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COMBINED EFFICACY OF ALIPHATIC HYDROCARBONS WITH NEONICOTENOIDS AGAINST *PHENACOCCUS SOLENOPSIS* (HEMIPTERA: PSEUDOCOCCIDAE) UNDER CONTROLLED CONDITIONS

[a]Mubashar Iqbal, [a]Shahbaz Ahmad, [b]Muhammad Dildar Gogi
[a] *Department of Entomology, University of the Punjab Lahore, Pakistan.*
[b] *Department of Entomology, University of Agriculture Faisalabad, Pakistan.*

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ABSTRACT

Phenacoccus solenopsis appeared as a major pest in recent years and caused huge losses to cotton. Control of cotton mealybug is difficult because of the waxy covering on the body which prevents penetration of insecticides in nymph and adult stages. Present study was carried out under laboratory conditions to evaluate the synergistic effects of minerals oil (Diver®) on the toxicity of diafenthiuron, nitenpyram and thiacloprid against different instars of cotton mealybug. Diafenthiuron, nitenpyram and thiacloprid alone demonstrated mortality in the range of 10.3-46.0%, 13.3-50% and 10.30-46.70% in 1st instar, 8.00-42%, 10.30-44% and 6.60-42.3% in 2nd instar while 7-38%, 7.30-38% and 3.3-32% in adult female of *P. solenopsis*, respectively. Admixing of 500 ml of Diver® with diafenthiuron administered 65.2-332.7% and 114.6-602.8%; 78.0-428.8% and 128.6-750.0%; and 94.6-457.1% and 147.3-852.9% increase in mortality of 1st instar, 2nd instar and adult female stages of *P. solenopsis,* respectively. Similarly, fraternization of 1000 ml of Diver® with diafenthiuron administered 42.0-202.6% and 100.0-490.2%; 60.6-261.4% and 127.3-600.2% and 79.8-336.6% and 154.4-854.9% increase in mortality of 1st instar, 2nd instar and adult female stages of *P. solenopsis* respectively. However, thiacloprid, when admixed with 500 ml and 1000 ml of Diver®, explained 16.7-114.3% and 114.3-633.3%; 24.9-103.6% and 132.3-900.9%; and 0.0-303.9% and 191.7-1990.9% increase in mortality of 1st instar, 2nd instar and adult female stages of *P. solenopsis,* respectively. In conclusion, Diver® can be exploited as synergist for resistance management of insecticides against *P. solenopsis*.

Corresponding Author: Mubashar Iqbal
Email: mubashar.ento@pu.edu.pk

INTRODUCTION

Cotton is an important fiber crop of Pakistan and a source of foreign exchange, but its yield is highly affected by many factors like insect pest attack, diseases, heavy rainfall, water shortage and nutritional deficiency (Anonymous, 2007). About 1326 species of insect pests attack cotton crop in the world (Atwal and Dhaliwal, 2002). Among these insect species, 93 species have been reported from Pakistan (Muhammad et al., 2022; Yunus and Yousuf, 1979) which cause 40-50% and 80-100% losses in cotton under sprayed and unsprayed conditions respectively (Huque, 1972; Naqvi, 1976; Yunus et al., 1980).

Cotton mealybug, *Phenacoccus solenopsis* (Hemiptera: Pseudococcidae) is a soft bodied insect that sucks the cell sap from phloem of soft parts of the plant (Iftikhar et al., 2018; Kakakhel, 2007; Saeed et al., 2007; Zhang et al., 2004) and produces honeydews that promotes development of sooty mold, blackening of leaves and reduction in rate of photosynthesis (Pruthi and Batra, 1960; Saeed et al., 2007). The direct infestation and feeding cause stunted plant growth, reduction in number and size of cotton bolls, yellowing and dropping of leaves and flowers (Dhawan et al., 1980; Mark and Gullan, 2005), shriveling of branches, death of plant if heavily infested (Abbas et al., 2007b; Abbas et al., 2007a; Arif et al., 2012; Arif et al., 2011). *P. solenopsis* emerged as a serious pest of cotton in Pakistan (Abbas et al., 2005; Abbas et al., 2006; CCRI, 2006; Hodgson et al., 2008; Muhammad, 2007; Parvez, 2008; Saeed et al., 2007; Zaka et al., 2006; Zhang et al., 2004) as well as in India (Nagrare et al., 2009) and china (Wang et al., 2009). It is a polyphagous pest (Vennila et al., 2010) of more than 154 plant species including field crops, vegetables, fruits, ornamentals, weeds, bushes and trees (Arif et al., 2012; Ben-Dov et al., 2009; Saini et al., 2009).

IPM is useful tool for the control of insect pests (Ahmad et al., 2003; Ahmed et al., 2012; Gogi et al., 2011) but chemical control is the most effective and rapid control as compared to biological control likes predators and parasitoids (Joshi et al., 2010; McKenzie, 1967). Insecticidal control of initial instars of *P. solenopsis* with profenofos, dimethoate, chlorpyriphos, thiamthoxam, imidacloprid (Suresh et al., 2010), some IGRs like buprofezin (Muthukrishnan et al., 2005), nicotine based insecticides (Daane et al., 2006) and non-conventional insecticides like petroleum spray, oils and soap sprays (JainHua, 2003) in crops and vineyards has been very effective as these instars lack waxy covering. However, later instars and adult females of *P. solenopsis* have thick layer of waxy covering and appear in the form of bunch infestation in crops. These factors interfere with the direct hit and penetration of insecticides through insect cuticle to kill the later instars. In Pakistan, its conventional control by the farmers mostly based on chemical control at two times over doses rather than ensuring efficient control of *P. solenopsis*, creates many problems like resistance, environmental hazards and resurgence of new pest species (Bhati et al., 1993; Walton and Pringle, 2001).

Insect pest control with petroleum spray oils and soap spray is good tactics for successful IPM module (Gogi et al., 2011; Mo-Jian and Mo, 2003; Mohyuddin et al., 1997). Ahmed et al. (2012) reported that insecticides when combined with mineral oils give effective control as compared to insecticides applied alone. The present study, therefore, was carried out to evaluate whether combinations of mineral oil (Diver®) with some neonecotinoids like Polo® 500 SC (Diafenthiuron), Pyramid® 10 SL (Nitenpyram) and Talent® 480 SC (Thiacloprid) synergize or antagonize their efficacy against different instars of cotton mealybug.

MATERIALS AND METHODS

Maintenance of mealybug culture

The culture of *P. solenopsis* was maintained on pumpkin fruits in rearing cages of dimensions of 40 cm × 40 cm × 40 cm in IPM Laboratory of the Department of Entomology, University of Agriculture Faisalabad, Pakistan. The branches of shoe-flower plants (*Rosa chinensis*) infested with *P. solenopsis* were clipped and placed in the transparent dome-shaped cages. Fresh and sterilized pumpkin fruits were also placed as food attractant for adult females as well as for young instars. After the infestation of pumpkin fruits with adult females and young instars, the infested pumpkins were shifted to rearing boxes. The male population was also aspirated from the dome-shaped cages and released into rearing cages. Cotton plug soaked with water and honey solution (10:1) in petri dish was also provided in rearing cages as food for adult male individuals. Both the foods (pumpkins and honey solution) were changed when required. The cages were maintained under laboratory conditions (30±2 ºC; 70±5% RH and 12 L: 12 D photoperiod) for mass-culturing of *P. solenopsis*.

Preparation of pesticide test solutions/dilutions

Three neonecotinoid insecticides most widely recommended for sucking insect pests viz. Polo® 500 SC (Diafenthiuron), Pyramid® 10 SL (Nitenpyram) and Talent® 480 SC (Thiacloprid) and mineral oil (Diver®) were selected for this study. Seven test concentrations (dilutions) of each insecticide (Table 1) were prepared from stock solution. The stock solution of the highest concentration (C_1) was prepared for each pesticide using formula $C_1V_1 = C_2V_2$ and the serial dilutions were made be taking half of the stock solution and diluting it with distilled water to the original volume in another measuring cylinder to make C_2. Successive dilutions were made by this method until seven dilutions for each of the pesticide were achieved along with control treatment (Table 1). Each concentration of each selected insecticide was admixed with two field dose levels (500 and 1000 ml/acre) and a zero level (insecticide alone) of Diver® and all sets of combinations were evaluated against *P. solenopsis* (Table 2).

Table 1: Detailed information about the insecticides to be used in the present study.

Chemical name	Trade name	FDR	Type	Mode of action	Concentrations
Diafenthiuron	Polo® (500 SC)	120 ml/a	Neonicotinoids	Contact and stomach poison, with systemic properties.	0.15, 0.3, 0.6, 1.2, 2.4, 4.8, 9.6
Nitenpyram	Pyramid® (10 SL)	200 ml/a	Neonicotinoids	Systemic insecticides with translamin, contact and stomach action.	0.25, 0.5, 1, 2, 4, 8, 16
Thiacloprid	Talent® (480 SC)	25 ml/a	Neonicotinoids	Contact and stomach poison, with systemic properties.	0.003125, 0.00625, 0.0125, 0.025, 0.05, 0.1, 0.2

Table 2: Treatment combinations of different concentration of each insecticide with three dose levels of Diver®.

Treatments	Concentrations of each insecticide	Dose levels of Diver® per acre		
		1st set of combinations	2nd set of combinations	3rd set of combinations
T_1	C1	500 ml	1000 ml	0 ml
T_2	C2	500 ml	1000 ml	0 ml
T_3	C3	500 ml	1000 ml	0 ml
T_4	C4	500 ml	1000 ml	0 ml
T_5	C5	500 ml	1000 ml	0 ml
T_6	C6	500 ml	1000 ml	0 ml
T_7	C7	500 ml	1000 ml	0 ml
T_8	C8 (only water)	C8 (only water)	C8 (water)	C8 (water)

Exposure of *P. solenopsis* to different test solutions

Twig-dip method was used for the exposure of 1st instars, 2nd instars and adult females of *P. solenopsis* to different concentrations of selected insecticides applied alone and in combinations with two levels of Diver®. In this method, small tender shoe-flower shoots of *R. chinensis* of 15 cm lengths were clipped with sharp budding knife, brought into the IPM laboratory, rinsed with water and kept in the laboratory for complete evaporation of water from the surface of the leaves of twig. This was done to make the twig free of any kind of contamination. The contaminated free twigs were dipped in each of the test solutions of different insecticide-Diver® combinations, separately in the beaker for 2 minutes and kept on filter paper separately for air drying in the laboratory. An experimental unit was prepared that consisted of two disposable cups each of 120 ml capacity having top diameter of 9 cm, bottom diameter of 5.5 cm and height of 9.9 cm. One cup (upper cup) was inverted and fitted on the other cup (lower cup). Lower cup of each experimental unit was filled with nutrient media having a mixture of boron, zinc, DAP and water (1: 1: 1: 1000). A hole for the insertion of twig was made in the lid of lower cup and pin holes for aeration were made in all surfaces of the upper cup. Each experimental unit had one twig treated with respective insecticide-Diver® combination, insecticide level alone, Diver® levels alone and water (control). Three sets, each having nine of such experimental unit, were prepared for whole of the experiment against each selected *P. solenopsis* stage. A counted number of one day old twenty individuals of each of 1st instar, 2nd instar and adult females were released on the treated twig of each experimental unit. For each stage of *P. solenopsis*, separate sets of experiment were maintained. Similarly, separate set of experiment for each insecticide, alone or in combination with Diver®, was carried out.

Whole of the experiment was laid out in Completely Randomized Design and repeated thrice.

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Data collection

Three days post treatment application, *P. solenopsis* individuals from each treatment were separated, counted and kept into a petri dish having fresh untreated shoe-flower twig for three days. The individuals were observed under microscope after three days. The individuals showing no movement in their appendages on slight but consistence touch for 15 seconds were considered dead. The data collected on dead individuals were transformed into percentage corrected mortality by Henderson and Tilton formula (Henderson and Tilton, 1955) given below.

$$\text{Corrected \% mortality} = \frac{\text{\% mortality in treatment} - \text{\% mortality in control}}{100 - \text{\% mortality in control}} \; X\, 100$$

Statistical analysis

Percentage corrected mortality data were analyzed by main effects one way ANOVA through Multivariate General Linear Model (MGLM) Technique (Tabachnick and Fidell, 2001), using Statistica program to determine the parameters of significance and mean values of different treatments. The means, if treatment showed significant variations, were compared with Tukey's Honestly Significant Difference (HSD) test at probability level of 5%, as performed by Danho et al. (2002). LC_{50}, chi-square, and confidence interval values for each insecticide against each stage of *P. solenopsis* were also calculated by probit analysis using the Minitab Statistical Program (Finney, 1971). Regression between mortality and insecticides' concentrations was also established, using linear regression and Pearson correlation analysis at 5% level of probability. Scattered diagrams for concentration of each insecticide (alone or in combination with Diver® at both doses) and mortality of 1st instar, 2nd instar and adult females of *P. solenopsis* were also drawn to construct fitted simple regression line of mortality on concentrations.

RESULTS

Mortality rates of different life stages of *Phenacoccus solenopsis* exposed to various concentrations of neonicotenoids insecticides alone or in combination with two different doses of Diver®

Significant variations in percentage mortality of adult females, 1st instar and 2nd instar stages of *P. solenopsis* were demonstrated by different concentrations of diafenthiuron, nitenpyram and thiacloprid when applied alone or in combinations with 500 ml/acre and 1000 ml/acre dose of Diver® ($P < 0.05$) (Table 3). A concentration dependent mortality in all tested life stages of *P. solenopsis* was explained by diafenthiuron, nitenpyram and thiacloprid when applied alone or in combinations with 500 ml/acre and 1000 ml/acre dose of Diver® (Figure 1, 2, 3). Diafenthiuron alone demonstrated mortality in the range of 10.3-46.0%. 8.0-42.3% and 7.0-38.7% in 1st instar, 2nd instar and adult female stage of *P. solenopsis*, respectively, being significantly higher at higher concentration (9.6%) and lower at lower concentration (0.15%).

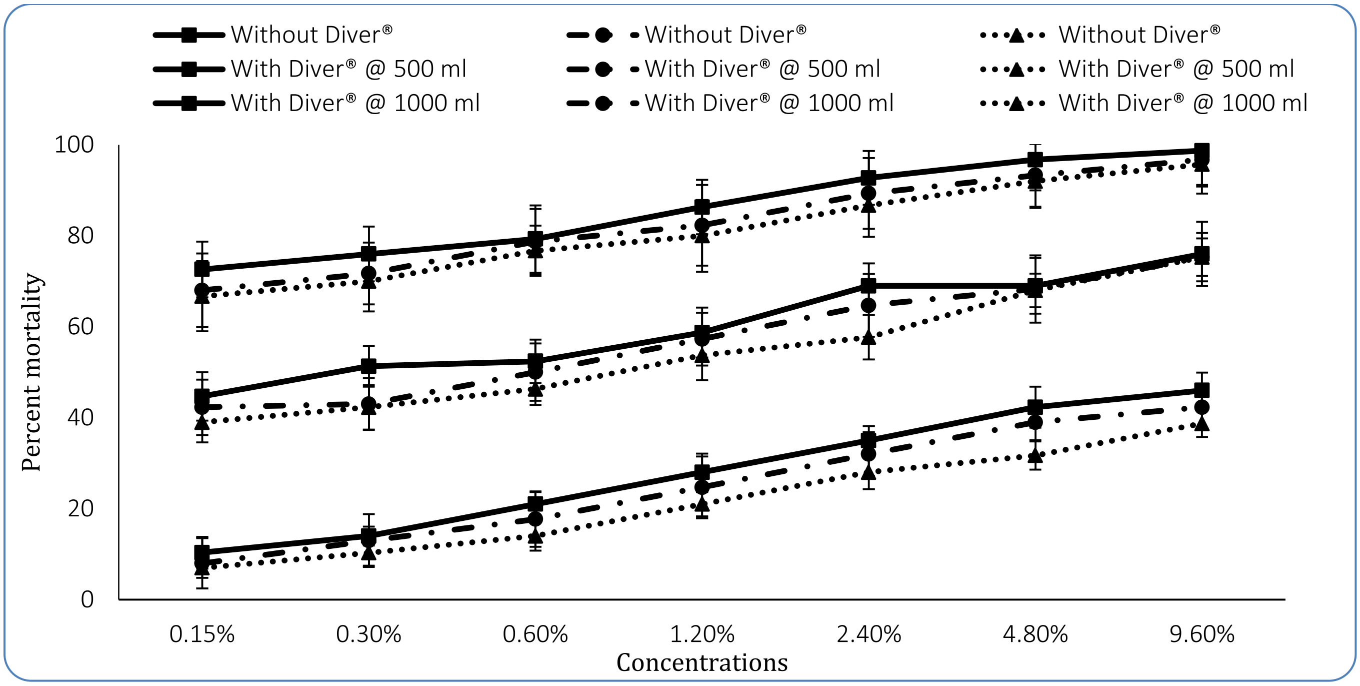

Figure 1: Percentage mortality of 1st instar (solid lines), 2nd instar (bar-dot lines) and adult female (dotted lines) mealybug, *Phenacoccus solenopsis* at different concentrations of diafenthiuron when applied alone (lower most cluster of line graph) or in combination with 500 ml/acre (middle cluster of line graph) and 1000 ml/acre dose (top most cluster of line graph) of Diver® (bars indicate ±SE).

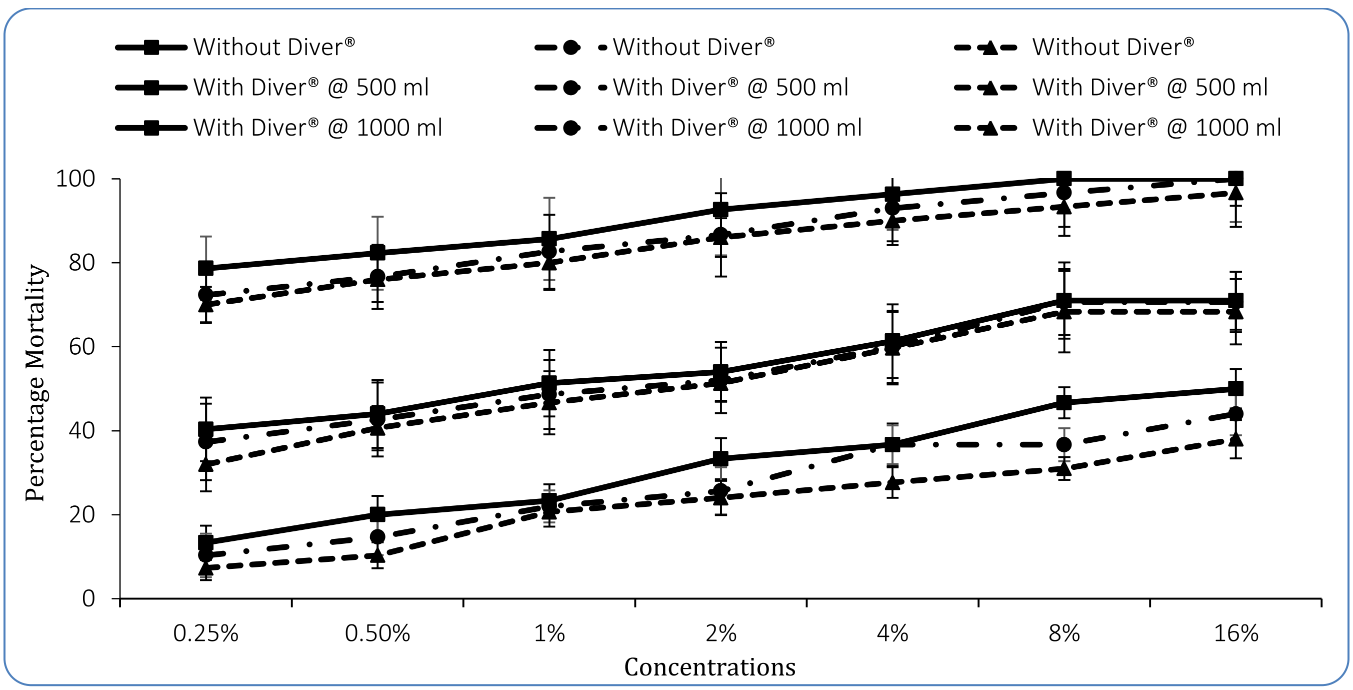

Figure 2: Percentage mortality of 1st instar (solid lines), 2nd instar (bar-dot lines) and adult female (dotted lines) mealybug, *Phenacoccus solenopsis* at different concentrations of nitenpyram when applied alone (lower most cluster of line graph) or in combination with 500 ml/acre (middle cluster of line graph) and 1000 ml/acre dose (top most cluster of line graph) of Diver® (bars indicate ±SE).

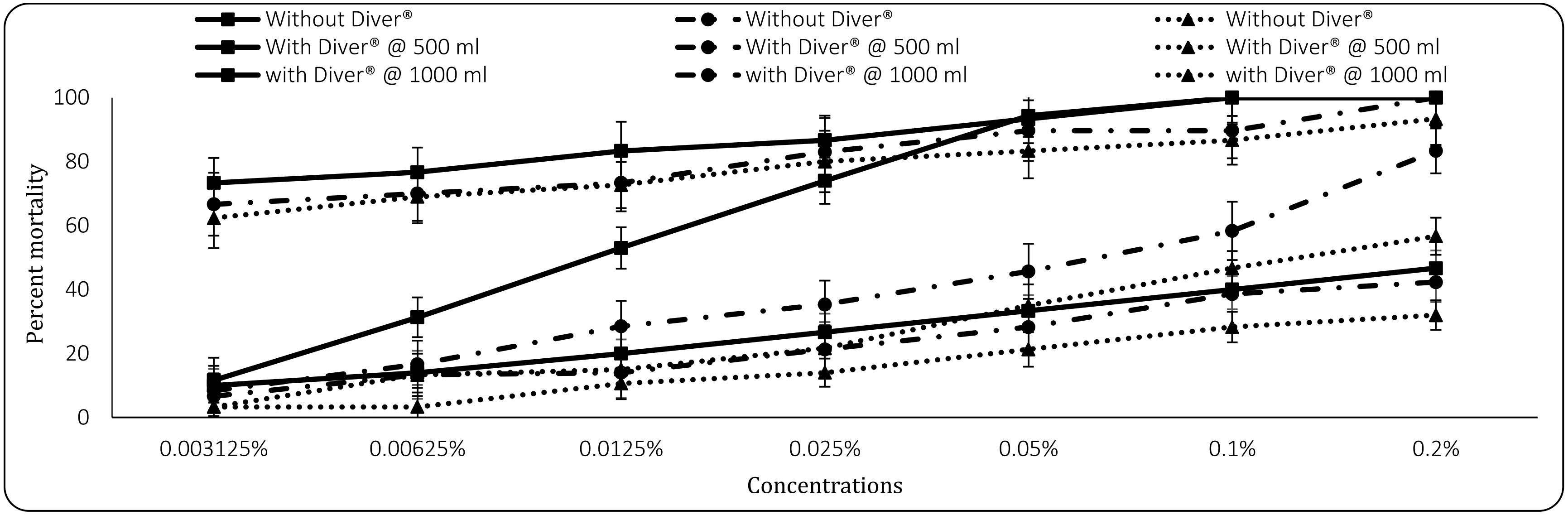

Figure 3: Percentage mortality of 1st instar (solid lines), 2nd instar (bar-dot lines) and adult female (dotted lines) mealybug, *Phenacoccus solenopsis* at different concentrations of thiacloprid when applied alone (lower most cluster of line graph) or in combination with 500 ml/acre (middle cluster of line graph) and 1000 ml/acre dose (top most cluster of line graph) of Diver® (bars indicate ±SE).

Table 3: ANOVA parameters regarding the variation in percent mortality of adult female, 1st instar and 2nd instar stages of *P. solenopsis* when exposed to different concentrations of neonicotenoid insecticides alone or in combination with Diver® (Total *df* = 23; *df* of replication = 2).

Dependent variable	Life stage of insect	Type of application	*Df*	Concentrations of Neonicotenoid Insecticides					
				Diafenthiuron		Nitenpyram		Thiacloprid	
				F	P	F	P	F	P
Percent mortality	1st instar	Insecticide alone	$7^a/14^b$	10.24	0.00**	21.18	0.00**	26.88	0.00**
		Insecticide + 500 ml Diver®	$7^a/14^b$	25.52	0.00**	53.03	0.00**	31.37	0.00**
		Insecticide + 1000 ml Diver®	$7^a/14^b$	75.63	0.00**	214.13	0.00**	93.72	0.00**
	2nd instar	Insecticide alone	$7^a/14^b$	18.11	0.00**	39.05	0.00**	7.97	0.00**
		Insecticide + 500 ml Diver®	$7^a/14^b$	41.32	0.00**	78.34	0.00**	28.81	0.00**
		Insecticide + 1000 ml Diver®	$7^a/14^b$	89.43	0.00**	169.44	0.00**	79.15	0.00**
	Adult female	Insecticide alone	$7^a/14^b$	31.11	0.00**	18.70	0.00**	20.42	0.00**
		Insecticide + 500 ml Diver®	$7^a/14^b$	43.26	0.00**	70.95	0.00**	223.89	0.00**
		Insecticide + 1000 ml Diver®	$7^a/14^b$	67.69	0.00**	172.07	0.00**	114.49	0.00**

Df = Degree of freedom; a = Degree of freedom of concentrations; b = Error Degree of freedom; F = Value of ; P = Probability value; ** = Highly significant at probability level of 0.05

Diafenthiuron demonstrated 44.7-76.0%, 42.3-75.5% and 39.0-75.3%, mortality when admixed with 500 ml/acre dose of Diver®, while explained 72.7-98.7%, 68.0-96.7% and 66.6-95.7% mortality when admixed with 1000 ml/acre dose of Diver®, in 1st instar, 2nd instar and adult female stages of *P. solenopsis,* respectively, being significantly higher at higher concentration (9.6%) and lower at lower concentration (0.15%) (Figure 1). Nitenpyram at its various concentrations (0.25%-16.0%) gave 13.3-50.0% mortality in 1st instar, 10.3-38.0% in 2nd instar and 7.3-44.0% mortality in adult females when Diver® was not admixed with nitenpyram. However, nitenpyram caused 40.3-71.0% and 78.7-100% mortality in 1st instar, 37.3-70.6% and 72.33-100% in 2nd instar and 32.0-68.3% and 70.0-96.6% mortality in adult female, being significantly higher at higher concentration (16%) and lower at lower concentration (0.25%) when nitenpyram was admixed with 500 ml/acre and 1000 ml/acre dose of Diver® respectively (Figure 2). At various concentrations, thiacloprid demonstrated 10.0-46.7%, 6.6-42.3% and 3.3-32.0% mortalities in 1st instar, 2nd instar and adult female stage, respectively being significantly higher at higher concentration (0.2%) and lower at lower concentration (0.003125%). Similarly, thiacloprid, when admixed with 500 ml/acre and 1000 ml/acre doses of Diver®, gave 11.6-100% and 73.3-100%, 8.3-83.3% and 66.6-100% and 3.3-56.7% and 62.3-93.3% mortalities in 1st instar, 2nd instar and adult female stage respectively (Figure 3). The mortality results (Figure 1, 2 and 3) also demonstrated that 1st instar was found comparatively more susceptible followed by 2nd instar and adult female stages of *P. solenopsis*. Admixing of 500 ml and 1000 ml of Diver® with diafenthiuron administered 65.2-332.7% and 114.6-602.8%; 78.0-428.8% and 128.6-750.0%; and 94.6-457.1% and 147.3-852.9% increases in mortalities of 1st instar, 2nd instar and adult female stages of *P. solenopsis* respectively. Similarly, 42.0-202.6% and 100.0-490.2%; 60.6-261.4% and 127.3-600.2% and 79.8-336.6% and 154.4-854.9% increases in mortalities of 1st instar, 2nd instar and adult female stages of *P. solenopsis* were demonstrated when nitenpyram was admixed with 500 ml and 1000 ml of Diver® respectively. However, thiacloprid, when admixed with 500 ml and 1000 ml of Diver®, explained 16.7-114.3% and 114.3-633.3%; 24.9-103.6% and 132.3-900.9%; and 0.0-303.9% and 191.7-1990.9% increases in mortalities of 1st instar, 2nd instar and adult female stages of *P. solenopsis* respectively. Increase in mortality of insecticide-with-Diver® over without-Diver® treatment for both doses of Diver® and all the life stages of *P. solenopsis* was observed higher at lower concentration and lower at higher concentration in case of diafenthiuron and nitenpyram; however, the same for thiacloprid was observed higher at lower concentration for 1000 ml dose of Diver® and fluctuating for concentrations at 500 ml dose of Diver® for all stages of *P. solenopsis* (Table 4).

LC_{50} values of neonicotenoid insecticides, alone or in combination with two different doses of Diver® for adult female, 1st instar and 2nd instar stages of *Phenacoccus solenopsis*

The performance of evaluated neonicotinoids, alone and in combinations with Diver®, varied significantly for adult female, 1st instar and 2nd instar stages as none of 95% fiducial limit overlapped each other (Table 4). LC_{50} values of diafenthiuron, nitenpyram and thiacloprid without coadministration of Diver® were 18.27, 41.68 and 2.1%; 9.76, 19.57 and 1.4%; and 12.42, 35.4 and 1.8% for adult females, 1st instar and 2nd instar stages respectively. Thiacloprid, without coadministration of Diver®, exhibited lowest LC_{50} value and proved to be the most effective among tested neonicotinoids against these life stages followed by diafenthiuron and nitenpyram. The coadministration of Diver® with tested neonicotinoids demonstrated a significant decrease in their LC_{50} values. The coadministration of Diver® at its half (500 ml/acre) and full recommended dose rate (1000 ml/acre) with diafenthiuron demonstrated approximately 83 and 203 times reduction; 65 and 195 times reduction; and 59 and 138 times reduction in its LC_{50} for adult females, 1st instar, 2nd instar stages, respectively.

Similarly, significant reductions of approximately 149 and 347 times; 89 and 280 times; and 142 and 295 times were obtained in LC_{50} of nitenpyram for adult female, 1st instar, 2nd instar stages when nitenpyram was coadministrated with Diver® at 500 and 1000 ml/acre doses respectively. Coadministration of Diver® at 500 and 1000 ml/acre doses with thiacloprid exhibited an approximate reduction of 30 and 111 times; 28 and 88 times; and 30 and 106 times in its LC_{50} for adult females, 1st instar and 2nd instar stages respectively. The results also described that coadministration of Diver® employed variable impacts on reduction of LC_{50} values of evaluated neonicotinoids and demonstrated least reduction in LC_{50} values of thiacloprid (Table 5).

Table 4: Percent increase in mortality of insecticide-with- Diver® over without-Diver® treatments for 1st instar, 2nd instar and adult female stages of *Phenacoccus solenopsis* when diafentiuron, nitenpyram and thiacloprid were admixed with 500 ml/acre and 1000 ml/acre does rates of Diver® (values inside the braces under columns titled "Increase in mortality of insecticide-with-Diver® over without- Diver® treatment (%)" indicate percent corrected mortalities)

Insecticides	Concentrations	1st instar			2nd instar			Adult female		
		Mortality Without Diver® (%)	Increase in mortality of insecticide-with-Diver® over without- Diver® treatment (%)		Mortality Without Diver® (%)	Increase in mortality of insecticide-with-Diver® over without- Diver® treatment (%)		Mortality Without Diver® (%)	Increase in mortality of insecticide-with-Diver® over without- Diver® treatment (%)	
			500 ml/acre Diver®	1000 ml/acre Diver®		500 ml/acre Diver®	1000 ml/acre Diver®		500 ml/acre Diver®	1000 ml/acre Diver®
Diafenthiuron	0.15%	10.3	333.9 (44.7)	602.8 (72.7)	8.0	428.8 (42.4)	750.0 (68.0)	7.0	457.1 (39.0)	852.9 (66.7)
	0.30%	14.0	266.4 (51.3)	442.9 (76.0)	13.0	230.8 (43.0)	451.5 (71.7)	10.3	310.7 (42.3)	579.6 (70.0)
	0.60%	21.0	149.5 (52.4)	277.6 (79.3)	17.7	182.5 (50.0)	344.6 (78.7)	14.0	230.7 (46.3)	447.9 (76.7)
	1.20%	28.0	109.6 (58.7)	208.2 (86.3)	24.7	131.9 (57.3)	233.2 (82.3)	21.0	155.7 (53.7)	280.9 (80.0)
	2.40%	35.0	97.1 (69.0)	164.9 (92.7)	32.0	102.2 (64.7)	179.1 (89.3)	28.0	106.1 (57.7)	209.6 (86.7)
	4.80%	42.3	63.1 (69.0)	128.6 (96.7)	39.0	75.1 (68.3)	139.2 (93.3)	31.7	114.5 (68.0)	190.2 (92.0)
	9.60%	46.0	65.2 (76.0)	114.6 (98.7)	42.3	78.0 (75.3)	128.6 (96.7)	38.7	94.6 (75.3)	147.3 (95.7)
Nitenpyram	0.25%	13.3	202.6 (40.3)	490.2 (78.7)	10.3	261.4 (37.3)	600.2 (72.3)	7.3	336.6 (32.0)	854.9 (70.0)
	0.50%	20.0	120.0 (44.0)	311.7 (82.3)	14.6	190.9 (42.7)	422.9 (76.7)	10.3	293.6 (40.7)	635.7 (76.0)
	1%	23.3	120.0 (51.3)	267.2 (85.7)	22.0	121.2 (48.7)	275.8 (82.7)	20.7	125.8 (46.8)	287.2 (80.0)
	2%	33.3	62.0 (54.0)	178.0 (92.6)	25.6	102.7 (52.0)	237.7 (86.7)	24.0	113.9 (51.3)	258.3 (86.0)
	4%	36.6	67.3 (61.3)	162.8 (96.3)	36.7	63.7 (60.0)	153.7 (93.0)	27.6	115.7 (59.7)	225.4 (90.0)
	8%	46.7	52.2 (71.0)	114.3 (100.0)	36.6	92.7 (70.6)	163.7 (96.7)	31.0	120.4 (68.3)	201.1 (93.3)
	16%	50.0	42.0 (71.0)	100.0 (100.0)	44.0	60.6 (70.8)	127.3(100.0)	38.0	79.8 (68.4)	154.4 (96.7)
Thiacloprid	0.003125%	10.0	16.7 (11.7)	633.3 (73.3)	6.6	25.1 (8.3)	900.9 (66.6)	3.3	0.0 (3.3)	1788.8(62.3)
	0.00625%	14.0	123.8 (31.3)	447.6 (76.7)	13.3	24.9 (16.6)	425.1 (70.0)	3.3	303.9 (13.3)	1990.9(69.0)
	0.0125 %	20.0	165.0 (53)	316.7 (83.3)	14.0	103.6 (28.5)	423.8 (73.3)	10.6	40.7 (15.0)	581.7 (72.7)
	0.025 %	26.6	177.5 (74.0)	225.1 (86.6)	21.3	65.9 (35.3)	289.7 (83.0)	14.0	54.7 (21.6)	471.4 (80.0)
	0.05%	33.3	183.0 (94.3)	180.0 (93.3)	28.3	61.3 (46.7)	216.8 (89.7)	21.3	64.3 (35.0)	291.2 (83.3)
	0.1%	40.0	150.0 (100.0)	150.0 (100.0)	38.6	51.1 (58.3)	132.3 (89.6)	28.3	64.9 (46.7)	206.2 (86.7)
	0.2%	46.7	114.3 (100.0)	114.3 (100.0)	42.3	96.9 (83.3)	136.4(100.0)	32.0	77.1 (56.7)	191.7 (93.3)

Table 5: LC_{50} values of tested insecticides for various life stages of *P. solenopsis* with and without co-administration of Diver®.

Life Stages	Insecticides	Application technique	LC_{50} (%)	(95%) FL	χ^2 (*df*, P)	Category (compared to FRD)
Adult female	Diafenthiuron (Polo® 500 SC with ai = 50%) (FRD = 120 ml/acre = 1.2%)	Insecticide only	18.27%	12.84-29.25	3.57 (5, 0.61)	Non-toxic (LC_{50} >FRD)
		Insecticide + 500 ml Diver®	0.22% (≈83)*	0.11-0.95	2.86 (5, 0.72)	Toxic (LC_{50} < FRD)
		Insecticide + 1000 ml Diver®	0.09% (≈203)*	0.01-0.22	2.05 (5, 0.8)	Toxic (LC_{50} < FRD)
	Nitenpyram (Pyramid® 10 SL with ai = 10%) (FRD = 200 ml/acre = 2%)	Insecticide only	41.68%	26.0-81.57	7.47 (5, 0.18)	Non-toxic (LC_{50} >FRD)
		Insecticide + 500 ml Diver®	0.28% (≈149)*	0.13-1.39	5.01 (5, 0.41)	Toxic (LC_{50} < FRD)
		Insecticide + 1000 ml Diver®	0.12% (≈347)*	0.06-0.19	11.62 (5, 0.04)	Toxic (LC_{50} < FRD)
	Thiacloprid (Talent® 480 SC with ai = 48%) (FRD = 25 ml/acre = 0.025%)	Insecticide only	2.1%	1.19-3.57	15.38 (5, 0.00)	Non-toxic (LC_{50} >FRD)
		Insecticide + 500 ml Diver®	0.07% (≈30)*	0.03-0.26	0.87 (5, 0.97)	Non-toxic (LC_{50} >FRD)
		Insecticide + 1000 ml Diver®	0.019% (≈111)*	0.004-0.31	20.71 (5, 0.00)	Toxic (LC_{50} < FRD)
1st Instar	Diafenthiuron (Polo® 500 SC) (FRD = 120 ml/acre = 1.2%)	Insecticide only	9.76%	7.40-13.85	5.31 (5, 0.37)	Non-toxic (LC_{50} >FRD)
		Insecticide + 500 ml Diver®	0.15% (≈65)*	0.08-0.71	2.42 (5, 0.78)	Toxic (LC_{50} < FRD)
		Insecticide + 1000 ml Diver®	0.05% (≈195)*	0.009-0.25	6.12 (5, 0.29)	Toxic (LC_{50} < FRD)
	Nitenpyram (Pyramid® 10 SL) (FRD = 200 ml/acre = 2%)	Insecticide only	19.57%	14.43-29.04	13.47 (5, 0.01)	Non-toxic (LC_{50} >FRD)
		Insecticide + 500 ml Diver®	0.22% (≈89)*	0.09-1.86	5.94 (5, 0.31)	Toxic (LC_{50} < FRD)
		Insecticide + 1000 ml Diver®	0.07% (≈280)*	0.003-0.31	0.81 (5, 0.97)	Toxic (LC_{50} < FRD)
	Thiacloprid	Insecticide only	1.4%	1.02-2.81	7.01	Non-toxic (LC_{50} >FRD)

	(Talent® 480 SC) (FRD = 25 ml/acre = 0.025%)				(5, 0.21)	
		Insecticide + 500 ml Diver®	0.05% (≈28)*	0.007-0.29	0.89 (5, 0.96)	Non-toxic (LC_{50} >FRD)
		Insecticide + 1000 ml Diver®	0.016% (≈88)*	0.006-0.07	21.13 (5, 0.00)	Toxic (LC_{50} < FRD)
2nd Instar	Diafenthiuron (Polo® 500 SC) (FRD = 120 ml/acre = 1.2%)	Insecticide only	12.42%	9.21-18.26	7.58 (5, 0.18)	Non-toxic (LC_{50} >FRD)
		Insecticide + 500 ml Diver®	0.21% (≈59)*	0.12-1.63	4.12 (5, 0.53)	Toxic (LC_{50} < FRD)
		Insecticide + 1000 ml Diver®	0.09% (≈138)*	0.01-0.21	5.13 (5, 0.40)	Toxic (LC_{50} < FRD)
	Nitenpyram (Pyramid® 10 SL) (FRD = 200 ml/acre = 2%)	Insecticide only	35.40%	26.65-52.81	4.54 (5, 0.47)	Non-toxic (LC_{50} >FRD)
		Insecticide + 500 ml Diver®	0.25% (≈142)*	0.09-1.65	3.93 (5, 0.55)	Toxic (LC_{50} < FRD)
		Insecticide + 1000 ml Diver®	0.12% (≈295)*	0.02-2.07	9.64 (5, 0.08)	Toxic (LC_{50} < FRD)
	Thiacloprid (Talent® 480 SC) (FRD = 25 ml/acre = 0.025%)	Insecticide only	1.8%	0.75-4.30	2.84 (5, 0.72)	Non-toxic (LC_{50} >FRD)
		Insecticide + 500 ml Diver®	0.06% (≈30)*	0.05-0.45	1.18 (5, 0.94)	Non-toxic (LC_{50} >FRD)
		Insecticide + 1000 ml Diver®	0.017% (≈106)*	0.008-0.07	3.11 (5, 0.68)	Toxic (LC_{50} < FRD)

FDR = Field recommended dose, * = values indicate x-times reduction in LC_{50} of tested insecticides admixed with Diver® over insecticide treatment without Diver®

Association of concentrations of diafenthiuron, nitenpyram and thiacloprid with mortality of different life stages of *P. solenopsis*

The probability value for correlation (P < 0.05) revealed that a relationship existed between percent mortality of 1st instar, 2nd instar and adult female stages of *P. solenopsis* and concentrations of diafenthiuron, nitenpyram and thiacloprid when were applied alone or in coadministration with 500 and 1000 ml doses of Diver® (Figure 1, 2 and 3).

The correlation coefficient values (r) and scatter diagrams (Figure 1, 2 and 3) showed that concentrations of three neonicotenoids applied alone or in coadministration with 500 and 1000 ml of Diver® had a high positive correlation with percent mortality of all the three life stages of *P. solenopsis* as coefficient of correlation values were very close to positive one (+1) value and data points were found scattered very close to a positively sloped line (Figure 1, 2 and 3). Regression parameters and scatter diagrams revealed that concentrations of three neonicotenoids, alone or in coadministration with 500 and 1000 ml of Diver®, had a significant linear relationship with and explained significant variability in percent mortality of all the three life stages of *P. solenopsis* (P < 0.05) (Figure 1, 2 and 3).

Coefficient of determination values ($100R^2$) illustrated that concentrations of diafenthiuron explained 71.9%, 80.53% and 86.74%; 69.67%,

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74.75% and 77.52% and 74.54%, 74.71% and 76.97% of total variability in percent mortality of 1st instar, 2nd instar and adult females stages of *P. solenopsis* when applied alone, coadministrated with 500 ml Diver® and coadministrated with 1000 ml Diver®, respectively (Figure 1).

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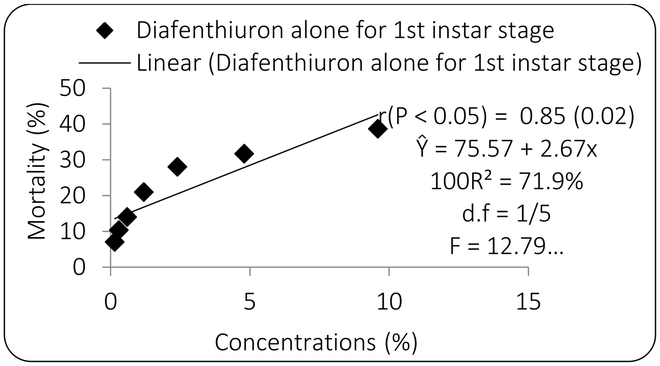
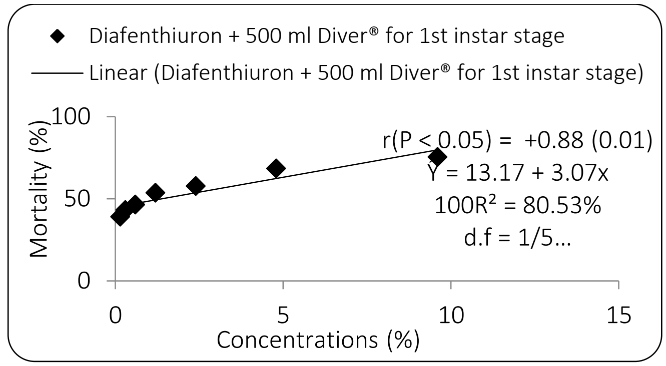
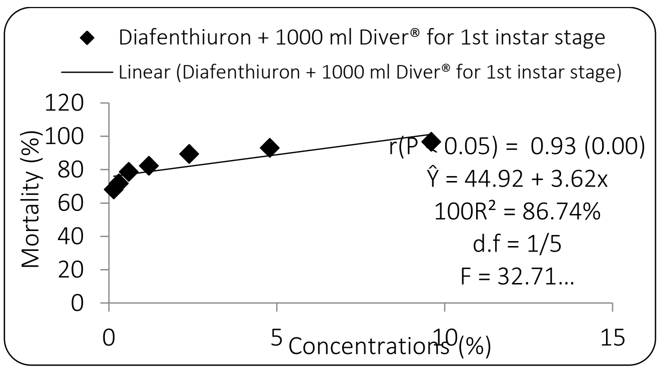
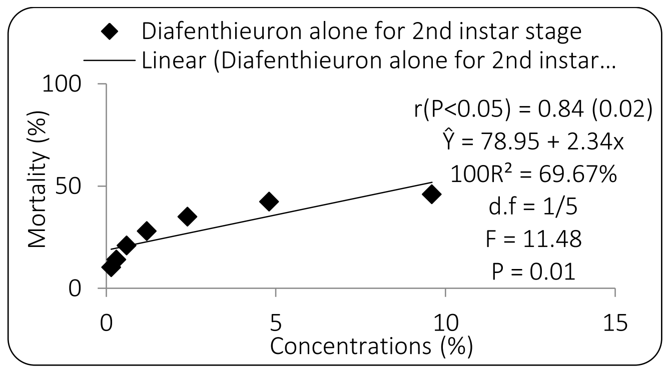
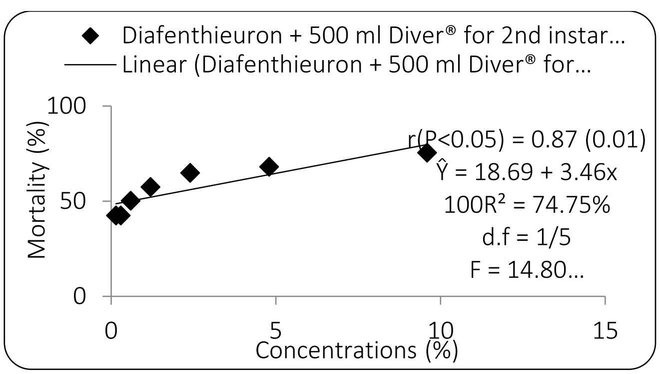
Similarly, concentrations of nitenpyram demonstrated 61.84%, 75.5% and 75.51%; 63.54%, 67.54% and 69.69%; and 71.87%, 73.56% and 75.52% of total variability; while concentrations of thiacloprid explained showed 68.97%, 70.02% and 78.05%; 69.3%, 73.8% and 79.99%; and 78.50%, 80.44% and 86.05% of total variability (Figure 3) in percent mortality of 1st instar, 2nd instar and adult females stages of *P. solenopsis* when applied alone, coadministrated with 500 and 1000 ml Diver®, respectively (Figure 4, 5 and 6).

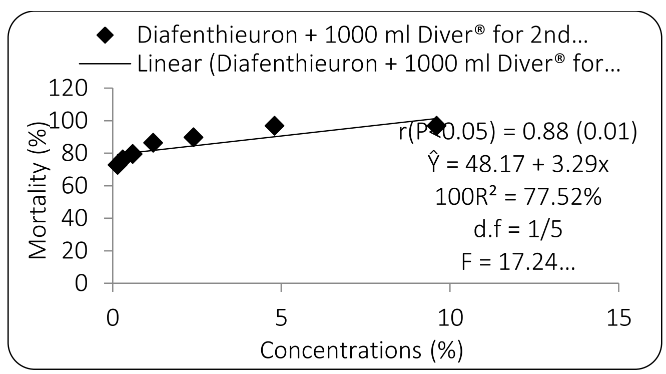


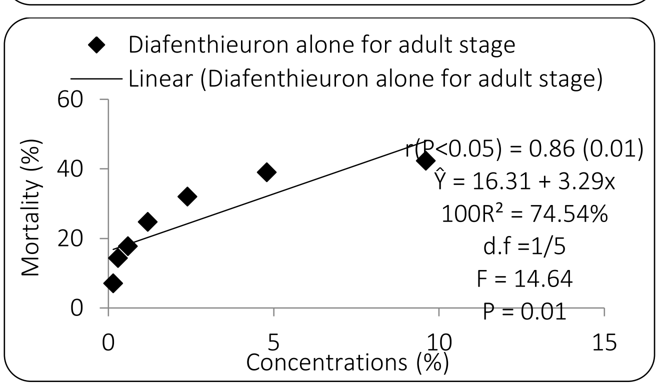


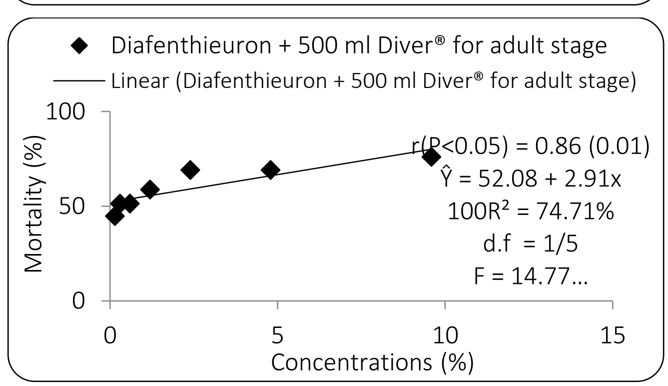












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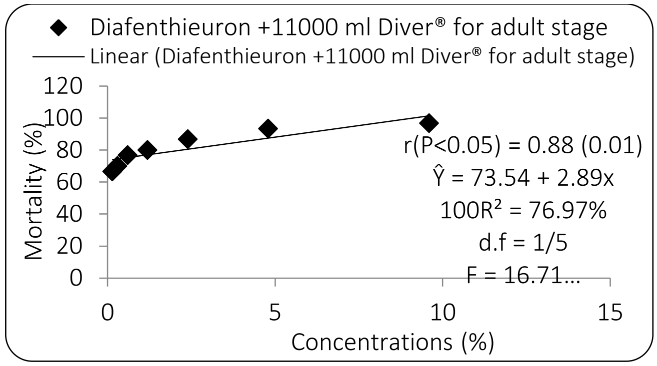
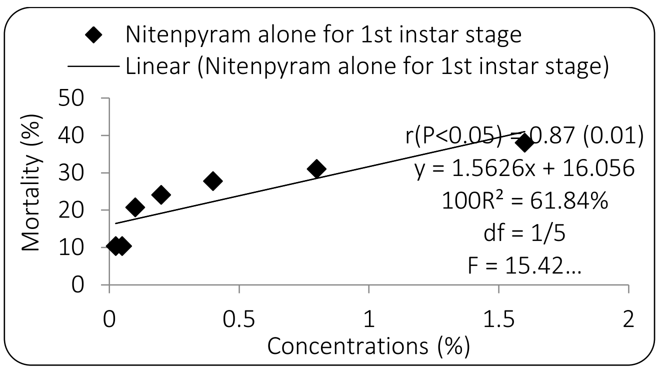
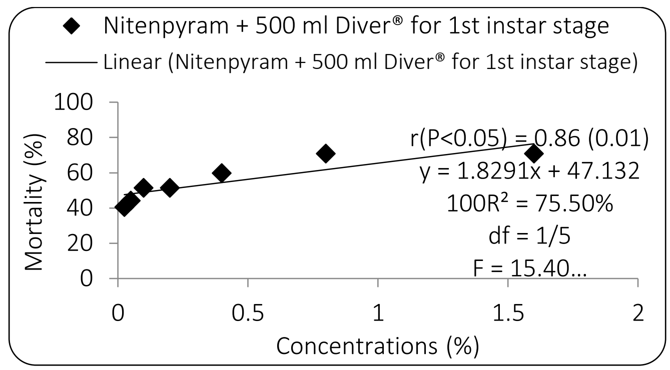
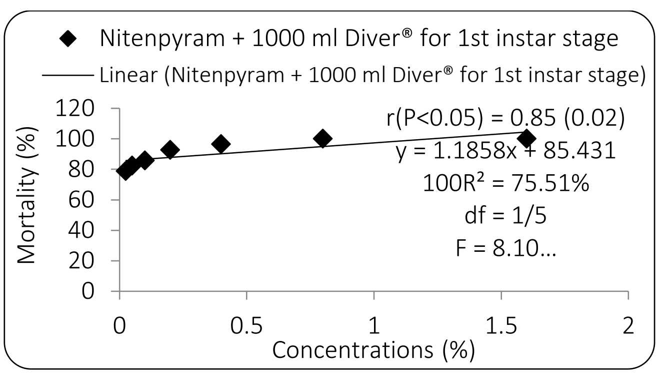
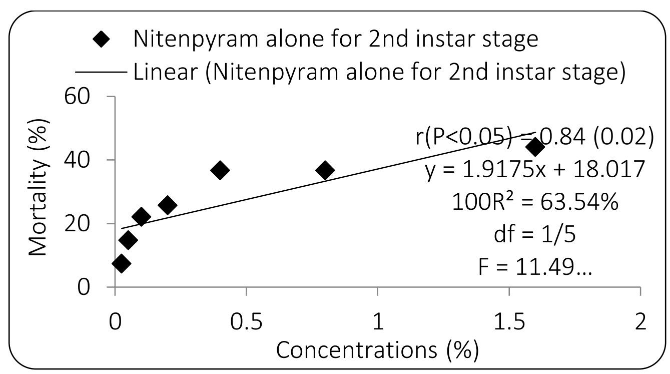


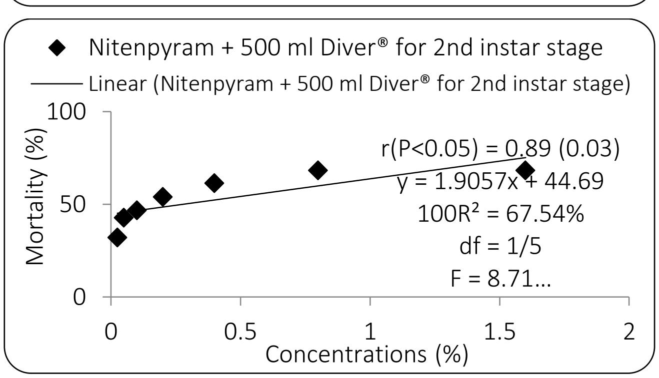
Figure 4: Correlation coefficient (r), linear regression equation ($\hat{Y}=b\pm ax$) and associated ANOVA parameters (*df*, F and P values), Coefficient of determination ($100R^2$) and scatter diagram showing the fitted simple regression line of $\hat{Y}$ (% mortality of 1st instar, 2nd instar and adult female stages of *Phenacoccus solenopsis*) on X (Concentrations of diafenthiuron alone as well as in coadministration with 500 ml and 1000 ml of Diver®) (P = Probability value, *d.f* = degree of freedom of concentration/degree of freedom of error, F = value of F calculated).

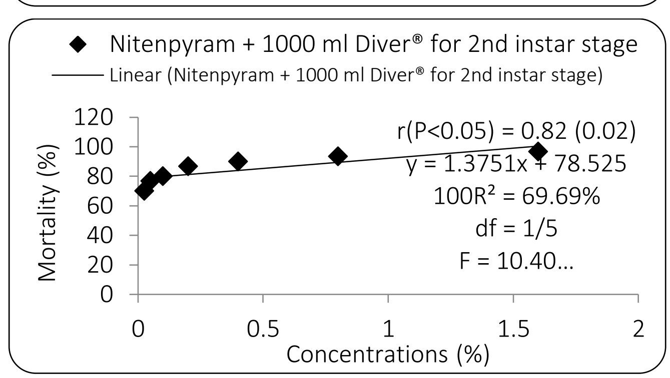












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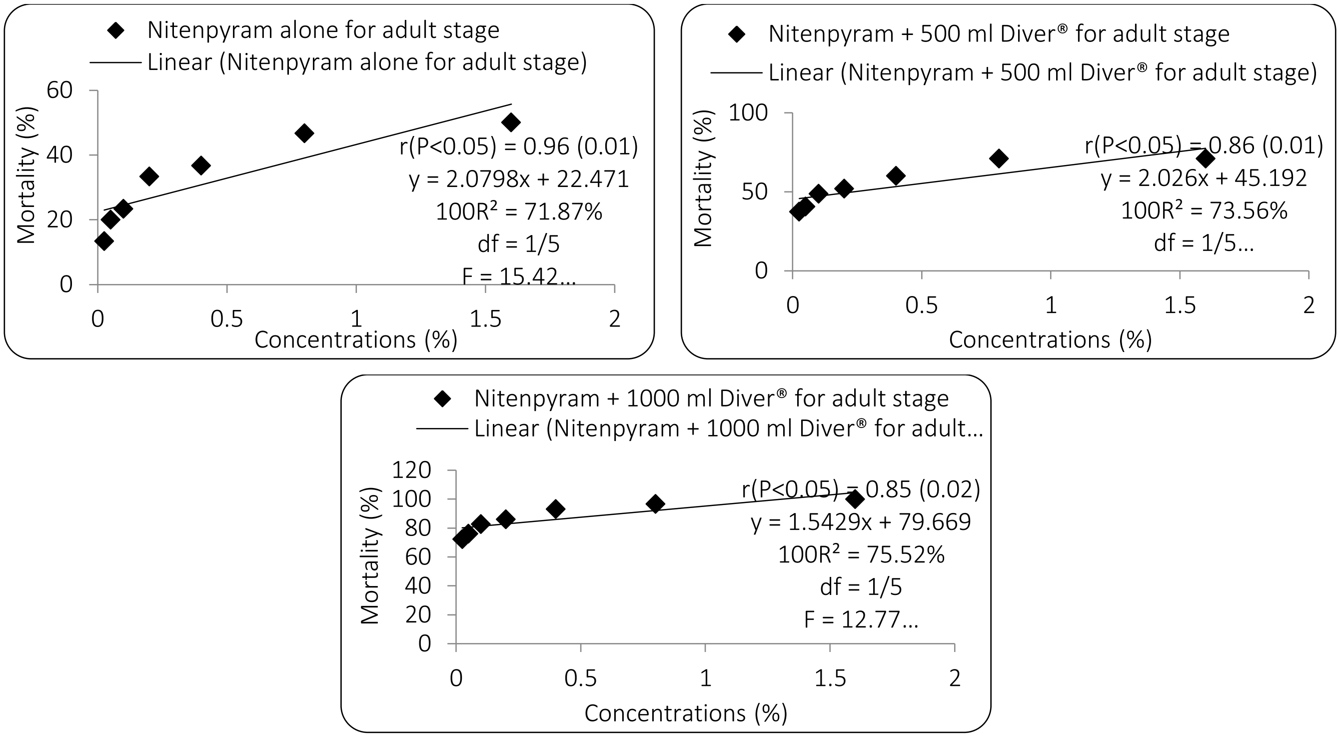
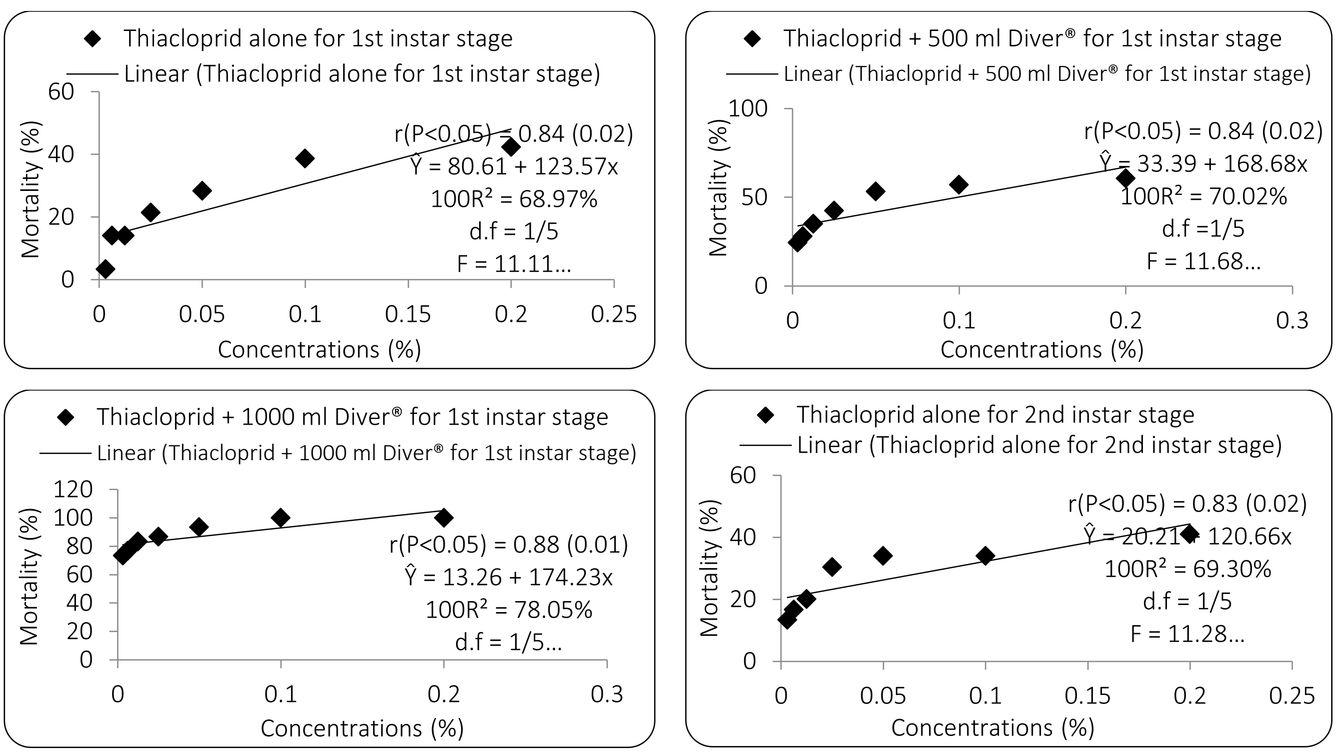


Figure 5: Correlation coefficient (r), linear regression equation ($\hat{Y}=b\pm ax$) and associated ANOVA parameters (*df*, F and P values), Coefficient of determination ($100R^2$) and scatter diagram showing the fitted simple regression line of $\hat{Y}$ (% mortality of 1st instar, 2nd instar and adult female stages of *Phenacoccus solenopsis*) on X (Concentrations of nitenpyram alone as well as in coadministration with 500 ml and 1000 ml of Diver®) (P = Probability value, *d.f* = degree of freedom of concentration/degree of freedom of error, F = value of F calculated).



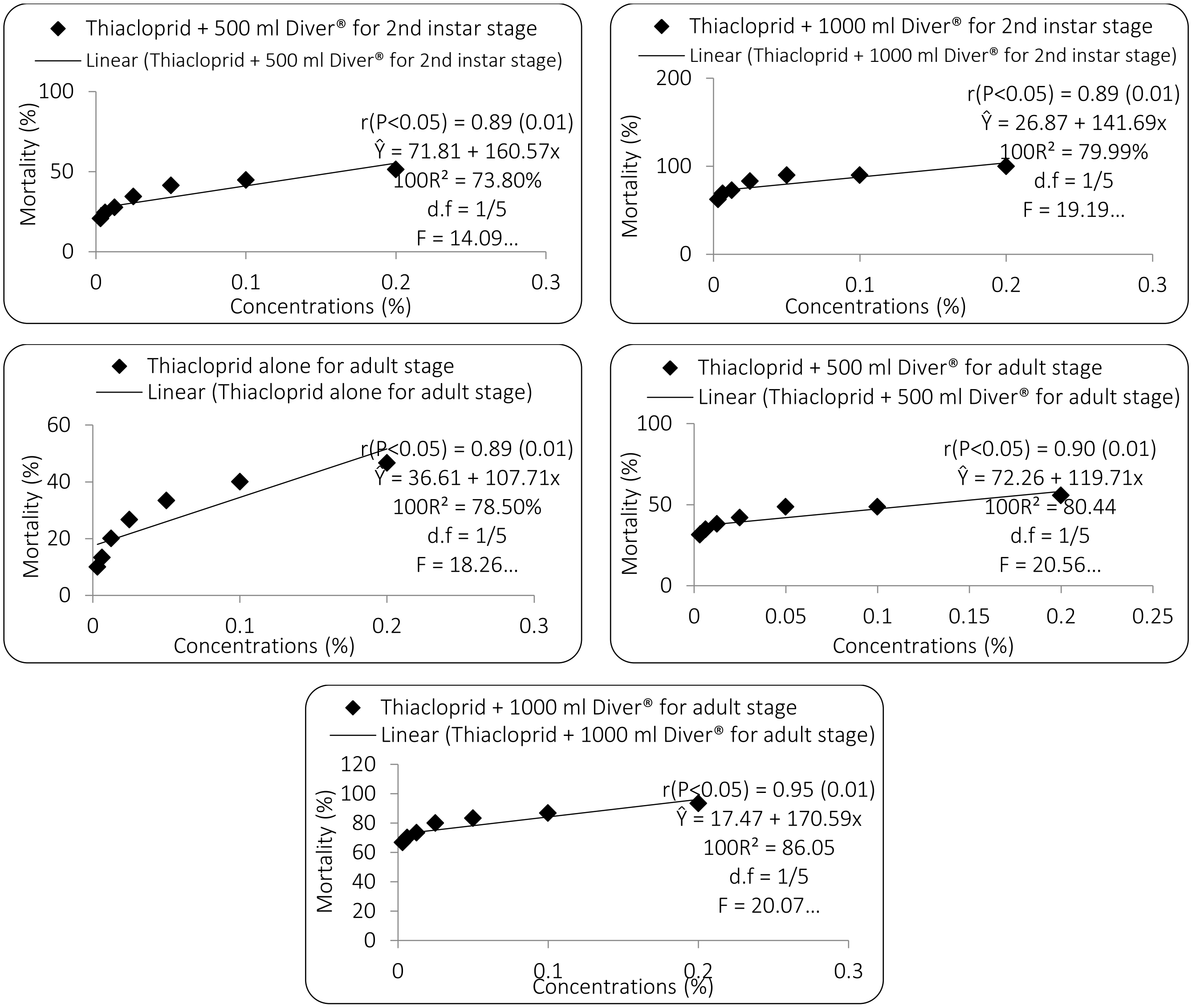


Figure 6: Correlation coefficient (r), linear regression equation ($\hat{Y}=b\pm ax$) and associated ANOVA parameters (*df,* F and P values), Coefficient of determination ($100R^2$) and scatter diagram showing the fitted simple regression line of $\hat{Y}$ (% mortality of 1st instar, 2nd instar and adult female stages of *Phenacoccus solenopsis*) on X (Concentrations of thiacloprid alone as well as in coadministration with 500 ml and 1000 ml of Diver®) (P = Probability value, *d.f* = degree of freedom of concentration/degree of freedom of error, F = value of F calculated).

Synergism, antagonism or additive effects of Diver® on the toxicity of neonicotenoid insecticides against *Phenacoccus solenopsis*

Co-Toxicity Coefficient (CTC) values, when Diver® was admixed at half dose rates with neonicotenoids, ranged between 80 and 120 for adult female, 1st instar and 2nd instar stages of *P. solenopsis*. These CTC values indicated that admixing of Diver® at half of its field recommended dose rate (500 ml/acre) with all tested neonicotenoids demonstrated additive effects on the toxicity of diafenthiuron, nitenpyram and thiacloprid for adult female (CTC = 117.96, 110.28 and 116.31, respectively), 1st instar (CTC = 124.65, 105.19 and 113.73, respectively) and 2nd instar (CTC = 102.43, 111.0 and 117.79, respectively) stage of *P. solenopsis* (Table 6).

DISCUSSION

Different life stages of *P. solenopsis* have developed resistance against commonly used contact and systemic insecticides (Saeed et al., 2007). Saturation (use of synergists) and multiple attacks (use of insecticide mixtures) are two ways being advocated for the management of insecticide resistance against insects (Pedigo and Rice, 2009).

Table 6: Estimation of Actual Toxicity Index of individual and mixtures of insecticides as well as Theoretical Toxicity Index, Co-Toxicity Coefficient and combined effects of blended insecticides against adult female, 1st instar and 2nd instar stages of *P. solenopsis*.

Treatments	Ratio (Insecticides:Diver®)	LC_{50} (%)	ATI	TTI(M)	CTC	Combined Effect
Adult stage						
Diver®	0:1	29.0	100			
Diafenthiuron	1:0	18.27	158.73			
Diafenthiuron + 500 ml Diver®	1:5 (20%:80%)	0.22	13181.82	11174.6	117.96	Additive*
Diafenthiuron + 1000 ml Diver®	1:10 (10%:90%)	0.09	32222.22	11174.6	304.34	Synergistic**
Nitenpyram	0:1	41.68	69.58			
Nitenpyram + 500 ml Diver®	1:5 (20%:80%)	0.28	10357.14	9391.56	110.28	Additive*
Nitenpyram + 1000 ml Diver®	1:10 (10%:90%)	0.12	24116.67	9695.78	249.24	Synergistic**
Thiacloprid	0:1	2.1	1380.95			
Thiacloprid + 500 ml Diver®	1:5 (20%:80%)	0.07	41428.57	35619.05	116.31	Additive*
Thiacloprid + 1000 ml Diver®	1:0 (10%:90%)	0.019	152631.6	22809.52	669.15	Synergistic**
1st instar stage						
Diver®	0:1	24.25	100			
Diafenthiuron	1:0	9.76	248.46			
Diafenthiuron + 500 ml Diver®	1:5 (20%:80%)	0.15	16166.67	12966.26	124.65	Additive*
Diafenthiuron + 1000 ml Diver®	1:10 (10%:90%)	0.05	48500.00	11484.63	422.30	Synergistic**
Nitenpyram	0:1	19.57	123.91			
Nitenpyram + 500 ml Diver®	1:5 (20%:80%)	0.22	11022.73	10478.28	105.19	Additive*
Nitenpyram + 1000 ml Diver®	1:10 (10%:90%)	0.07	34642.86	20547.62	338.33	Synergistic**
Thiacloprid	0:1	1.4	1732.14			
Thiacloprid + 500 ml Diver®	1:5 (20%:80%)	0.05	48500.00	42642.86	113.73	Additive*
Thiacloprid + 1000 ml Diver®	1:10 (10%:90%)	0.016	151562.5	20547.62	737.61	Synergistic**
2nd instar stage						
Diver®	0:1	26.33	100			
Diafenthiuron	1:0	12.42	211.99			
Diafenthiuron + 500 ml Diver®	1:5 (20%:80%)	0.21	12538.10	12239.94	102.43	Additive*

Diafenthiuron + 1000 ml Diver®	1:10 (10%:90%)	0.09	29255.56	11119.97	263.09	Synergistic**
Nitenpyram	0:1	35.4	74.38			
Nitenpyram + 500 ml Diver®	1:5 (20%:80%)	0.25	10532.00	9487.57	111.00	Additive*
Nitenpyram + 1000 ml Diver®	1:10 (10%:90%)	0.12	21941.67	9743.79	255.19	Synergistic**
Thiacloprid	0:1	1.8	1462.78			
Thiacloprid + 500 ml Diver®	1:5 (20%:80%)	0.06	43883.33	37255.56	117.79	Additive*
Thiacloprid + 1000 ml Diver®	1:10 (10%:90%)	0.017	154882.4	23627.78	655.51	Synergistic**

LC_{50} = Lethal Concentration causing 50% mortality; ATI = Actual Toxicity of Insecticides; TTI(M) = Theoretical Toxicity Index of blended insecticides; CTC = Co-toxicity Coefficient; * = Additive effect if CTC value exists between 80 and 120; ** = synergistic effect if CTC value > 120

A laboratory study was carried out to assess the interactive effects of a mineral oil, Diver®, on the toxicity of three commonly used neonecotenoid (diafenthiuron, nitenpyram and thiacloprid) against 1st instar, 2nd instar and adult female stages of *P. solenopsis*. A significant variation in percentage mortality of adult female, 1st instar and 2nd instar stages of *P. solenopsis* was demonstrated by different concentrations of diafenthiuron, nitenpyram and thiacloprid when applied alone or in combinations with 500 ml/acre and 1000 ml/acre doses of Diver®. Admixing Diver® with these insecticides enhanced the mortality in all the three *P. solenopsis* stages and toxicity of these insecticides many folds as compared to their alone applications. The results are in confirmation to the results of Ahmed et al. (2012) who assessed the efficacy of mineral oil in combination with lower doses of insecticides against cotton mealybug, *P. solenopsis* and reported comparative higher mortality and more effective control of *P. solenopsis* when combined with mineral oils as compared to insecticides applied alone. This enhanced toxicity and mortality may be attributed to additive or synergistic effects of Diver® on the tested insecticides. The results also showed that Diver® demonstrated additive effects on the toxicity of selected insecticides against all the three stages of *P. solenopsis* when admixed at its half dose rate while same explained synergistic effects on the toxicity of selected insecticides against all the three stages of *P. solenopsis* when admixed at its full dose rate. These effects of Diver® may be due to its physical mode of action (spiracle blockage and/or waxy cuticular layer disruption) as reported by Rinehold and Jenkins (2006) and Gogi et al. (2011) who also concluded that oil based adjuvants are not only compatible with insecticides but also improve their coverage, penetration through insects cuticle and effective against insect pests. However, exact interactions behind the mechanism of additivity and synergism of Diver® with three neonicotenoids have not been studied in present research and need to be explored. However, the reports of Shaban and Al Mallah (1993) and Sun and Johnson (1960) explained that synergist materials have motivating or inhibitory effects on the enzymes as well as on the chemical composition of pesticides. While Wilkinson (1979) documented that the additives materials have inhibitory effects on those enzymes that are responsible for primary metabolism, excretion and accumulation of active ingredient as well as for enzymatic metabolic reactions leading to mortality of insects. These reports lead to reasoning that Diver® may have motivating or inhibitory effect on the enzymes as well as on the chemical composition of tested neonecotinoids at full dose rate while the same has inhibitory effects on the enzymes involved in regulating primary detoxifying metabolic reactions. However, such type of study has not been carried out in the present study and need to be investigated in future. The results of present study revealed that mortality of the three stages of *P. solenopsis* depend upon the mixing ratio between insecticides and Diver®. A higher morality of three stages was observed on insecticides: Diver® ratio of 1:10 and lower mortality on ratio 1:5. These results are in consistent with the results of Karso and Al Mallah (2014) who reported variable mortality of *Trogoderma* *granarium* larvae for different ratios between insecticides and oils. In conclusion, Diver® can be exploited as synergist for resistance management of insecticides against *P. solenopsis*.

AUTHORS' CONTRIBUTION

MI and MDG designed and conceptualized the studies, MI did experimental work and collected the data, MI and SA wrote the part paper, prepared graphs, carried out statistical analysis, participated in manuscript writing and did final editing of the work, MDG co-supervised the work, All the authors proofread the article.

CONFLICT OF INTEREST

The authors declare no conflict of interest

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