Variation in the susceptibility of stored-product insects from five locations to two diatomaceous earth and botanical based formulations

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Abstract

The objective of this study was to determine the susceptibility of the local populations of *Tribolium castaneum*, *Rhyzopertha dominica* and *Sitophilus oryzae* at five different locations (Canada, Iran, Pakistan and Serbia (2 locations) using two new DE based formulations made from various combinations of amorphous silicon dioxide (diatomaceous earth and silica gel), bay leaf powder, milled sesame seeds, baits and pyrethrin. The LD_{50}'s ranged from 78 to over 1000 ppm. In general, bay leaf formulation caused less mortality than pyrethrin formulation. There was greater mortality in tests conducted in Canada and Iran than in Pakistan and Serbia. For example, the LD_{50} was about 3 to 5 times higher for tests conducted in Pakistan and Belgrade than Canada and Iran for *S. oryzae* after 14 d exposure to the bay leaf formulation. Offspring production was reduced at the higher concentrations. The possible reasons for these differences; wheat varieties, insect strain or insecticide degradation are discussed. The implications of this work reveal that it may be difficult to predict the efficacy of an insecticide for use around the world. We recommend testing in several laboratories before making label recommendations.

Keywords: insecticides, susceptibility, insects, local populations, wheat varieties

1. Introduction

Stored-product insects cause significant damage to stored cereals around the world (Rees, 2004). There are a number of ways to prevent loss in quality or quantity of cereals in storage. Low temperature can reduce or eliminate populations and this is a common practice using ambient air to cool grain in temperate regions (Fields, 1992). Fumigation with phosphine, carbon dioxide or methyl bromide is one of the most common control methods (White and Leesch, 1995; Small, 2007; Phillips et al., 2012). Contact insecticides such as methoprene, malathion, deltamethrin, chlorpyrifos-methyl, fenitrothion, diatomaceous earth, spinosad provide long term protection (Snelson, 1987; Subramanyam and Roesli, 2000; Kljajić and Perić, 2009; Vayias et al., 2009). Combinations of synthetic insecticides increase efficacy and broaden spectrum of species controlled (Daglish, 1998); deltamethrin combined with chlorpyrifos-methyl (Arthur, 1994); combinations of bioresmethrin combined with chlorpyrifos-methyl or pirimiphos methyl (Bengston et al., 1980). These combinations can work better for a variety of reasons, for example the combination of organophosphate and pyrethroids allows for the control of both *Sitophilus* sp which are susceptible to organophosphates and *Rhyzopertha dominica* (F.) which is susceptible to pyrethroids. The combination of insecticides from these two groups has the potential advantage that resistance would be more difficult to develop because of the presence of two insecticides (Korunic and Fields, 1995; Daglish, 1998). Combinations of DE with synthetic or natural insecticides have been shown to be effective; silica aerogel (Korunic and Fields, 1995); methoprene (Arthur, 2004); deltamethrin (Korunic and Rozman 2010); plant extract bitterbarkomycin (Korunic 2001, Athanassiou and Korunic 2007); spinosad (Vayias et al., 2009), thiamethoxam (Wakil et al., 2013); *Metarhizum anisopliae* (Metschnikoff) (Kavallieratos et al., 2006); *Beauveria bassiana* (Vassilakos et al., 2006; Wakil et al., 2012); *Paecilomyces fumosorroseus* (Wise) Brown and Smith (Michalaki et al., 2007). The combinations of these insecticides often enhance the effectiveness of DE, thus reducing DE dosages, which mitigate DE's adverse effects on grain bulk density (test weight) and grain flow rate.

Most experiments with stored-product insecticides are carried out with a single strain in a single lab. However, there a few studies that raised concerns over the ability of this type of testing to predict efficacy in the field for the stored-product insects found in different regions of the world (Rigaux et al., 2001; Fields et al., 2002; Arnaud et al., 2005; Korunic, 2007; Vayias et al., 2009).

The objective of this study was to determine the susceptibility of the local populations of *Tribolium castaneum* (Herbst), *Rhyzopertha dominica* (F.), and *Sitophilus oryzae* (L.) at five different laboratories. The study was conducted in Canada, Iran, Pakistan and Serbia (2 locations) using two new formulations made from various combinations of amorphous silicon dioxide (diatomaceous earth and silica gel), Bay leaves powder, milled sesame seeds, baits and pyrethrin.

2. Materials and Methods

2.1. Test insects

Local strains *of S. oryzae,* the rice weevil, *R. dominica*, the lesser grain borer and *T. castaneum*, the red flour beetle were used in the experiments. *Sitophilus oryzae* and *R. dominica* were reared on whole wheat between 12 and 14% m.c. (moisture content) at 25±1°C, 60±10% r.h. (relative humidity). *T. castaneum* was reared on 95% wheat flour with 5% brewers' yeast mixture at the same temperature and humidity. Fifty unsexed, 7 to 21 day-old adults per replicate were used in the experiments.

2.2. Wheat

Each laboratory used locally available wheat variety (*Triticum aestivum* L.). Before the experiment started, wheat was sifted and there were no dockage or broken kernels. The same clean wheat grain was used for insect's rearing and the experiments. Grain moisture content was determined by using RF dielectric grain moisture meters. In the tests the initial grain moisture contents ranged from 12 to 13%. The grain was un-infested and previously without any contact with insecticides. *Sitophilus oryzae* and *R. dominica* were tested on whole grain. *Tribolium castaneum* was tested on whole kernel wheat with 1% (by weight) cracked wheat. The cracked kernel wheat was sieved over a sieve with 1 mm openings to remove any flour. The variety of wheat were also obtained locally: Canada used hard red spring wheat, Iran used hard wheat, variety Charman, Pakistan used soft wheat, variety Inqilab-91, Belgrade used soft wheat, cultivar Pobeda, and Novi Sad used soft wheat, variety NS-40 S.

2.3. Insecticides

Two insecticide powder formulations were developed by Diatom Research and Consulting in Canada. One formulation (bay leaf formulation) contained diatomaceous earth (DE, EP Minerals DE Celatom Mn 51), silica gel (Sipernat 50S is Degussa Canada, Ltd. Burlington, ON, Canada), dried powder of bay leaves (*Laurus nobilis* L.), milled sesame seeds, and baits. All substances are food grade. The other formulation (pyrethrin formulation) contained DE, silica gel, sesame seed extract, bait (all substances are food grade) and pyrethrin (MGK, USA product containing 20% active ingredient). There was no piperonyl butoxide in the pyrethrin formulation.

The laboratories in Canada, Iran, Pakistan and Serbia (Novi Sad), treated grain with the following concentrations of the two formulations: bay leaf formulation; *S. oryzae* was tested with 0, 50, 100, 150, 200 and 250 ppm and *R. dominica* and *T. castaneum* with 0, 100, 200, 300, 400 and 500 ppm; Pyrethrin formulation; *R. dominica* was tested with 0, 50, 100, 150, 200 and 250 ppm and *S. oryzae* and *T. castaneum* with 0, 100, 200, 300, 400 and 500 ppm. The laboratory in Belgrade applied these concentrations: bay leaf formulation; *S. oryzae* was tested with 0, 50, 100, 200, 300, 400, 500, 600, 700, 800, 900 and 1000 ppm and *R. dominica* and *T. castaneum* with 0, 100, 200, 300, 400, 500, 600, 700, 800, 900 and 1000 ppm; pyrethrin formulation; *S. oryzae* with 0, 100, 200, 300, 400, 500, 600 and 700 ppm and *R. dominica* and *T. castaneum* with 0, 50, 100, 150, 200, 250, 300, 400 and 500 ppm.

2.4. Mixing

Laboratories in Canada and Belgrade for each insect species and each determined rate used 2, 100 g replications. For each replicate a determined rate was separately applied. The laboratory in Iran added the appropriate weights of formulations to 300 g of grain, the laboratory in Pakistan added determined concentrations into 200 g of grain and the lab in Novi Sad added determined concentration into 1000 g of wheat. The grain and powder were shaken in jars by hand for several minutes. After mixing, the treated grain was divided into three 100 g sub-samples (Iran), two 100 g sub-samples (Pakistan), and four 250 g sub-samples (Novi Sad) (one for each replicate). After 7 d the entire contents of the jar were placed on a tray, the number of live and dead adults was noted, and after removing dead adults the entire contents were returned to the vials. After 14 d from the beginning of the experiment, the same procedure was repeated, but at this time all dead and live adults were removed and the entire contents, grain and dust were again put into vials. Grain was held for 7 weeks and the emerged adults counted to estimate offspring production. The bioassays were conducted at 25 ± 1°C and 60 ± 10% r.h.

2.5. Hard vs soft wheat

To determine the effect of soft or hard wheat, grain of these two types were treated with 400 ppm DE (DE used in the formulations without the additives), 100 g per replicate, and three replicates with either fifty adults of *S. oryzae* or *T. castaneum.* Insects were held for 2 to 5 d at 28°C and 65% r.h. and the number of live and dead noted. The hard wheat had 13.2% m.c., whereas the soft wheat had 12.7% m.c.

2.6. Statistical analysis

Probit analysis was conducted on the mortality (probit transformed) with dose (log transformed) using PoloPlus (Version 2.0, LeOra Software). Linear correlations were run between untransformed data of the offspring production as percentage of untreated controls. Differences in mortality between hard and soft wheat were tested using a t-test on mortality data transformed using the arcsine-square root transformation.

3. Results

The different locations had different efficacy. In general, there was greater mortality in tests conducted in Canada and Iran than the other locations. For example, the LD_{50} is about three to five times higher for tests conducted in Pakistan and Belgrade, than Canada and Iran for *S. oryzae* after 14 d exposure to the bay leaf formulation (Table 1). Tests conducted at Novi Sad had only 2% mortality at 250 ppm, the highest dose they tested.

In general, bay leaf formulation (Table 1) caused less mortality of the three species tested than pyrethrin formulation (Table 2). For example, in Pakistan the LD_{50} of bay leaf formulation was 326 Offspring production was reduced at the higher concentrations of the formulations (Table 3, Fig. 1). The reduction in offspring production with increased dose often had a good fit with a straight line, with r^2 values as high as 96% (Table 3). In a few instances, there was a low correlation (*R. dominica*, pyrethrin formulation, Canada and Belgrade, Table 3). This is probably due to the almost no offspring production at the lowest dose of 50 ppm. Different locations had different slopes. For example, data from Belgrade (Serbia) had a lower slope compared with other countries for the bay leaf formulation with both *S. oryzae* and *R. dominica*.

In Canada and Iran, hard wheat was used for tests whereas; in the other locations the soft wheat varieties were used. Hard and soft wheat varieties from Canada were tested at the same time, and hard wheat had higher mortalities than soft wheat when treated with 400 ppm of DE for both *T. castaneum* and *S. oryzae* (Table 4).

4. Discussion

In this study, there were significant differences between the locations, sometimes as high as a ten-fold difference in LD_{95} for the same species and the same formulation. There are several possible reasons for these differences; wheat varieties, insect strain insecticide degradation, differences among the laboratories in the method of mixing grain with insecticides or other factors. Fields et al. (2002) developed a standard protocol that could be use as a base line for studying DE tested four different formulations in three laboratories. In general, there was a good agreement between laboratories with the exception of higher efficacy of the laboratory using hard red spring, whereas the other laboratories used soft wheat varieties. In this study the laboratories from Pakistan, Belgrade and Novi Sad used soft wheat, whereas Canada and Iran used hard wheat. The countries using the soft wheat had lower efficacy. Korunic (2007) showed that there was lower mortality to DE against *R. dominica*, *S. oryzae* and *T. castaneum* on soft wheat than on hard wheat. Finally, in a side by side test in this study there was 13 to 20% greater in mortality in hard wheat versus soft wheat.

However, it is doubtful that the large differences between locations in our results could be attributed solely to kernel hardness, given the differences seen in a side-by-side test in this study. There may have been degradation of the formulations in transit from Canada to the other locations. The DE component of the formulation will not degrade, but the natural products may have, either in transit or in storage before the tests. Further work on degradation should be conducted to determine the stability of these formulations. The differences we saw between locations could be due to the different insect strains or rearing methods used in the different locations. Other studies have shown differences in susceptibility to insecticides between different geographical strains. There was as much as a six-fold difference in susceptibility for *S. oryzae* and *T. castaneum* to lindane and malathion (Parkin 1965). Rigaux et al. (2001) showed a two-fold difference in susceptibility to DE between strains of *T. castaneum*. Arnaud et al. (2005) showed differences in susceptibility to DE of populations of *T. castaneum* originated from Canada, Ivory Coast, Japan, Pakistan, Thailand and the USA. Vayias et al. (2009) using DE and spinosad showed differences in *T. castaneum* originating from Denmark, Greece and Portugal. With the synthetic insecticides, there could be localized selection for resistance genes (Opit et al., 2012). For the DE, this was probably not caused by exposure and selection to DE, as there is not wide spread use of DE.

The implications of this work and other similar studies (Rigaux et al., 2001; Fields et al., 2002; Athanassiou et al., 2003; Arnaud et al., 2005; Korunic, 2007; Vayias et al., 2009) reveal that it may be difficult to predict the efficacy of an insecticide for use around the world. Commercial insecticides often have application rates above the LD_{95}, to allow for differences in application rate, commodities and degradation. We recommend that testing in several laboratories be done when making label recommendations.

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Table 1 The LD_{50} and LD_{95} of the population of various insects exposed to bay leaf formulation after 7 or 14 days.

Duration of Exposure (d)	Insect	Lab	Lethal Dose (ppm)					
			LD_{50}	95% CL	LD_{95}	95% CL	h[a]	Slope ± SE
7	*S. oryzae*	Canada	183	154-229	782	499-1894	1.83	2.6 ± 0.3
		Iran	245	221-280	918	685-1409	0.24	2.9 ± 0.3
		Pakistan	703	444-2255	4315	1576-59851	0.47	2.1 ± 0.5
		Belgrade	1072	898-1540	3005	1918-8843	4.24	3.7 ± 0.4
		Novi Sad[b]	-		-			
	R. dominica	Canada	200	150-246	1347	829-3753	1.68	2.0 ± 0.2
		Iran	236	212-260	578	493-723	2.21	4.2 ± 0.3
		Pakistan	343	287-432	4460	2216-17132	0.85	1.5 ± 0.2
		Belgrade	452	398-510	1505	1202-2097	2.56	3.2 ± 0.2
		Novi Sad	812	598-1928	2756	1374-23176	3.92	3.1 ± 0.4
	T. castaneum	Canada	214	159-268	1627	926-5875	1.82	1.9 ± 0.2
		Iran	233	219-247	559	507-631	0.74	4.3 ± 0.3
		Pakistan	495	396-725	7169	3053-41130	0.4	1.4 ± 0.3
		Belgrade	797	676-1042	1806	1269-5009	9.87	4.6 ± 0.4
		Novi Sad	593	555-674	822	711-1112	0.59	11.6 ± 2.1
14	*S. oryzae*	Canada	76	59-91	206	165-303	2.64	3.8 ± 0.3
		Iran	111	101-122	442	362-580	1.03	2.8 ± 0.2
		Pakistan	326	267-473	1548	872-4953	0.56	2.4 ± 0.4
		Belgrade	573	427-740	1200	876-3321	22.31	5.1 ± 0.3
		Novi Sad[c]	-		-			
	R. dominica	Canada	78	27-118	487	339-1185	2.08	2.1 ± 0.3
		Iran	122	109-133	301	268-348	1.08	4.2 ± 0.3
		Pakistan	157	121-188	1581	1021-3412	0.95	1.6 ± 0.2
		Belgrade	304	266-340	834	712-1034	2.24	3.8 ± 0.2
		Novi Sad	314	283-351	1040	815-1501	2.35	3.2 ± 0.2
	T. castaneum	Canada	140	87-181	440	323-878	4.20	3.3 ± 0.3
		Iran	133	122-144	336	304-380	0.61	4.1 ± 0.3
		Pakistan	260	217-310	3069	1697-9196	0.87	1.5 ± 0.2
		Belgrade	621	546-700	1109	929-1568	8.49	6.5 ± 0.4
		Novi Sad	429	368-545	1139	791-2553	6.28	3.9 ± 0.3

a. h = Heterogeneity, b. 0% mortality at 250 ppm., c. 2% mortality at 250 ppm

Table 2 The LD_{50} and LD_{95} of the population of various insects exposed to pyrethrin formulation after 7 or 14 days.

Duration of Exposure (d)	Insect	Lab	Lethal Dose (ppm)					
			LD_{50}	95% CL	LD_{95}	95% CL	h[a]	slope
7	*S. oryzae*	Canada	199	170-226	445	374-582	2.30	4.7 ±0.4
		Iran	231	202-260	592	488-794	3.08	4.0 ± 0.3
		Pakistan	227	188-267	2343	1404-5837	0.54	1.6 ± 0.2
		Belgrade	257	229-283	482	424-580		6.0 ± 0.4
		Novi Sad[b]	-		-			
	R. dominica	Canada	162	126-232	5548	1688-ud	0.88	1.1 ± 0.3
		Iran	178	160-201	716	536-1103	1.26	2.7 ± 0.2
		Pakistan	209	163-323	2770	1098-27,399	1.23	1.5 ± 0.3
		Belgrade	184	103-300	10,470	2259- -		0.9 ± 0.2
		Novi Sad[c]	-		-			
	T. castaneum	Canada	218	178-245	443	385-574	1.21	5.4 ± 0.8
		Iran	255	229-282	536	462-669	3.03	5.1 ± 0.3
		Pakistan	284	242-336	2803	1628-7377	0.35	1.7 ± 0.3
		Belgrade	223	196-254	677	532-994		3.4 ± 0.2
		Novi Sad	550[d]		-			
14	*S. oryzae*	Canada	100	90-108	174	154-211	0.30	6.8 ± 1.0
		Iran	193	160-221	468	389-633	3.34	4.3 ± 0.3
		Pakistan	100	66-125	284	225-432	2.54	3.6 ± 0.4
		Belgrade	225	200-249	409	358-503	2.07	6.4 ± 0.5
		Novi Sad	223	205-253	434	348-676	3.47	5.7 ± 0.5
	R. dominica	Canada[d]	25[d]		50[4]			
		Iran	124	107-143	577	418-981	1.93	2.5 ± 0.2
		Pakistan	69	51-83	339	253-565	1.37	2.4 ± 0.3
		Belgrade[d]	25		50		0.39	
		Novi Sad	458	406-579	981	707-2633	5.06	5.0 ± 0.5
	T. castaneum	Canada	113	93-129	279	240-347	1.01	4.2 ± 0.5
		Iran	190	169-208	419	371-495	1.71	4.8 ± 0.5
		Pakistan	116	84-142	498	391-745	1.41	2.6 ± 0.3
		Belgrade	154	136-170	257	225-319	3.95	7.4 ± 0.5
		Novi Sad[e]	-		-			

a. h = Heterogeneity.
b. 8% mortality at 250 ppm.
c. 3% mortality at 500 ppm.
d. Estimated graphically.
e. 3% mortality at 200 ppm

Table 3 Correlations between offspring production (% of control) and DE dose for *S. oryzae* Figure 1[a].

Species	Formulation	Lab	R^2	Slope +SEM	Intercept +SEM	N (doses)	P	Number offspring in controls
S. oryzae	Bay leaf	Canada	0.75	-0.15+0.03	93+8	12	<0.001	620
		Pakistan	0.96	-0.19 + 0.01	99 + 2	12	<0.001	92
		Belgrade	0.87	-0.08+0.01	92+4	24	<0.001	1061
		Novi Sad	0.59	-0.14+0.03	92+4	24	<0.001	1946
	Pyrethrin	Canada	0.77	-0.22+0.04	87+11	12	<0.001	620
		Pakistan	0.80	-0.18 + 0.03	75 + 9	12	<0.001	98
		Belgrade	0.71	-0.14+0.02	76+10	16	<0.001	1061
		Novi Sad	0.61	-0.17+0.03	107+5	24	<0.001	1773
R. dominica	Bay leaf	Canada	0.52	-0.13+0.04	85+11	12	0.008	142
		Pakistan	0.89	-0.18 + 0.02	83 + 6	12	<0.001	68
		Belgrade	0.75	-0.07+0.01	67+5	20	<0.001	196
		Novi Sad	0.60	-0.14+0.03	68+8	24	<0.001	863
	Pyrethrin	Canada	0.39	-0.29+0.12	53+18	11	0.04	142
		Pakistan	0.85	-0.38 + 0.05	80 + 8	12	<0.001	74
		Belgrade	0.25	-0.10 + 0.04	33 + 12	18	0.04	196
		Novi Sad	0.76	-0.15 + 0.02	74 + 6	24	<0.001	1001

a. Control offspring to calculate% reduction in offspring. For *Tribolium castaneum* in all locations there were few offspring in controls, and for Iran there were few offspring in controls of all species.

Table 4 Effects of hard or soft wheat on efficacy of 400 ppm DE, mortality after 2 or 5 days of *Tribolium castaneum* and *Sitophilus oryzae*.

Species	Duration (d)	Mortality (x + SEM)		t-value	P[a]
		Hard Wheat	Soft Wheat		
Sitophilus oryzae	2	45 + 9	27 + 2	2.12	0.05
	5	100 + 0	85 + 6	4.02	0.008
Tribolium castaneum	2	20 + 3	7 + 4	2.14	0.05
	5	55 + 4	35 + 4	3.39	0.01

a. One-tailed p values.

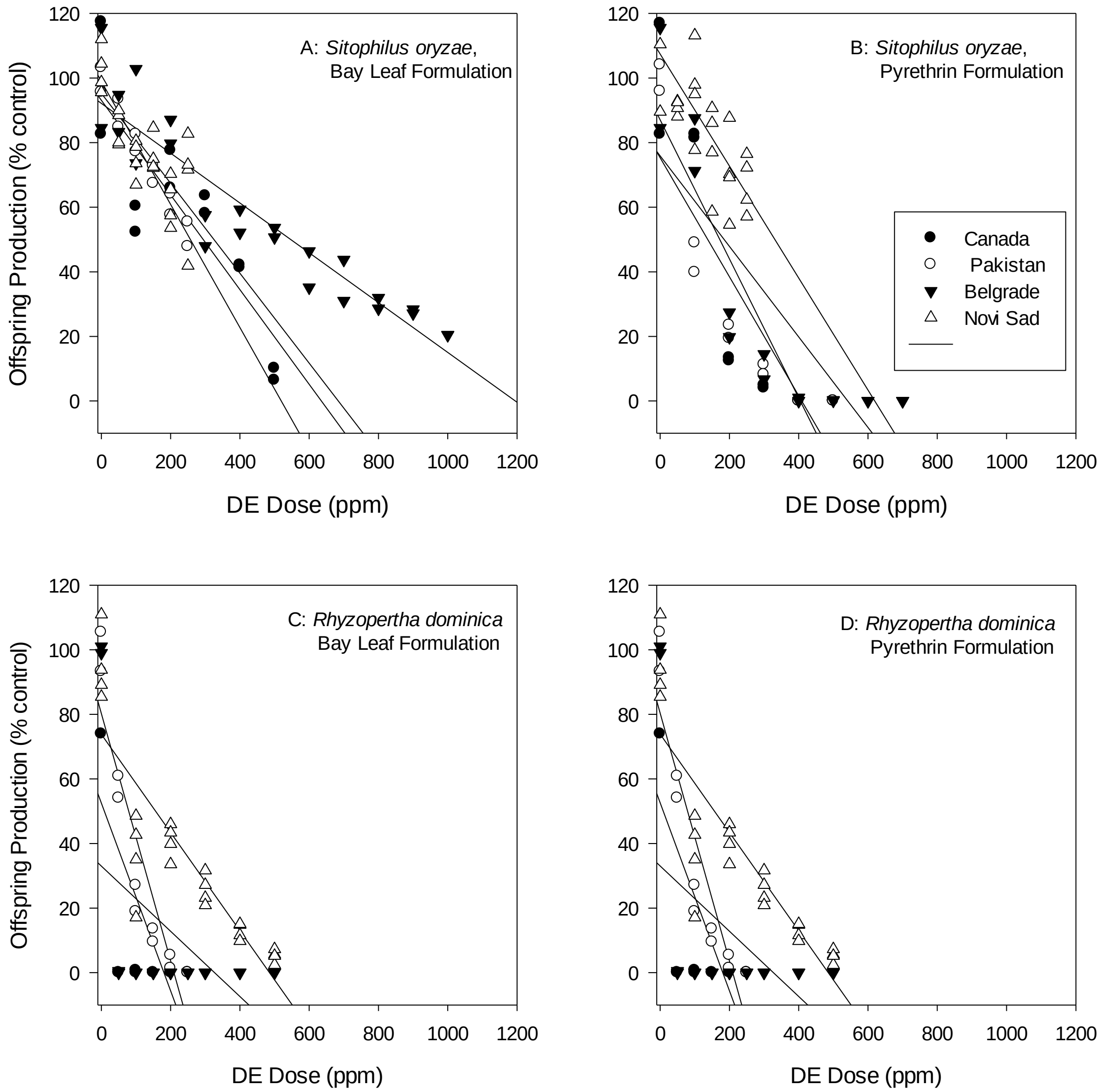

Figure 1 The reduction of offspring (% control) with increasing dose of the DE formulations: A. *Sitophilus oryzae* with bay leaf formulation; B: *Sitophilus oryzae* with pyrethrin formulation; C: *Rhyzopertha dominica* with bay leaf formulation; D: *Rhyzopertha dominica* with pyrethrin formulation. Laboratory experiments were conducted at different locations, details on the correlations are found in Table 3.