthropogenic disturbances that produce physical and chemical stresses can reduce taxonomic and functional diversity of soil organisms like Collembola, and affect resistance and resilience of ecosystem.

The IGR pesticides are supposed to have high specificity for insect pests, and low toxicity to non-target organisms, because they act as chitin synthesis inhibitors, ecdysone agonists or as juvenile hormone analogues. Insects treated with chitin synthesis inhibitors failed to moult, and were unable to become adults and to reproduce (Tunaz and Uygun, 2004). However, a few authors have demonstrated biological ill effects of IGRs on non-target organisms, which suggest ecological consequences. There are reports that showed ill effects of several IGRs on growth and reproduction in worker bumblebees *Bombus terrestris* (Mommaerts et al., 2006), in housefly *Musca domestica* (Abo El-Mahasen et al., 2010), and in earthworm, *Eisenia fetida* (Wang et al., 2012). But comparable information is lacking on ecologically important soil animals. According to Hammock and Quistad (1981) toxicity of teflubenzuron and hexaflumuron on *F. Candida* (Collembola) was probably due to inhibition of chitin synthetase, the final enzyme in the pathway of chitin synthesis from glucose. Campiche et al. (2006) 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 *F. candida* at environmentally relevant concentrations. The present microcosm study showed that sub-lethal effects of Applaud and Takumi on growth and reproduction in *C. javanus* were comparable with the effects of Colt (cypermethrin). The pyrethroids are typical contact poisons, and so, cypermethrin may decrease the population of sensitive groups like Collembola. 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 Collembola would cause imbalances in the structure and function of soil ecosystem.

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References

[1] Abo El-Mahasen, M.M., Assar, A.A., Khalil, M.E. and Mahmoud, S.H., 2010. Biological effects of some insect growth regulators on the house fly, *Musca domestica* (Diptera: Muscidae). *Egypt. Acad. J. Biolog. Sci.*, 3(2): 95-105.

[2] Campiche, S., Becker-van, S.K., Ridreau, C. and Tarradellas, J., 2006. Effects of insect growth regulators on the non target soil arthropod *Folsomia candida* (Collembola). *Ecotoxicol. Environ. Saf.*, 63(2): 216-225.

[3] Choi, W., Neher, D.A. and Ryoo, M., 2008. Life-history trade-offs of *Paronychiurus kimi* (Lee) (Collembola: Onychiuridae) populations exposed to paraquat. *Ecotoxicol. Environ. Saf.*, 69: 227-232.

[4] Deng, L., Xu, M., Cao, H. and Dai, J., 2008. Ecotoxicological effects of buprofezin on fecundity, growth, development, and predation of the wolf spider *Pirata piratoides* (Schenkel). *Arch. Environ. Contam. Toxicol.*, 55: 652–658.

[5] Eijsackers, H., 1980. Assessment of the toxic effects of the herbicide 2,4,5-T on the soil fauna by laboratory tests. In: Soil biology as related to land use practices. (Ed) D.L. Dindal., Proc. VIIth, Int. Soil Zool. Colloq., Int. Soc. Soil Sci., Syracuse. pp. 427-440.

[6] Fountain, M.T. and Hopkin, S.P., 2005.*Folsomia candida* (Collembola): A “standard” soil arthropod. *Ann. Review. Entomol*. 50: 201-222.

[7] Gorney, M., 1975. Studies on the influence of industrial pollution on soil animals in pine stands: aims and methods of the soil block model experiment. In: Progress in soil zoology. (Ed) J. Vanek., Proc. Vth Int. Colloq. Soil Zool., Academia, Prague. pp. 357-362.

[8] Hammock, B.D. and Quistad, G.B., 1981. Metabolism and mode of action of juvenile hormone, juvenoids, and other insect growth regulators. In: Progress in pesticide biochemistry. Vol.1. John Wiley and Sons, Ltd.

[9] Hopkin, S., 1997. Biology of the springtails (Insects: Collembola). Oxford University Press. Oxford. 330 pp.

[10] Ijumba, J.N., Mtana, R.R. and Suya, T.B., 2010. Bio-efficacy of novaluron, a chitin-synthesis inhibitor against larval stages of *Culex quinquefasciatus* and *Anopheles gambiae SensuLato. Euro. J. Sci. Res*., 47(3): 484-493.

[11] Ishaaya, I. and Horowitz, A.R., 1997. Insecticides with novel mode of actions: Overview., pp. 1-39. In: "Insecticides with novel mode of actions, Mechanisms and application" (Eds.) Ishaaya, I. and Degheele, D., Berlin.

[12] ISO., 1999. Soil quality-Inhibition of reproduction of Collembola (*Folsomia candida*) by soil polluants. Geneva: International Standards Organisation.

[13] Joy, V.C. and Chakravorty, P.P., 1991. Impact of insecticides on non target microarthropod fauna in agricultural soil. *Ecotoxicol. Environ. Saf*. Academic press, 22: 8-16.

[14] Lebrun, Ph., 1980. A preliminary study of the use of some soil mites in bioassays for pesticide residue detection. In: Soil biology as related to land use practices. (Ed) D.L. Dindal., Proc. Viith Int. Soil Zool. Colloq., Int. Soc. Soil Sci., Syracuse. pp. 42-55.

[15] Lintelmann, J., Katayama, A., Kurihara, N., Shore, L. and Wenzel, A., 2003. Endocrine disruptors in the environment (IUPAC Technical Report). *Pure. Appl. Chem*. 75(5): 631-681.

[16] Mommaerts, V., Sterk, G. and Smagghe, G., 2006. Hazards and uptake of chitin synthesis inhibitors in bumble bees *Bombus terrestris*. *Pest Manage. Sci*., 62: 752-758.

[17] Novak, P., Vopravil, J. and Lagova, J., 2010. Assessment of the soil quality as a complex of productive and environmental soil function potentials. *Soil & Water. Res*. 5(3): 113-119.

[18] Praveena, Y.V., Kotikal, Y.K., Awaknavar, J.S., Kenchanagoudar, P.V. and Somashekar, 2011. Evaluation of newer insecticides against groundnut leaf miner *Aproaerema modicella* Deventer. *Karnataka J. Agric. Sci*., 24(4): 542-545.

[19] Rajasekar, P. And Jebanesan, A., 2012. Efficacy of IGRs compound novaluron and buprofezin against *Culex quinquefasciatus* mosquito larvae and pupal control in pools, drains and tanks. *Int. J. Res. Biol. Sci.*, 2(1): 45-47.

[20] Riepert, F., 1995. First report of the second international ring test on a method for determining the effects of chemicals or soil contaminants on the reproduction of Collembola. Report of the Biologische bundesanstaltfu¨ r Land- und Forstwirtschaft Institutfu¨ r Chemikalienpru ¨ fung.

[21] Tripathi, G. and Sharma, B.M., 2005. Effects of habitats and pesticides on aerobic capacity and survival of soil fauna. *Biomed. Environ. Sci.*, 18: 169-175.

[22] Tunaz, H. and Uygun, N., 2004. Insect growth regulators for insect pest control. *Turk J. Agric. For.*, 28: 377–387.

[23] Wang, Y., Cang, T., Zhao, X., Yu, R., Chen, L., Wu, C. and Wang, Q., 2012. Comparative acute toxicity of twenty-four insecticides to earthworm, *Eisenia fetida. Ecotoxicol. Environ. Saf.*, doi:10.1016/j.ecoenv.2011.12.016.

[24] Wardle, D.A., Bonner, K.I. and Barker, G.M., 2000. Stability of ecosystem properties in response to above-ground functional group richness and composition. *Oikos*. 89: 11–23.