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ACUTE CONTACT TOXICITY OF SIX PESTICIDES IN HONEYBEES (*APIS MELLIFERA MEDA*) IN IRAN

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ABSTRACT

Pollination has an important role in both agricultural production and wild plant reproduction. For the pollination of crops, agriculture relies largely on managed colonies of the honeybee *Apis mellifera*. Worker bees are primarily affected by pesticides. The symptoms of poisoning vary depending on the developmental stage of the individual bee and kind of chemical employed. The acute contact toxicity of insecticides (phosalone and pirimicarb), acaricide (propargite), insecticide and acaricide (fenpropathrin), fungicides and bactericides (copper oxychloride and bordeaux mixture) was assessed in Iran through laboratory experiments. The median lethal concentrations (LC_{50}-24h, LC_{50}-48h and LC_{50}-72h) were evaluated for the purposes of this research. Results showed that fenpropathrin had high toxicity; LC_{50}-24h, LC_{50}-48h and LC_{50}-72h were 5.7, 3.2 and 2.9 ppm respectively. Additionally, the bordeaux mixture had the minimum contact toxicity on honeybees with LC_{50}-24h, LC_{50}-48h and LC_{50}-72h being 79,926; 69,552 and 69,045 ppm respectively and was safe and non-toxic in honeybees.

Keywords: *Apis mellifera* meda, Contact Toxicity, LC_{50}, Pesticide

INTRODUCTION

Crop pollination relies largely on managed colonies of *Apis mellifera* (Gallai et al., 2009). Unfortunately, recently all over the worldcolonies have disappeared, better known as "Colony Collapse Disorder" (CCD) (Mullin et al., 2010). Factors such as parasites and pesticides or a combination of these factors might be responsible for a decline in the health of honeybees (VanEngelsdorp et al., 2009). To date, contact toxicity of some pesticides has been tested on honeybees, and those such as synthetic organophosphate, carbamate and pyrethroid insecticides have a significant impact on bees and pollination.

Honeybees are estimated to provide the agriculture industry annual pollination services worth US $4.1 billion. Each year, dozens of beekeepers with hundreds of bee colonies move to various hills and valleys of Himachal Pradesh and apple farmers pay them IRs 800 (US $18) for one colony of honeybees to provide pollination services during each flowering season (Hepburn & Radloff, 2011).

Pesticides are important for ensuring both crop quality and quantity in sustainable agricultural production. The use of pesticides is one of the most effective practices in controlling pests (Kevan, 1999). In the assessment and evaluation of toxic characteristics of substances, the acute toxicity in honeybees needs to be determined. The acute toxicity test is conducted to determine the inherent toxicity of pesticides and other chemicals to honeybees. In particular, this method can be used in stepwise programs for evaluating the hazards of pesticides to bees, based on sequential progression from laboratory toxicity tests to semi-field and field experiments (OECD, 1998; OEPP/EPPO, 1993). Therefore, active substances and formulated pesticides currently undergo various tests for the assessment of their risk to honey bees, before they are allowed to be used in agriculture. For this study, the European and Mediterranean Plant Protection Organization guidelines

Fig. 1 Spray tower for contact toxicity experiments

No. 170 (OEPP/EPPO, 2010a) and the relative risk assessment scheme (OEPP/EPPO, 2010b) were followed.

Insecticides are normally designed to control insect pests but they can also affect non-target organisms, including the honeybee an insect of agro-environmental, economic and scientific importance (Gallai et al., 2009; Gauthier, 2010; Srinivasan, 2011). The EFSA Guidance Document suggests a tiered risk assessment scheme with a simple and cost-effective first tier to more complex higher tier studies under field conditions. Each of the tiers will have to ensure that the appropriate level of protection is achieved (EFSA, 2013).

Since there is no published information on the precise acute toxicity (LC_{50}) of phosalone, pirimicarb, propargite, fenpropathrin, copper oxychloride and bordeaux mixture to *Apis mellifera meda*, LC_{50}-24h, LC_{50}-48h and LC50-72h were evaluated to fill this gap in the data. The aim of this study was to calculate LC_{50}-24h, LC_{50}-48h and LC50-72h of six pesticides.

MATERIAL AND METHODS

Commercial formulations available in Iran were used. These formulations contained Phosalone (Zolone® 35.0% EC), Pirimicarb (Pirimor®, 50% WP), Propargite (Omite®, D-014®, BPPS®, Comite®, 57% EC), Fenpropathrin (Danitol®, 10% EC), Copper oxychloride (Cupravit, 355®, 35% WP) and Bordeaux mixture (Bordeaux Fix®, 18% EC). Bioassay experiments were conducted in 2015. Worker honeybees were used for contact toxicity, honeybee foragers were obtained from one adequately fed, healthy, disease-free and queen-right colony, and forager honeybees were sampled from one colony.

Pretest experiments were conducted then six concentrations were prepared for each pesticide. Honeybees were anaesthetized with CO2 gas, and fifteen worker honey bees were transferred to each petri dish. Three petri dishes were used as replications for each concentration. 2 ml volume of each treatment was sprayed on the dorsal surface of honeybees with a spray tower (with two bar pressure) (Fig. 1). 270 worker honeybees (four to six weeks age) were used for each pesticide and 45 workers for each control. After treatment, three cages (30 cm high and 20 cm wide) were used in each concentration and one cage for control. The fifteen worker honeybees of each replication were laid in each cage and fed with pure sucrose solution in water (50%w/v). Mesh-like nets in parts of the cages provided ventilation. A sleeve - like net was applied to transfer the treated petri dish into the cage.

The mortality rates were logged at 24, 48 and 72h after the start of the test. The tests were performed in a dark room at 25-30°C and 45-55% relative humidity (Laurino et al., 2010; 2011; 2013). Experiments lasted until the time (day) that control mortality did not exceed 10 percent (≤10%) (OECD 1998; Laurino et al., 2013). In our experiment, control mortality exceeded 10 percent after 72 hours (≥10%), so we did not continue observation records to 96 hours. LC_{50}-24h, LC_{50}-48h LC_{50}-72h were calculated using Polo-PC software. Probit regressions were plotted by SPSS ver. 18. A sig-

Table 1.

Comparisons of LC_{10}, LC_{50} and LC_{90} of commonly used pesticides in Iran

pesticide	LC_{50} (ppm)			Slop±SE			Chi-square			LC_{10} (ppm)			LC_{90} (ppm)		
	24h	48h	72h	24h	48h	72h	24h	48h	72h	24h	48h	72h	24h	48h	72h
Phosalone	965.5	773.7	762.8	12.5 ±2.5	8.6 ±1.2	9.4 ±1.5	2.98	2.1	5.7	763.1	550.9	557.3	1221.4	1086.6	1044.1
Pirimicarb	790.28	705.6	701.9	15.1 ±2.5	11.6 ±2.1	13.5±2	0.97	2.5	5.3	650.5	547.9	564.4	960.06	908.6	873.1
Propargite	31283	26659	25049	13.5 ±1.6	11 ±1.3	12.2±1.3	2.7	2.4	6.9	25159	20427	19699	38898	34793	31852
Fen-propath-rin	5.7	3.2	2.9	5.1 ±0.6	2.6 ±0.34	3.1 ±0.3	1.4	1.9	7.7	3.18	1.03	1.1	10.06	9.76	7.4
Copper oxychlo-ride	29396	24444	23819	8.7 ±1.2	7.8 ±1.1	8.8 ±1.1	1.6	1.7	3.6	20982	16783	17091	41184	35601	33196
Bordeaux mixture	79926	69552	69045	12.1 ±1.7	9.9 ±1.4	11.7±1.6	2.1	2.3	5.01	62718	51749	53665	101860	93479	88832

nificant comparison between LC_{50}-24h and LC_{50}-48h was conducted by lethal dose ratio method (Robertson & Preisler, 1992). Concentrations used to determine the LC_{50} were Copper oxychloride- 16,000; 20,000; 25,000; 30,000; 35,000 and 40,000 ppm (active ingredient); Phosalone- 500, 700, 900, 1000, 1100 and 1250 ppm (active ingredient); Primicarb- 400, 600, 700, 800, 900 and 1100ppm (active ingredient); Propargite- 20,000; 25,000; 28,000; 32,000; 35,000 and 38,000 ppm (active ingredient); Fenpropathrin- 1, 3, 5, 7, 9 and 10 ppm (active ingredient); Bordeaux mixture- 50,000; 60,000; 70,000; 80,000; 90,000 and 100,000 ppm (active ingredient);

RESULTS:

Results showed that fenpropathrin had the highest toxicity compared to the other pesticides (LC_{50}-24h=5.7 ppm) meaning it must be not applied during the flowering time of crops and trees. Even, 0.01 ml/L (10 ppm) of fenpropathrin could have caused 90% mortality within 24h after the experiment. Also, LC_{50} of fenpropathrin decreased from 24h to 72h after experiment, and there was significant difference between LC_{50} at 24h and LC_{50} at 48h (lethal dose ratio=0.4-0.6) (Tab. 1&2). Results indicated that regression slops between the log of concentration and mortality probit decreased from 24h to 48h in all studied pesticides (Tab. 1 & Fig. 3). The

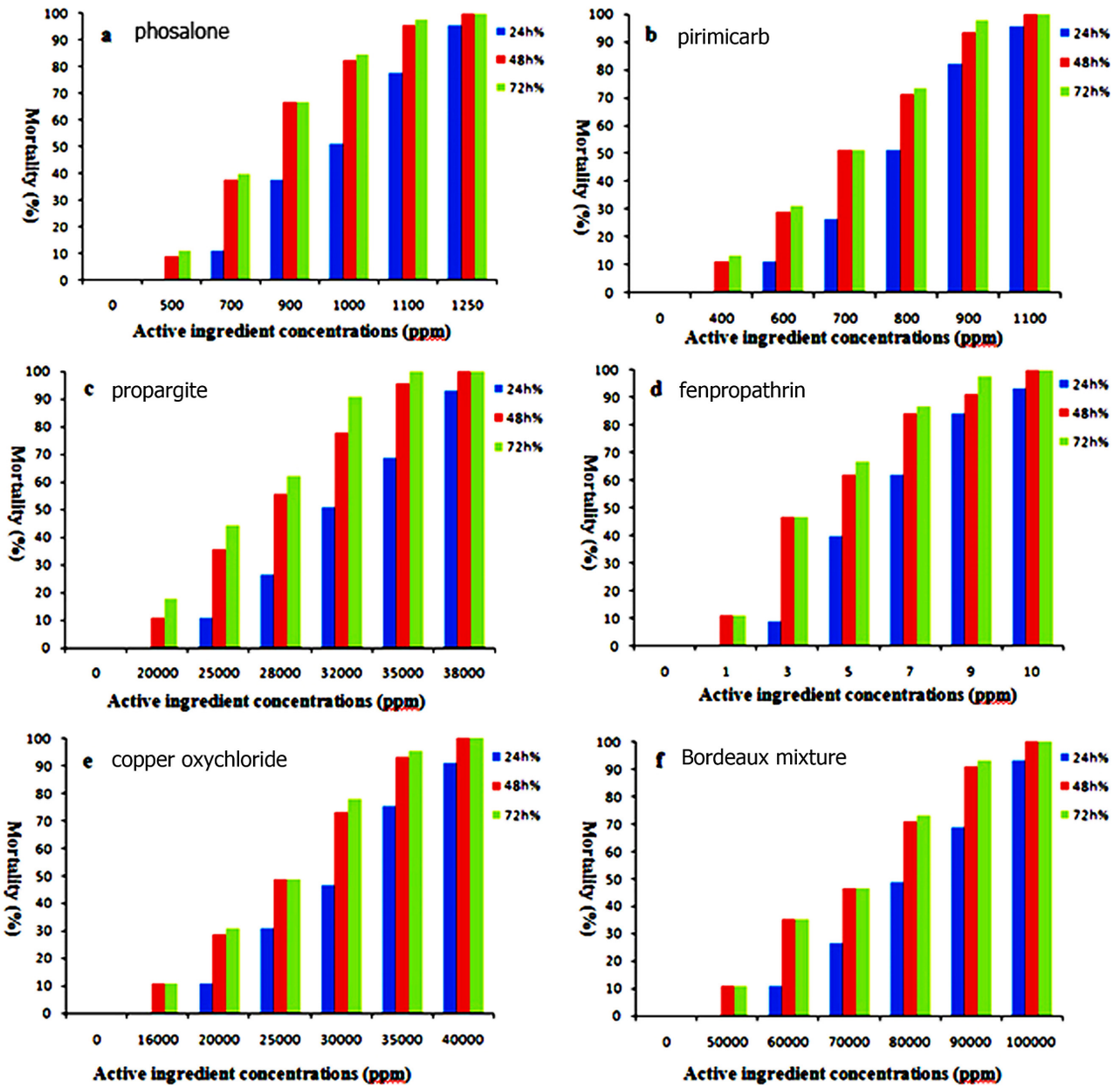

Fig. 2 Mortality comparisons of worker honeybees in different concentrations (active ingredient) at 24h, 48h and 72h. a- phosalone, b- pirimicarb, c- propargite, d- fenpropathrin, e- copper oxychloride, f- Bordeaux mixture

bordeaux mixture and copper oxychloride had high LC_{50}-24 which proved they were non-toxic for foraging honeybees. The application of almost 31.2 ml/L propargite (acaricide) caused 50% mortality in 24h after the experiment demonstrating it was safe for common usage in flowering time (Tab. 2). Additionally, LC_{50} decreased from 24h to 72h after the experiment in all pesticides. LC_{90} decreased from 24h to 72h after experiment in all pesticides while LC_{10} decreased from 24h to 72h only in fenpropathrin and propargite (Tab.1). LC_{50} comparisons indicated that there was significant difference between LC_{50}-24h and LC_{50}-48h in all pesticides but not between LC_{50}-48h and LC_{50}-72h. We found only a significant parallelism between LC_{50}-24 and LC_{50}-48 in fenpropathrin. Our results showed that pirimicarb as a specific toxic to aphids was moderately safe to honeybees meaning it could be used safely with foraging pollinator bees. Also, phosalone was moderately toxic so it should be not used during flowering (Tab. 2). There was a significant difference between the mortalities of applied concentra-

Table 2.
Significant evaluation and parallelism hypothesis between LC_{50}-24 hours, LC_{50}-48 hours and 72 hours of pesticides

pesticides	LC_{50}			Parallelism (Chi-square)		df	Lethal Dose Ratio (lower-upper limits)	
	24 hours	48 hours	72 hours	24 hours with 48 hours	72 hours with 48 hours		24 hours with 48 hours	72 hours with 48 hours
Phosalone	965.5	773.7	762.8	1.64 (P=0.2)*	0 (P=0.95)	1	0.7-0.8**	0.9-1.1
Pirimicarb	790.28	705.6	701.9	0.49 (P=0.4)	0.03 (P=0.85)	1	0.8-0.9	0.9-1.07
Propargite	31283	26659	25049	0.92 (P=0.33)	0.04 (P=0.84)	1	0.80-0.89	1-1.12
Fenpropathrin	5.7	3.2	2.9	9.49 (P=0.002)	0.35 (P=0.55)	1	0.4-0.6	0.8-1.3
Copper oxychloride	29396	24444	23819	1.03 (P=0.33)	0.05 (P=0.82)	1	0.7-0.9	0.9-1.1
Bordeaux mixture	79926	69552	69045	1.4 (P=0.23)	0.06 (P=0.8)	1	0.8-0.9	0.9-1.08

* Parallelism hypothesis is not rejected in P> 0.05
** If 95% confidence interval includes 1, then LD_{50}-24h and LD_{50}-48h are not significantly different.
*** Lethal dose ratio is a method for statistical comparisons of LC_{50}

tions of propargite at 24h after the experiment (F=120.7, P=0.00) as well as between those of applied concentrations of copper oxychloride at 24h (F=106.7, P=0.00). Results demonstrated that concentrations of 10; 1100; 1250; 38,000; 40,000 and 100,000 ppm caused 100% mortalities at 48h and 72h in fenpropathrin, pirimicarb, phosalone, propargite, copper oxychloride and bordeaux mixture respectively (Fig. 2).

DISCUSSION

Despite the efforts of many researchers, the question of the possible causes of CCD has not yet been fully resolved, but one of the important causes is believed to be the indiscriminate use of insecticides in agricultural practice. This investigation is focused on only one aspect of the effect of these substances on the insect's body thatcould provide a solution to the current threat to Apis mellifera. On the other hand, the individual honeybees s have weakly developed immune and detoxifying systems because the amount of monooxygenase coding genes P450 or glutation-S-transferase of this species is nearly 50% lower than in other representatives of the insects (Piechowicz et al., 2013).

Our results showed that phosalone, pirimicarb (insecticides) and fenpropathrin (insecticide and acaricide) were more toxic than fungicides and bactericides because copper oxychloride and bordeaux mixture have different mode of action than insectides. Copper oxychloride and bordeaux mixture seem to be more toxic through oral exposure (LC_{50}= 5,408 ppm and 4,469 ppm respectively, Rasuli et al., 2015) than through contact probably because they can penetrate more easily via the midgut than via the external cuticular layer.

Fenpropathrin was the most toxic toApis mellifera workers (LC_{50} 24h = 5.7 ppm) in comparison to phosalone and pirimicarb. The chemical family of fenpropathrin are pyrethroids and has different mode of action than carbamates (pirimicarb)and organophosphates (phosalone). These inhibit the enzyme acetylcholinesterase which serves to interrupt the transmission of nerve impulses whereas fenpropathrin acts as an axonic poison by interfering with the sodium channels of both the peripheral and central nervous system thereby stimulating repetitive nervous discharges and leading to paralysis.

Furthermore, results showed that propargite (acaricide) with LC_{50}-24h=31,283 ppm was non-toxic for honeybees because its mode of

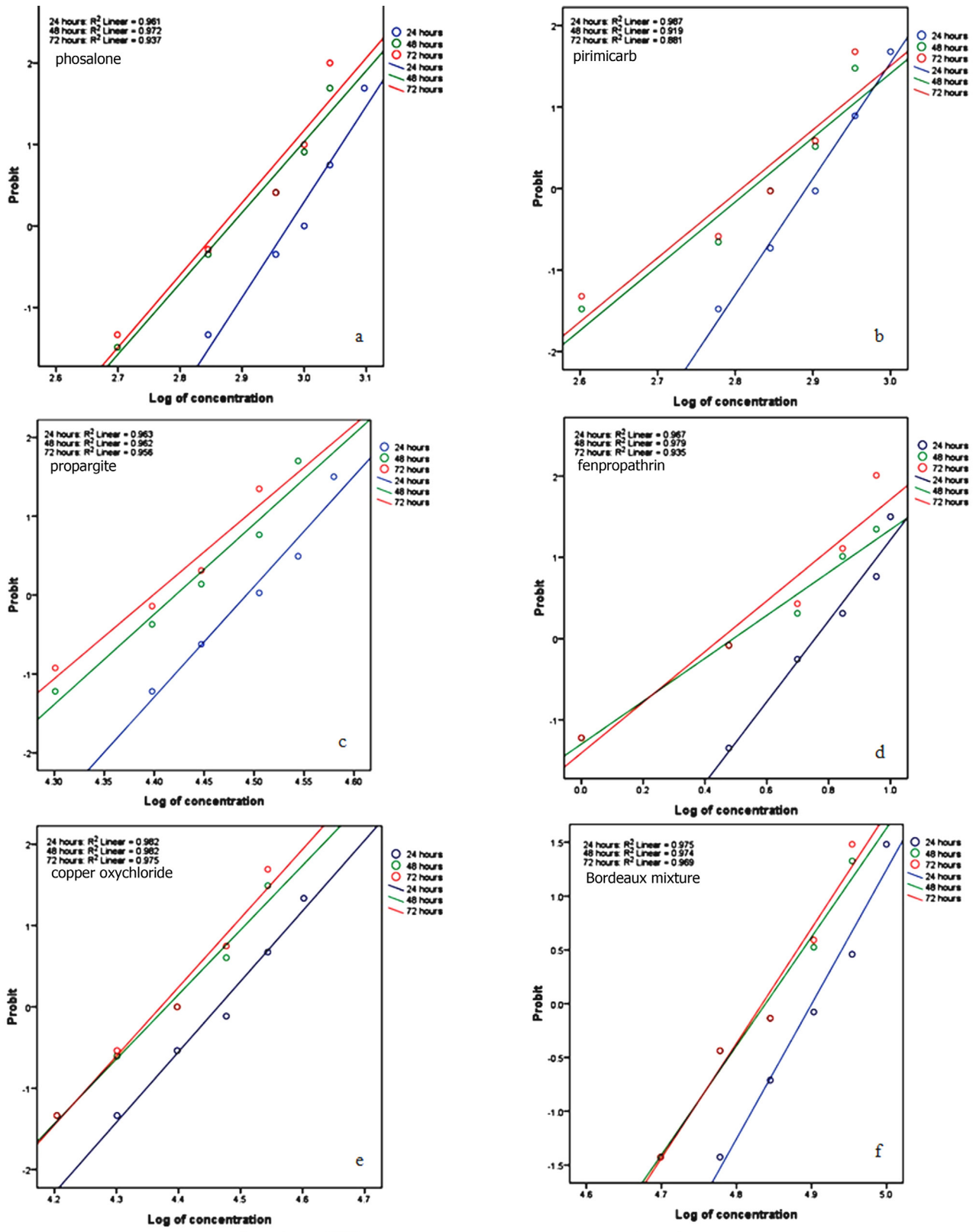

Fig 3. Probit regression lines of commonly used pesticides in 24 hours, 48 hours and 72 hours; a- phosalone, b-pirimicarb, c-propargite, d- fenpropathrin, e- copper oxychloride, f- bordeaux mixture

action (inhibitors of mitochondrial ATP) differed from other insecticides. Our research showed that LC_{50}-24h and LC_{50}-48h of phosalone was 966 and 774 ppm, respectively. Additionally, LC_{90}-24h was 1221 ppm. The usage instruction of the trade formulation of phosalone 35% is 1500 ppm for controlling pests, so this insecticide is moderately toxic to honeybees. There is only fragmented data regarding moderate toxicity of phosalone in honeybees (Mayer et al., 1999; Sanford, 2009; Adams & Bartholomew, 2012).

There is not any precise information concerning acute contact toxicity in fungicides and bactericides of copper oxychloride and Bordeaux mixture. Our research showed that the LC_{50}-24h of copper oxychloride and Bordeaux mixture were 29.396 and 79.926 ppm of the active ingredient, respectively. The usage instruction of trade formulation of copper oxychloride 35% and bordeaux mixture 18% were 2000 to 5000 ppm in Iran, hence these pesticides were non-toxic to honeybees. Tesoriero et al., (2003) showed that 1µl copper oxychloride did not have a toxic effect on adult *Osmia cornuta* (Latreille) but at 1µl per egg caused 40% mortality in eggs. Individual studies of fungicides showed that they had little effect on honeybees. However Vandame & Belzunces (1998) examined the combined effect of a fungicide and the insecticide deltamethrin and found a significant effect on honeybee thermoregulation. Our results showed that pirimicarb was safe for *Apis mellifera* and because of its specific toxicity to aphids it could be used safely with pollinator bees.

Risk assessments of the six pesticides showed that Fenpropathrin (acaricide and insecticide) was highly toxic in honeybees and must not be used during the foraging of honeybees. Furthermore, propargite (acaricide), Copper oxychloride and Bordeaux mixture (fungicides and bactericides) were non-toxic for honeybees therefore could be used safely during the foraging of honeybees (*Apis mellifera*). Additionally, Pirimicarb was particularly toxic to aphids and could be used safely with pollinator bees.

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