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Mortality response of wheat aphid (*Rhopalosiphum padi*) against most commonly used insecticides in Pakistan

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Abstract

Rhopalosiphum padi is one of the most common wheat inhabiting pest and reduce the crop yield up to `15%. This pest species was collected from the wheat fields located in the vicinity of Faisalabad. The mortality response of said pest was find out in the different insecticides by standard nymphal dip method. The results showed that LC_{50} values of 9 tested insecticides against *R. padi* are statically different (5% probability level) and varied from 1.33±0.52 ppm for Imidacloprid to119.17± 16.55 ppm for Charphyrifos. Imidacloprid which caused 100% mortality at 64, 32 and 16 ppm more than 80% mortality at 8, 4 and 2 ppm was proved to be the most toxic insecticide against the *R. padi*. The Charphyrifos was least toxic as it caused only 41.53±3.21% mortality even at highest concentration. This study recommended the minimum dose recommendation in ppm for controlling this damaging insect. This study could be used as a reference for resistance monitoring or other related researches.

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Introduction

Climatic conditions in Pakistan favor the production of verities of crops. Wheat is the most important cash crop of country with production of 26.346 million tons annually (PAR, 2015). It provide 20% food calories to 40% of world population (Shah *et al*., 2007). Unfortunately, per acre yield of wheat is much more less than the other countries due to number of factors including; low yield verities (Khan *et al*., 2012), improper uses of fertilizers and irrigation (Kibe *et al*., 2006), number of undesired weeds (Memon *et al*., 2013) and pest population (Khattak *et al*., 2007).

Among various pests, 29 species of only aphids attack on wheat (Kuroli, 2000). These soft bodied creatures are herbivores in nature and found in the growing parts of plants including flowers and tips (Mushtaq *et al*., 2013). The *Rhopalosiphum padi* is the most common wheat inhabiting aphid species (Khan *et al*., 2006). It is polyphagus sucking insect worldwide in distribution (Taheri *et al*., 2010) and reduce the wheat yield up to 15% at flowering stage only (Oerke, 2006). The incidence time is start of spring in wheat fields where it mainly sucks the sap from shoots and leaves found responsible for distortion, curling and chlorosis of leaves growth (Akhtar *et al*., 2003). It also transmitted the viral and fungal diseases reducing the yield up to 80% due to diseases (Goggin, 2007; Rossing *et al*., 1994; Sadeghi *et al*., 2009). It not only acts as vector of barley yellow dwarf virus (BYDV) but also causes the direct injury to plants by injecting the chemical present in the saliva, and removal of sap and indirectly through excess production of honeydew damaging the whole crops(Brault *et al*., 2007).

The control of this insect pest is very necessary. Several methods have been documented including physical, cultural, mechanical, host plant resistance, biological and chemical control. In the current scenario, the chemical pesticide is effective tool in controlling this damaging aphid species (Pathak *et al*., 2013). It was also investigated the pests develop resistance against a pesticide if required dose was not previously applied (Ghafoor *et al*., 2011). Therefore, dose recommendation is very important parameter for the control of the *R. padi* in the agro-ecosystems. In the country like Pakistan where the influence of this damaging pest is very large in the wheat crop, the selection and dose recommendation of suitable is of prime importance for the control of aphid economically. Therefore, this study was aimed to quantify the recommended dose of 9 most extensively used pesticides in Pakistan under laboratory conditions.

Materials and methods

Insecticides

The insecticides with purity were Bifentherin (98%), Endosulfan (94.2%), abamectin (94.4%), imidacloprid (96.4%), acetamiprid (96.4%), profenofos (89%), Trebon (90%), Imidacloprid (95%) and Methomyl (98%) purchased from the local chemical distributors in Pakistan from sigma Aldrich and Merck (Germany).

Sample collection and Bioassay test

The study area was fields of wheat, located district Faisalabad and vicinity. The experiment was conducted for a period of three months during the severe aphid attack on wheat. The aphids were collected from the open fields randomly by hand picking method brought to the Bioassay Lab Government college university Faisalabad along with some stem and leaves of the wheat so that aphids may not die due to starvation.

The control laboratory conditions were maintained at 26±2 °C temperature, 75±5% R.H and 16:8 light and dark photoperiods.

A stock solution of each tested compound was prepared and subsequent serial concentrations were made by diluting with water (v/v) or (w/v) to give the necessary concentrations ppm inducing (20–80%) mortality for each material. Concentration of each insecticide was prepared from time to time as needed. Six concentrations plus one control (water) were used for bioassay in case of each insecticide. Five petridishes were used for each concentration as unit of replication and each test was repeated for five times. A thin layer of agar was set in the petri plates and small pieces of leaves were put in the petri plates. All the aphids were subjected to the Nymphal Dip Method against each concentration.

Ten treated aphids were kept in each Petri plate on the leaves while ten aphids were kept untreated i.e. as a control. Mortality was assessed after 24, 48 and 72 h of treatment to determine the percent mortality and LC_{50} against each concentration of the given insecticide. Aphids that seemed extremely lethargic or did not show any sign of movement when lightly touched with a needle were recorded as dead.

Data was presented as Mean ±SD. The mortality data was analyzed by probit analysis for LC_{50} values. The significant difference was recorded by Tukey's comparison post hoc test using Minitab (v.17) among the insecticides groups.

Results and discussion

R. padi caused significant economic damage to wheat plants. In most of the studies, it was noted that aphids showed quite different reactions against the tested compounds. Thus, the LC_{50} values were used to evaluate the insecticides toxicity. The mortality of adult *R. padi* against each insecticide against is shown in Table-1. The results revealed that Imidacloprid was the most efficient insecticide in all the exposure time (24, 48 and 72 h) followed by Acetamprid, Profenophas, Abamectin Endosulfan, Bifentherin, Trebon, Methomyl and Charphyrifos against *R. padi.* Lower LC_{50} value for Imidacloprid showed its high toxicity at very low concentration (1.33±0.52) during the 24, 48 h exposure and same was observed in the case of 72 h (table-1).

Table 1. Lethal concentration of 9 different pesticides against wheat aphid during 24, 48 and 72 hours.

Pesticides group	LC_{50} Value (ppm)		
	24 h	48h	72h
Control	0.0±0.0^{H}	0.0±0.0^{H}	0.0±0.0^{J}
Bifentherin	26.33±2.64^{D}	12.4011± 1.11^{E}	10. 12±2.091^{E}
Endosulfan	17.99±1.81^{E}	13.267±1.71^{D}	13.20±1.47^{C}
Profenophas	11.31±0.97^{F}	9.62±0.86^{F}	5.84±0.65^{F}
Abamectin	13.06±5.33^{D}	2.21±0.45^{H}	4.61±3.63^{G}
Acetamprid	5.62±1.34^{G}	3.38±0.73^{G}	3.102±1.17^{I}
Imidacloprid	5.23±0.92^{G}	1.33±0.52^{I}	3.68±0.41^{H}
Charphyrifos	119.17±16.55^{A}	104.72±13.73^{A}	33.72±7.21^{A}
Trebon	31.19±2.86^{C}	23.29±2.45^{C}	23.68 ±2.43^{B}
Methomyl	54.52±13.33^{B}	30.15±8.07^{B}	11.75± 1.62^{D}

Values are mean ±SE of five replicates

Values in the same column with same letter are not significantly different at 5% probability level.

The acute toxicity of Imidacloprid was very high, and thus the LC_{50} value obtained was very low (5.23± 0.92, 1.33±0.52 and 3.68± 0.41 ppm for 24h, 48 and 72h respectively). The LC_{50} value for Acetamprid (5.23±0.92 ppm) was slightly higher than Imidacloprid but sharing the same toxicity. The higher LC_{50} values of Charphyrifos (119.17±16.55, 104.72±13.73, 11.75±1.62 for 24, 48 and 72 h respectively) suggest this insecticide is less effective against the *R. padi* compared to the other insecticides (Table-1). Tang *et al.*, (2013) test the similar insecticide and found the low LC_{50} value against the *Aphis craccivora* suggesting that this group is extremely toxic to the *A. craccivora*. But in our study the higher LC_{50} values suggested that the *R. padi* has developed resistance against this group and usually high dose is required for controlling the pest. One possible explanation for this changed fate in both species might be due to the fact that different species showed different behavior to same insecticide group (Table-1). However, in contrast to our findings, in the studies of Tang *et al.* (2013) chlorpyrifos was found to be the most toxic to *R. maidis* with the LC_{50} value of 1.03 mg/L when test with other insecticides group. One possible fact might be due to the development of the resistance of *R. padi* against the chlorpyrifos.

Table 2. Mean percent mortality of *R. padi* under the 48 hours exposure of 9 insecticides with different concentration (ppm).

Insecticides	Percent mortality (%)						
	0 ppm	64 ppm	32 ppm	16 ppm	8 ppm	4 ppm	2 ppm
Bifentherin	0±0.00^{F}	98.21±0.03^{B}	96.01±0.01^{C}	86.01±0.23^{B}	26.25±1.56^{I}	30.41±2.71^{F}	21.43±1.36^{F}
Endosulfan	10.2±2.67^{A}	87.03±1.45^{F}	80.22±1.21^{F}	54.58±2.27^{F}	46.41±4.11^{F}	46.27±3.52^{D}	42.23±2.37^{D}
Profenophas	2.6±0.83^{C}	97.16±0.02^{C}	89.06±2.05^{E}	72.39±5.29^{E}	52.62±3.15^{D}	24.50±2.45^{G}	28.55±1.54^{E}
Abamectin	0±0.00^{F}	88.07±0.04^{E}	90.15±0.35^{D}	84.24±2.13^{C}	80.81±3.34^{C}	74.19±46^{B}	62.52±4.42^{C}
Acetamprid	0±0.00^{F}	100±0.00^{A}	96.23±0.03^{B}	96.08±0.05^{A}	92.59±1.00^{B}	72.27±4.28^{C}	66.33±4.35^{B}
Imidacloprid	2.41±1.23^{D}	100±0.00^{A}	100±0.00^{A}	96.10±0.03^{A}	96.13±0.05^{A}	84.06±3.35^{A}	80.82±5.53^{A}
Charphyrifos	2.93±.0.21^{B}	41.53±3.21^{H}	22.88±2.34^{I}	30.73±2.21^{H}	26.39±3.43^{H}	22.47±1.93^{H}	12.98±1.05^{I}
Trebon	2.30±0.11^{D}	88.81±0.21^{D}	72.91±1.71^{H}	46.92±3.56^{G}	30.81±4.29^{G}	21.11±2.05^{I}	19.27±2.16^{G}
Methomyl	2.01±0.02^{E}	86.35±0.54^{G}	78.72±2.56^{G}	74.35±6.26^{D}	46.46±4.52^{E}	34.57±3.61^{E}	18.76±1.67^{H}

Values are Mean ±SD of five replicates

Values in the same column with same letter are not significantly different at 5% probability level.

The tale-2 and 3 represents the toxicity of each insecticide group against the *R. padi* after 48 and 72 h respectively. The setting of baseline toxicity is the basic requirement for monitoring the resistance in insect pests against particular insecticide. It also provided the information regarding the strategy of resistance management. Following this, Zhu *et al.* (2000) found out the baseline of toxicity for organophosphate insecticide by using the glass residual film method for *Schizaphis graminum* (green bug) as test organism. Similarly, Liu *et al.*, (2001) determined the baseline toxicity of aphicides against the Kalten bach (*Schizaphis graminum*).

In this study, we determined that LC_{50} values varied from 2.21±0.45 to104.72±13.73 ppm for 72 h toxicity assay of 9 different insecticides (Table-1).

The LC_{50} values for Imidacloprid reported (3.68±0.4 to 5.32±0.93 ppm) was much lesser then the actual concentration applied in the fields, where up to 500 ppm concentration is being used (Jam *et al.*, 2014). So this study also defined the effective doses of Imidacloprid against this pest to reduce the economic losses and possible hazardous to other beneficial insects. Among the nine insecticides tested, Imidacloprid and Acetamprid were found to be the most toxic to *R. padi* (Table-2). Both insecticides cause 100% mortality and nearly 100% at 64 ppm and 32 ppm. The Imidacloprid showed above 80% mortality even at low doses (16 ppm, 8 ppm, 4 ppm and 2 ppm) suggesting the one of the suitable insecticides group for controlling the wheat aphid (*R. padi*). In our study, the Charphyrifos caused less mortality 41.53% even at higher dose (64 ppm) compared to the other insecticides (Table 2, 3).

Exposure time is also important factor beside the concentration. It also influenced the outcomes of bioassay tests against particular aphid (Yanhui *et al.*, 2009). Stable mortality was seen at 48 and 72 hours in the case of most insecticides (Huang *et al.*, 2006). Considering this, we recorded the mortality data after 24, 48 and 72 h of exposure to monitor the resistance of aphid against these 9 insecticides. The results in the table-1 showed significantly different values for the same insecticides groups which was the proof that exposure time also plays a significant role beside the concentration.

Table 3. Mean percent mortality of *R. padi* under the 72 hours exposure of 9 insecticides with different concentration (ppm).

Insecticides	Percent mortality (%)						
	0 ppm	64 ppm	32 ppm	16 ppm	8 ppm	4 ppm	2 ppm
Bifentherin	0±0.00^{D}	100±0.00^{A}	98.87±4.00^{B}	94.73±3.30^{B}	36.65±1.50^{G}	30±2.09^{G}	29±2.44^{F}
Endosulfan	2±0.02^{C}	88±3.63^{E}	86±0.00^{F}	57±2.00^{F}	49±2.10^{F}	49±2.55^{D}	48±2.70^{D}
Profenophas	2±0.02^{C}	99±3.25^{AB}	94±3.10^{C}	77.39±5.99^{E}	62.62±3.06^{D}	43.50±2.45^{E}	38.55±1.54^{E}
Abamectin	2±0.02^{C}	94.07±4.04^{D}	94.15±2.75^{C}	88.24±4.13^{C}	85.81±4.77^{B}	80.19±4.60^{B}	72.52±4.42^{C}
Acetamprid	0±0.00^{A}	100±0.00^{A}	100±0.03^{A}	98.08±2.05^{A}	97.59±4.00^{A}	82.27±5.78^{B}	75.33±4.37^{B}
Imidacloprid	2.41±1.02^{A}	100±0.00^{A}	100±2.11^{A}	99.56±3.03^{A}	98.13±3.05^{A}	94.06±6.39^{A}	92.82±5.76^{A}
Charphyrifos	2.±0.0.01^{C}	52.53±2.55^{F}	42.88±2.36	40.73±1.21^{G}	36.39±3.49^{G}	35.47±2.34^{G}	21.98±1.43^{H}
Trebon	2.30±0.11^{B}	98.81±3.33^{BC}	92.91±4.71^{D}	76.92±3.56^{E}	53.81±4.20^{E}	43.11±2.11^{E}	25.27±2.97^{G}
Methomyl	2.01±0.0^{C}	97.35±2.50^{C}	89.72±2.56^{E}	84.35±6.26^{D}	66.46±5.12^{C}	57.57±3.39^{C}	27.76±1.45^{F}

Values are Mean ±SD of five replicates

Values in the same column with same letter are not significantly different at 5% probability level.

In the second phase of the study, all insecticides were tested against the *R. padi* at low concentration of 2 ppm. Imidacloprid was again found most efficient causing the 90% and above mortality in test pest following the Acetamprid and Abamectin with 75% mortality responses in each. The other insecticides caused low or moderate toxicity (fig.1). Joshi *et al.* (2009) found the similar results in the Imidacloprid when applied against the wheat aphid. They used the confidor alone and with the fungicides. However the results of the study showed that imidacloprid alone was more effective than mixed with Tilt. Another study of Zhang *et al.* (2015) showed that the combination of imidacloprid and clothianidin had significant control against the wheat aphid throughout the season.

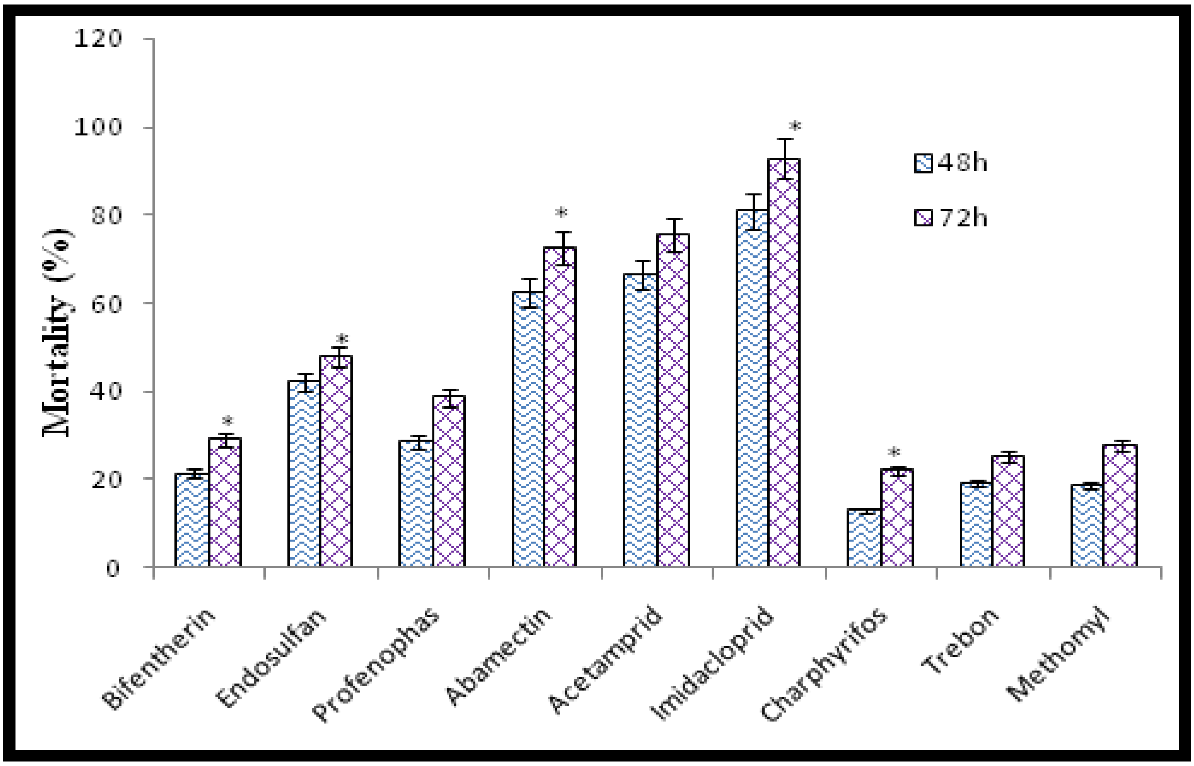

Fig. 1. Lethality percentage (%) of different insecticides at low concentration (2ppm) during 48 and 72 hours exposure (Asterisk represents the significantly different values ($p<0.05$) among the insecticides groups).

In conclusion it was revealed that Imidacloprid is most effective insecticide against *R. padi* due to its lower LC_{50} values. Charphyrifos is least effective due to its lower mortality even at high dose (64 ppm). The authors' further don't recommend Endosulfan for practical control of *R. padi* in the wheat fields due to is huge number of side effects. This study further revealed that the continuous and nonselective use of the insectaries creates the resistance against different groups as shown by Ghafoor *et al.* (2011). Therefore, different researcher uses biological control measures for controlling the pests in agricultural lands (Ghafoor *et al.*, 2011; Khan *et al.*, 2015; Khuhro *et al.*, 2012; Mohsin *et al.*, 2015).

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