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Toxicity of natural insecticides on the larvae of wheat head armyworm, *Dargida diffusa* (Lepidoptera: Noctuidae)

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ABSTRACT

The wheat head armyworm, *Dargida* (previously *Faronta*) *diffusa* (Walker) (Lepidoptera: Noctuidae), is widely distributed in North American grasslands and is most common on the Great Plains, where it is often a serious pest of corn and cereal crops. Six commercially available botanical or microbial insecticides used against *D. diffusa* were tested in the laboratory: Entrust® WP (spinosad 80%), Mycotrol® ESO (*Beauveria bassiana* GHA), Aza-Direct® (azadirachtin), Met52® EC (*Metarhizium brunneum* F52), Xpectro® OD (*Beauveria bassiana* GHA + pyrethrins), and Xpulse® OD (*Beauveria bassiana* GHA + azadirachtin). Concentrations of 0.1, 0.5, 1.0 and 2.0 fold the lowest labelled rates of formulated products were tested for all products, while for Entrust WP additional concentrations of 0.001 and 0.01 fold the label rates were also assessed. Survival rates were determined from larval mortality at 1–9 days post treatment application. We found that among the tested chemicals, Entrust® (spinosad) was the most effective, causing 83–100% mortality (0–17% survival rate) at day 3 across all concentrations. The others, in order of efficacy from most to least, were Xpectro® (*B. bassiana* GHA + pyrethrins), Xpulse® OD (*B. bassiana* GHA + azadirachtin), Aza-Direct® (azadirachtin), Met52® EC (*M. brunneum* F52), and Mycotrol® ESO (*B. bassiana* GHA). These products and entomopathogenic fungi caused 70–100% mortality (0–30% survivability) from days 7 to 9. The tested products and entomopathogenic fungi can be used in management of *D. diffusa*.

1. Introduction

The wheat head armyworm, *Dargida* (previously *Faronta*) *diffusa* (Walker) (Lepidoptera: Noctuidae), while usually a minor pest, can sporadically cause important crop damage (Peairs et al., 2010). This species is similar in appearance to its congener *Dargida terrapictalis* (Buckett) (Lepidoptera: Noctuidae), with which it is often confused (Rodriguez and Angulo, 2005). Both species were moved from *Faronta* to *Dargida* by Rodriguez and Angulo (2005). *Dargida diffusa* feeds on a range of grasses and cereal crops and appears to prefer seed heads (Watts and Bellotti, 1967), making it a pest of cereal grains throughout the Midwest and Great Plains of North America (Covell, 1984). Although its host range and pest status are not well studied (Michaud et al., 2007), crop damage seems to occur both in the field and during grain storage. There are currently no integrated pest management thresholds or recommended treatments for this pest due to its sporadic late season appearance (Peairs et al., 2010).

Dargida diffusa larvae pupate and overwinter in the soil, and adults mate within a few days of emerging. Females then lay eggs on developing wheat or barley (Powell and Opler, 2009). Larvae occur on wheat heads by June. Larvae and adults are typically nocturnal (Michaud et al., 2007; Royer, 2007). In more northern regions, *D. diffusa* has two generations per year and adults fly in late August. A 35% yield loss in spring wheat due to *D. diffusa* has been reported in Washington State (Roberts, 2009). Meanwhile, Rondon et al. (2011) found both *D. diffusa* and *D. terrapictalis* to cause crop damage in Idaho and Oregon.

Concern over this pest increased with the occurrence of increased percentages of insect-damaged kernels (IDK) in 2014 in wheat harvest in the Golden Triangle area of Montana. Underhill et al. (1977) reported that *D. diffusa* responds to lures baited with a combination of the sex attractant compounds Z11-16Ac and Z11-16Ald. Such pheromone lures are being used to detect and monitor adults in wheat fields (Landolt et al., 2011). However, the use of these lures is limited to monitoring, and control is based on use of insecticides, even though such applications may not be advisable near harvest.

Sustainable insect pest management (SIPM) products are intended to be safe alternatives to conventional insecticides, and some are both effective and harmless to the environment (Peshin

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Table 1
Insecticide treatments and concentrations used.

Treatments	Insecticide concentration[a]						
	0*X*	0.001*X*	0.01*X*	0.1*X*	0.5*X*	1*X*	2*X*
Mycotrol ESO[b]	0			0.072	0.36	0.72	1.44
Met52 EC[c]	0			0.072	0.36	0.72	1.44
Aza-Direct[d]	0			0.144	0.72	1.44	2.88
Xpulse OD[e]	0			0.072	0.36	0.72	1.44
Xpectro OD[f]	0			0.25	1.25	2.5	2.5
Entrust WP[g]	0	0.000091	0.00091	0.0091	0.0455	0.091	0.182

[a] Insecticide concentration: 0*X*, control (water); 0.001*X*, 0.01*X*, 0.1*X*, 0.5*X*, 1*X*, and 2*X* of the lowest label application rate.
[b] Mycotrol ESO: 0.1*X* = 0.072 ml/L (0.007848 g a.i./L); 0.5*X* = 0.36 ml/L (0.03924 g a. i./L); 1*X* = 0.72 ml/L (0.07848 g a.i./L); 2*X* = 1.44 ml/L (0.15696 g a.i./L).
[c] Met52 EC: 0.1*X* = 0.072 ml/L (0.00792 g a.i./L); 0.5*X* = 0.36 ml/L (0.0396 g a. i./L); 1*X* = 0.72 ml/L (0.0792 g a.i./L); 2*X* = 1.44 ml/L (0.1584 g a.i./L).
[d] Aza-Direct: 0.1*X* = 0.072 ml/L (0.01728 g a.i./L); 0.5*X* = 0.72 ml/L (0.0864 g a.i./L); 1*X* = 1.44 ml/L (0.1728 g a.i./L); 2*X* = 2.88 ml/L (0.03456 g a.i./L).
[e] Xpulse OD: 0.1*X* = 0.072 ml/L (0.0072432 g a.i./L); 0.5*X* = 0.36 ml/L (0.036216 g a.i./L); 1*X* = 0.72 ml/L (0.072432 g a.i./L); 2*X* = 1.44 ml/L (0.144864 g a.i./L).
[f] Xpectro OD: 0.1*X* = 0.25 ml/L (0.002025 g a.i./L); 0.5*X* = 1.25 ml/L (0.010125 g a.i./L); 1*X* = 2.5 ml/L (0.02025 g a.i./L); 2*X* = 5 ml/L (0.0405 g a.i./L).
[g] Entrust WP: 0.001*X* = 0.000091 ml/L (0.0000728 g a.i./L); 0.01*X* = 0.00091 ml/L (0.000728 g a.i./L); 0.1*X* = 0.0091 ml/L (0.00728 g a.i./L); 0.5*X* = 0.0455 ml/L (0.0364 g a.i./L); 1*X* = 0.091 ml/L (0.0728 g a.i./L); 2*X* = 0.182 ml/L (0.1456 g a.i./L).

and Dhawan, 2009; Murray et al., 2013; Bailey et al., 2013). To date, no attempt has been made to find materials with these attributes for use against *D. diffusa*. Here we present results from a laboratory bioassay to evaluate the efficacy of several commercially available biorational products against larvae of *D. diffusa*.

2. Materials and methods

2.1. Insects

Larvae of *D. diffusa* were collected from wheat fields near Valier, MT, USA, using sweep nets, in June and July, 2015. Larvae were taken to the laboratory and placed in collapsible cages (12 cm × 10 cm × 10 cm), where they were fed wheat seed heads, and held at 21 ± 2 °C, 70–80% relative humidity, and an approximately 14:10 h L:D photoperiod. Field-collected larvae were separated by instar and ranged from first to four instars. For all experiments, second instars were used for laboratory bioassays.

2.2. Insecticides

Insecticides used were commercial formulations of (1) Entrust WP (spinosad 80%, Dow AgroSciences Indianapolis, IN), (2) Mycotrol ESO (*Beauveria bassiana* GHA, Lam International, Butte, MT), (3) Aza-Direct (azadirachtin, Gowan Company, Yuma, AZ), (4) Met52 EC (*Metarhizium brunneum* F52, Novozymes Biologicals, Salem, VA), (5) Xpectro OD (*Beauveria bassiana* GHA + pyrethrins, Lam International, Butte, MT), and (6) Xpulse OD (*Beauveria bassiana* GHA + azadirachtin, Lam International, Butte, MT). Cultures of *M. brunneum* F52 (a commercialized isolate previously identified as *M. anisopliae*) conidial powders were stored dry at 4–5 °C until formulated for use. The concentrations tested were 0.1, 0.5, 1.0 and 2.0 fold lowest label rate, while for Entrust additional concentrations of 0.001 and 0.01 fold the label rate were also prepared (Table 1).

2.3. Laboratory tests

Laboratory tests were carried out from July and August of 2015 via contact application of various concentrations of the test materials (see Table 1 for exact concentrations tested). For each replicate, five second instar larvae were transferred onto a disk of Whatman No. 1 filter paper (9 cm diam, Whatman® quantitative filter paper, ashless, Sigma–Aldrich, St. Louis, MO, USA) in a 9 cm disposable Petri dish. Each Petri dish received three wheat stems about 5 cm long, each with 8–10 leaves as food for the larvae. Six replicate Petri dishes, containing a total of 30 larvae (5 per dish), were treated (using a 473 ml capacity Plant & Garden Sprayer, Sprayco, Livonia, MI) with 1 ml of the relevant test material (Reddy et al., 2014). Controls were sprayed with 1.0 ml tap water. Following application, dishes were held under the same laboratory conditions used for rearing, and larval mortality was assessed daily for 9 days.

2.4. Statistical analyses

The data were analyzed with SAS 9.4 (SAS Institute, 2015). Mortality rates were corrected using Abbott's formula (Abbott, 1925; Perry et al., 1998; Antwi et al., 2007a) to adjust for control mortality. Mortality rates were regressed on concentrations, days, with treatment as categorical variable using logistic function in the general linear model (GLM). Based on the logistic function the effect and significance of concentration, day, and treatment on mortality were assessed. Survival rates were also determined from the mortality rates and graphs of survival rate (%) against log concentration were plotted with Sigma Plot 13.0 (SPSS Inc., Chicago, IL). Survival rates were regressed on log concentration using PROC REG. Lethal values (LC_{50}) were determined with PROC PROBIT. Differences in lethal values between treatments were determined by comparison of the 95% confidence limits (Finney, 1971; Robertson et al., 2007; Antwi and Peterson, 2009). Poor fit models were accounted for by multiplying the variances by the heterogeneity factor ($\chi^2/k-2$), where k is the number of concentrations to account for extra binomial variations due to genetic and environmental influences that caused poor fit (SAS Institute, 2015; Antwi and Peterson, 2009).

3. Results

3.1. Mortality

The results of contact bioassays with tested materials against second instars of *D. diffusa*, shown in Table 2, and Fig. 1. Entrust caused high mortality to larvae, acting rapidly and reaching 83–100% mortality (0–17% survival rate) at day 3 across all concentrations (Table 2, Fig. 1.). Mortalities were 66.7–100% (0–33.3% survival rate) for Xpectro, 42.5–100% (0–57.5% survival rate) for Xpulse, 30.8–100% (0–69.2% survival rate) for Aza-Direct across all concentrations from days 4 to 9. Across all the concentrations from days 5 to 9 mortalities were 10–100% (0–90% survival rate) for Mycotrol, 30–100% (0–70% survival rate) for Met52 (Table 2, Fig. 1).

Effects of concentration, day and treatment on mortalities are shown in Tables 3 and 4. Concentration and day effects were significant (Table 3). Among the regression models that were fitted Eq. (2) was the best model (Table 3). Eq. (2) from Table 3 indicates that among the treatments Entrust was the most effective and this had

Table 2
Time–concentration–mortality response of *Dargida diffusa* larvae to reduced risk insecticides.

Treatments	DAT[a]	Insecticide concentration[b]						
		0*X*	0.001*X*	0.01*X*	0.1*X* % Mortality[c]	0.5*X*	1*X*	2*X*
Mycotrol ESO	1	0			0	0	0	0
Met52 EC	1	0			0	0	3.3	0
Aza-Direct	1	0			0	0	0	0
Entrust	1	0	26.7	36.7	90	96.7	100	100
Xpulse OD	1	0			0	0	0	0
Xpectro OD	1	0			0	0	6.7	36.7
Water	1	0	0	0	0	0	0	0
Mycotrol ESO	2	0			0	0	0	0
Met52 EC	2	0			0	0	3.3	0
Aza-Direct	2	0			0	0	6.7	16.7
Entrust	2	0	63.3	86.7	100	100	100	100
Xpulse OD	2	0			0	3.3	13.3	30
Xpectro OD	2	0			13.3	26.7	60	93.3
Water	2	0	0	0	0	0	0	0
Mycotrol ESO	3	0			0	0	0	0
Met52 EC	3	0			0	0	3.3	13.3
Aza-Direct	3	0			0	5.8	22.5	59.2
Entrust	3	0	83.3	93.3	100	100	100	100
Xpulse OD	3	0			10	26.7	60.8	96.7
Xpectro OD	3	0			43.3	80	90	100
Water	3	0	0	0	0	0	0	0
Mycotrol ESO	4	0			0	0	2.5	14.2
Met52 EC	4	0			0	12.5	26.7	69.2
Aza-Direct	4	0			30.8	44.17	66.7	100
Entrust	4	0	86.67	93.33	100	100	100	100
Xpulse OD	4	0			42.5	71.7	95.83	100
Xpectro OD	4	0			66.67	100	100	100
Water	4	0	0	0	0	0	0	0
Mycotrol ESO	5	0			22.5	10	40.8	52.5
Met52 EC	5	0			30	52.5	70	100
Aza-Direct	5	0			69.2	78.3	88.3	100
Entrust	5	0	86.7	93.3	100	100	100	100
Xpulse OD	5	0			90	96.7	100	100
Xpectro OD	5	0			90	100	100	100
Water	5	0	0	0	0	0	0	0
Mycotrol ESO	6	0			37.5	35.8	80	80
Met52 EC	6	0			59.2	77.5	95.8	100
Aza-Direct	6	0			100	100	100	100
Entrust	6	0	86.7	93.3	100	100	100	100
Xpulse OD	6	0			100	100	100	100
Xpectro OD	6	0			100	100	100	100
Water	6	0	0	0	0	0	0	0
Mycotrol ESO	7	0			73.3	84.2	100	91.7
Met52EC	7	0			88.9	100	100	100
Aza-Direct	7	0			100	100	100	100
Entrust	7	0	83.3	93.3	100	100	100	100
Xpulse OD	7	0			100	100	100	100
Xpectro OD	7	0			100	100	100	100
Water	7	0	0	0	0	0	0	0
Mycotrol ESO	8	0			91.1	100	100	91.7
Met52 EC	8	0			100	100	100	100
Aza-Direct	8	0			100	100	100	100
Entrust	8	0	83.3	91.7	100	100	100	100
Xpulse OD	8	0			100	100	100	100
Xpectro OD	8	0			100	100	100	100
Water	8	0	0	0	0	0	0	0
Mycotrol ESO	9	0			94.4	100	100	88.9
Met52 EC	9	0			100	100	100	100
Aza-Direct	9	0			100	100	100	100
Entrust	9	0	83.3	91.7	100	100	100	100
Xpulse OD	9	0			100	100	100	100
Xpectro OD	9	0			100	100	100	100
Water	9	0	0	0	0	0	0	0

[a] DAT, days after treatment.
[b] Insecticide concentration: 1*X* the lowest label application rate equals Mycotrol ESO, 0.72 ml/L; Met 52 EC, 0.72 ml/L; Aza-Direct, 1.44 ml/L; Entrust, 0.091 ml/L; Xpulse OD, 0.72 ml/L; Xpectro OD, 2.5 ml/L.
[c] Mortalities were adjusted for using the Abbott method (Abbott, 1925).
[d]Water, control.

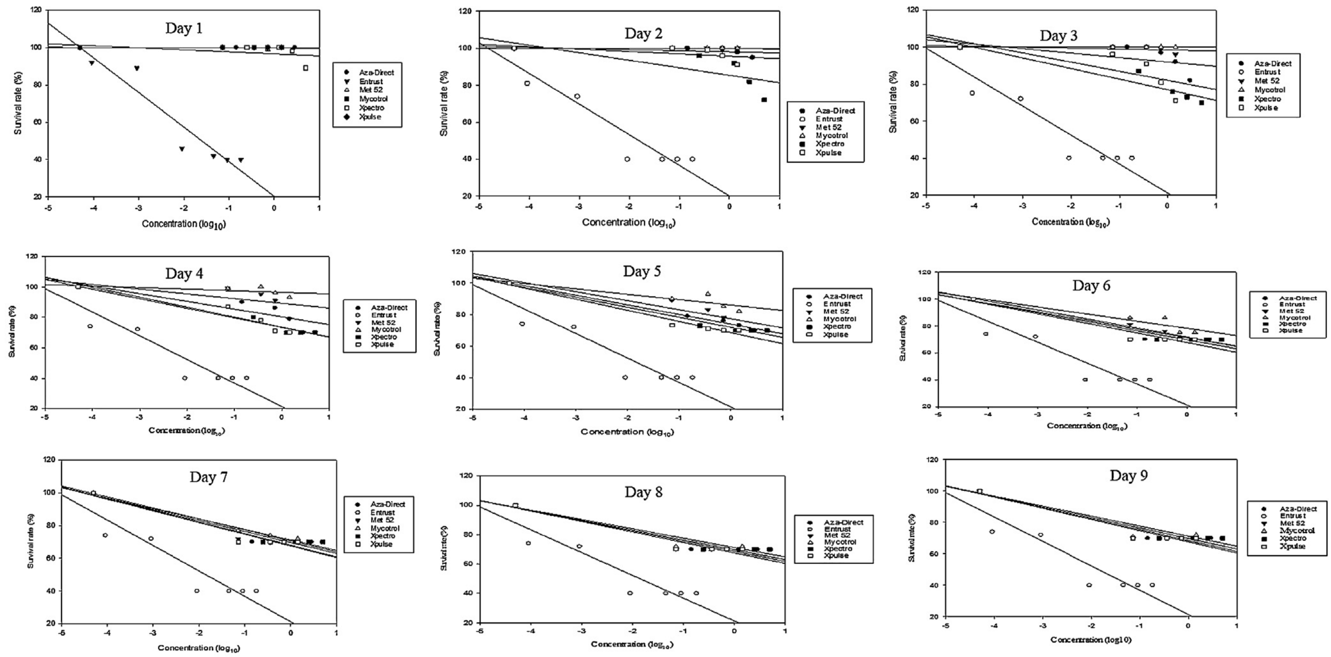

Fig. 1. Survival rate of *Dargida diffusa* larvae versus log concentration of Aza-Direct, Entrust, Met52, Mycotrol, Xpectro, and Xpulse at days 1–9.

Table 3
Effect of concentrations, days, and treatments on mortalities of *Dargida diffusa*.

Fitted equation	Logistic regression model[a,b,c]	Variables	S.E.[d]	*t* Value	*P*[e]
1	$Y = -2.42 + 1.57X_1 + 0.22X_2$[a]	Intercept	0.39221	−6.172	2.31×10^{-9}***
		Concentration	0.31581	4.971	1.15×10^{-6}***
		Day	0.06084	3.618	0.000351***
2	$Y = -1.16 + 1.47X_1 - 0.21X_2 - 0.45X_3 + 0.33X_4 + 0 \times .15X_5$[b]	Intercept	0.3373	−3.448	0.000652***
		Entrust	0.4104	3.582	0.000401***
		Met52	0.4929	−0.423	0.672928
		Mycotrol	0.5137	−0.869	0.385469
		Xpectro	0.4611	0.713	0.476385
		Xpulse	0.4698	0.311	0.756286
3	$Y = -2.03 \times 10^{15} + 8.79 \times 10^{14}X_1 + 2.51 \times 10^{14}X_2 + 3.30 \times 10^{14}X_3 + 1.82 \times 10^{14}X_4 + 1.56 \times 10^{14}X_5 - 2.82 \times 10^{14}X_6 + 4.60 \times 10^{14}X_7$[c]	Intercept	4.411×10^{14}	−4.598	6.47×10^{-6}***
		Concentration	1.764×10^{14}	4.985	1.09×10^{-6}***
		Day	5.244×10^{13}	4.786	2.76×10^{-6}***
		Entrust	4.431×10^{14}	0.744	0.457
		Met52	4.838×10^{14}	0.376	0.707
		Mycotrol	4.838×10^{14}	0.321	0.748
		Xpectro	4.862×10^{14}	−0.580	0.562
		Xpulse	4.838×10^{14}	0.952	0.342

[a] Logistic regression model: Y = mortality (%); X_1 = concentration (ml/L); X_2 = day.
[b] Logistic regression model: Y = mortality (%); X_1 = entrust; X_2 = Met52; X_3 = mycotrol; X_4 = Xpectro; X_5 = Xpulse.
[c] Logistic regression model: Y = mortality (%); X_1 = concentration (ml/L); X_2 = day; X_3 = entrust; X_4 = Met52; X_5 = mycotrol; X_6 = Xpectro; X_7 = Xpulse.
[d] S.E. = standard error.
[e] ***Highly significant effect at $P \leq 0.05$.

the most significant effect on *D. diffusa*. Concentration effects were significant for Mycotrol, Met52, Aza-Direct, and Xpulse (Table 4). Entrust had a *P* value of 1 for the concentration and intercept, due to the death rate being approximately close to 1 as most organisms were dead within day 1 (Table 4). Day effects were significant for Xpulse, and Entrust treatments (Table 4).

Lethal concentrations for each test material are presented in Table 5. Generally, there was a good fit to the model assumptions. Entrust was the most effective insecticide compared to Mycotrol, Met52, Aza-Direct, Xpulse, and Xpectro, since Entrust had a steep slope of mortality over time (i.e., it killed rapidly) (Fig. 1). Table 6, show the regression relationship between survival rates of *D. diffusa* and log concentration of tested materials (Mycotrol, Met52, Aza-Direct, Xpulse, Xpectro, and Entrust).

For Mycotrol the models explained 22.78–90.17% of the total survival rate variation for *D. diffusa* for days 1–9 (Table 6). The regression models explained 3.67–99.44% of the *D. diffusa* survival in the Met52 treatment from days 1 to 9 (Table 6), and 24.29–99.30% of the total survival rate response variation for days 1–9 in the Aza-Direct treatment (Table 6). Regression models explained 24.29–98.33% of the total survival rate of *D. diffusa* to Xpulse from days 1 to 9 (Table 6). Xpectro treatment to *D. diffusa*

Table 4
Effect of concentrations, and days for each treatment on mortalities of *Dargida diffusa*.

Treatment	Logistic regression model[ab]	Variables	S.E.[c]	*t* Value	*P*[d]
Mycotrol	$Y = -7.73 \times 10^{14} - 1.61 \times 10^{14} X_1$ [a]	Intercept	5.782×10^{14}	−1.338	0.188
		Day	1.013×10^{14}	−1.586	0.120
	$Y = -2.41 + 2.16X_1$ [b]	Intercept	0.4877	−4.932	$1.27 \times 10^{-5***}$
		Concentration	0.6859	3.142	0.00303**
Met52	$Y = -7.45 \times 10^{14} + 6.23 \times 10^{13} X_1$ [a]	Intercept	6.594×10^{14}	−1.131	0.265
		Day	1.155×10^{14}	0.539	0.593
	$Y = -2.28 + 2.64X_1$ [b]	Intercept	0.4870	−4.688	$2.79 \times 10^{-5***}$
		Concentration	0.7959	3.312	0.00188**
Aza-Direct	$Y = -5.57 \times 10^{13} - 4.29 \times 10^{13} X_1$ [a]	Intercept	7.181×10^{14}	−0.078	0.938
		Day	1.263×10^{14}	−0.340	0.736
	$Y = -2.14 + 1.58X_1$ [b]	Intercept	0.4897	−4.361	$7.94 \times 10^{-5***}$
		Concentration	0.4949	3.189	0.00266**
Entrust	$Y = 2.45 \times 10^{15} - 4.03 \times 10^{14} X_1$ [a]	Intercept	8.703×10^{14}	2.814	0.00658**
		Day	1.532×10^{14}	−2.629	0.01083*
	$Y = -1.63 + 1.05X_1 \times 10^3 X_1$ [b]	Intercept	1.299×10^5	0	1[e]
		Concentration	2.964×10^8	0	1
Xpectro	$Y = -1.61 \times 10^{15} + 1.71 \times 10^{14} X_1$ [a]	Intercept	8.250×10^{14}	−1.951	0.0576
		Day	1.445×10^{14}	1.184	0.2431
	$Y = -2.14 + 1.69X_1$ [b]	Intercept	0.9591	−2.235	0.0307*
		Concentration	0.9780	1.726	0.0915
Xpulse	$Y = -3.21 \times 10^{15} + 3.34 \times 10^{14} X_1$ [a]	Intercept	7.722×10^{14}	−4.159	0.00015***
		Day	1.352×10^{14}	2.472	0.01745*
	$Y = -2.07 + 3.74X_1$ [b]	Intercept	0.5405	−3.827	0.000416***
		Concentration	1.3062	2.863	0.006460**

a Logistic regression model: $X_1{}^a$ = day; $X_1{}^b$ = concentration (ml/L).
b Logistic regression model: $X_1{}^a$ = day; $X_1{}^b$ = concentration (ml/L).
c S.E. = standard error.
d *Significant, **very significant, ***highly significant effect at $P \le 0.05$.
e Due to most death within day 1, the death rate was close to one, the generalized linear model could not converge with fitted probabilities close to one.

Table 5
Lethal concentrations of *Dargida diffusa* larvae to reduced risk insecticides.

Treatment	Day	LC_{50} (g a.i./L)	C.I. (95%)	$P > \chi^2$
Mycotrol ESO[a]	5	0.10968	0.042–164.62	0.0834
Met52 EC[b]	5	0.01880	0.0094–0.029	0.0382
Aza-Direct	5	0.0004042	ND[c]	0.0100
Xpulse OD[d]	5	0.0007180	1.0733E-26–0.0035	0.7012
Xpectro OD	5	0.00177	0.0017–0.0019	1.0000
Entrust WP	5	8.11E-6[e]	1.70179E-8–0.000037	0.8477

a Weight estimate of 4.78×10^{-12} g/spore (2×10^{13} viable spores/quart).
b Contains 5.5×10^9 colony forming units (CFU)/g of product (5×10^{10} viable conidia/g of active ingredient.
c ND, no data as confidence interval could not be determined by statistical analysis.
d *Beauveria bassiana* Strain GHA (0.06%) contains not less than 1×10^{11} viable spores/quart.
e 8.11E-6 = 8.11×10^{-6}.

resulted in total survival rate response variation of 24.29–97.67% at days 1–9 (Table 6). Entrust treatment also resulted in the models explaining survival rate of *D. diffusa* variation from 35.02 to 61.09% at days 1–9 (Table 6).

For Mycotrol the slopes varied from −10.56 to 2.12 at days 1–9 (Table 6). For Met52 the slopes ranged from −17.70 to 2.12 at days 1–9 (Table 6). Aza-Direct treatment resulted in slopes ranging from −9.11 to 1.12 at days 1–9 (Table 6). Xpulse treatment resulted in slopes varying from −19.77 to 2.12 from days 1 to 9 (Table 6). At days 1 to 9 for Xpectro treatment the slopes varied from −5.49 to 0.67 (Table 6). From days 1 to 9 for Entrust treatment the slopes ranged from −275.06 to 16.51 (Table 6).

Lethal concentrations at 5 days post treatment were determined for Entrust (8.11×10^{-6} g a.i./L), Aza-Direct (0.0004042 g a.i./L), Xpulse (0.0007180 g a.i./L), Xpectro (0.00177 g a.i./L), Met52 (0.01880 g a.i./L), and Mycotrol (0.10968 g a.i./L) (Table 3). Based on the lethal concentrations Entrust was the most toxic among the treatments to *D. diffusa*.

4. Discussion

Of the six biological insecticides tested against *D. diffusa* in the laboratory, only Entrust (spinosad 80%), caused high rates of mortality to larvae, with 100% of larvae dying by 9 days after treatment. The other materials were virtually indistinguishable in final rates of mortality at day 9 but some acted more quickly, with Xpectro (*B. bassiana* GHA + pyrethrins) was the next most toxic followed by Xpulse, Aza-Direct, Met52 and Mycotrol. Spinosad, the active ingredient in Entrust, is a broad-spectrum insecticide, relatively fast acting and toxic to wide variety of insects (Salgado, 1998; Simon, 2009; Sparks et al., 1998). Studies by Cleveland et al. (2001) and Morandin et al. (2005) showed through acute oral and contact toxicity that spinosad is highly toxic to bees. During our study, Entrust caused lower survivability within 24 h after treatment, which may make this product advantageous to use whenever sudden pest outbreaks occur. On the other hand, repeated Entrust applications may be necessary, since spinosad loses its toxicity after 7 days

Table 6
Relationship between survival rate of *Dargida diffusa* and log concentration of Aza-Direct, Entrust, Met 52, Mycotrol, Xpectro, and Xpulse.

Treatment	Day	Regression model[a]	F	R^2	P
Mycotrol ESO	1	$Y = -2.27 + 2.12X$	2.67	0.4711	0.2007
	2	$Y = 100.00 + 0X$	ND[b]	ND	ND
	3	$Y = 100.00 + 0X$	ND	ND	ND
	4	$Y = 100.00 + 0X$	ND	ND	ND
	5	$Y = 100.15 - 4.93X$	27.51	0.9017	0.0135
	6	$Y = 95.26 - 10.15X$	7.71	0.7198	0.0692
	7	$Y = 91.75 - 14.18X$	5.67	0.6541	0.0975
	8	$Y = 85.15 - 12.63X$	1.75	0.3681	0.2779
	9	$Y = 82.27 - 10.56X$	0.88	0.2278	0.4163
Met52 EC	1	$Y = -2.27 + 2.12X$	2.67	0.4711	0.2007
	2	$Y = 99.88 - 0.15X$	0.11	0.0367	0.7575
	3	$Y = 99.88 - 0.15X$	0.11	0.0367	0.7575
	4	$Y = 100.46 - 2.81X$	30.63	0.9108	0.0116
	5	$Y = 100.27 - 14.41X$	530.11	0.9944	0.0002
	6	$Y = 93.18 - 17.70X$	16.10	0.8430	0.0278
	7	$Y = 87.55 - 15.34X$	3.59	0.5447	0.1545
	8	$Y = 82.56 - 11.89X$	1.16	0.2796	0.3595
	9	$Y = 81.83 - 11.24X$	0.96	0.2429	0.3989
Aza-Direct	1	$Y = -2.09 + 1.12X$	2.50	0.4550	0.2117
	2	$Y = 100.00 + 0X$	ND	ND	ND
	3	$Y = 100.48 - 1.81X$	52.20	0.9457	0.0055
	4	$Y = 100.84 - 6.41X$	423.48	0.9930	0.0003
	5	$Y = 94.45 - 9.11X$	26.06	0.8968	0.0145
	6	$Y = 87.07 - 7.20X$	3.09	0.5074	0.1770
	7	$Y = 81.83 - 5.62X$	0.96	0.2429	0.3989
	8	$Y = 81.83 - 5.62X$	0.96	0.2429	0.3989
	9	$Y = 81.83 - 5.62X$	0.96	0.2429	0.3989
Xpulse OD	1	$Y = -2.27 + 2.12X$	2.67	0.4711	0.2007
	2	$Y = 100.00 + 0X$	ND	ND	ND
	3	$Y = 100.55 - 6.47X$	176.49	0.9833	0.0009
	4	$Y = 98.05 - 19.77X$	114.71	0.9745	0.0017
	5	$Y = 90.27 - 17.50X$	6.26	0.6759	0.0876
	6	$Y = 83.19 - 12.33X$	1.35	0.3096	0.3300
	7	$Y = 81.83 - 11.24X$	0.96	0.2429	0.3989
	8	$Y = 81.83 - 11.24X$	0.96	0.2429	0.3989
	9	$Y = 81.83 - 11.24X$	0.96	0.2429	0.3989
Xpectro OD	1	$Y = -1.94 + 0.67X$	2.39	0.4437	0.2197
	2	$Y = 101.35 - 2.19X$	22.18	0.8808	0.0181
	3	$Y = 98.29 - 5.49X$	125.61	0.9767	0.0015
	4	$Y = 90.00 - 4.89X$	5.75	0.6573	0.0960
	5	$Y = 85.50 - 4.17X$	2.23	0.4260	0.2325
	6	$Y = 82.93 - 3.52X$	1.28	0.2984	0.3408
	7	$Y = 81.83 - 3.24X$	0.96	0.2429	0.3989
	8	$Y = 81.83 - 3.24X$	0.96	0.2429	0.3989
	9	$Y = 81.83 - 3.24X$	0.96	0.2429	0.3989
Entrust WP	1	$Y = -3.14 + 16.51X$	7.85	0.6109	0.0379
	2	$Y = 77.06 - 275.06X$	4.20	0.4563	0.0958
	3	$Y = 69.80 - 224.03X$	2.92	0.3683	0.1484
	4	$Y = 68.04 - 210.76X$	2.72	0.3525	0.1599
	5	$Y = 67.82 - 209.10X$	2.70	0.3502	0.1616
	6	$Y = 67.82 - 209.10X$	2.70	0.3502	0.1616
	7	$Y = 67.82 - 209.10X$	2.70	0.3502	0.1616
	8	$Y = 67.82 - 209.10X$	2.70	0.3502	0.1616
	9	$Y = 67.82 - 209.10X$	2.70	0.3502	0.1616

[a] Regression model: Y = survival rate (%); X = concentration ($\log_{10}$).
[b] ND = No data due to insufficient variation in the data to create a density plot.

and it may therefore be necessary to reapply if new larvae hatch. Rizk et al. (2014) suggested that this might be because the major route for spinosad degradation is photolysis. Several other reports (Brunner and Doerr, 1996; Liu et al., 1999; Antwi et al., 2007b) have found that Entrust (spinosad) applied to field crops largely loses activity after a week due to degradation when exposed to sunlight (Saunders and Bret, 1997). However, we found that none of the other treatments provided similar levels of control to that of the Entrust treatment (Fig. 1, Tables 5 and 6).

Aza-Direct (azadirachtin), while having no immediate knock-down effect on pests, has been found to reduce feeding and cause death within several days (Rizwan-Ul-Haq et al., 2009;Roy and Gurusubramanian, 2011). Foliar spray applications of commercial neem formulations have been found to persist for 5–7 days under field conditions (Schmutterer, 1990).

Met52 (*M. brunneum* F52) and Mycotrol (*B. bassiana* GHA), compared to spinosad, caused 73.3–100% (0–26.7 survival rate), and 88.9–100% (0–11.1% survival rate), respectively from days 8 to 9.

This is consistent with the mode of action of fungi, which require time for infections to develop and become lethal (Schapovaloff et al., 2014; Wu et al., 2014). While they act more slowly than spinosad, the data indicates that entomopathogenic fungi can be used in the management of *D. diffusa*. However, additional work is needed to determine the efficacy of these products under field conditions.

Conflict of interest

None declared.

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