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TOXICITY OF LUFENURON AND THIAMETHOXAM AGAINST FIVE PAKISTANI STRAINS OF *SITOPHILUS ORYZAE* ON WHEAT, RICE AND MAIZE

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ABSTRACT

The efficacies of two biorational insecticides (lufenuron and thiamethoxam) were evaluated against the five Pakistani strains of *Sitophilus oryzae*. The insecticides were applied to untreated wheat, rice and maize grains with the following application doses: 2ppm, 4ppm, 6ppm, 8ppm and 10ppm. Mortality was evaluated after 21 days of exposure to treated grain commodities. Generally, higher concentrations resulted in the higher efficacy of both insecticides, but thiamethoxam was significantly more effective than lufenuron against all the tested strains of *S. oryzae.* Statistically, significant differences in mortality on three grain commodities and among the five strains of *S. oryzae* were detected. With lufenuron, the highest mortality was 99% on maize against Multan strain while in case of thiamethoxam, almost all strains on all grain commodities showed 100% mortality. Only Bahawalpur and Rawalpindi strains on wheat didn't show complete mortality. The study revealed the potential of new environment friendly pesticides which could be considered in management of stored grain insect pests.

Keywords: Biorational Insecticides, Lufenuron, Thiamethoxam, Stored grain-insect pests.

INTRODUCTION

The rice weevil, *Sitophilus oryzae* (L.) is one of the most destructive pests of stored grains worldwide. This species as an internal feeder is competent to infest the whole grain easily which is not possible for other stored grain insects (Aitken, 1975). Various species of insects feed on the endosperm of grain, resulting in the quality and weight loss whereas some species nourish on the germ and led to the reduced seed germination (Malek and Parveen, 1989; Santos *et al*., 1990). Behavior of insects related to different species depends on the selection of appropriate food materials for their consumption (Slansky and Rodriguez, 1987). Insects that feed on food with high moisture content generally developed rapidly and have large body size (Baker and Loschiavo, 1987). Mostly external grain feeders as compared to internal feeders are differentiating as they are able to reproduce on a variety of processed and whole stored foodstuffs (Loschiavo, 1976; Levinson and Levinson, 1978). Studies on *Sitophilus granarius* showed that when they feed on five different types of grains, their developmental period was longest on maize as compared to rice, while their progeny development was lowest on oats and largest on barley (Schwartz and Burkholder, 1991). The growth rate reflects the food quality that is consumed by an insect (Slansky and Scriber, 1985). As *S. oryzae* is more competent to consume wheat as a food and have high ability to increase population than *S. granarius* (Baker, 1974; Evans, 1977a; Evans 1977b). The use of residual insecticides is a common tactic to control insects in stored food (Arthur, 1996) and the main intention to use these insecticides is to safe the food grains from insects for longer time period after harvesting. The most commonly used insecticides are pyrethroids and organophosphates (Collins, 2006; Nighat *et al*., 2007), but with the increase in residual free food demand, the acceptability of the grains treated with these insecticides has reduced. Insect's resistance against different insecticides (Irshad and Gillani, 1992), their impact on the environment and on human health have increased the consideration for the development of new insecticides (Athanassiou *et al*., 2008a; Kavallieratos *et al*., 2009; Khan *et*

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al., 2016a, 2017). In this regard, the group of insecticide derive from natural products have been developed which can disturbed the different body functions of the insect pest (Thompson *et al*., 2000; Smagghe *et al*., 2003). These insecticides act on biological processes like moulting of insects that does not exist in human beings. Grafton-Cardwell *et al*. (2005) revealed that an insecticide that contains natural products have greater effects on targeted insects as compared to non targeted or natural insects which are highly effected by the use of other synthetic insecticide. Insect growth regulators (IGRs), contain substances that disturb the complete life cycle of targeted pest (Mondal and Parween, 2000). Thiamethoxam has been used as a second generation contact insecticide of the neonicotinoid group (Hofer and Brandl, 1999) for seed treatment with very low toxicity towards mammals. It was initially evaluated as an effective protectant for wheat and maize grains by Arthur *et al.* (2004) against *Sitophilus oryzae and Rhyzopertha dominica.* Chitin synthesis inhibitors (CSIs) interfere with the synthesis of new chitin by preventing the moult process in insects (Ishaaya and Casida, 1974; Hammock and Quistad, 1981) and disturb the complete life phase (Verloop and Ferrell, 1977). For example, Lufenuron is a chitin synthesis inhibitor, and it controls various insect species including Lepidoptera (Sáenz-de-cabezón *et al*., 2006) and Coleoptera (Ahire, 2008). In mammals, chitin and insects harmones responsible for the moulting process are not present; therefore IGRS are safe for humans and can be helpful for grain protection (Sammour *et al*., 2008). The efficacies of biorational insecticides are affected by a number of factors such as the exposure interval of insects to insecticide dose rate, and the population of target insects collected from different locations and grains type. Athanassiou *et al*, (2008a) observed the effect of geographical locations on the tolerance level of stored grain insects to insecticides and they found a significant difference in the susceptibility level of the larvae and adults of six European populations of *Tribolium confusum* to spinosad dust.

The purpose of this research was to evaluate the efficacy of two biorational insecticides (thiamethoxam, lufenuron) against different strains of rice weevil on wheat, maize and rice grains.

MATERIALS AND METHODS

Collection of *Sitophilus oryzae*

S. oryzae adults used in the experiment were collected from Multan (Longitude: 71° 26' E; Latitude: 30 °12' N; Altitude: 123m), Bahawalpur (Longitude: 73°15′12″ E; Latitude: 29°59′55″ N; Altitude: 159 m), Faisalabad (Longitude: 73° 5' 0" E; Latitude: 31° 25' 0" N; Altitude: 192m), Rawalpindi (Longitude: 73°04′04″ E ; Latitude: 33°36′02″ N; Altitude: 493m) and Lahore (Longitude: 74.3436◦ E; Latitude: 31.5497◦ N; Altitude: 216m) located in Punjab Province, Pakistan. Insects were kept in the laboratory at 26±1°C and 70±5% R.H, on whole wheat, rice and maize grains. *Sitophilus oryzae* adults used in the experiments were 2-3-weeks old and mixed sex (Athanassiou *et al*., 2011).

Insecticide formulations and commodities

The insecticide formulations used in the current study were Actara WG containing 25% thiamethoxam and Match EC containing 5.2% lufenuron provided by Syngenta. Wheat, rice and maize grains used in the bioassays were untreated and these grains were collected from grain markets of Lahore.

Bioassay

Lufenuron and thiamethoxam were applied at 2, 4, 6, 8, 10 ppm as a solution in distilled water by following the methodology of Athanassiou *et al*. (2011) and Khan *et al.* (2016b). For wheat, maize and rice, lot of 900g grains was required at each concentration. To make the required concentration, an appropriate quantity of each insecticide was added in distilled water. A thin layer of 100g grains was spread on a tray and treated with 1ml insecticide solution. For uniform insecticide distribution, the grains were manually shaken for 5 minutes (Athanassiou *et al*., 2011) and then the grains were allowed to dry. The treated grains were placed in jars and 30 insects of *S. oryaze* were added in each single jar. The jars were covered with a muslin cloth to facilitate aeration and to prevent the insects escaping. The same procedure was carried out three different times by preparing new lots for each grain. One jar containing grains treated with distilled water was served as control. The treated jars were maintained at 30°C and mortality data were recorded after 21 days.

Statistical analysis

The mortality data were analyzed using ANOVA in Statistix (8.1) version. The means were separated by Fisher's LSD test at $P<0.05$. However, percent mortality and standard errors are shown in the tables.

RESULTS

When lufenuron applied, the mortality level of *S. oryzae* was significantly affected by the interaction of strains and dose ($F_{16,32} = 5.15$, P=0.00). The interactions between strain x commodity ($F_{8,32} = 0.65$, P = 0.73), dose x commodities ($F_{8,32} = 2.55$, P = 0.28) were not statistically significant. All main effects, strain ($F_{4, 32} = 838$, P = 0.000), dose ($F_{4, 32} = 13.3$, P=0.0000), commodities ($F_{2, 32} = 9.03$, P=0.0008) for mortality of *S. oryzae* were significantly different (Table 1). When thiamethoxam applied to control *S. oryzae,* main effects like strain ($F_{4, 32} = 16.01$, P = 0.0000) and dose ($F_{4,32} = 432.2$, P = 0.0000) were statistically significant. The interactions between strain x dose ($F_{16,32} = 4.99$, P = 0.0001), strain and commodities ($F_{8,32} = 2.50$, P = 0.031), dose and commodities ($F_{8,32} = 2.43$, P = 0.035) were significant (Table 1). In case of the Faisalabad strain, when lufenuron applied at 2 ppm the highest mortality rate of *S. oryzae* was observed on rice (43%) followed by wheat (40%) and maize (38%). At 4 ppm there was high mortality on rice (55%) and on wheat and maize mortality rate was same (53%). When applied at 6ppm, the mortality on maize grains was recorded the highest as compared to other commodities. At the higher dose rate of 8ppm, the lowest mortality was on wheat grains, while at 10pm the lowest mortality was observed on maize. When lufenuron applied at 2ppm on wheat, rice and maize grains against Multan strain, the mortality rate in wheat was observed (46%), while on maize and rice grains mortality was same (45%). The highest mortality was recorded on wheat (59%) when lufenuron applied at 4 ppm, as compared to other commodities. At the higher dose rate of 6ppm, 8ppm, 10ppm, lowest mortality was observed on wheat (71%, 79%, 92%) respectively. The highest mortality (99%) on maize was recorded at 10ppm (Table 2)

With Bahawalpur strain, lufenuron at 2ppm resulted in highest mortality (52%) on maize, while at this concentration the mortality on wheat and rice grains was the same. There was significantly highest mortality (67%) on maize at 4ppm, followed by rice (64%) and wheat (62%). At 6ppm and 8ppm the mortality rate was highest on maize (76%, 88%) respectively, while at 8ppm mortality on wheat and rice was same (86%). Against Lahore strain, lufenuron at 2ppm resulted in the significantly lowest (43%) mortality on wheat as compared to rice and maize on which mortality was 51%. At 4ppm and 6ppm the mortality on maize was higher 70% and 77%, while mortality was observed on wheat 51% and 69% respectively. The mortality on wheat, maize and rice at 8ppm was 88%, 86% and 88% respectively. At the highest concentration of lufenuron 95% mortality was recorded on maize. With Rawalpindi strains, the concentration of 2ppm resulted in the lowest mortality on maize 41%, while the mortality on wheat and rice was observed 43% and 44%. On the other, 4 ppm and 6ppm resulted in the lower mortality of 51% and 69% on wheat when compared with others. At 8ppm, significantly higher mortality (86%) on wheat and at 10ppm (93%) mortality on rice was observed (Table 2). The overall efficacy of Lufenuron against *S. oryzae* on wheat, rice and maize were demonstrated separately in Figure 1.

Table 1

ANOVA for mortality of different strains of *Sitophilus oryzae* on wheat, rice and maize treated with lufenuron and thiamethoxam.

Insecticides	Main effects	Df	F	P
Lufenuron	Strains	4	838	0.0000
	Commodities	2	9.03	0.0008
	Dose	4	13.34	0.0000
	Strains x commodities	8	0.65	0.7333
	Strains x dose	16	5.15	0.0000
	commodities x doses	8	2.55	0.286
	Error	32		
	Total	74		
Thiamethoxam	Cities	4	16.01	0.0000
	Commodities	2	2.70	0.0828
	Dose	4	432.27	0.000
	Strains x Commodities	8	2.50	0.0311
	Strains x Doses	16	4.99	0.0001
	Commodities x Doses	8	2.43	0.0355
	Error	32		
	Total	74		

Table 2

Percent mortality (±SE) of different strains of *Sitophilus oryzae* in three commodities treated with five dose rates of lufenuron.

Strains	Commodity	2ppm	4ppm	6ppm	8ppm	10ppm
Fais alabad	Wheat	39.9±2.7c	53.3±2.7b	58.8±4.5b	86.6±2.7a	95.5±1.8a
	Rice	43.3±0.0d	55.5±1.8c	61.1±0.8c	88.8±1.8b	95.5±1.8a
	Maize	37.7±1.8d	53.4±1.6c	63.3±2.7b	88.8±0.9a	93.3±0.0a
Multan	Wheat	46.7±3.1d	59.1±3.3c	71.3±3.5b	79±0.8b	92.2±0.8a
	Rice	45.6±2.4c	52.3±1.9c	74±2.4b	86.6±1.5a	93.3±0.0a
	Maize	45.5±3.2e	55±1.8d	72.3±3.2c	81.8±0.8b	99±0.8a
Bhawalpur	Wheat	47.7±1.8e	62.3±1.9d	70±1.4c	86.6±0.8b	92.2±0.8a
	Rice	47.7±3.2e	64.6±1.9d	72±0.8c	86.6±0.8b	93.4±1.6a
	Maize	52.2±0.8d	67±0.0c	76.7±4.0b	88.8±1.8a	95.5±1.8a
Lahore	Wheat	43.3±4.1d	51.3±2.4c	69±0.8b	86.6±1.5a	88.8±1.8a
	Rice	51.1±0.8d	65.5±1.8c	72±0.8b	88.8±1.8a	89.6±1.5a
	Maize	51.1±0.8e	70±0.0d	77.7±3.2c	87.7±0.9b	95.5±1.8a
Rawalpindi	Wheat	43.3±4.1d	51.3±0.8c	69±1.5b	86.7±1.5a	88.8±1.8a
	Rice	44.4±3.6c	53.3±2.7c	75.6±1.0b	83.3±0.0b	93.3±3.1a
	Maize	41.0±1.8e	53±0.0d	72±0.8c	84.4±0.8b	92.2±0.8a

Means followed by the same upper-case letter within row are not significantly different; in all cases df = 4, 14, LSD test at $P < 0.05$.

Thiamethoxam at the dose of 2ppm resulted in higher mortality of Faisalabad strain on wheat (68%) while on rice the lowest mortality (63%) was observed. On the other side the higher mortality 80% and 87%, at 4ppm and 6ppm respectively was observed on rice. At 8ppm on rice and maize, 95% mortality was recorded whereas at 10ppm, 100% was achieved on all grain commodities (Table 3).

Table 3

Percent mortality (±SE) of different strains of *Sitophilus oryzae* in three commodities treated with five dose rates of thiamethoxam.

Strains	Commodity	2ppm	4ppm	6ppm	8ppm	10ppm
Faisalabad	Wheat	68 ± 0.8e	78± 0.8d	84.4± 0.8 c	88.8±1.8b	100± 0.0a
	Rice	63.3± 2.7d	80 ±0.0c	87.8 ±3.7bc	95.5± 1.8ab	100 ±0.0a
	Maize	65.5 ±1.8c	75.5±1.8b	84.4 ±3.6b	95.5 ±3.6a	100± 0.0a
Multan	Wheat	70± 0.0d	79.9±2.7c	90.1± 3.2b	92.3± 2.4ab	100 ±0.0a
	Rice	67 ±0.0d	79.9±3.1c	88.8 ±3.6bc	95.5± 3.6ab	100± 0.0a
	Maize	71.2 ±1.7c	78 ±0.8c	87.8± 3.7b	97.7 ±1.8a	100 ±0.0a
Bahawalpur	Wheat	58± 4.0c	77 ±0.0b	88.8± 0.9a	90.1± 4.2a	99 ±0.8 a
	Rice	64.6± 1.9d	79± 0. 8c	91.1± 0.8b	95.5 ±1.8ab	100 ±0.0a
	Maize	70.3± 2.7d	82.2±1.7c	91.0 ±1.8b	93.3 ±0.0b	100 ±0.0a
Lahore	Wheat	78 ±0.8d	81.1±0.8cd	86.6± 3.1bc	92.3 ±1.9b	100 ±0.0a
	Rice	70.3 ±2.7d	78± 0.8c	81.2 ±2.3 c	92.3± 1.9b	100 ±0.0 a
	Maize	73.6 ±2.7d	78.8 ±2.4 cd	84.4 ±1.7bc	90.0 ±2.8b	100 ±0.0 a
Rawalpindi	Wheat	47.7± 1.8e	70± 0.0d	81.8 ±0.8c	90± 2.8b	97.7± 1.8a
	Rice	53.3± 2.7c	77± 0.0b	79.9 ±2.7b	95.5± 1.8a	100 ±0.0a
	Maize	65.5 ±1.8c	73.4 ±1.6b	77.7 ±3.2b	92.3 ±1.9a	100 ± 0.0a

Means followed by the same upper-case letter within row are not significantly different; in all cases df = 4, 14, LSD test at $P < 0.05$.

When thiamethoxam applied against the strains of Multan, 71% mortality was observed followed by wheat (70%) and rice (67%) at 2ppm. With the application rate of 4ppm, mortality on wheat and rice reached (79%), while on maize (78%) was observed. Mortality was higher at 6ppm on wheat (90%) followed by rice (88%) and maize (87%). The highest mortality of (97%) was recorded on maize at 8ppm, whereas on maize the low mortality (92%) was observed at 8ppm as compared to other grains. At the higher dose of 10 ppm, 100 % mortality was achieved on wheat, rice and maize. The Bahawalpur strain at 2ppm, showed the statistically lowest mortality on wheat (58%), as compared to rice (64%) and maize (70%). Mortality at 4ppm was observed highest on maize (82%), while at 6ppm rice and maize showed the same mortality rate (91%). On rice at 8ppm (95%) mortality was recorded followed by maize (92%) and wheat (90%). Total mortality of 100% was recorded at 10ppm dose, on rice and maize while mortality on wheat was recorded (99%) .With Lahore strain, at 2ppm mortality on rice (70%) was significantly lower as compared to wheat on which mortality was 78%. At 4ppm and 6ppm, wheat showed the highest mortality of 81% and 86% respectively when compared with other commodities. Wheat and rice showed the same mortality rate 92% at 8ppm while on after maize 90% mortality was observed. Complete mortality was achieved at 10ppm on all grain commodities. Thiamethoxam applied at 2ppm against Rawalpindi strain, caused significant lower mortality (47%) on wheat, while the highest mortality was observed in maize (65%). Mortality on rice was highest 77%, while on wheat 70% and on maize 73% was observed. At 6ppm, 81% mortality was achieved on wheat, while at 8ppm mortality reached at highest rate of 95% on rice. At 10ppm thiamethoxam reached at the highest efficacy of 100 % on rice and maize, as compared to wheat on which mortality was 97% (Table 3).Overall mortality due to thiamethoxam of different strains on wheat, rice and maize are displayed separately (Fig. 2).

DISCUSSION

The result of this study indicates that thiamethoxam is more efficient as compared to lufenuron and it can be used successfully against complete control of *S. oryzae.* The efficacies of insecticides are affected by a number of factors such as exposure interval, dose rate, and the population of target insects and commodity of grains. Many studies revealed that IGRSs generally acts against the immature developmental stages of insects and may have a little or no lethal effect on the insect's adults (Cogburn, 1988; Daglish and Nayak, 2010; Mohandass *et al*., 2006; Oberlander and Silhacek, 2000). But the results of current study appeared to contradict this statement because all the strains of insects showed mortality after contact with grains treated with IGRs. However, all results showed that mortality was dose dependent. The association between dose and mortality has been studied by many researchers. When grains treated with fenoxycarb dose range from 0.001-10ppm, the moratlity of *S. zeamais* after contact increased from 4 - 45.5% (Letellier *et al*., 1995). Mortality of *S. oryzae* adults increased from 24-55% after their exposure in treated wheat with flufenoxuron

dose rate from 0.5-25ppm (Ammar 1988). According to the findings of present study, lufenuron applied at 2ppm resulted in 39-52% mortality while at 4ppm, the highest mortality of 70% was observed in Rawalpindi strain on maize. With the increase in dose rate, mortality also increases. Overall, the efficacy of lufenuron was highest at the dose rate of 10ppm against the strain of Multan on maize with 99% mortality.

In the present study, thiamethoxam applied at five different doses against five strains of *S. oryaze* at 30°C. Mortality was recorded after 21 days and the results indicate that at low dose, rate of mortality was low as compared to high doses. Aurthur *et al*. (2004) observed the efficacy of thiamethoxam at dose rates ranging from 1- 4mg/kg against *S. oryzae, T. castaneum, R. dominica and S. zeamais* on wheat and maize grains .They reported that mortality reached approximately 100% with the increased in exposure interval from 2 days to 6 days, but they did not report any association between the efficacy of thiamethoxam and grain type. The effect of different grain types on efficacy has been studied for different insecticide like spinosad (Huang and Subramanyam, 2007), abamectin (Kavallieratos *et al*., 2009) and thiamethoxam (Wakil *et al.,* 2013). Studies done (Arthur, 2002; Vayias *et al*., 2009) on the efficacy of insecticides on different grains revealed that insecticides proved to be more effective on maize as compared to wheat. In the present study, whereas, thiamethoxam was found more efficient on rice and maize with mortality reached to 100% at the high dose range of 10 ppm while in Bhawalpur and Rawalpindi strain on wheat, the mortality was not completely controlled. All results proved that mortality depends on the dose rate of an insecticide. The physiochemical properties of the different grains possibly affect the exposure or residual activity of an insecticide in different ways. Strains of insects which are originating from different geographical localities may be different in their susceptibility to a different insecticide .Different studies showed that populations from fields were found less tolerant to applied insecticides as compared to laboratory populations (Huang *et al*., 2004; Rahman *et al*., 2007; Ali *et al*., 2007). In this research effectiveness of thiamethoxam and lufenuron among *S. oryzae* populations originating from different cities of the Punjab province reported. The results are in agreement with those reported by Wakil *et al*. (2013) against *R. dominica*.

CONCLUSION

In summary, thiamethoxam and lufenuron can be used to protect the stored grains from *S. oryzae* in festation. The difference in the mortality rate among local populations of *S. oryzae* to thiamethoxam and lufenuron was detected in this research. Thiamethoxam and lufenuron are not yet registered in Pakistan, as grain protectants. However, the results of the present study could provide a window to include these insecticides in the management plans for stored grain insect

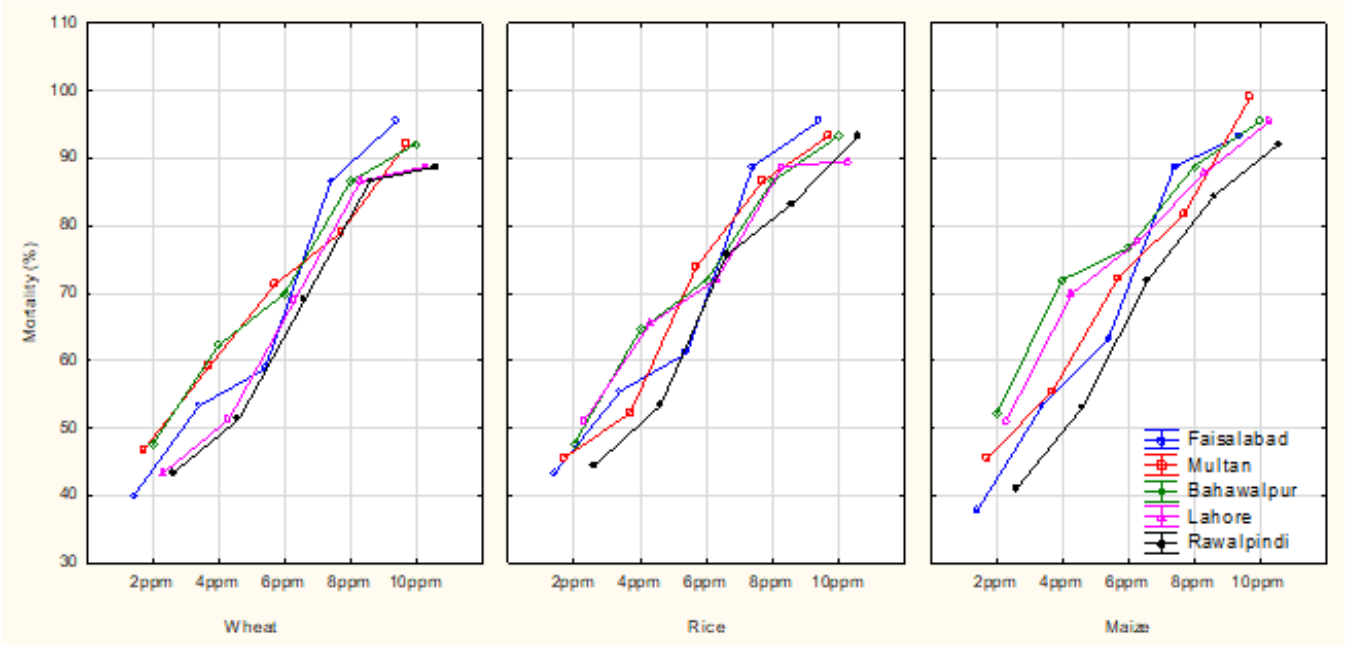

Fig 1.

Comparison of five strains of *Sitophilus oryzae* by percentage mortality of adults in wheat, rice and maize treated with lufenuron at five dose rates (2ppm, 4ppm, 6ppm, 8ppm, 10ppm).

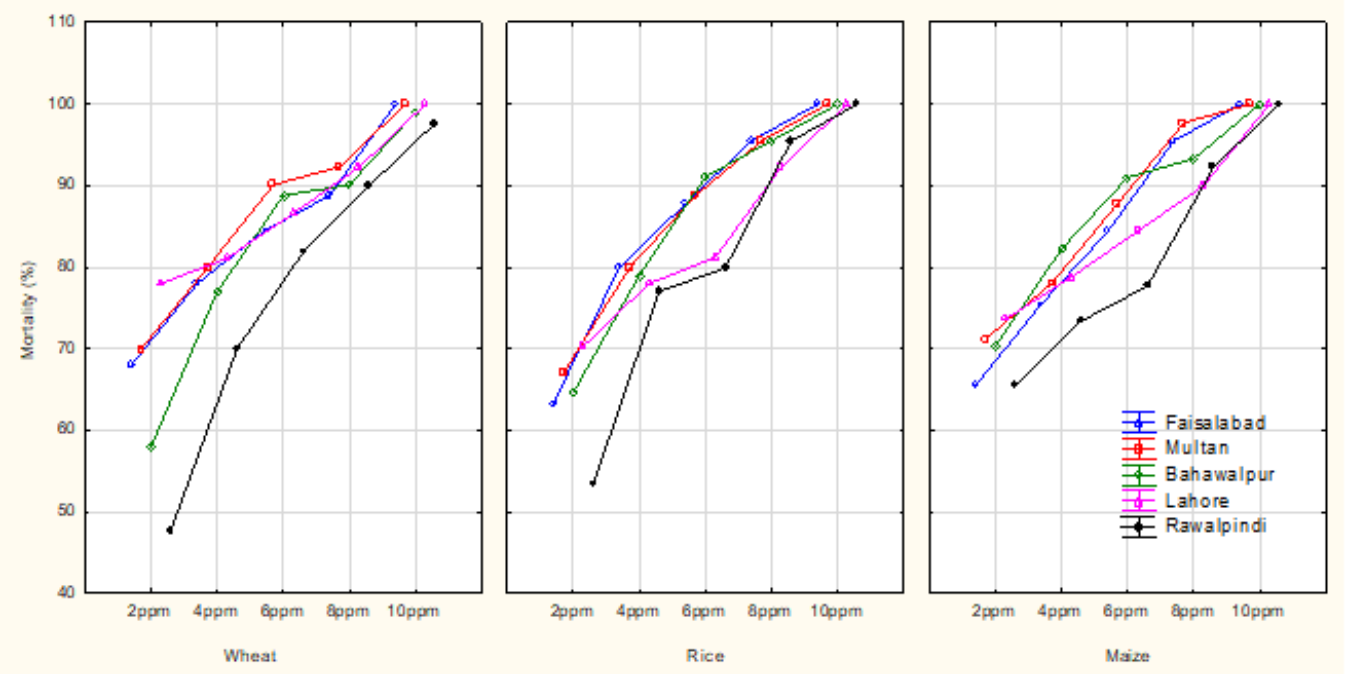

Fig. 2

Comparison of five strains of *sitophilus oryzae* by percentage mortality of adults in wheat, rice and maize treated with thiamethoxam at five dose rates (2ppm, 4ppm, 6ppm, 8ppm, 10ppm).

pests.

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