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Effects of combined thiamethoxam and diatomaceous earth on mortality and progeny production of four Pakistani populations of *Rhyzopertha dominica* (Coleoptera: Bostrichidae) on wheat, rice and maize

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ABSTRACT

Bioassays were conducted to evaluate the effects of combining thiamethoxam at 0.25, 0.5 and 0.75 mg/kg of active ingredient with the diatomaceous earth (DE) formulation, SilicoSec, at the rate of 100 mg/kg against four Pakistan populations of the lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae). The tests were carried out with adult beetles on wheat, maize, and rice. Mortality increased with increasing application rates and exposure intervals for each population. Individually, thiamethoxam alone was more effective at the high dose rate than DE alone, but after 14 days of exposure in most cases, there was greater mortality with DE than with the low dose of thiamethoxam. There was greater mortality in wheat than in rice or maize. Populations differed in susceptibility to treatments and production of progeny.

1. Introduction

The use of residual insecticides as grain protectants and seed treatments is a common strategy for stored product protection against insect pests (Arthur, 1996). The main purpose for the application of grain protectants is to ensure the safety and integrity of the seed and food grains for extended periods after harvesting. Among various residual insecticides, organophosphates and pyrethroids are the most relied upon (Zettler and Arthur, 2000; Collins, 2006; Nighat et al., 2007). But the residues of these synthetic insecticides reduce consumer acceptability as there is increasing demand of residue-free food. Moreover, resistance development in insect pests to widely used insecticides (Arthur and Zettler, 1992; Irshad and Gillani, 1992), environmental concerns, and health hazards have necessitated the assessment of new substances for stored grain pest management. Several new molecules of existing insecticidal groups have been investigated in this regard (Hussain et al., 2005; Arthur and Campbell, 2008; Athanassiou et al., 2008a; Kavallieratos et al., 2009, 2010a, 2011). While alternatives to chemical insecticides such as diatomaceous earths have also been evaluated with good insecticidal efficacy (Subramanyam and Hagstrum, 2000), residual grain protectants continue to have their inevitable role in stored product pest management (Arthur, 1996).

Thiamethoxam is the broad spectrum contact insecticide from the second generation of the neonicotinoid group that has been applied as a seed treatment (Hofer and Brandl, 1999) and that does not possess any known teratogenic or mutagenic effects (Lawson et al., 1999). It is very effective against sucking insect pests (Zang et al., 1999), termites such as *Trinervitermes trinervius Rambur*, *Odontotermes smeathmani Fuller*, and *Amitermes evuncifer Silvestri* (Delgarde and Rouland-Lefevre, 2002), blueberry maggot flies (*Rhagoletis mendax* Curran) (Ayyappath et al., 2000), urban insect pests such as house flies (*Musca domestica*) and cockroaches (*Blattella germanica*) (Eremina and Lopatina, 2005) and insect pests of cowpea (Soliman, 2011). Thiamethoxam was evaluated for its grain protection potential for the first time by Arthur et al. (2004) on wheat and maize against *Sitophilus zeamais* Motschulsky (maize weevil), *Oryzaephilus surinamensis* (L.) (sawtoothed grain beetle) and *Tribolium castaneum* (Herbst) (red flour beetle). In addition to dose rates, exposure interval and temperature factors affected the efficacy both in wheat and maize.

Diatomaceous earths (DEs) are naturally occurring substances that have been certified as organic insecticides and are non-toxic and ecologically benign (Ross, 1981). They have a physical mode

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of action and offer the advantage of being applicable in a variety of ways including dusts and slurries (Subramanyam and Roesli, 2000). Proper use of diatomaceous earth does not affect grain quality (Simioni et al., 2010). Various DE formulations have been successfully evaluated against different stored grain insect or mite species (Athanassiou et al., 2004b, 2005, 2008b; Iatrou et al., 2010; Kavallieratos et al., 2010b; Riasat et al., 2011; Wakil et al., 2010, 2011, 2012), including in mixes with low concentrations of insecticides (Athanassiou and Kavallieratos, 2005), demonstrating the potential for use of DE in combination with insecticides in stored product protection. Although DE's possess various advantages, negative effects on grain flowability by high concentrations is a limiting factor (Korunic, 1998) that can be overcome by integrating with insecticides to enhance effectiveness at low concentrations.

The purpose of this study was to evaluate the efficacy of thiamethoxam as grain protectant in combination with DE on three grain commodities, wheat, rice and maize. The bioassays were performed against four Pakistani populations of an internally feeding primary pest of grains, the lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae). In addition to adult mortality, progeny production of treated *R. dominica* was evaluated.

2. Materials and methods

2.1. Insecticide and DE formulations

The thiamethoxam formulation used was Actara (Syngenta Pakistan Limited, Karachi, Pakistan), which is a wettable granule (WG) and contains 250 g/kg of active ingredient. The DE formulation used was SilicoSec (Biofa GmbH, Münsingen, Germany), a DE of fresh water origin that contains 92% SiO_2, 3% Al_2O_3, 1% Fe_2O_3, and 1% Na_2O.

2.2. Insects

R. dominica adults used in the experiments were collected from four different locations in Pakistan and kept in the laboratory for up to four generations on wheat at 25 ± 2 °C and 65 ± 5% RH. The populations were initially collected from wheat in different storage conditions (bulk and bag storage etc.) of four districts, viz. Faisalabad (Latitude: 31° 30′ 0″ N; Longitude: 73° 6′ 0″ E; Altitude: 184 m); Toba Tek Singh (Latitude: 30° 59′ 0″ N; Longitude: 72° 28′ 0″ E; Altitude: 156 m); Jhang (Latitude: 31° 16′ 0″ N; Longitude: 72° 19′ 0″ E; Altitude: 153 m) and Bahawalpur (Latitude: 29° 24′ 0″ N; Longitude: 71° 40′ 0″ E; Altitude: 115 m) located in Punjab province, Pakistan. Adults aged less than 2 wk from emergence were used in the tests.

2.3. Commodities

The tested commodities were clean, untreated, and infestation-free wheat (var. Inqlab-91), rice (var. Super Kernel), and maize (var. Pioneer 32-F-10). The moisture contents of all tested commodities as determined by Dickey-John moisture meter (Dickey–John Multigrain CAC II; Dickey–John Co., USA) were 11.25, 10.50, and 11.65% for wheat, rice and maize, respectively.

2.4. Bioassays

Eight 1 kg lots of each grain commodity, namely, wheat, rice and maize, were prepared in plastic jars. For each commodity, thiamethoxam at the rate of 0.25, 0.5, and 0.75 mg/kg was applied separately to two lots, i.e., a total six lots were treated with thiamethoxam. One lot (lot seven) of each grain was treated with diatomaceous earth (SilicoSec) at the rate of 100 mg/kg, and the remaining lot (lot eight) was un-treated and served as a control for each grain. The grain lots were spread in trays in thin layers so that thiamethoxam could be applied uniformly. Then 5 ml of thiamethoxam from each solution or water for controls was sprayed per kg of each grain using a hand sprayer, and grains were allowed to dry before being placed in incubators set at 25 °C and 65% RH for 2 d to equilibrate moisture (Kavallieratos et al., 2009). For each, three of the six lots treated with thiamethoxam were admixed with diatomaceous earth formulation at 100 mg/kg. They were manually shaken for 5 min to obtain a uniform distribution of the DE particles with the grains. From each grain lot, a group of four 50 g samples served as an experimental unit. They were placed in small vials (11 cm height and 6.5 cm width) provided with the hole at the top (10 mm in diameter) covered with a muslin cloth to facilitate air exchange between the inside and outside of the vial while preventing insects from escaping. Fifty adult *R. dominica* were placed in each vial, and vials were placed in incubators maintained at 30 °C and 65% RH. The same procedure was repeated for the remaining three field populations of *R. dominica*. This process was carried out three different times, i.e., three temporal replications, by preparing new lots each time for each grain commodity. The desired relative humidity (65% RH) was maintained using saturated KI solution (Greenspan, 1977). Adult mortality counts were conducted on all vials at 1, 2, 7 and 14 d after treatment by dumping out the treated grains into a tray and returning after removing dead beetles. At 14 d post-exposure, all dead and live adults were removed from the vials after the last count (14 d). All vials were maintained under the same conditions (30 °C and 65% RH) for 60 d to assess the emergence of adult progeny for each population. In summary, there were eight treatments with three temporal replicates of four vials for each of three commodities and four populations.

2.5. Data analysis

Mortalities were adjusted for control mortality with Abbott's formula (Abbott, 1925). Repeated-measures analysis was conducted by population, with insecticide treatment and commodity as main effects, and adult mortality as the response variable. Progeny production was analyzed with ANOVA with the response variable being number of emerging adults per vial while insecticide treatment and commodity were the main effects. Means for mortality and progeny were compared using Tukey–Kramer (HSD) test at $\alpha = 0.05$ (Sokal and Rohlf, 1995). Analyses were done with Minitab 13.2 (Minitab 2002 Software Inc., Northampton, MA).

3. Results

3.1. Mortality of R. dominica

Mortality of adult beetles from all four populations was significantly affected by the exposure intervals (Faisalabad: $F_{3,\,248} = 184$, $P < 0.001$; Bahawalpur: $F_{3,\,248} = 197$, $P < 0.001$; Jhang: $F_{3,\,248} = 177$, $P < 0.001$; Toba Tek Singh: $F_{3,\,248} = 158$, $P < 0.001$). Main effects for mortality levels of all tested populations were significant while their associated interaction was significant within exposure interval only for the Bahawalpur population at 1 and 2 d and the Faisalabad and Toba Tek Singh populations at 14 d (Table 1).

For the Bahawalpur population at 7 d post-treatment, mortality was significantly greater on wheat than on maize for all treatments except for SilicoSec combined with 0.75 mg/kg of thiamethoxam (Table 2). The combination of SilicoSec with 0.5 or 0.75 mg/kg of thiamethoxam had significantly greater mortality than either material alone at the respective concentrations on all grains at all exposure periods of more than 1 d except on wheat, where some of the mortality differences were not statistically significant.

Table 1
ANOVA parameters for adult mortality of diverse populations of *R. dominica* on wheat, maize and rice treated with thiamethoxam and DE.

Populations	Source	df	24 h		48 h		7 d		14 d	
			F	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Bahawalpur	Commodity	2	64.40	<0.001	56.54	<0.001	63.98	<0.001	40.78	<0.001
	Treatment	6	38.41	<0.001	91.89	<0.001	204.93	<0.001	126.51	<0.001
	Commodity × treatment	12	5.49	<0.001	2.11	0.037	0.50	0.905	0.74	0.701
Faisalabad	Commodity	2	22.00	<0.001	60.35	<0.001	79.32	<0.001	86.34	<0.001
	Treatment	6	59.84	<0.001	154.73	<0.001	203.83	<0.001	126.08	<0.001
	Commodity × treatment	12	0.47	0.918	1.01	0.459	0.52	0.891	3.09	0.003
Jhang	Commodity	2	23.17	<0.001	81.63	<0.001	79.84	<0.001	77.01	<0.001
	Treatment	6	82.88	<0.001	220.06	<0.001	165.83	<0.001	111.00	<0.001
	Commodity × treatment	12	1.11	0.377	1.66	0.111	1.56	0.165	1.67	0.108
Toba Tek Singh	Commodity	2	64.08	<0.001	128.01	<0.001	126.73	<0.001	72.10	<0.001
	Treatment	6	136.07	<0.001	322.93	<0.001	243.80	<0.001	117.89	<0.001
	Commodity × treatment	12	1.87	0.067	1.85	0.071	1.61	0.125	3.66	<0.001

The trends for the Faisalabad population were similar to those of the Bahawalpur population with mortality at 7 and 14 d post-treatment on wheat being significantly greater than that on maize for all treatments and significantly greater than that on rice for seven of the 14 exposure period–treatment combinations (Table 3). At 7 d on all grains, there was significantly greater mortality for the SilicoSec with 0.5 or 0.75 mg/kg of thiamethoxam combinations than any of the components alone at the respective concentrations. On wheat at 14 d, there was greater mortality with all combination treatments than with the components at the same doses except for 0.75 mg/kg thiamethoxam. On maize and rice at 14 d, there was significantly greater mortality for the SilicoSec with 0.5 or 0.75 mg/kg of thiamethoxam than the components alone at the respective doses.

With the Jhang population, mortality at 2 d, 7 d, and 14 d was significantly greater on wheat than maize for all treatments except for 100 mg/kg SilicoSec at 2 d and 0.5 mg/kg thiamethoxam at 7 d (Table 4). At 7 and 14 d, the mortalities for SilicoSec with 0.25 mg/kg thiamethoxam were not significantly different than the mortalities for SilicoSec alone on all grains. However, on all grains at 7 or 14 d, both 0.5 and 0.75 mg/kg thiamethoxam with SilicoSec had significantly greater mortalities than the components at the same doses alone except at 14 d for 0.75 mg/kg thiamethoxam on wheat with or without SilicoSec, wherein the respective mortalities were 100 and 93.1%.

For the Toba Tek Singh population, the mortality on wheat was significantly greater than that on maize except for the 1 d exposure to SilicoSec alone or 0.25 mg/kg thiamethoxam alone and the 14 d exposure to SilicoSec with 0.75 mg/kg thiamethoxam, in which case all mortalities were 100%. For 11 of the 28 exposure interval–treatment combinations, the mortalities were significantly greater on wheat than on rice. At 14 d, the mortalities of Toba Tek Singh beetles were significantly greater for all of the combination treatments than the components at the respective doses except that on rice the mortality for 100 mg/kg SilicoSec alone did not differ significantly from that for SilicoSec with 0.25 mg/kg thiamethoxam, and on rice or wheat the mortality for SilicoSec with 0.75 mg/kg thiamethoxam did not differ significantly from the mortality with 0.75 mg/kg thiamethoxam alone (Table 5).

3.2. Progeny production

The mean progeny production by population, treatment, and grain commodity is displayed in Table 6. Overall progeny production and mortality by population are displayed in Fig. 1. For progeny production, all main effects (commodity: Bahawalpur $F_{2, 62} = 5.05$, $P = 0.009$; Faisalabad $F_{2, 62} = 12.57$, $P < 0.001$; Jhang $F_{2, 62} = 13.00$, $P < 0.001$; Toba Tek Singh $F_{2, 62} = 10.56$, $P < 0.001$; dose: Bahawalpur $F_{6, 62} = 34.45$, $P < 0.001$; Faisalabad $F_{6, 62} = 12.11$, $P < 0.001$; Jhang $F_{6, 62} = 13.43$, $P < 0.001$; Toba Tek Singh $F_{6, 62} = 13.78$, $P < 0.001$) and their associated interaction, commodity × dose (Bahawalpur $F_{12, 62} = 7.52$, $P < 0.001$; Faisalabad $F_{12, 62} = 25.81$, $P < 0.001$; Jhang $F_{12, 62} = 3.99$, $P < 0.001$; Toba Tek Singh $F_{12, 62} = 8.72$, $P < 0.001$) were significant. There was significantly greater progeny production by the Bahawalpur and Faisalabad populations than by the Jhang and Toba Tek Singh ($F_{3, 250} = 30.4$; $P = 0.001$, Fig. 1). The progeny emergence was reduced significantly more on wheat than on maize in most cases. The combinations of SilicoSec with 0.25, 0.5, or 0.75 mg/kg thiamethoxam had significantly lower progeny production than the respective components

Table 2
Percent mortality (±SE) of Bahawalpur population of *R. dominica* in three commodities treated with one dose rate of SilicoSec (100 mg/kg), three dose rates of thiamethoxam (0.25, 0.5 and 0.75 mg/kg) applied alone or in combination with DE. Means followed by the same lower-case letter within row and means followed by the same upper-case letter within exposure interval and column are not significantly different; HSD test at $P < 0.05$.

Exposure interval (d)	Commodity	Dose rate (mg/kg) (SilicoSec + thiamethoxam)						
		100 + 0	0 + 0.25	0 + 0.5	0 + 0.75	100 + 0.25	100 + 0.5	100 + 0.75
1	Wheat	5.3 ± 0.9Ac	6.6 ± 1.1Abc	12.6 ± 1.4Aabc	18.7 ± 2.6Aa	6.6 ± 1.7Abc	15.3 ± 1.3Aab	20.7 ± 4.0Aa
	Rice	3.5 ± 1.76Ac	5.2 ± 2.1Abc	10.3 ± 1.2Aabc	14.7 ± 1.9ABab	4.8 ± 2.5Abc	11.3 ± 3.1Aabc	17.3 ± 2.5Aa
	Maize	2.6 ± 0.9Ab	4.2 ± 2.7Aab	7.2 ± 1.5Aab	9.0 ± 1.2Bab	3.5 ± 2.6Aab	7.6 ± 1.5Aab	10.4 ± 1.2Aa
2	Wheat	9.2 ± 0.3Ae	15.1 ± 2.8Ade	22.0 ± 2.8Acd	35.1 ± 2.7Aab	10.7 ± 3.2Ae	31.5 ± 2.4Abc	43.1 ± 1.4Aa
	Rice	5.6 ± 1.0Ae	10.1 ± 1.8Ade	16.5 ± 2.1ABcd	23.4 ± 1.0Bbc	7.0 ± 1.2Ae	26.8 ± 2.7ABb	35.7 ± 1.8Ba
	Maize	4.1 ± 1.2Ad	6.2 ± 2.1Ad	10.0 ± 2.0Bcd	16.6 ± 1.5Bbc	5.4 ± 1.2Ad	20.1 ± 2.5Bab	26.0 ± 3.1Ca
7	Wheat	40.0 ± 2.8Acd	35.1 ± 2.3Ad	48.1 ± 2.8Ac	69.2 ± 3.0Ab	46.3 ± 1.2Acd	75.8 ± 2.0Aab	87.6 ± 3.2Aa
	Rice	32.2 ± 1.9ABcd	28.7 ± 2.8ABd	37.7 ± 2.5ABcd	59.1 ± 2.6ABb	40.5 ± 1.7ABc	68.4 ± 2.3ABb	82.5 ± 2.5Aa
	Maize	27.6 ± 1.4Bde	21.6 ± 1.1Be	32.1 ± 1.9Bd	48.7 ± 2.4Bc	34.6 ± 2.7Bd	61.4 ± 2.0Bb	74.8 ± 1.4Aa
14	Wheat	66.4 ± 2.9Ab	48.6 ± 2.1Ac	60.7 ± 2.2Abc	83.8 ± 3.2Aa	71.6 ± 2.1Ab	86.1 ± 3.2Aa	94.4 ± 1.5Aa
	Rice	58.1 ± 2.3Ad	43.9 ± 1.9ABe	55.4 ± 2.4ABd	72.3 ± 1.9Bbc	64.3 ± 2.3Acd	81.4 ± 1.5ABb	92.5 ± 2.0Aa
	Maize	56.9 ± 2.6Acd	38.0 ± 2.8Be	46.6 ± 2.4Bde	65.4 ± 1.6Bbc	60.8 ± 3.6Ac	74.6 ± 1.5Bab	85.2 ± 3.2Aa

Table 3
Percent mortality (±SE) of Faisalabad population of *R. dominica* in three commodities treated with one dose rate of SilicoSec (100 mg/kg), three dose rates of thiamethoxam (0.25, 0.5 and 0.75 mg/kg) applied alone or in combination with DE. Means followed by the same lower-case letter within row and means followed by the same upper-case letter within exposure interval and column are not significantly different; HSD test at $P < 0.05$.

Exposure interval (d)	Commodity	Dose rate (mg/kg) (SilicoSec + thiamethoxam)						
		100 + 0	0 + 0.25	0 + 0.5	0 + 0.75	100 + 0.25	100 + 0.5	100 + 0.75
1	Wheat	8.4 ± 1.9Ae	15.6 ± 1.3Acde	22.6 ± 2.2Abcd	29.4 ± 2.5Aab	12.2 ± 3.2Ade	26.3 ± 1.7Aabc	35.6 ± 2.5Aa
	Rice	7.5 ± 2.0Ad	11.5 ± 1.7ABcd	18.9 ± 1.3Abc	27.6 ± 2.5Aab	10.5 ± 2.2Acd	21.6 ± 1.6ABab	29.0 ± 2.1Aa
	Maize	5.5 ± 0.9Ad	7.9 ± 1.9Bcd	16.0 ± 1.4Abc	21.2 ± 1.7Aab	4.5 ± 2.0Ad	18.4 ± 1.2Bb	27.1 ± 2.6Aa
2	Wheat	14.7 ± 1.4Ae	27.5 ± 2.3Ad	41.0 ± 3.4Ac	57.5 ± 2.1Aab	23.0 ± 2.2Ade	48.0 ± 2.4Abc	63.3 ± 2.7Aa
	Rice	11.9 ± 2.8Ad	23.8 ± 1.5Ac	36.2 ± 2.4Ab	45.9 ± 2.1Bab	16.1 ± 1.2ABcd	41.8 ± 2.1ABb	56.3 ± 3.2ABa
	Maize	7.7 ± 1.0Ac	15.2 ± 1.8Bc	29.6 ± 2.8Ab	36.1 ± 1.7Cb	10.0 ± 2.1Bc	35.2 ± 1.5Bb	47.0 ± 2.5Ba
7	Wheat	52.6 ± 2.4Ac	40.7 ± 1.8Ad	58.5 ± 1.8Ac	73.9 ± 2.5Ab	56.4 ± 1.9Ac	80.6 ± 2.1Ab	91.4 ± 1.5Aa
	Rice	41.4 ± 2.5Bcd	33.7 ± 2.5ABd	46.3 ± 1.8Bc	64.5 ± 1.5ABb	44.9 ± 2.4Bcd	73.2 ± 2.3ABb	86.5 ± 3.4ABa
	Maize	34.6 ± 2.7Bcd	28.7 ± 2.8Bd	41.2 ± 1.5Bc	57.0 ± 2.5Bb	40.1 ± 2.1Bc	67.1 ± 2.8Bb	80.5 ± 1.8Ba
14	Wheat	78.3 ± 2.0Ab	64.9 ± 3.3Ac	75.4 ± 2.8Ab	91.0 ± 1.8Aa	93.5 ± 2.0Aa	100 ± 0.0Aa	100 ± 0.0Aa
	Rice	66.6 ± 2.1Bcd	57.1 ± 2.6ABd	68.7 ± 1.5ABbc	77.7 ± 2.1Bb	72.1 ± 2.3Bbc	90.3 ± 2.5Ba	100 ± 0.0Aa
	Maize	64.2 ± 2.9Bcd	50.8 ± 1.8Be	63.2 ± 2.6Bd	74.2 ± 2.6Bbc	70.5 ± 1.5Bbcd	81.1 ± 2.0Cb	96.0 ± 1.4Ba

in three, five, and one instance respectively of the 12 cases each. Progeny production means (±SE) for untreated control insects in wheat, rice and maize respectively were 83.1 ± 2.4, 72.4 ± 2.1, 76.8 ± 2.2 for Bahawalpur; 91.4 ± 6.3, 82.1 ± 5.6, 77.5 ± 4.3 for Faisalabad; 82.9 ± 1.8, 73.5 ± 4.1, 70.6 ± 3.4 for Jhang; and 89.5 ± 3.5, 77.4 ± 3.9, 75.2 ± 4.1 for Toba Tek Singh. Progeny production means (±SE) for untreated control insects taken over all *R. dominica* populations were 91.4 ± 84.1 for wheat, 82.1 ± 71.6 for rice, and 77.5 ± 68.6 for maize.

4. Discussion

The results of this study indicate that DE and thiamethoxam can be used with success against *R. dominica*, but their efficacies are influenced by several factors such as dose rates, exposure interval, commodity, and the population of target insect. Thiamethoxam at a rate of 0.75 mg/kg gave greater than 65% mortality of all four *R. dominica* populations by 14 d from application, while beetle mortality exceeded 85% when 100 mg/kg of DE was combined with it. Previously, Arthur et al. (2004) reported that thiamethoxam is effective against *T. castaneum*, *Sitophilus oryzae* (L.), *S. zeamais*, and *R. dominica* and that the mortality of *S. oryzae* and *R. dominica* was less than 60% when exposed on treated wheat for 1 and 2 d, but it increased to approximately 100% after 6 d of exposure. However, in that study the thiamethoxam was applied at rates ranging from 1 to 4 mg/kg, which are considerably greater than our dose rates of 0.25–0.75 mg/kg. The difference in the application rates may be mitigated by the longer exposures of 7 or 14 d, which increase mortality. This stands in accordance with previous reports dealing with different commercially available grain protectants (Athanassiou et al., 2004a; Kavallieratos et al., 2010a). Importantly, it demonstrates that good efficacy of the treatments may be achieved with reduced application rates.

Several published reports indicate satisfactory efficacy of DE for various insect pests in stored grain commodities (Arthur, 2002; Athanassiou et al., 2003, 2004b, 2007; Collins and Cook, 2006; Ferizli and Beris, 2005; Kavallieratos et al., 2007a; Wakil et al., 2010). However, *R. dominica* is considered to be among the most tolerant stored-grain insect species to DE formulation (Fields and Korunic, 2000). According to Vardeman et al. (2006), *R. dominica* is one of the least susceptible species to DE due to the low mobility of its adults that reduces their chances to come into contact with DE particles. As an alternative to the use of high DE dose rates, the combination of DE formulations with other IPM-compatible control measures has proven very effective against various stored-product insect pests (Lord, 2005; Athanassiou, 2006; Chintzoglou et al., 2008). The results of the present study demonstrate the efficacy for *R. dominica* of thiamethoxam combined with DE.

The effectiveness of DE is different on different grains (Athanassiou et al., 2003, 2009; Kavallieratos et al., 2005, 2010b; Palyvos et al., 2006). The DE formulation, PyriSec, proved to be more effective against *R. dominica* in wheat than seven other grain types tested (Athanassiou and Kavallieratos, 2005). Similarly, Athanassiou et al. (2008b) evaluated the efficacy of three DE formulations, PyriSec, Protect-It, and DEBBM, against *S. oryzae* reared on wheat, barley, or maize and found the maize-reared

Table 4
Percent mortality (±SE) of Jhang population of *R. dominica* in three commodities treated with one dose rate of SilicoSec (100 mg/kg), three dose rates of thiamethoxam (0.25, 0.5 and 0.75 mg/kg) applied alone or in combination with DE. Means followed by the same lower-case letter within row and means followed by the same upper-case letter within exposure interval and column are not significantly different; HSD test at $P < 0.05$.

Exposure interval (d)	Commodity	Dose rate (mg/kg) (SilicoSec + thiamethoxam)						
		100 + 0	0 + 0.25	0 + 0.5	0 + 0.75	100 + 0.25	100 + 0.5	100 + 0.75
1	Wheat	7.3 ± 1.8Ad	18.7 ± 3.8Acd	29.4 ± 1.9Abc	36.3 ± 1.1Aab	10.1 ± 2.4Ad	34.2 ± 2.7Aab	42.2 ± 2.5Aa
	Rice	5.9 ± 1.7Ad	15.0 ± 1.9Acd	24.4 ± 2.5Abc	30.2 ± 1.0ABab	8.9 ± 1.9Ad	28.5 ± 1.4ABab	35.2 ± 2.4ABa
	Maize	7.6 ± 2.6Ab	9.0 ± 3.2Ab	21.2 ± 1.2Aa	27.5 ± 4.5Ba	5.6 ± 0.5Ab	22.2 ± 1.2Ba	30.5 ± 1.3Ba
2	Wheat	16.5 ± 1.7Ae	32.3 ± 1.7Ad	49.0 ± 2.14Ac	65.5 ± 1.9Aab	28.1 ± 2.2Ad	57.1 ± 2.4Abc	70.0 ± 2.5Aa
	Rice	10.4 ± 2.0Ae	25.1 ± 2.9ABd	39.1 ± 2.4Ac	54.2 ± 2.6Bb	21.0 ± 1.6Ad	45.3 ± 1.0Bbc	69.3 ± 1.6Aa
	Maize	8.8 ± 3.0Ad	19.0 ± 2.3Bcd	27.3 ± 3.5Bc	45.5 ± 2.7Bab	12.4 ± 0.9Bd	40.6 ± 2.8Bb	56.4 ± 1.9Ba
7	Wheat	58.1 ± 3.0Ac	43.4 ± 3.1Ad	59.2 ± 1.9Ac	77.8 ± 1.8Ab	65.1 ± 3.1Ac	87.2 ± 2.1Aab	96.6 ± 2.4Aa
	Rice	49.4 ± 2.1Ad	38.8 ± 2.7ABe	52.3 ± 2.4Acd	61.1 ± 1.7Bc	54.4 ± 1.8Acd	80.3 ± 1.5Ab	91.2 ± 1.6ABa
	Maize	36.6 ± 2.8Bde	31.7 ± 0.72Be	48.6 ± 3.5Acd	59.3 ± 2.4Bbc	42.6 ± 2.6Bde	69.2 ± 3.1Bb	83.1 ± 2.5Ba
14	Wheat	80.4 ± 1.5Acd	74.1 ± 1.3Ad	85.3 ± 2.7Ac	93.1 ± 1.6Aab	87.1 ± 1.4Abc	100 ± 0.0Aa	100 ± 0.0Aa
	Rice	71.6 ± 1.4ABcd	65.8 ± 1.5Ad	78.4 ± 2.4ABbc	84.4 ± 1.5ABb	79.0 ± 2.7ABbc	95.0 ± 2.2ABa	100 ± 0.0Aa
	Maize	66.3 ± 2.8Bc	53.7 ± 2.7Bd	70.7 ± 2.0Bbc	79.3 ± 2.6Bb	73.5 ± 1.5Bbc	90.8 ± 1.9Ba	94.4 ± 2.1Ba

Table 5
Percent mortality (±SE) of Toba Tek Singh population of *R. dominica* in three commodities treated with one dose rate of SilicoSec (100 mg/kg), three dose rates of thiamethoxam (0.25, 0.5 and 0.75 mg/kg) applied alone or in combination with DE. Means followed by the same lower-case letter within row and means followed by the same upper-case letter within exposure interval and column are not significantly different; HSD test at $P < 0.05$.

Exposure interval (d)	Commodity	Dose rate (mg/kg) (SilicoSec + thiamethoxam)						
		100 + 0	0 + 0.25	0 + 0.5	0 + 0.75	100 + 0.25	100 + 0.5	100 + 0.75
1	Wheat	10.7 ± 1.2Ad	21.3 ± 0.7Ac	35.4 ± 1.7Ab	41.2 ± 1.7Ab	17.3 ± 2.3Acd	43.3 ± 2.5Ab	54.2 ± 0.6Aa
	Rice	7.9 ± 1.2Ad	18.4 ± 2.7Acd	27.3 ± 3.0ABbc	36.9 ± 1.1Aab	10.3 ± 2.3Bd	35.1 ± 2.6ABab	40.2 ± 1.8Bab
	Maize	6.0 ± 1.4Ab	11.1 ± 2.8Ab	25.3 ± 1.8Ba	29.0 ± 1.8Ba	5.3 ± 0.9Bb	29.0 ± 2.9Ba	33.6 ± 1.8Ba
2	Wheat	19.7 ± 1.6Ae	40.0 ± 2.4Ad	61.0 ± 1.8Ac	73.2 ± 2.5Aab	34.4 ± 1.8Ad	67.6 ± 2.0Abc	80.5 ± 1.8Aa
	Rice	11.7 ± 2.5ABe	29.6 ± 1.6Bd	43.3 ± 2.3Bc	62.5 ± 2.1Bb	25.5 ± 1.7Bd	54.5 ± 2.1Bb	73.4 ± 2.6ABa
	Maize	8.1 ± 2.2Be	23.4 ± 1.4Bd	36.3 ± 1.3Bc	49.1 ± 1.9Cb	19.6 ± 2.5Bd	47.1 ± 1.8Bb	68.4 ± 2.3Ba
7	Wheat	63.1 ± 2.1Ad	52.2 ± 2.5Ae	74.0 ± 1.7Ac	86.7 ± 1.6Ab	71.6 ± 1.5Acd	93.1 ± 1.6Aab	100 ± 0.0Aa
	Rice	51.5 ± 1.2Bde	43.1 ± 2.7ABe	65.2 ± 2.3Bc	79.0 ± 2.8Ab	61.0 ± 1.8Bcd	86.4 ± 1.7Aab	96.2 ± 1.6ABa
	Maize	44.1 ± 2.2Bcd	39.9 ± 1.5Bd	53.3 ± 1.5Cc	68.1 ± 2.5Bb	50.1 ± 2.7Cc	75.1 ± 1.6Bb	90.6 ± 1.8Ba
14	Wheat	87.4 ± 1.9Ac	76.4 ± 1.2Ad	90.2 ± 2.7Abc	100 ± 0.0Aa	96.2 ± 2.4Aab	100 ± 0.0Aa	100 ± 0.0Aa
	Rice	78.3 ± 7.2Bcd	69.2 ± 2.2Ad	85.3 ± 2.8ABbc	93.6 ± 2.0ABab	87.2 ± 1.9ABbc	100 ± 0.0Aa	100 ± 0.0Aa
	Maize	70.4 ± 1.6Be	58.6 ± 2.1Bf	76.0 ± 1.8Bde	87.1 ± 2.6Bbc	80.2 ± 2.3Bcd	95.2 ± 1.6Bab	100 ± 0.0Aa

insects to be the most susceptible. Our results show greater efficacy of SilicoSec on wheat than on rice and maize. Two factors that are believed to be the responsible for the efficacy of any DE formulation are strength of adhesion of DE particles to insects and the rate at which these particles are picked up by the insects (Aldryhim, 1993). Additionally, DE particles adhere to maize kernels less frequently than to other grains (Kavallieratos et al., 2005), and grain adhesion is reported to correlate with efficacy of DEs (Korunic et al., 1997). However, according to Kavallieratos et al. (2005, 2010b) DE's adherence is not always positively correlated with their efficacy. The poorer efficacy of DE on maize may also be due to the fact that maize kernels have high oil content in their pericarp that reduces the lipid absorption capacity of DE particles.

The DEs are slow acting insecticides that take time to achieve dehydration after attachment to an insect's epicuticle. This was confirmed in this study, as with DE alone mortalities were significantly less than those for the lowest dose rate of thiamethoxam at 1 d in most cases, but after that period the mean mortality from DE treatment increased to exceed that for the low rate of thiamethoxam. Unlike the case of DE, there is no data that describe the effect of grain commodities on the activity of thiamethoxam. Arthur et al. (2004) tested thiamethoxam at different dose rates on wheat and maize but did not report a correlation between its efficacy and the type of grain. The effect of grain type on efficacy has been studied for several insecticides such as spinosad (Huang and Subramanyam, 2007), abamectin (Kavallieratos et al., 2009), fipronil (Kavallieratos et al., 2010a), and chlorfenapyr (Kavallieratos et al., 2011). In most of the studies, the insecticides appeared to be more effective in maize than in wheat (Arthur, 2002; Huang and Subramanyam, 2007; Vayias et al., 2009). In the present study, thiamethoxam was more effective on wheat than maize. The physiochemical characteristics of the grains probably affect the coverage and residual activity of thiamethoxam in different ways.

Insect strains originating from different geographical locations vary in their susceptibility or tolerance to insecticides. Furthermore, in most studies, the field populations were found less susceptible than laboratory cultures to the applied chemicals (Subramanyam et al., 1989; Subramanyam and Hagstrum, 1995; Huang et al., 2004; Ali et al., 2007; Rahman et al., 2007). Ali et al. (2007) evaluated the efficacy of mixtures of organophosphate malathion and a synthetic pyrethroid deltamethrin against different field populations (C, K, W, S, L and M) of *R. dominica* being collected from different locations of Punjab, Pakistan. The strain C was found to be more resistant to malathion and M strain the least susceptible with LC_{50} values of 115.50 and 12.40 ppm, respectively. Similarly, when bioassays were conducted to determine the susceptibility of field populations of *Plodia interpunctella* (Hubner), *T. castaneum* (Herbst), *R. dominica* (F.), and *Cryptolestes ferrugineus* (Stephens) and corresponding insecticide susceptible laboratory strains, then field strains of most of the test insect species were relatively more resistant as compared with their respective laboratory strains (Huang et al., 2004). In our experiments, comparatively low mortality levels of different strains of *R. dominica* compared with those of Arthur et al. (2004) may be attributable to field populations being used in the bioassays as well as dose differences. The genetic diversity and associated vigor of the field

Table 6
Number (±SE) of *R. dominica* adult progeny in grain commodities treated with SilicoSec (100 mg/kg) or three dose rates of thiamethoxam (0.25, 0.5, 0.75 mg/kg) applied alone and in combination with DE. Means within row followed by the same lower-case letter and within column and population followed by the same upper-case letter are not significantly different; HSD test at $P < 0.05$.

Population	Commodity	Dose rate (mg/kg) (SilicoSec + thiamethoxam)						
		100 + 0	0 + 0.25	0 + 0.5	0 + 0.75	100 + 0.25	100 + 0.5	100 + 0.75
Bahawalpur	Wheat	25.5 ± 1.8Ca	18.8 ± 1.5Bb	10.4 ± 1.6Bc	6.7 ± 0.9Bcd	17.9 ± 1.4Bb	7.5 ± 0.6Acd	3.2 ± 0.7Ad
	Rice	41.1 ± 1.0Ba	26.4 ± 1.6Ab	13.9 ± 1.4Bc	8.0 ± 1.8ABcd	32.4 ± 1.3Ab	9.5 ± 0.9Acd	5.2 ± 1.5Ad
	Maize	48.5 ± 1.4Aa	32.0 ± 1.7Ab	21.2 ± 1.5Ac	13.5 ± 1.8Acd	36.8 ± 1.3Ab	11.7 ± 2.1Ad	9.1 ± 1.8Ad
Faisalabad	Wheat	18.0 ± 1.5Ca	11.5 ± 1.2Bb	7.4 ± 1.3Bbcd	4.8 ± 1.1Bcde	9.5 ± 1.3Bbc	3.9 ± 0.8Bde	0.0 ± 0.0Ce
	Rice	28.4 ± 1.2Ba	13.9 ± 1.6Bb	10.0 ± 1.3Bbc	6.8 ± 1.2Bcd	14.6 ± 2.9Bb	7.4 ± 0.9ABcd	3.4 ± 0.3Bd
	Maize	48.8 ± 1.7Aa	31.7 ± 1.1Ac	21.5 ± 1.7Ad	13.0 ± 0.8Ae	38.9 ± 1.4Ab	12.0 ± 1.6Ae	8.1 ± 0.8Ae
Toba Tek Singh	Wheat	3.5 ± 0.5Cb	7.5 ± 0.7Ca	2.6 ± 0.6Bb	0.0 ± 0.0Bc	0.0 ± 0.0Bc	0.0 ± 0.0Bc	0.0 ± 0.0c
	Rice	6.5 ± 0.5Bb	9.4 ± 0.6Ba	5.6 ± 0.6ABb	0.0 ± 0.0Bc	0.0 ± 0.0Bc	0.0 ± 0.0Bc	0.0 ± 0.0c
	Maize	10.0 ± 0.8Ab	16.4 ± 1.6Aa	7.7 ± 1.0Abc	5.2 ± 0.5Abc	9.5 ± 1.2Ab	3.1 ± 0.7Acd	0.0 ± 0.0d
Jhang	Wheat	5.5 ± 0.8Bb	9.0 ± 1.4Ba	3.5 ± 0.3Bb	0.0 ± 0.0Bc	2.4 ± 0.6Cbc	0.0 ± 0.0Bc	0.0 ± 0.0Bc
	Rice	8.1 ± 1.7Bb	13.4 ± 1.1Ba	7.0 ± 0.9Abc	2.5 ± 0.6Bde	6.5 ± 1.0Bbcd	2.8 ± 0.6Bcde	0.0 ± 0.0Be
	Maize	14.4 ± 1.6Ab	21.6 ± 1.2Aa	9.1 ± 0.7Acd	5.5 ± 0.8Ade	11.9 ± 1.1Abc	8.5 ± 1.1Acd	2.0 ± 0.3Ae

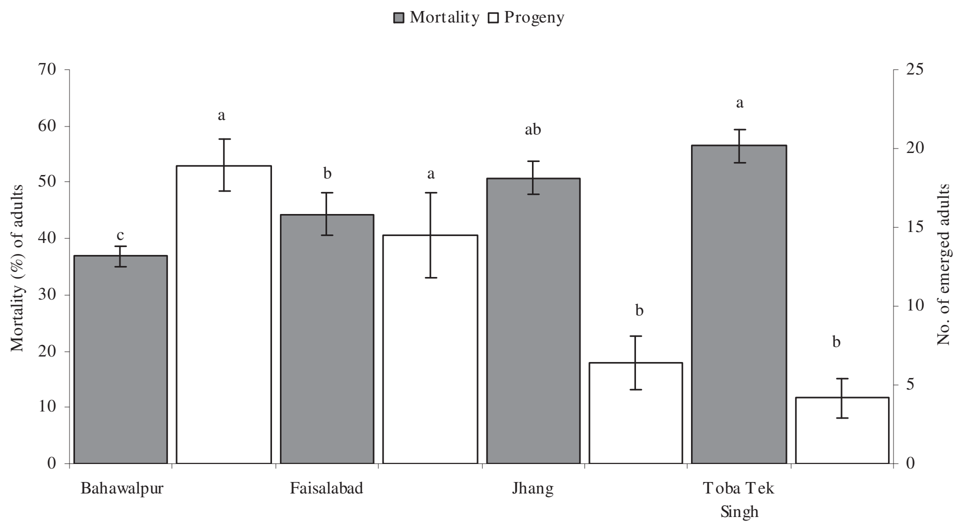

Fig. 1. Comparison of four populations of *R. dominica* by mortality (±SE) of adult beetles and progeny production in wheat, rice and maize treated with DE (100 mg/kg) or three dose rates of thiamethoxam (0.25, 0.5, 0.75 mg/kg) applied alone and in combination with DE. Means within bars for mortality ($F_{3,\ 1007} = 22.2$; $P = 0.001$) and within progeny emergence having same letter are not significantly different ($F_{3,\ 250} = 30.4$; $P = 0.001$).

strains could explain their greater tolerance of the insecticides. The effect of geographical location on the susceptibility levels of different stored grain insect pests to test chemical insecticides has also been documented by Athanassiou et al. (2008a) who found significant differences in susceptibility of the adults and larvae of six European populations of *Tribolium confusum* Jacquelin du Val to spinosad dust. The residual effect of malathion and deltamethrin against different populations of *Sitophilus granarius* (L.) was investigated by Kljajic and Peric (2009) who found significant variation among the population responses to malathion and deltamethrin. Similarly, Kavallieratos et al. (2007b) and Vayias et al. (2006) found differences in the vulnerabilities of different European populations of *T. confusum* to several DE formulations. Our study is the first in which differential efficacy of thiamethoxam among *R. dominica* populations originating from diverse geographical locations is reported. These findings revealed significant difference among the populations even within a limited geographical area, Punjab (Pakistan). The population from Bahawalpur (district) was more tolerant to DE and thiamethoxam than the other three populations.

In Pakistan, resistance to traditionally used control measures (e.g. phosphine) has been observed in various insect pests of stored products (Alam et al., 1999). Differences in the natural tolerance to thiamethoxam suggest that resistance development would develop at very different rates in different localities. In case of DE, the differential mobility of various strains may correspond to differential particle accumulation on their bodies (Rigaux et al., 2001) and therefore differential DE efficacy.

Reduction of progeny production is major function of any control measure as it is crucial for long-term stored-products protection. In our assays, thiamethoxam alone at a dose rate of 0.75 mg/kg and in combination with DE, eliminated or greatly reduced progeny production of *R. dominica* on wheat, rice and maize, presumably because of high adult mortality.

In summary, this is the first study in which thiamethoxam has been evaluated as grain protectant or seed treatment in combination with DE. Variable susceptibility among local populations of *R. dominica* to thiamethoxam and DE was detected and may affect resistance development. Thiamethoxam is not yet registered in Pakistan, and this study demonstrates that it can have a role in Pakistani stored-product IPM. The baseline data presented here for the various populations of *R. dominica* can be used to monitor changes in susceptibility of this species in Pakistan to DE, and thiamethoxam, when it is registered. The reduced use rates can help registrants in efforts to expand the label to include its use as a grain protectant. Thiamethoxam is currently labeled in the United States for seed treatment of grains such as corn and barley at an application rate ca. 4 mg/kg, which is well above the rates used in this study. However, maximum residue limits may need to be established or revised if thiamethoxam were to be developed as a grain protectant for grain destined for animal or human consumption.

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