ISJ 15: 31-38, 2018 ISSN 1824-307X

RESEARCH REPORT

Differential impact of pesticides and biopesticides on edaphic invertebrate communities in a citrus agroecosystem

MZ Majeed[1,2], M Naveed[2], MA Riaz[2,3], C-S Ma[1], M Afzal[2]

[1]State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, PR China
[2]Department of Entomology, College of Agriculture, University of Sargodha, 40100, Sargodha, Pakistan
[3]Department of Entomology, University of Georgia, Athens, GA 30602, USA

Accepted January 08, 2018

Abstract

Edaphic invertebrate fauna is usually exposed directly or indirectly to a wide range of pesticides in agroecosystems worldwide. Very few studies have assessed the negative effects of these pesticides on the diversity and population dynamics of soil invertebrates. In this study, the effect of most commonly used pesticides *viz*; bifenthrin (a synthetic pyrethroid), spinosad (a bio-insecticide), Aliette (a synthetic fungicide) and *Trichoderma harzianum* formulation ($30x10^6$ cells mL^{-1}; a bio-fungicide) was assessed on soil invertebrate fauna in a citrus agroecosystem. Secondary objective was to compare the impact of synthetic versus biological pesticides and insecticides versus fungicides. There was a significant effect of all pesticides on the population abundance of springtails ($F_{4,14}$ = 16.53; p<0.001), mites ($F_{4,14}$ = 12.07; p<0.001) and ants ($F_{4,14}$ = 16.28; p<0.001). By and large, soil fauna got recovered after two to three weeks post-treatment. Insecticides were more suppressive for soil invertebrates than fungicides. Overall, biological pesticides *i.e.* spinosad and *T. harzianum* formulation were less disruptive to soil invertebrate fauna than synthetic conventional pesticides. Hence, keeping in view the key role of soil invertebrates in soil sustainability and crop productivity, the utilization of biopesticides should be encouraged.

Key Words: bio-pesticides; fungicides; insecticides; population abundance; soil invertebrates

Introduction

Citrus is an important fruit crop around the globe. However, its production is hampered by numerous species of insect pests including psyllids, leafminers, fruit flies and scales, and diseases including canker, greening and downy mildews (Anjum and Javaid, 2005; Tahir *et al.,* 2015). In order to control these pests and to protect their crop and yield, farmers indiscriminately and recurrently use a wide range of synthetic pesticides including insecticides and fungicides (Monzo *et al.,* 2014).

Although these pesticides provide the control of insect pests and diseases because of their rapid knockdown effect and save considerable yield loss by reducing the pest infestation, but concomitantly these agro-chemicals have many non-target effects including disruption of beneficial organisms, insecticide resistance, pest resurgence and human health hazards (Edwards, 2013; Monzo *et al.,* 2014). Most of the pesticides being used by citrus growers in Indo-Pak region are broad spectrum and highly persistent in the environment diminishing many beneficial fauna along with the target pests (Ashraf *et al.,* 2014). Regarding pesticide side effects, soil invertebrates are one of the non-target organisms which may be exposed to all pesticides either directly to spray splashes and drift or indirectly by contacting pesticidal residues on foliage, soil, litter and thatch (Bünemann *et al.,* 2006; Larson *et al.,* 2014).

Agricultural soils harbor a considerable diversity of invertebrates often classified as micro and mesofauna including collembolans, mites and tiny insects etc., and macro-fauna including earthworms, termites, ants, beetles and spiders etc. (Lavelle *et al.,* 1997; Kocourek *et al.,* 2013). These soil invertebrates play a crucial role in different ecological processes such as organic matter decomposition and nutrients cycling, and are indispensable for soil biological functioning and

Corresponding author:
Muhammad Zeeshan Majeed
State Key Laboratory for Biology of Plant Diseases and Insect Pests
Institute of Plant Protection
Chinese Academy of Agricultural Sciences
Beijing 100193, PR China
E-mail: zeeshan.majeed@uos.edu.pk

Table 1 List of treatments used in this study

Treatment No.	Treatment	Application rate
1	Bifenthrin (synthetic pyrethroid)	625 ml ha^{-1}
2	spinosad (microbial bio-insecticide)	150 ml ha^{-1}
3	Aliette (fosetyl-Al; synthetic fungicide)	7 kg ha^{-1}
4	*Trichoderma harzianum* (bio-fungicide)	$30x10^6$ conidia mL^{-1}
5	Control (water)*	-

*water used in control plots was same as used for preparation of pesticides spray mixtures.

sustained crop productivity in agro-ecosystems (Lavelle *et al.,* 1997; Lardo *et al.,* 2012; Majeed, 2012; Bagyaraj *et al.,* 2016). Soil fauna, particularly meso- and macro-fauna, improve soil physico-chemical conditions through their feeding (ingestion, digestion and ejection) and foraging (tunneling, boring, mining, movement) activities (Lee and Pankhurst, 1992; Edwards and Bohlen, 1996; Lavelle *at al.,* 1997; Jouquet *et al.,* 2006; Coleman and Wall, 2015; Bagyaraj *et al.,* 2016).

Keeping in view the ecological importance of soil invertebrates and lack of information regarding the side effects of most commonly and widely used pesticides in indigenous citrus agroecosystems on the soil invertebrate fauna, this study sought to compare the impact of different type of pesticides (insecticides and fungicides) on the density (abundance) and diversity (community assemblage) of non-target soil invertebrates. Our *a priori* hypothesis was that conventional pesticides would have more severe effects on soil fauna than bio-pesticides. To achieve these objectives, different pesticides were applied according their label-recommended dose rates on the under canopy area of citrus plants and pre- and post-application data regarding soil invertebrate fauna was collected for different time intervals up to two months.

Materials and Methods

The study area is situated in the district Sargodha of the province of Punjab (Pakistan) and is characterized by semi-arid sub-tropical climatic conditions with mean annual temperature and precipitation of 23 °C and 450 mm, respectively. Citrus is the principal fruit crop of Sargodha region. Study was conducted in private citrus orchards of kinnow mandarin (cv. *Citrus reticulata*) (32°05'N and 72°40'E) situated in the vicinity of the College of Agriculture, University of Sargodha. Orchard age was approximately 12 years and was not treated with any pesticide for last nine weeks. Fifteen healthy and equal sized citrus trees were selected and tagged at random leaving rows of plants on all sides of the orchard as buffer zone to avoid edge effect. Five treatments as given in Table 1 including four most commonly used pesticides by indigenous citrus growers and one control (sprayed only with tap water) were applied on 30 April, 2017 on the under-canopy soil area of the citrus trees according to randomized complete block (RCB) design with five replications for each treatment. Pesticides were applied according to their label-recommended dose rates using a back-mounted knapsack pump sprayer from a height of 1.5 m to mimic the pesticide spray drift. Physico-chemical characteristics of the study soil were also determined for pre-treatment soil samples (Table 2).

Pesticides included bifenthrin (a synthetic pyrethroid insecticide), spinosad (a microbial bio-insecticide), Aliette (an aluminium based (fosetyl-Al) synthetic fungicide) and formulation of *Trichoderma harzianum* (a bio-fungicide). There were two motives behind the selection of these pesticides. First was to have a comparative assessment of the impact of different pesticides (insecticides and fungicides) and different pesticide groups (*i.e*. synthetic and biological) on soil non-target fauna.

Table 2 Physico-chemical characteristics of the soil of citrus orchards studied

Soil characteristic	Condition/Content
Soil texture	Sandy loam
pH	7.8 (0.03)
ECe (µS cm^{-1})	2410.0 (221.4)
Soil organic matter (g kg^{-1})	8.1 (0.29)
Soil organic-C (g kg^{-1} soil)	4.7 (0.16)
Total soil N (mg kg^{-1})	408.6 (15.01)
$NaHCO_3$ extractable-P (mg kg^{-1} soil)	7.9 (0.40)
Extractable-K (mg kg^{-1} soil)	163.4 (5.65)

Values are means of five independent soil composite samples along with standard errors within parenthesis.

Table 3 Diversity indices of different edaphic faunal (invertebrate) groups in citrus orchard soils treated with different types of pesticides

Diversity Indices	Shannon Wiener's Diversity Index					Richness Index					Evenness Index				
Treatments	1DBT	3DAT	15DAT	30DAT	60DAT	1DBT	3DAT	15DAT	30DAT	60DAT	1DBT	3DAT	15DAT	30DAT	60DAT
Insecticide	1.56	1.54	1.22	1.44	1.40	10.00	7.00	7.00	10.00	7.00	0.68	0.79	0.63	0.62	0.72
Bio-insecticide	1.39	1.53	1.13	1.23	1.51	9.00	9.00	7.00	10.00	9.00	0.63	0.70	0.58	0.53	0.69
Fungicide	1.44	1.35	1.20	1.59	1.51	9.00	8.00	9.00	9.00	10.00	0.65	0.65	0.55	0.72	0.66
Bio-fungicide	1.44	1.44	1.04	1.70	1.46	10.00	9.00	7.00	11.00	8.00	0.63	0.66	0.53	0.71	0.70
Control	1.47	1.55	1.74	1.90	1.68	9.00	9.00	9.00	11.00	10.00	0.67	0.71	0.79	0.79	0.73

DBT: Days before treatment; DAT = Days after treatment

Second criterion was that these pesticides were the most commonly used by citrus famers in Sargodha region as assessed from a preliminary survey of local pesticide dealers and citrus growers. Three 0 - 15 cm deep soil samples were collected from each treatment 2 days before, 3, 15, 30 and 60 days after application of treatments. Each sample (weighing about 1,750 g) was the composite of four sub-samples taken randomly from four sides of the treated plant using 10x10 cm metallic soil corer. These samples were brought to the laboratory of the Department of Entomology and data regarding soil invertebrate fauna was taken. Macro-invertebrates were collected and enumerated from each sample manually, while meso- and micro-invertebrates were extracted from samples by installing them on Tullgren-Berlese funnel for 24 h. Extracted invertebrates were preserved in 50% ethanol solution in transparent 20 ml plastic vials for their further identification and enumeration under light microscope up to higher taxonomic (order, genus or family) level.

Statistica® version 7.1 (StatSoft®, France) was used for statistical interpretation of data. Normality of data was checked and data were transformed by $\log_{10}$ (X+2) before further analyses where normality was not met. Data regarding soil fauna was subjected to 2-way factorial analysis of variance with treatment and time interval as factors and two-sample student's T-tests were used to compare pesticides and/or their groups. For effect of each pesticide on soil invertebrates, one-way ANOVA was applied at 95% confidence level followed by Tukey's highest significant difference (HSD) tests to compare treatment means. For faunal (invertebrates) community assemblage determination, Shannon-Wiener's index, faunal group richness and evenness indices were calculated as described by Ahmed *et al.* (2017) along with the graphical presentation (pie charts) of data. Moreover, multivariate analysis of significance was also run to assess the impact of pesticides on soil invertebrate fauna.

Results

Impact of pesticides on the diversity of soil faunal groups

Results have demonstrated that Shannon-Wiener diversity index, which estimates the relative richness and abundance of different groups or species of organisms collected and sampled from different locations (Shannon-Wiener, 1963), fluctuated among different time intervals for all treatments (Table 3). Maximum diversity index (1.90) was found for control treatment at 30DAT (days after treatment) while minimum was recorded for bio-fungicide at 15DAT (Table 3) For all pesticide treatments, diversity of soil invertebrate faunal groups reduced for the first two weeks post-treatment as compared to the diversity index of control treatment which was least perturbed during the entire period of experiment. After 15 days post-treatment, Shannon-Wiener index increased to its maximum at 30 DAT for all treatments but then reduced to normal level at 60DAT.

Pesticide treatments had a differential impact on the evenness index of soil faunal groups. Evenness index measures the relative abundance of species or organismal groups of an area. In case of insecticide (bifenthrin), maximum evenness (0.79) was observed just after three days of spray but then reduced slightly as compared to control (before spray) value. In case of bio-insecticide (spinosad), minimum evenness value (0.58) was observed at 30DAT and then returned to normal (control) value after one month. In case of fungicides, both synthetic (Aliette) and bio-fungicide (*T. harzianum* formulation) showed similar response regarding their effect on fauna group evenness with minimum values (0.55 and 0.53, respectively) at 15DAT (Table 3). Similarly, soil faunal (invertebrate) groups' richness was reduced up to 15DAT, then increased to a maximum value of 11.00 at 30DAT, and again decreased up to normal level at 60DAT (Table 3). Among treatments, synthetic fungicide showed minimum values of richness of soil faunal groups as compared to others, while insecticide (bifenthrin) showed maximum fluctuation in faunal group richness index (Table 3).

Effect of pesticides on community assemblages of invertebrate faunal groups

Gross higher-level taxonomic composition of treated soil samples have been presented in the form of pie charts (Fig. 1). According to this graphical presentation of invertebrate fauna encountered in soil samples, collembolans and mites were the most abundant faunal groups found in all samples, followed by ants, spiders and rove beetles. The least dominant groups were oligochaeta (earthworms) and carabid beetles. The

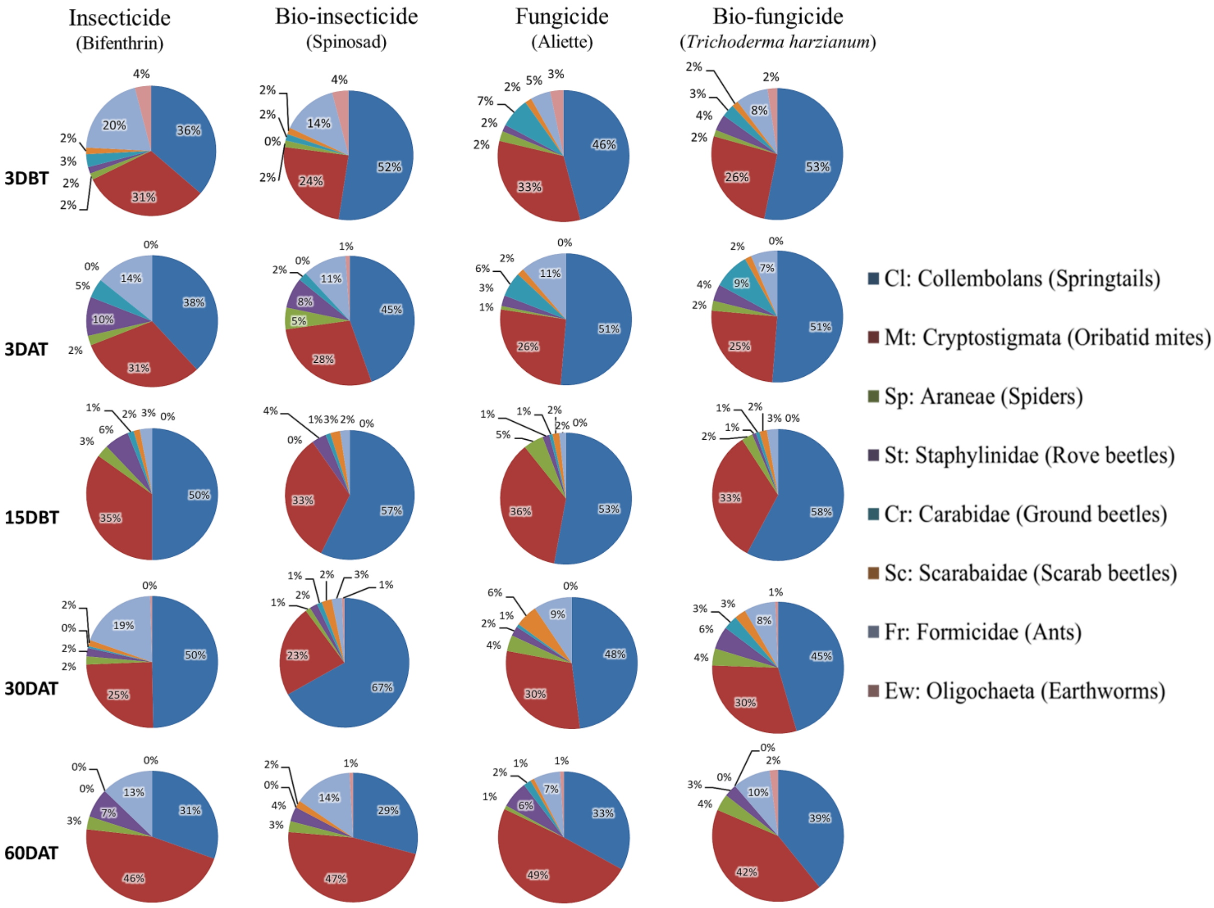

Fig. 1 Pie-charts showing community assemblages of edaphic faunal (invertebrate) groups in a citrus agroecosystem at different time intervals in response to application of different types of pesticides. (DBT = days before treatment; DAT = days after treatment).

most prominent change occurred in the community assemblages of soils treated with insecticide (bifenthrin) and bio-insecticide (spinosad) till 15 days post-exposure. The most effected faunal groups were ants and collembolans (Fig. 1). For the first month of experiment, the faunal community assemblages remained dominant by collembolans (springtails) in all samples while oribatid mites (cryptostigmata) outnumbered all other invertebrate faunal groups in the last observation at 60 days post treatment.

Impact of pesticides on population abundance of different soil faunal groups

Collembola (springtails), cryptostigmata (oribatid mites), formicidae (ants) and araneae (spiders) were the most abundant and dominant faunal groups in the study soils of citrus orchard. Therefore, the response of only these faunal groups towards different pesticides was further analyzed statistically apart from its graphical representation (Fig. 2). In case of micro-invertebrates (collembola and cryptostigmata), insecticide (bifenthrin) reduced three times the average population of springtails and oribatid mites at 3DAT than their control population (*i.e.* 15.0 springtails and 13.3 mites sample^{-1}). The maximum population of springtails and mites reached at 30DAT (*i.e.* 35.0 springtails and 16.3 mites sample^{-1}). However, in case of all treatments including control, population of springtails suddenly decreased at 60DAT except mites (Fig. 2). Similarly, bio-insecticide (spinosad) had a little but significant effect while fungicide and bio-fungicide had no significant effect on population abundance of springtails and oribatid mites.

Similarly, regarding macro-invertebrates (formicidae and araneae), a similar trend had been observed for ants. While in case of spiders, there was no clear-cut trend regarding the impact of pesticides on their population dynamics. Maximum population of ants and spiders (*i.e.* 8.67 ants and 3.67 spiders sample^{-1}) were recorded at 30DAT and 60DAT respectively, while the minimum ones (*i.e.* 0.67 ants and 0.33 mites sample^{-1}) at 15DAT and 60DAT, respectively. In general, population of all faunal groups in control soils, which were treated with water only, gradually increased till 30DAT, and then decreased at 60DAT for all groups except oribatid mites which continued to increase till 60DAT. However, in control (water) treatment, spider population increased gradually from start to the end of experiment at 60DAT (Fig. 2).

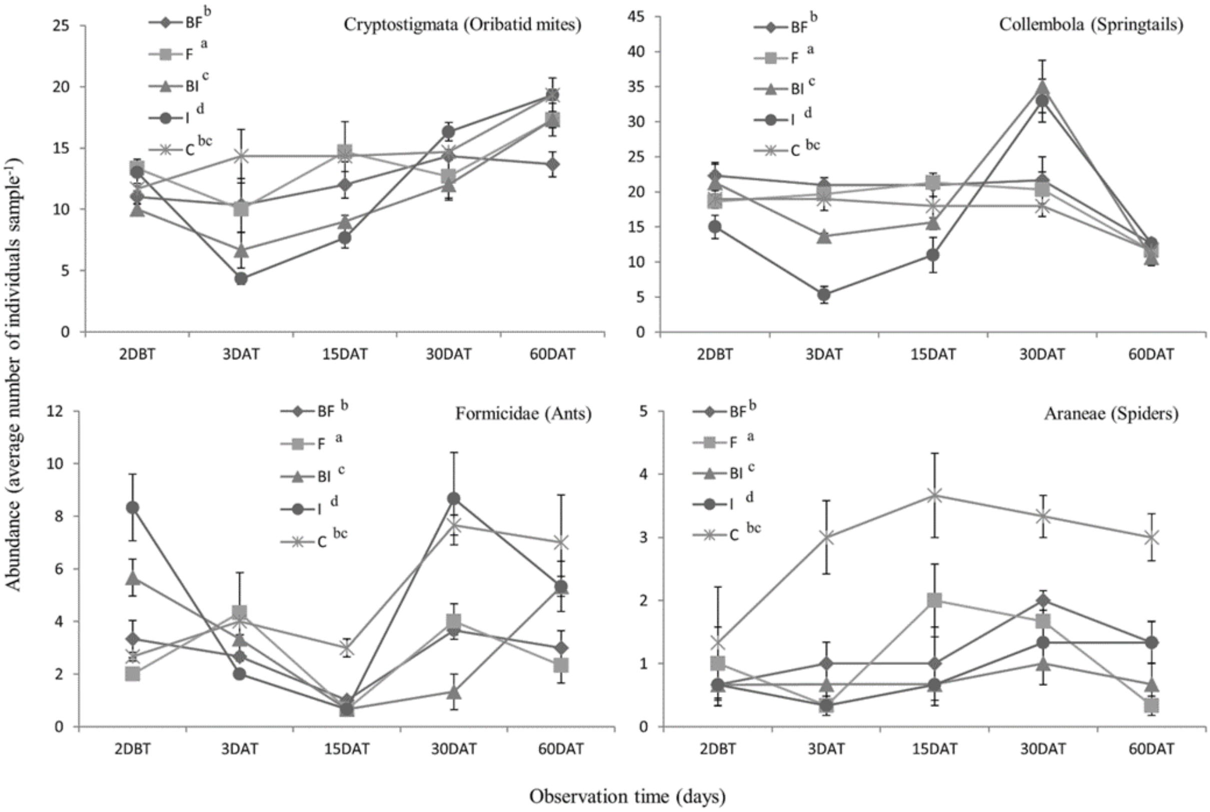

Fig. 2 Impact of different types of pesticides on the population abundance of major edaphic invertebrate groups in a citrus agroecosystem. Data points represent population means ± standard error (n = 4). For each faunal group, treatments bearing same superscripted letters are not statistically different from each other (factorial (two factor) ANOVA; $\alpha = 0.05$). (DBT = days before treatment; DAT = days after treatment; BF = bio-fungicide; F = fungicide; BI = bio-insecticide; I = Insecticide; C = control).

According to two-way factorial analysis of variance, insecticide (bifenthrin) had the most drastic and significant effect on population abundance of all edaphic faunal groups followed by bio-insecticide (spinosad), while the least disturbing pesticide for soil invertebrate fauna was formulation *T. harzianum* (bio-fungicide) (Fig. 2). Fungicide (Aliette) exhibited an intermediate response (Fig. 2). Overall, there was a significant effect of pesticides on the population abundance of springtails ($F_{4,14} = 16.53$; $p<0.001$), oribatid mites ($F_{4,14} = 12.07$; $p<0.001$), formicid ants ($F_{4,14} = 16.28$; $p<0.001$) but non-significant for the population abundance of spiders ($F_{4,14} = 1.51$; $p=0.212$). Similarly, observation times had a significant effect on the population abundance of springtails ($F_{4,14} = 4.46$; $p<0.01$), oribatid mites ($F_{4,14} = 3.01$; $p=0.027$), formicid ants ($F_{4,14} = 6.60$; $p<0.001$) and spiders ($F_{4,14} = 9.27$; $p<0.001$). However, the interaction of treatment and time exhibited significant effect on the population of all faunal groups except spiders (araneae). Nevertheless, according to multivariate analysis, all treatments (pesticides) and time intervals exhibited a significant ($p < 0.05$) effect on overall population abundance of soil invertebrate fauna encountered in soil samples of different treatments, except for time effect of bio-fungicide ($p = 0.2455$; Table 4).

Discussion

In sustainable agriculture, one of the contemporary ecological issues regarding extensive use of pesticides is their deleterious effects on non-target organisms including soil invertebrate fauna (Edwards, 2013; Pisa *et al.,* 2015). Beneficial edaphic invertebrate fauna is usually exposed directly or indirectly to a wide range of pesticides (Frampton and van den Brink, 2006; Adamski *et al.,* 2009; Larson *et al.,* 2014). This study was carried out to determine the impact of different types of pesticides on edaphic soil fauna in a citrus orchard. Two types of pesticides *i.e.* insecticides and fungicides with different origins *i.e.* synthetic and biological were evaluated under field conditions. Most commonly used insecticides selected in this study were bifenthrin and spinosad, while fungicides were Aliette and *T. harzianum* formulation containing 30×10^6 cells mL^{-1}. Soil fauna was sampled, identified up to group or order level and enumerated at regular time intervals for two months post treatment. The overall effect of these pesticides on diversity of soil invertebrate fauna was evaluated by calculating three diversity indices *i.e.* Shannon-Wiener diversity index, faunal group evenness index and faunal group richness index, while the abundance of major soil faunal groups was compared

Table 4 Multivariate analysis of significance for the effect of different types of pesticides on the abundance of edaphic faunal (invertebrate) groups in citrus orchard soils

Treatments/Effect	Wilk's Lambda value	*F*-value	Hypothesis df	Error df	*p*-value
Insecticide	0.000182	2061.87	8	3.00	0.0000 ***
Observation time	0.000026	6.51	32	12.66	0.0005 ***
Bio-insecticide	0.001722	331.34	7	4.00	0.0000 ***
Observation time	0.002005	2.60	28	15.84	0.0246 *
Fungicide	0.000939	608.02	7	4.00	0.0001 ***
Observation time	0.001874	2.66	28	15.84	0.0222 *
Bio-fungicide	0.001143	499.34	7	4.00	0.0000 ***
Observation time	0.011285	1.39	28	15.84	0.2455

*** $p < 0.001$; * $p < 0.01$; * $p < 0.05$; multivariate analysis (effective decomposition of the hypothesis) at α = 0.05

with control (water) treatment and with pre-treatment data of soil fauna as well.

Study results revealed that the insecticide bifenthrin, a synthetic pyrethroid, exerted considerable reduction of soil invertebrate fauna particularly of springtails, mites, ants and earthworms. Similar findings have been reported by Frampton and van den Brink (2007) and Larson *et al.* (2014) that pyrethroid insecticides like cypermethrin and bifenthrin had more pronounced negative effects on soil-dwelling fauna as compared to organophosphate (chlorpyriphos) and neonicotinoids (clothianidin). Similar effects of pyrethroid insecticides (deltamethrin and cypermethrin) on soil-inhabiting spiders have been described by Pekár and Beneš (2008). Nevertheless, Jänsch *et al.* (2006) also reported the most pronounced deleterious effects of pyrethroid and organophosphate insecticides to earthworms, spiders, mites and springtails. Hence, the continuous use of such toxic insecticides may reduce the diversity and abundance of soil non-target invertebrates (Bünemann *et al.,* 2006; Frampton and van den Brink, 2006; Adamski *et al.,* 2009).

Although spinosad, which is a bio-insecticide derived from metabolites of soil-borne actinomycete *Saccharopolyspora spinosa*, reduced the diversity and abundance of soil fauna particularly of springtails, mites and ants, however, it exhibited relatively less effect on soil fauna as compared to bifenthrin. Insecticides of biological origin such as spinosad are replacing conventional insecticides due to their higher target specificity, quick environmental biodegradation and more biorational and eco-friendly nature (Williams *et al.,* 2003; Biondi *et al.,* 2012). Nevertheless, the combined effect of insecticide and bio-insecticide on soil non-target fauna was also significant. Both type of chemicals suppressed population abundance and community assemblage of all soil faunal groups up to 15 days post-application. These observations are in accordance with those of Pekár and Beneš (2008) who demonstrated up to 100 % mortality of soil-inhabiting spiders by their exposure to few hours to 20 days old residues of insecticides (chlorpyrifos, cypermethrin and deltamethrin).

On the other hand, fungicide Aliette (fosetyl aluminum) exerted very little reduction of some soil fauna only for three days post-application while *T. harzianum* formulation did not affect soil fauna as much as insecticides. Al-Assiuty *et al.* (2014) described similar findings that bio-fungicides have less severe effect on community structure and population size of oribatid mites than synthetic fungicides. However, possible reasons for the observed little or no significant effects on non-targets in case of fungicides might be due to the poor diversity of invertebrates in study soils as well as the application of treatments on limited soil patches close to citrus plants as manifested by Babendreier *et al.* (2015). Nevertheless, the effects of both fungicides were non-significant. This observation is in context with the findings of Moreby *et al.* (1997) and Jänsch *et al.* (2006) who found that fungicides have less negative effect on soil dwelling invertebrates than insecticides. However, prolonged exposure to fungicides may reduce the population abundance of soil fauna either by exerting immediate changes in soil food web system (Jänsch *et al.,* 2006) or by affecting the behavior and long-term survival of soil invertebrates (Evans *et al.,* 2010).

Regarding community assemblages of different soil faunal groups, the most prominent changes were observed in case of micro-invertebrates (springtails and mites) and ants in response to insecticides. In case of fungicides, community assemblages of soil fauna remained almost identical at all sampling dates. This trend corroborates the findings of Moreby *et al.* (1997) and Al-Assiuty *et al.* (2014). Nevertheless, diversity of different edaphic faunal groups collected and identified during the study was influenced by the application of pesticides. In general, all diversity indices *i.e.* Shannon-Wiener' index, Species (faunal group) evenness and richness indices were lowered in the first two weeks of application and then either recovered to normal or increased from 15DAT to 30DAT levels. The same pattern of recovery was dominant in both micro-invertebrates (springtails and mites) and macro-invertebrates (ants, spiders, rove beetles and earthworms). Soil invertebrates usually have high recovery rates following the pesticidal application but repeated use of pesticides may exacerbate the situation of soil biological functioning (Desneux *et al.,* 2007; Iloba and Ekrakene, 2008; Evans *et al.,* 2010). However, there was a sudden decline in the diversity and abundance as well for all the treatments including control one. This trend might be due to the increased temperature in the month of June. Only mite population exhibited an increasing trend till 60DAT, which may be due the fact that mites survive better in hot and dry environment than other invertebrates (Behan *et al.,* 2003; Al-Assiuty *et al.,* 2014). Nevertheless, some insecticides such as neonicotinoids have shown a positive effect on the population abundance of mites (Pisa *et al.,* 2014).

Apart from four major faunal groups, *i.e.* springtails (collembola), mites (cryptostigmata), ants (formicidae) and spiders (araneae), rove (staphylinidae) and ground (carabaiedae) beetles were also encountered in all soil samples. Nevertheless, rove beetles showed the minimum impact of pesticides while ground beetles population showed a decreasing trend in all pesticidal treatments. These results are in line with those of Huusela-Veistola *et al.* (1996) who demonstrated that ground beetles are very sensitive to agrochemicals than other macro-invertebrates, particularly staphylinidae beetles (Honěk *et al.,* 2012).

Conclusion

The principal finding of this study is that synthetic pesticides (insecticides and fungicides) have more adverse effects on soil biological components such as soil invertebrate fauna than pesticides of biological origin. Therefore, keeping in view the key role of soil fauna in soil sustainability and crop productivity and the ecological implications of pesticides in citrus agroecosystems, farmers should opt for more eco-friendly plant protection options such as biopesticides.

References

Adamski Z, Błoszyk J, Piosik K, Tomczak K. Effects of diflubenzuron and mancozeb on soil microarthropods: a long-term study. Biol. Lett. 46: 3-13, 2009.

Al-Assiuty ANI, Khalil MA, Ismail AWA, van Straalen NM, Ageba MF. Effects of fungicides and biofungicides on population density and community structure of soil oribatid mites. Sci. Total Environ. 466: 412-420, 2014.

Anjum T, Javaid A. Major diseases of citrus in Pakistan: A review. Int. J. Biol. Biotechnol. 2: 793-796, 2005.

Ashraf S, Khan GA, Ali S, Iftikhar M, Mehmood N. Managing insect pests & diseases of citrus: on farm analysis from Pakistan. Pak. J. Phytopathol. 26: 301-307, 2014.

Babendreier D, Jeanneret P, Pilz C, Toepfer S. Non-target effects of insecticides, entomopathogenic fungi and nematodes applied against western corn rootworm larvae in maize. J. Appl. Entomol. 139: 457-467, 2015.

Bagyaraj DJ, Nethravathi CJ, Nitin KS. Soil biodiversity and arthropods: role in soil fertility. In: Chakravarthy AK, Sridhara S. (eds) Economic and ecological significance of arthropods in diversified ecosystems. Springer, Singapore: pp 17-51, 2016.

Biondi A, Mommaerts V, Smagghe G, Viñuela E, Zappalà L, Desneux N. The non-target impact of spinosyns on beneficial arthropods. Pest Manage. Sci. 68: 1523-1536, 2012.

Bogya S, Markó V. Effect of pest management systems on ground-dwelling spider assemblages in an apple orchard in Hungary. Agric. Ecosys. Environ. 73: 7-18, 1999.

Bünemann EK, Schwenke GD, Van Zwieten L. Impact of agricultural inputs on soil organisms-a review. Soil Res. 44: 379-406, 2006.

Chelinho S, Domene X, Andrés P, Natal-da-Luz T, Norte C, Rufino C, *et al*. Soil microarthropod community testing: A new approach to increase the ecological relevance of effect data for pesticide risk assessment. Appl. Soil Ecol. 83: 200-209, 2014.

Coleman DC, Wall DH. Soil fauna: occurrence, biodiversity, and roles in ecosystem function. Soil Microbiol. Ecol. Biochem. 111-149, 2015.

Desneux N, Decourtye A, Delpuech JM. The sublethal effects of pesticides on beneficial arthropods. Ann. Rev. Entomol. 52: 81-106, 2007.

Edwards, CA, ed. Environmental pollution by pesticides. Vol. 3. Springer Science & Business Media, 2013.

El-Naggar JB, Zidan NEHA. Field evaluation of imidacloprid and thiamethoxam against sucking insects and their side effects on soil fauna. J. Plant Prot. Res. 53: 375-387, 2013.

Evans SC, Shaw EM, Rypstra AL. Exposure to a glyphosate-based herbicide affects agrobiont predatory arthropod behaviour and long-term survival. Ecotoxicology. 19: 1249-1257, 2010.

Everts JW, Aukema B, Hengeveld R, Koeman JH. Side-effects of pesticides on ground-dwelling predatory arthropods in arable ecosystems. Environ. Pollut. 59: 203-225, 1989.

Frampton GK, van den Brink PJ. Collembola and macroarthropod community responses to carbamate, organophosphate and synthetic pyrethroid insecticides: direct and indirect effects. Environ. Pollut. 147: 14-25, 2007.

Francesena N, Desneux N, de Campos MR, Schneider MI. Side effects of spirotetramat on pupae and adults of a Neotropical strain of *Eretmocerus mundus* (Hymenoptera: Aphelinidae). Effects on the life parameters and demography. Environ. Sci. Pollut. Res. 1-12, 2017.

Gill HK, Garg H. Pesticides: environmental impacts and management strategies. Pesticides-Toxic Effects. In Tech. Rijeka, Croatia, pp 187-230, 2014.

Honěk A, Kocian M, Martinkova Z. Rove beetles (Coleoptera: Staphylinidae) in an apple orchard. Plant Protec. Sci. 48: 116-122, 2012.

Huusela-Veistola E. Effects of pesticide use and cultivation techniques on ground beetles (Col, Carabidae) in cereal fields. Ann. Zool. Fenn. 197-205, 1996.

Jänsch S, Frampton GK, Römbke J, Van den Brink PJ, Scott-Fordsmand JJ. Effects of pesticides on soil invertebrates in model ecosystem and field studies: a review and comparison with laboratory toxicity data. Environ. Toxicol. Chem. 25: 2490-2501, 2006.

Kocourek F, SaSKa P, Řezáč M. Diversity of carabid beetles (Coleoptera: Carabidae) under three different control strategies against European corn borer in maize. Plant Protec. Sci. 49: 146-153, 2013.

Lardo E, Coll P, Le Cadre E, Palese AM, Villenave C, Xiloyannis C, *et al.* Electromagnetic induction (EMI) measurements as a proxy of earthworm presence in Southern French vineyards. Appl. Soil Ecol. 61: 76-84, 2012.

Larson JL, Redmond CT, Potter DA. Impacts of a neonicotinoid, neonicotinoid-pyrethroid premix, and anthranilic diamide insecticide on four species of turf-inhabiting beneficial insects. Ecotoxicology 23: 252-259, 2014.

Lee KE, Pankhurst CE. Soil organisms and sustainable productivity. Soil Res. 30: 855-892, 1992.

Majeed MZ. Nitrous oxide emissions by tropical soil macrofauna: impact of their feeding guilds and functional microbial communities involved in these emissions. [PhD Thesis.] Universite Montpellier II, Montpellier, France: pp 235, 2012.

Monzo C, Qureshi JA, Stansly PA. Insecticide sprays, natural enemy assemblages and predation on Asian citrus psyllid, *Diaphorina citri* (Hemiptera: Psyllidae). Bull. Entomol. Res. 104: 576-585, 2014.

Moreby SJ, Sotherton NW, Jepson PC. The effects of pesticides on species of non-target Heteroptera inhabiting cereal fields in southern England. Pestic. Sci. 51: 39-48, 1997.

Pekár S, Beneš J. Aged pesticide residues are detrimental to agrobiont spiders (Araneae). J. Appl. Entomol. 132: 614-622, 2008.

Pisa LW, Amaral-Rogers V, Belzunces LP, Bonmatin JM, Downs CA, Goulson D, *et al.* Effects of neonicotinoids and fipronil on non-target invertebrates. Environ. Sci. Pollut. Res. 22: 68-102, 2015.

Stork NE, Eggleton P. Invertebrates as determinants and indicators of soil quality. Am. J. Alternative Agr. 7: 38-47, 1992.

Tahir HM, Nazarat I, Naseem S, Butt A, Yaqoob R, Mukhtar MK, *et al.* Seasonal dynamics of spiders and insect pests in citrus orchards of district Sargodha, Pakistan. Pak. J. Zool. 47: 1673-1681, 2015.

Williams T, Valle J, Viñuela E. Is the naturally derived insecticide Spinosad® compatible with insect natural enemies? Biocontrol Sci. Technol. 13: 459-475, 2003.