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PESTICIDES TOXICITY FOR *Neoseiulus barkeri* (ACARI: PHYTOSEIIDAE) AND NON-TARGET ORGANISMS

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Phytoseiidae have been used successfully as bio-control agents of sucking insect pests worldwide. *Neoseiulus barkeri* is potential predator of whiteflies, thrips and spider mites. To minimize toxic effects and develop IPM, integration of pesticides and predators in a manner which is safer for other organisms is key factor. The present study was planned for screening out pesticides being used against sucking pests *i.e*., buprofezin, spirotetramate, dimethoate, hexithiazox and imidacloprid for *N. barkeri* to find out compatibility. Leaf dip bioassay was conducted and pesticides were tested at five serial dilutions under laboratory conditions. Maximum mortality 17.5, 45, 82.5, 35 and 17.5 percent was observed after 144 hours exposure to pesticides at field relevant dose, respectively. Repellent effects of pesticides revealed that escape from arena was highest in the start and then gradually decreased. Lowest hemolytic activity (78.56%) for imidacloprid and highest (97.76%) for dimethoate, and *Staphylococcus aureus* biofilm inhibition 43% for buprofezin and 23% for dimethoate was observed. LC_{50} of imidacloprid (26526) and buprofezin (7209) declared it safer while spirotetramate and hexithiazox were moderately harmful to *N. barkeri*. Dimethoate was highly hazardous for *N. barkeri* due to highest mortality and lowest LT_{50} (27.75), hence is not recommended in IPM module.

Keywords: *Neoseiulus barkeri*, reduced-risk pesticides, mortality, biofilm inhibition, pest management.

INTRODUCTION

Protection of crops from insect pests is the main focusing approach for maximizing existing crop yields and economic returns. The use of pesticides has become an integral part of our pest management approach (Lechenet *et al*., 2014). These chemicals are giving satisfactory results but in parallel they also impose serious threats like environmental pollution, risk to beneficial fauna and human health. Due to injudicious use of pesticides, there are many reports for the development of pesticides resistance in insect pests (Abang *et al*., 2013; Attia *et al.*, 2015). This adverse situation directs the agricultural experts to rely on human and ecosystem friendly alternate approach. Biological control is considered as the most promising for the conservation of natural fauna and reduction of toxic residues in our ecosystem (Ajinath *et al*., 2013). The predatory mites are being commercially used in various parts of the world and are considered as potential predators in biological systems (Chant and McMurtry, 2007; Mallik *et al*., 2010; Szabo *et al*., 2014). In recent years, the use of chemical and biological control in an integrated manner has become a popular IPM approach. The main objective of such tactic is to use lower doses of pesticides or apply safer pesticides along with predators, to lessen the pesticides burden on ecosystems, decrease pesticide resistance in pests, and to increase existing crop yields and ensure good human health (Hosny *et al*., 2009).

In Pakistan major threat of sucking insect-pests, particularly whitefly and spider mites, has become very crucial (Rafiq *et al*., 2008; Ahmad *et al*., 2010; Mamoon-ur-Rashid, 2011) and for their control huge investments are being spent on pesticides (Malik, 2014). The predatory mite, *Neoseiulus barkeri* Hughes (Acari: Phytoseiidae) is a generalist predator (McMurtry *et al*., 2013). Number of studies revealed the predatory potential of *N. barkeri* against wide range of pests including *Thrips tabaci* (Hansen, 1988; Bakker and Sabelis, 1989; Bonde, 1989; Jafari *et al*., 2013), broad mite, *Polyphagotarsonemus latus* (Fan and Petitt, 1994), bulb scale mites, *Steneotarsonemus laticeps* (Messelink and Holstein-Saj, 2007; Messelink, 2012), date mite, *Oligonychus afrasiaticus* (Negm *et al*., 2014) and *Aleuroglyphus ovatus* (Xia *et al*., 2012). Keeping in view, the predatory potential of this predatory mite, the focus is to use selective pesticides along with it which are least toxic for this predator. Safer and compatible pesticides are the main pillars of biological and chemical control programs in IPM approaches. Now a day, it became evident that the newer pesticides fit the reduced-risk profile, but these may not be necessarily safer for predatory mites (Villanueva and Walgenbach, 2005; Bostanian *et al*., 2009; Lefebvre *et al*., 2012). The varying level of toxicity

ranging from harmless to harmful effects of pesticides against predatory mites has been reported (Jansen, 2010). Imidacloprid, fenbutain oxide, acetamiprid, buprofezin, fenobucarb, dinotefuran, validamycin, carbendazim, hexithiazox and sulfur were safer against different predatory mites (Castagnoli *et al*., 2005; Kongchuensin and Takafuji, 2006; Sanatgar *et al*., 2011; Fiedler and Sosnowska, 2014). Pesticides such as etofenprox, spinosad, chloropyriphos and dimethoate had harmful effects (Bostanian and Akalach, 2006; Alzoubi and Cobanoglu, 2008), while thiamethoxam was low to moderately harmful for predatory mites (Tirello *et al*., 2013). Emmamectin benzoate was reported to be highly toxic, while indoxacarb was a safer pesticide against tested predatory mite (Bernard *et al*., 2010). Different pesticides have proved to be toxic for human beings and other non-target organisms and reported to affect human health adversely (Son *et al*., 2010; Alvanja and Bonner, 2012). Imidacloprid, thiamethoxam, clothianidin and acetamiprid were found highly toxic to beneficial fauna in comparison with spirotetramate, buprofezin and fipronil (Kumar *et al*., 2012). Dimethoate had significantly decreased the body size, haemocyte counts and morphometric factors in carabid beetle (Giglio *et al*., 2011). Sulfonylurea herbicides-chlorsulfuron, metasulfuron methyl and thifensulfuron methyl reduced the growth rate of fluorescent bacteria *Pseudomonas* strains (Boldt and Jacobson, 1998). Methyl isothiocyanate caused an increase in gram positive bacteria and decrease in gram negative bacteria (Ibekwe *et al*., 2001).

In Pakistan, no work has been carried out to screen the safer pesticides for predators and to study the impact of pesticides on human and some non-target organisms present in nature.

MATERIALS AND METHODS

***Source of mites*:** The native predatory mite *N. barkeri* was collected from cotton fields of University of Agriculture, Faisalabad, Pakistan and reared in the laboratory since 2010, having no exposure to pesticides used for experimentation. Stock culture was reared on stored grain mite *Rhizoglyphus tritici* in growth chamber at 26±2°C temperature, 65±5% relative humidity and 12:12 (L:D) photoperiod. The culture was kept in small petri dishes of 5.5 cm diameter placed on foam of 12 cm diameter which was soaked in water in large petri dishes (14 cm diameter).

***Pesticides*:** The pesticides were selected from diverse groups which are being commonly used against sucking insect pests (Table 1). These were purchased from the local market. Serial dilutions were prepared in acetone starting from the field relevant dose.

***Toxicity to adults*:** Leaf discs of 1.7cm diameter were prepared from three months old brinjal, *Solanum melongena* (Solanaceae) leaves with the help of cork borer. Arenas were prepared by keeping foam (12cm diameter) in 14cm diameter petri dish containing water as barrier to prevent escape of predatory mites. The leaf discs were dipped in different concentrations of pesticides for 10 seconds. These discs were allowed to dry for 30 minutes at room temperature (Kongchuensin and Takafuji, 2006). Predatory mites were fed with respective diet before experimentation to ensure mortality occurred due to pesticide not due to starvation. Ten newly developed females were placed on each leaf disc. Immatures of *R. tritici* were offered as food source daily. These mites were added in the arenas to replace the consumed preys to keep the predator prey ratio (1:3) constant. Moreover, absconded predatory mites were excluded from data. Those predatory mites were considered dead which showed no response when touched with a fine needle. Mortality and escape data was recorded after every 24 hours till 144 hours.

***Statistical analysis*:** Data were analyzed statistically by calculating means, standard errors, percentages and two way analysis of variance (ANOVA) and comparison of means were separated by least significant difference (LSD) ($P<0.05$). LC_{50} and LT_{50} values for all tested pesticides were calculated with probit analysis by using statistical software Minitab17. Toxicity categories of tested pesticides were evaluated according to IOBC (International Organization for Biological and Integrated Control) criteria (Jansen, 2010).

RESULTS

***Toxicity to adult female Neoseiulus barkeri*:** Pesticides at different concentrations and time intervals were evaluated against *N. barkeri*, and different effects were observed. Highly significant differences in mortality at different concentrations ($F = 9.01, 119.03, 268.29, 76.16, 20.33$, $df = 5,108$, $P \leq 0.000$) and time intervals ($F = 11.07, 9.49, 31.05, 7.33, 23.02$, $df = 5,108$, $P \leq 0.000$) were observed for buprofezin, spirotetramate, dimethoate, hexithiazox and imidacloprid respectively. Escape of *N. barkeri* from leaf

Table 1. Pesticides along with trade names, groups, concentration (ppm) and field recommended dose rates.

Name of pesticide	Trade name	Group	Concentration in sprayable material (ppm)	Recommended dose/acre/100 L water
buprofezin	Starter 25% WP	Insect Growth Regulator	1250	500 GM
spirotetramate	Movinto	Keto-enolen	720	150 ML
dimethoate	Sanitox 40 EC	Organophosphate	1600	400 ML
hexithiazox	Nissuron	Thiazolidine and Carboxamide	125	125 GM
imidacloprid	Confidor 20% SC	Neonicotinoid	500	250 ML

arena depends upon toxicity of tested pesticides, it was highest in the start of experiment but gradually decreased with the passage of time due to acclimatization. It was significantly different at different concentrations (F = 12.65, df = 5,108 P≤ 0.000) and time intervals (F = 3.74, df = 5,108 P = 0.004) for buprofezin (Table 2b). It was not different at different time intervals (F=1.76, 1.64, df =5,108 P=0.127, 0.156) and significantly different at different concentrations (F= 10.24, 8.42, df =5,108 P≤ 0.000) for spirotetramate and dimethoate respectively (Table 3b, 4b). It was significantly different at

Table 2a. Mortality (%) of *N. barkeri* (n=10) observed on leaf arenas at different time intervals for buprofezin (Mean±SE).

Time	Dose (ppm)						Mean
(hours)	1250	625	312.50	156.25	78.12	Control	
24	2.50 ± 2.50	2.50 ± 2.50	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.83 ± 0.58E
48	5.00 ± 2.89	5.00 ± 2.89	2.50 ± 2.50	2.50 ± 2.50	0.00 ± 0.00	2.50 ± 2.50	2.92 ± 0.95DE
72	5.00 ± 2.89	5.00 ± 2.89	2.50 ± 2.50	2.50 ± 2.50	2.50 ± 2.50	5.00 ± 2.89	3.75 ± 1.01CD
96	12.50 ± 2.50	7.50 ± 2.50	5.00 ± 2.89	2.50 ± 2.50	2.50 ± 2.50	5.00 ± 2.89	5.83 ± 1.19BC
120	15.00 ± 2.89	12.50 ± 2.50	10.00 ± 0.00	2.50 ± 2.50	5.00 ± 2.89	5.00 ± 2.89	8.33 ± 1.30AB
144	17.50 ± 2.50	12.50 ± 2.50	10.00 ± 0.00	2.50 ± 2.50	5.00 ± 2.89	7.50 ± 2.50	9.17 ± 1.33A
Total	9.58 ± 1.53A	7.50 ± 1.24AB	5.00 ± 1.04BC	2.08 ± 0.85D	2.50 ± 0.90CD	4.17 ± 1.03CD	

Means with the same letter in a row or in a column are not significantly different by Fisher's LSD (P>0.05); T1= Field relevant dose (1250 ppm), T2=1/2 of field relevant dose (625 ppm), T3= 1/4 of field relevant dose (312.50 ppm), T4=1/8 of field relevant dose (156.25 ppm), T5= 1/16 of field relevant dose (78.12 ppm) and T6= Control (Acetone)

Table 2b. Rate of escape (%) of *N. barkeri* (n=10) at different time intervals for buprofezin (Mean±SE).

Time	Dose (ppm)						Mean
(hours)	1250	625	312.50	156.25	78.12	Control	
24	10.00 ± 0.00	10.00 ± 0.00	12.50 ± 2.50	7.50 ± 2.50	7.50 ± 2.50	5.00 ± 2.89	8.75 ± 0.92C
48	12.50 ± 2.50	12.50 ± 2.50	12.50 ± 2.50	10.00 ± 0.00	10.00 ± 0.00	5.00 ± 2.89	10.42 ± 0.95BC
72	12.50 ± 2.50	15.00 ± 2.89	12.50 ± 2.50	10.00 ± 0.00	10.00 ± 0.00	7.50 ± 2.50	11.25 ± 0.92ABC
96	15.00 ± 2.89	17.50 ± 2.50	12.50 ± 2.50	10.00 ± 0.00	10.00 ± 0.00	7.50 ± 2.50	12.08 ± 1.04AB
120	17.50 ± 2.50	17.50 ± 2.50	15.00 ± 2.89	12.50 ± 2.50	10.00 ± 0.00	7.50 ± 2.50	13.33 ± 1.15A
144	17.50 ± 2.50	17.50 ± 2.50	15.00 ± 2.89	12.50 ± 2.50	12.50 ± 2.50	5.00 ± 2.89	13.33 ± 1.30A
Total	14.17 ± 1.03A	15.00 ± 1.04A	13.33 ± 0.98A	10.42 ± 0.73B	10.00 ± 0.60B	6.25 ± 1.01C	

Means with the same letter in a row or in a column are not significantly different by Fisher's LSD (P>0.05); T1= Field relevant dose (1250 ppm), T2=1/2 of field relevant dose (625 ppm), T3= 1/4 of field relevant dose (312.50 ppm), T4=1/8 of field relevant dose (156.25 ppm), T5= 1/16 of field relevant dose (78.12 ppm) and T6= Control (Acetone)

Table 3a. Mortality (%) of *N. barkeri* (n=10) observed on leaf arenas at different time intervals for spirotetramate (Mean±SE).

Time	Dose (ppm)						Mean
(hours)	720	360	180	90	45	Control	
24	22.50 ± 2.50	15.00 ± 2.89	12.50 ± 2.50	7.50 ± 2.50	5.00 ± 2.89	0.00 ± 0.00	10.42 ± 1.75C
48	32.50 ± 4.79	25.00 ± 2.89	15.00 ± 2.89	10.00 ± 0.00	7.50 ± 2.50	0.00 ± 0.00	15.00 ± 2.48B
72	37.50 ± 4.79	30.00 ± 4.08	15.00 ± 2.89	10.00 ± 0.00	7.50 ± 2.50	0.00 ± 0.00	16.67 ± 2.93AB
96	42.50 ± 4.79	30.00 ± 4.08	17.50 ± 2.50	12.50 ± 2.50	7.50 ± 2.50	5.00 ± 2.89	19.17 ± 3.00A
120	45.00 ± 2.89	32.50 ± 4.79	17.50 ± 2.50	12.50 ± 2.50	7.50 ± 2.50	5.00 ± 2.89	20.00 ± 3.19A
144	45.00 ± 2.89	32.50 ± 4.79	17.50 ± 2.50	12.50 ± 2.50	7.50 ± 2.50	5.00 ± 2.89	20.00 ± 3.19A
Total	37.50 ± 2.19A	27.50 ± 1.93B	15.83 ± 1.03C	10.83 ± 0.83D	7.08 ± 0.95E	2.50 ± 0.90F	

Means with the same letter in a row or in a column are not significantly different by Fisher's LSD (P>0.05).

Table 3b. Rate of escape (%) of *N. barkeri* (n=10) at different time intervals for spirotetramate (Mean±SE).

Time	Dose (ppm)						Mean
(hours)	720	360	180	90	45	Control	
24	10.00 ± 0.00	10.00 ± 0.00	10.00 ± 0.00	12.50 ± 2.50	10.00 ± 0.00	5.00 ± 2.89	9.58 ± 0.73A
48	10.00 ± 0.00	12.50 ± 2.50	10.00 ± 0.00	12.50 ± 2.50	10.00 ± 0.00	5.00 ± 2.89	10.00 ± 0.85A
72	12.50 ± 2.50	12.50 ± 2.50	12.50 ± 2.50	15.00 ± 2.89	10.00 ± 0.00	5.00 ± 2.89	11.25 ± 1.10A
96	12.50 ± 2.50	12.50 ± 2.50	12.50 ± 2.50	15.00 ± 2.89	12.50 ± 2.50	5.00 ± 2.89	11.67 ± 1.15A
120	12.50 ± 2.50	12.50 ± 2.50	12.50 ± 2.50	17.50 ± 2.50	12.50 ± 2.50	7.50 ± 2.50	12.50 ± 1.09A
144	12.50 ± 2.50	12.50 ± 2.50	12.50 ± 2.50	17.50 ± 2.50	12.50 ± 2.50	7.50 ± 2.50	12.50 ± 1.09A
Total	11.67 ± 0.78B	12.08 ± 0.85B	11.67 ± 0.78B	15.00 ± 1.04A	11.25 ± 0.69B	5.83 ± 1.03C	

Means with the same letter in a row or in a column are not significantly different by Fisher's LSD (P>0.05).

Table 4a. Mmortality (%) of *N. barkeri* (n=10) observed on leaf arenas at different time intervals for dimethoate (Mean±SE).

Time	Dose (ppm)						Mean
(hours)	1600	800	400	200	100	Control	
24	37.50 ± 2.50f	25.00 ± 2.89ghi	15.00 ± 2.89jkl	7.50 ± 2.50lmn	5.00 ± 2.89mn	0.00 ± 0.00n	15.00 ± 2.82E
48	65.00 ± 2.89bc	50.00 ± 4.08e	27.50 ± 6.29gh	17.50 ± 2.50ijk	5.00 ± 2.89mn	2.50 ± 2.50mn	27.92 ± 4.96C
72	70.00 ± 4.08b	52.50 ± 2.50de	27.50 ± 6.29gh	17.50 ± 2.50ijk	7.50 ± 2.50lmn	5.00 ± 2.89mn	30.00 ± 5.14BC
96	72.50 ± 2.50b	55.00 ± 2.89de	30.00 ± 4.08fg	20.00 ± 4.08hij	10.00 ± 0.00klm	5.00 ± 2.89mn	32.08 ± 5.18AB
120	82.50 ± 2.50a	60.00 ± 4.08cd	30.00 ± 4.08fg	20.00 ± 4.08hij	10.00 ± 0.00klm	7.50 ± 2.50lmn	35.00 ± 5.84A
144	82.50 ± 2.50a	65.00 ± 2.89bc	30.00 ± 4.08fg	20.00 ± 4.08hij	10.00 ± 0.00klm	7.50 ± 2.50lmn	35.83 ± 4.58D
Total	68.33 ± 5.93A	51.25 ± 2.91A	26.67 ± 2.06B	17.08 ± 1.53C	7.92 ± 0.85D	4.58 ± 1.04D	

Means with the same letter in a row or in a column are not significantly different by Fisher's LSD (P>0.05).

Table 4b. Rate of escape (%) of *N. barkeri* (n=10) at different time intervals for dimethoate (Mean±SE).

Time	Dose (ppm)						Mean
(hours)	1600	800	400	200	100	Control	
24	15.00 ± 2.89ab	10.00 ± 0.00bcd	10.00 ± 0.00bcd	10.00 ± 0.00bcd	12.50 ± 2.50abc	5.00 ± 2.89de	10.42 ± 0.95A
48	15.00 ± 2.89ab	12.50 ± 2.50abc	10.00 ± 0.00bcd	10.00 ± 0.00bcd	12.50 ± 2.50abc	5.00 ± 2.89de	10.83 ± 1.03A
72	17.50 ± 2.50a	15.00 ± 2.89ab	12.50 ± 2.50abc	12.50 ± 2.50abc	12.50 ± 2.50abc	5.00 ± 2.89de	12.50 ± 1.24A
96	17.50 ± 2.50a	15.00 ± 2.89ab	12.50 ± 2.50abc	12.50 ± 2.50abc	15.00 ± 2.89ab	7.50 ± 2.50cd	13.33 ± 1.15A
120	17.50 ± 2.50a	15.00 ± 2.89ab	12.50 ± 2.50abc	12.50 ± 2.50abc	15.00 ± 2.89ab	7.50 ± 2.50cd	13.33 ± 1.15A
144	17.50 ± 2.50a	15.00 ± 2.89ab	15.00 ± 2.89ab	15.00 ± 2.89ab	15.00 ± 2.89ab	7.50 ± 2.50cd	14.17 ± 1.51A
Total	16.67 ± 1.57B	13.75 ± 1.01AB	12.08 ± 0.85AB	12.08 ± 0.85A	13.75 ± 1.01A	6.25 ± 1.01AB	

Means with the same letter in a row or in a column are not significantly different by Fisher's LSD (P>0.05).

Table 5a. Mortality (%) of *N. barkeri* (n=10) observed on leaf arenas at different time intervals for hexithiazox (Mean±SE).

Time	Dose (ppm)						Mean
(hours)	125	62.50	31.25	15.62	7.81	Control	
24	15.00 ± 2.89	10.00 ± 0.00	5.00 ± 2.89	2.50 ± 2.50	2.50 ± 2.50	0.00 ± 0.00	5.83 ± 1.33C
48	25.00 ± 2.89	15.00 ± 2.89	10.00 ± 0.00	5.00 ± 2.89	2.50 ± 2.50	2.50 ± 2.50	10.00 ± 1.90B
72	27.50 ± 2.50	17.50 ± 4.79	10.00 ± 0.00	7.50 ± 2.50	2.50 ± 2.50	2.50 ± 2.50	11.25 ± 2.11AB
96	30.00 ± 4.08	20.00 ± 4.08	10.00 ± 0.00	7.50 ± 2.50	2.50 ± 2.50	5.00 ± 2.89	12.50 ± 2.27AB
120	35.00 ± 2.89	20.00 ± 4.08	10.00 ± 0.00	7.50 ± 2.50	5.00 ± 2.89	5.00 ± 2.89	13.75 ± 2.47A
144	35.00 ± 2.89	20.00 ± 4.08	10.00 ± 0.00	7.50 ± 2.50	5.00 ± 2.89	5.00 ± 2.89	13.75 ± 2.47A
Total	27.92 ± 1.80A	17.08 ± 1.53B	9.17 ± 0.58C	6.25 ± 1.01CD	3.33 ± 0.98D	3.33 ± 0.98D	

Means with the same letter in a row or in a column are not significantly different by Fisher's LSD (P>0.05).

Table 5b. Rate of escape (%) of *N. barkeri* (n=10) at different time intervals for hexithiazox (Mean±SE).

Time	Dose (ppm)						Mean
(hours)	125	62.50	31.25	15.62	7.81	Control	
24	10.00 ± 0.00	10.00 ± 0.00	7.50 ± 2.50	7.50 ± 2.50	7.50 ± 2.50	5.00 ± 2.89	7.92 ± 0.85B
48	10.00 ± 0.00	10.00 ± 0.00	7.50 ± 2.50	7.50 ± 2.50	7.50 ± 2.50	5.00 ± 2.89	7.92 ± 0.85B
72	12.50 ± 2.50	12.50 ± 2.50	10.00 ± 0.00	7.50 ± 2.50	7.50 ± 2.50	5.00 ± 2.89	9.17 ± 1.03AB
96	15.00 ± 2.89	15.00 ± 2.89	10.00 ± 0.00	10.00 ± 0.00	7.50 ± 2.50	5.00 ± 2.89	10.42 ± 1.12AB
120	15.00 ± 2.89	15.00 ± 2.89	12.50 ± 2.50	10.00 ± 0.00	10.00 ± 0.00	7.50 ± 2.50	11.67 ± 0.98A
144	15.00 ± 2.89	15.00 ± 2.89	12.50 ± 2.50	10.00 ± 0.00	10.00 ± 0.00	7.50 ± 2.50	11.67 ± 0.98A
Total	12.92 ± 0.95A	12.92 ± 0.95A	10.00 ± 0.85B	8.75 ± 0.69B	8.33 ± 0.78BC	5.83 ± 1.03C	

Means with the same letter in a row or in a column are not significantly different by Fisher's LSD (P>0.05).

different time intervals (F=3.62, 2.40 df=5,108, P=0.005, 0.042) and concentrations (F=9.38, 7.69, df=5,108, P≤ 0.000) for hexithiazox and imidacloprid respectively (Table 5b, 6b). Buprofezin was harmless for *N. barkeri*, maximum mortality (17.50%) was observed at field-relevant dose (1250 ppm) after 144 hours, while minimum mortality (2.50%) at same concentration and at 625 ppm after 24 hours (Table 2a). There was non-significant interaction of time and concentrations for mites escape (F = 0.38, df = 25,108, P= 0.996) and mortality (F = 0.82, df =25, 108, P=0.699) were observed (Table 2a, b). Spirotetramate caused maximum mortality (45%) at field relevant dose (720 ppm) after 120 hours and minimum mortality (5.00%) at 45 ppm after 24 hours (Table 3a). There was non-significant interaction of time and spirotetramate

Table 6a. Mortality (%) of *N. barkeri* (n=10) observed on leaf arenas at different time intervals for imidacloprid (Mean±SE).

Time (hours)	Dose (ppm)						Mean
	500	250	125	62.50	31.25	Control	
24	2.50 ± 2.50	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.42 ± 0.42D
48	12.50 ± 2.50	7.50 ± 2.50	5.00 ± 2.89	2.50 ± 2.50	0.00 ± 0.00	2.50 ± 2.50	5.00 ± 1.20C
72	15.00 ± 2.89	12.50 ± 2.50	7.50 ± 2.50	5.00 ± 2.89	2.50 ± 2.50	2.50 ± 2.50	7.50 ± 1.38BC
96	17.50 ± 2.50	12.50 ± 2.50	10.00 ± 0.00	10.00 ± 0.00	5.00 ± 2.89	2.50 ± 2.50	9.58 ± 1.27AB
120	17.50 ± 2.50	15.00 ± 2.89	12.50 ± 2.50	10.00 ± 0.00	7.50 ± 2.50	5.00 ± 2.89	11.25 ± 1.25A
144	17.50 ± 2.50	15.00 ± 2.89	12.50 ± 2.50	10.00 ± 0.00	10.00 ± 0.00	5.00 ± 2.89	11.67 ± 1.15A
Total	13.75 ± 1.45A	10.42 ± 1.41B	7.92 ± 1.20BC	6.25 ± 1.01CD	4.17 ± 1.03DE	2.92 ± 0.95E	

Means with the same letter in a row or in a column are not significantly different by Fisher's LSD (P>0.05).

Table 6b. Rate of escape (%) of *N. barkeri* (n=10) at different time intervals for imidacloprid (Mean±SE).

Time (hours)	Dose (ppm)						Mean
	500	250	125	62.50	31.25	Control	
24	10.00 ± 0.00	10.00 ± 0.00	10.00 ± 0.00	7.50 ± 2.50	7.50 ± 2.50	7.50 ± 2.50	8.75 ± 0.69C
48	12.50 ± 2.50	10.00 ± 0.00	10.00 ± 0.00	7.50 ± 2.50	7.50 ± 2.50	7.50 ± 2.50	9.17 ± 0.83BC
72	12.50 ± 2.50	10.00 ± 0.00	10.00 ± 0.00	10.00 ± 0.00	7.50 ± 2.50	7.50 ± 2.50	9.58 ± 0.73ABC
96	15.00 ± 2.89	12.50 ± 2.50	12.50 ± 2.50	10.00 ± 0.00	10.00 ± 0.00	7.50 ± 2.50	11.25 ± 0.92AB
120	15.00 ± 2.89	12.50 ± 2.50	12.50 ± 2.50	10.00 ± 0.00	10.00 ± 0.00	7.50 ± 2.50	11.25 ± 0.92AB
144	17.50 ± 2.50	12.50 ± 2.50	12.50 ± 2.50	10.00 ± 0.00	10.00 ± 0.00	7.50 ± 2.50	11.67 ± 0.98A
Total	13.75 ± 1.01A	11.25 ± 0.69B	11.25 ± 0.69B	9.17 ± 0.58BC	8.75 ± 0.69C	7.50 ± 0.90C	

Means with the same letter in a row or in a column are not significantly different by Fisher's LSD (P>0.05).

Table 7. Categories of tested pesticides against *N. barkeri*.

Tested pesticides	Observed mortality (%) after 144 hours	Categories of pesticides according to IOBC (Jansen, 2010)	Categories of tested pesticides
buprofezin	17.5	0-25% (Harmless)	Harmless
imidacloprid	17.5	0-25% (Harmless)	Harmless
hexithiazox	35.0	25-50% (Slightly harmful)	Slightly harmful
spirotetramate	45.0	25-50% (Slightly harmful)	Slightly harmful
dimethoate	82.5	>75% (Harmful)	Harmful

Table 8. LC_{50} values of tested pesticides against *N. barkeri* after 144 hours.

Pesticides	LC_{50}	SE	95% Fiducial CI		Chi-square	P-Value
			Upper	Lower		
buprofezin	7209.62	4944.55	2846.91	91452.50	3.231	0.357
imidacloprid	26526.66	60019.92	2335.84	1.8063E+17	0.249	0.969
spirotetramate	682.31	109.15	520.78	1009.77	0.647	0.886
*dimethoate	–	–	–	–	–	–
hexithiazox	179.59	36.63	129.33	305.73	1.312	0.726

CI: Confidence Interval, P: Probability, * No *Neoseiulus barkeri* survived on arena after 120 hours (82.50% dead; 17.50% escaped).

concentrations for escape (F = 0.14, df = 25,108, P = 1.000) and mortality (F=1.25, df =25,108, P=0.2154) (Table 3a, b).

Dimethoate was harmful for *N. barkeri* and complete mortality was observed at field relevant dose (1600 ppm) after 120 hours while minimum mortality 5% was observed at 100 ppm after 24 and 48 hours interval (Table 4a). Significant interaction of time and concentrations for escape of mites (F = 1.73, df = 25,108, P = 0.028) and mortality (F = 17.17, df = 25,108, P≤ 0.000) was observed (Table 4a, b).

Maximum mortality of *N. barkeri* (35%) was reported at field relevant dose (125 ppm) of hexithiazox after 120 hours while minimum mortality (2.50%) at 15.62 and 7.81 ppm after 24 and 96 hours respectively (Table 5a). There was no interaction between time and concentrations for escape of mites (F = 0.182, df = 25,108, P = 1.000) and mortality (F = 0.885, df = 25,108, P= 0.624) (Table 5a, b).

Imidacloprid was harmless and caused maximum mortality (17.5%) at field relevant dose (500 ppm) after 96 hours, while minimum mortality (2.5%) at same concentration after 24 hours (Table 6a). There was no interaction between time and concentrations for escape (F = 0.24, df = 25,108, P = 0.999) and mortality (F = 0.85, df = 25,108 P = 0.668) (Table 6a, b).

Table 9. LT_{50} values of tested pesticides against *N. barkeri*.

Pesticides	LT_{50}	SE	95% Fiducial CI		Chi square	P Value
			Upper	Lower		
buprofezin	411.35	111.03	275.13	901.38	6.132	0.804
imidacloprid	332.38	70.63	239.63	594.80	9.810	0.457
spirotetramate	113.30	12.10	94.07	146.33	1.713	0.998
dimethoate	27.76	1.80	24.05	31.12	14.775	0.039
hexithiazox	203.59	34.09	156.10	317.21	1.078	1.000

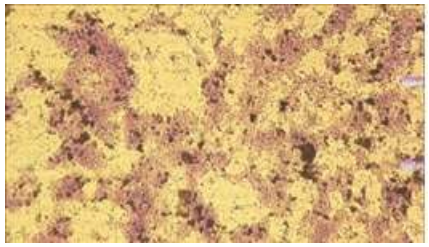
Table 10. Biofilm inhibition and hemolytic activities of tested pesticides.

Name of Pesticide	*Staphylococcus aureus* (%)	Hemolytic activity (%)
imidacloprid	26	78.56
buprofezin	43	94.57
spirotetramate	26	87.05
hexithiazox	36	88.20
dimethoate	23	97.76
Rimpacin	87.43	-
PBS	-	0.086
Triton-x-100	-	98.85

Values (mean ± SD) are average of three samples of each formulated pesticides, analyzed individually in triplicate (n = 1x3 x 3), ($P < 0.05$); PBS: Phosphate Buffer Saline

According to IOBC category of tested pesticides against beneficial arthropods (Jansen, 2010) imidacloprid and buprofezin were harmless while spirotetramate and hexithiazox were slightly harmful and dimethoate was harmful for *N. barkeri* (Table 7). After 144 hours LC_{50} values were 26526, 7209, 682 and 179 for imidacloprid, buprofezin, spirotetramate and hexithiazox respectively. The complete mortality due to dimethoate after 120 hours declared it highly toxic for *N. barkeri* and this pesticide cannot be recommended in IPM programs along with this predator (Table 8). However, LC_{50} did not indicate the clear mechanism of mortality due to difference of field relevant dose/ppm of all tested pesticides. So, LT_{50} were also calculated, which varied significantly: dimethoate (27.76) < spirotetramate (113.30) < hexithiazox (203.59) < imidacloprid (332.38) < buprofezin (411.35) (Table 9). For biofilm inhibition rimpacin was used as positive control. *Staphylococcus aureus* biofilm inhibition effect was lowest for dimethoate and highest for buprofezin as, 23, 26, 26, 36 and 43 percent for dimethoate, spirotetramate, imidacloprid, hexithiazox and buprofezin, respectively. The results indicated that gram-positive bacterium *S. aureus* biofilm inhibition was high due to the absence of slime layer. Erythrocytes lysis for each pesticide sample showed the least cytotoxicity (78.56%) due to imidacloprid, while the highest cytotoxicity (97.76%) due to dimethoate in contrast to positive control (Triton-x-100) (98.85%) (Table 10). Biofilm inhibition microscopy of tested pesticides showed highest biofilm inhibition due to buprofezin as treatment when tested against *S. aureus* positive (rimpacin), negative (growth) and sample (growth) (Fig. 1).

MICROSCOPY



Treatment (Buprofezin)

Negative control (Simple nutrient agar microbes growth)

Positive control (Rimpacin)

Figure 1. Phase contrast microscopic view of inhibition of *Staphylococcus aureus* biofilm by pesticides nanoparticles at 100x. Positive (Triton-x-100), biofilm treated S. aureus growth by pesticides nanoparticles dissolved.

DISCUSSION

Mortality and repellent effects due to tested pesticides varied significantly for *N. barkeri* (Acari: Phytoseiidae). Buprofezin in the present study proved to be least harmful pesticide at field relevant dose. These outcomes agree with the findings of Kongchuensin and Takafuji (2006) who observed least mortality (4.9%) of *Neoseiulus longispinosis* due to buprofezin after 48 hours and declared harmless pesticide. The present results are also in agreement with IOBC/wprs recommendations (Boller *et al*., 2006) that buprofezin at dose rate 250 g/L was harmless against *Typhlodromus pyri* and *Phytoseiulus persimilis*. Spirotetramate had slightly harmful affect against *N. barkeri*. Present findings are in consistence with outcomes of Beers and Schmidt (2014) who tested spirotetramate against *Galendromus occidentalis* at different dose rates *i.e*., 328(2x), 164(x), 16(0.1x) and 0 mg active ingredients per liter, and found 52, 20.83, 48 and 12.5% mortality after 48 hours, respectively. Beers and Schmidt (2014) declared spirotetramate as slightly harmful against predatory mites like the results presented here. The present study confirmed that dimethoate was harmful. Similarly, Bostanian and Akalach (2006) tested dimethoate at dose rate 0.190 grams active ingredients per liter against *P. persimilis* and *Amblyseius fallacis* and found 100% and 94% mortality after 168 hours, respectively. Their outcomes are in consistence to present findings. Alzoubi and Cobanoglu (2008) tested dimethoate at dose rate 450 ppm against *Amblyseius californicus* and *P. persimilis* and observed LC_{50} 33.76 and 5.54 respectively. Their findings agree with the present results that dimethoate had lethal effects against predatory mites. Dimethoate was also found harmful for *T. pyri* in field conditions and for *P. persimilis* in laboratory conditions according to IOBC/wprs. These results indicated its higher toxicity for predatory mites and dimethoate is not recommended in IPM module.

Hexithiazox at dose rate 50 ppm tested against *A. californicus* and *P. persimilis* by Alzoubi and Cobanoglu (2008), who observed LC_{50}, 280.98, 50.61 and 184.48, 28.92 after 24 and 72 hours respectively. Their conclusion is in consistence to present findings that hexithizox is suitable for IPM programs due to slightly harmful effects against tested predatory mite. Sanatgar *et al*. (2011) also observed the least toxicity of hexithiazox for different generations of *P. persimilis* in consistence to present findings. Fiedler and Sosnowska (2014) tested hexithiazox at 0.02% dose rate against *Amblyseius swiriskii, Amblyseius andersoni* and *P. persimilis* and found 8, 12 and 20% mortality after 168 hours, respectively. Hexithiazox tested by IOBC/ wprs at dose rate 200 grams per liter against *T. pyri* in field conditions and *P. persimilis* in laboratory conditions was found slightly harmful. Present findings revealed that imidacloprid was least harmful, which agrees with the outcomes of Castagnoli *et al*. (2005) who tested imidacloprid at 13.3g a.i./hl against *Neoseiulus californicus* and found 1.27% mortality after 72 hours. Their results are different from present findings due to difference of dose and tested species. Imidacloprid was tested at 50 ppm dose rate against *Neoseiulus longispinosis* by Kongchuensin and Takafuji (2006), who reported 20.2% mortality in 48 hours. Their findings are somewhat in agreement with present results because imidacloprid falls in harmless category of IOBC in both studies. Beers and Schmidt (2014) tested imidacloprid against *G. occidentalis* at dose rate of 120 mg active ingredients per liter (x) and found 80, 68 and 12% mortality at 2x, 1x, 0.1x dose rates along with 8% mortality at control after 48 hours. Present results are not in agreement with their findings due to difference of species and conditions, but somewhat similar because the author also found 5% mortality in control conditions. Villanueva and Walgenbach (2005) tested imidacloprid at 60 ppm dose rate against *Neoseiulus fallacis* and found 1.3% mortality and 8.8% escape after 24 hours, while 20% mortality and escape reported after 96 hours. Their results showed the similar trend of mortality and missing as present findings. Fiedler and Sosnowska (2014) tested imidacloprid at 0.075% dose rate against *A. swiriskii, Amblyseius andersoni* and *P. persimilis* and found 92, 98 and 100% mortality after 168 hours, respectively. Moreover, according to IOBC/wprs, imidacloprid was found harmless against *T. pyri* and harmful for *P. persimilis*. Present results are also in disagreement with IOBC due to difference of species used in both cases.

Present study is the first to screen out reduced risk pesticides against *N. barkeri* under laboratory conditions. Buprofezin and imidacloprid caused the lowest mortality, while dimethoate resulted highest mortality. However, further investigations are still needed to study sub-lethal effects of these pesticides on subsequent generations of *N. barkeri*. Present study declared hexithiazox and spirotetramate to be slightly harmful against *N. barkeri* in laboratory conditions. Resultantly, buprofezin and imidacloprid can be recommended for use at their field relevant dose rates, hexithiazox and spirotetramate can be used at lower dose rates, while dimethoate is not recommended in IPM module.

***Conclusion*:** Hexithiazox and spirotetramate declared slightly harmful against *Neoseiulus barkeri* under laboratory conditions. Buprofezin and imidacloprid can be recommended for use at their field relevant dose rates, hexithiazox and spirotetramate can be used at lower dose rates, while dimethoate is not recommended in IPM module. Biochemical analysis also revealed that imidacloprid and buprofezin were the safest pesticides while dimethoate declared as highly toxic for non-target organisms.

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