

Efficacy of Entomopathogenic Fungi and Nematodes, and Low Risk Insecticides Against Wheat Stem Sawfly, *Cephus cinctus* (Hymenoptera: Cephidae)

Khanobporn Tangtrakulwanich¹, Gadi V. P. Reddy¹, Shaohui Wu¹, John H. Miller¹, Victoria L. Ophus¹ & Julie Prewett¹

¹ Western Triangle Agricultural Research Center, Montana State University, Conrad, Montana, USA

Correspondence: Gadi V. P. Reddy, Western Triangle Agricultural Research Center, Montana State University, Conrad, MT., Montana 59425, USA. Tel: 1-406-278-7707. E-mail: reddy@montana.edu

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Abstract

Entomopathogenic nematodes, fungi, and low risk insecticides were evaluated for the management of the wheat stem sawfly, *Cephus cinctus* Norton, in winter wheat at two locations (Devon and Western Triangle Ag Research center) in the Golden Triangle area of Montana (USA) in 2013. Two fungi (*Beauveria bassiana* and *Metarhizium brunneum*), four nematodes species (*Steinernema carpocapsae*, *Steinernema kraussei*, *Steinernema feltiae*, and *Heterorhabditis bacteriophora*), an insect growth regulator (diflubenzuron/dimilin), and a botanical-based chemical (azadirachtin/Aza-direct) were used as foliar sprays. These control agents significantly reduced damage caused by *C. cinctus* larvae, compared to the untreated control or treatment with water alone. No yield differences were observed among entomopathogenic fungi, nematodes, and low risk insecticides. The effectiveness of azadirachtin, diflubenzuron, the entomopathogenic fungi, and the nematodes persisted at the 28th day post application, by which time the wheat had been harvested. Stubbles collected after harvest showed significantly fewer sawfly larvae in the plots treated with entomopathogenic fungi, nematodes, diflubenzuron, and azadirachtin compared to the untreated and water spray plots, indicating that these biorational pesticides have potential to be used as alternatives to conventional pesticides for controlling the wheat stem sawfly larvae.

Keywords: *Cephus cinctus*, entomopathogenic fungus, nematode, diflubenzuron, azadirachtin

1. Introduction

The wheat stem sawfly, *Cephus cinctus* Norton (Hymenoptera: Cephidae), is one of the most important pests of wheat, *Triticum aestivum* L. (Cyperales: Poaceae), especially in the Northern Great Plains of the United States and Canada (Weiss & Morill, 1992; Morill et al., 1993). Annual losses from this pest exceed \$600 million (Beres et al., 2011). Sawfly larvae overwinter underground in dead stubbles, and adults emerge in late spring (Ainslie, 1929; Weiss & Morill, 1992; Gress et al., 2013). Males are haploid with nine chromosomes and typically emerge before females (Holmes, 1979). Mating takes place soon after emergence except for periods of strong wind or rain, which interfere with mating behaviors (Wallace & McNeal, 1966). Females usually fly near wheat plants and lay one egg per stem per visit, mostly in the upper developing internode (Holmes & Peterson, 1960). However, there can be multiple eggs deposited in a stem by different females (Buteler et al., 2009). One female can lay 30 to 50 eggs (Wenda-Piesik et al., 2009). The larvae feed on parenchymous and vascular tissues inside wheat stems, causing damage to the wheat by reducing head weight. As the wheat plant matures, the larva moves down to the base of the stem where it cuts a notch at ground level, leading to lodging before harvest, further reducing yield (Beres et al., 2007).

Attempts to control the wheat stem sawfly with conventional pesticides have been either ineffective or cost more than the economic yield return (Knodel et al., 2009). Alternative approaches such as biological control have therefore been considered for the management of this pest. Wenda-Piesik et al. (2009) demonstrated that *Fusarium* isolates caused mortality in both diapausing wheat stem sawfly larvae in a topical bioassay and developing larvae feeding in infested stems in a greenhouse experiment. Several commercially available fungi and nematodes have been used for biological control of various insect pests. These include *Beauveria bassiana* (Bals.-Criv.) Vuill (McGuire et al., 2005; Bhadauria et al., 2013), *Metarhizium anisopliae* (Metschnikoff) Sorokin (Bhat et al., 2010;

Larramendy et al., 2011), *S. carpocapsae* Weiser (Cossentine et al., 2002; Chambers et al., 2010), *S. kraussei* Steiner (Haukeland & Lola-Luz, 2010), *S. feltiae* Filipjev (Shapiro-Ilan et al., 2004; Navaneethan et al., 2010), and *Heterorhabditis bacteriophora* Poinar (Toledo et al., 2005; Koppenhöfer et al., 2006). Dimilin is an insect growth regulator which has been reported to be effective in mosquito control (Msangi et al., 2011). Neem (Aza-direct) has also been reported to be effective against some insects (Mamoon-ur-Rashid et al., 2013, Sivasakthi et al., 2013). This study was aimed to investigate the potential use of these biorational control agents for the management of *C. cinctus*.

2. Materials and Methods

2.1 Trial Design and Location

Two trials were conducted, one at the Montana State University Western Triangle Agricultural Research Center (WTARC) (N48°18'24.88" W111°55'28.45") and the other at Devon, Montana (N48°33'14.94"W111°23'42.96"). The distance between the two locations was 58 miles. The experiments were carried out from May-September 2013. Winter wheat Yellowstone variety was used for these trials. The wheat was seeded at the rate of 194 live seeds per m². In both trials, the wheat was planted in four rows, with 30 cm between rows. Glyphosate (Roundup Powermax) was applied at the rate of 2.5 L/ha (active ingredient of 540 g/L of acid glyphosate) before the wheat was seeded to control weed growth. Fertilizer N, P, and K ratio at 224.2, 0, and 22.4 kg/ha was broadcasted while planting, and an additional application of 12.3, 25.2, and 0 kg/ha of these three nutrients were placed through seed plot drill. The treatment plots were arranged in a complete randomized design (CRD) with four replicates.

Treatment plots were 8 m × 4 m and there was 2 m-distance between adjacent treatment plots to avoid spray drift. Each plot consisted of four rows. Standing plants were counted after seed germination. There were approximately 142 standing plants per m² in each plot. In each plot, control agents (Table 1) were sprayed after stem elongation had begun. The spray of each treatment was conducted only once at this plant stage. Treatment materials were mixed in a Chapin Lawn & Garden Sprayer, and sprayed at 297 L water per hectare, pressure ranges 241 to 310 Kpa.

Table 1. Materials and rates applied in each treatment

Treatment	Active Ingredient	Dose	Source
T1: Control (no spray)	No treatment	-	-
T2: Water spray	-	-	-
T3: Mycotrol [®]	<i>Beauveria bassiana</i> Strain GHA (2.11×10 ¹⁰ viable spores/ml)	712 ml/ha	Laverlam International Corporation, Butte, MT
T4: Met 52G	<i>Metarhizium brunneum</i> Strain F52 (5×10 ⁹ viable conidia/g)	1484 g/ha	Novozymes, Davis, CA
T5: Millenium [®]	<i>Steinernema carpocapsae</i>	2.3×10 ⁷ infective juveniles (IJs)/ha	Becker Underwood Ames, Iowa
T6: Nemasys [®] L	<i>Steinernema kraussei</i>	2.3×10 ⁷ IJs/ha	Becker Underwood Ames, Iowa
T7: Nemasys [®]	<i>Steinernema feltiae</i>	2.3×10 ⁷ IJs/ha	Becker Underwood Ames, Iowa
T8: Nemasys [®] G	<i>Heterorhabditis bacteriophora</i>	2.3×10 ⁷ IJs/ha	Becker Underwood Ames, Iowa
T9: Growth Hormone (Dimilin)	Benzoylurea-type insecticide of benzamide class	148 g/ha	Chemtura company, Middlebury, CT
T10: Neem (Aza-direct)	Azadirachtin 1.2%	1484 ml/ha	Gowan Company, Yuma, AZ

Stem damage was assessed weekly after treatment. Treatments were applied on 07/25/2013 and data were collected on 08/01/2013, 08/06/2013, 08/12/2013, and 08/19/2013, respectively, by counting the number of stems lodged in randomly selected 1 m² area. A 1 m² quadrat was randomly thrown into each plot to choose the area to

assess the stem damage (Reddy, 2011). The wheat was harvested in late August in 2013. A Hedge 140 plot combine was used to thresh the wheat plots and the grain yield was recorded as grain weight produced in each plot divided by the plot area. To measure pest density after harvest, ten pieces of wheat stubbles were randomly uprooted from each plot at 1st, 2nd, 3rd and 4th week after harvest (08/28/2013, 09/5/13, 09/12/13, 09/23/13) and the number of *C. cinctus* larvae inside stems was recorded.

2.2 Statistical Analyses

Analyses of variance (ANOVA) was used to analyze differences among treatments in yield, number of stems damaged, and number of sawfly larvae per stubble. The locations were treated as random effect (Random, 2 levels) and the treatments were treated as fixed effect (Fixed, 10 levels). Homogeneity of variances among treatments was tested by using Bartlett's test and no transformed data was conducted. Means were compared using least square difference (LSD) test. Values of $P < 0.05$ were considered significant. All analyses were conducted using SAS version 9.3 (SAS Institute 2011).

3. Results

3.1 Stem Damage

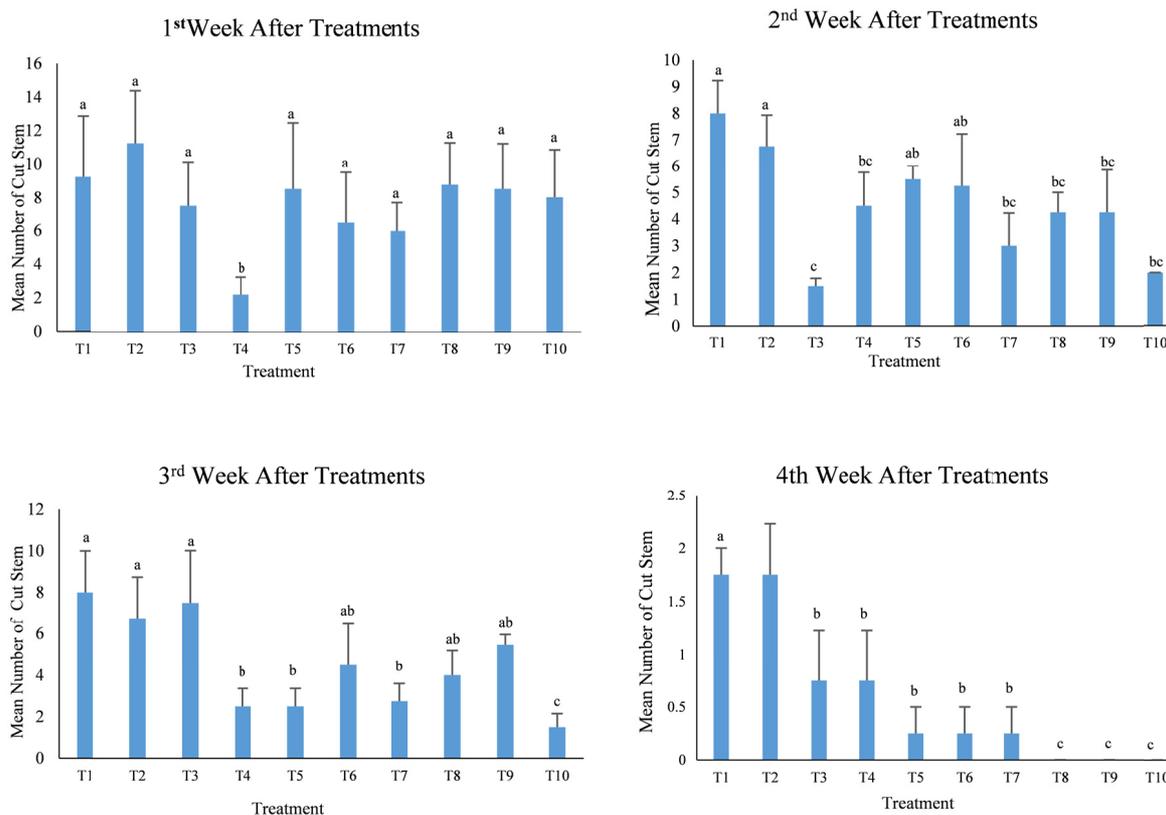


Figure 1. Number of cut stem in plots treated with control materials for *Cephus cinctus* (mean \pm SEM). Different letters indicate significant differences (Two-way ANOVA, LSD test, $\alpha = 0.05$). T1 = control (no treatment); T2 = control (water spray); T3 = *Beauveria bassiana*; T4 = *Metarhizium brunneum*; T5 = *Steinernema carpocapsae*; T6 = *Steinernema kraussei*; T7 = *Steinernema feltiae*; T8 = *Heterorhabditis bacteriophora*; T9 = Dimilin; T10 = Neem (Aza-direct). Data were recorded on 08/01/2013, 08/06/2013, 08/12/2013, and 08/19/2013

In the first week after treatment, there were no significant differences among the untreated control, water spray, *B. bassiana*, *S. carpocapsae*, *S. kraussei*, *S. feltiae*, *H. bacteriophora*, Dimilin, and Aza-direct ($F_{8,63} = 0.50$, $P > 0.05$; Figure 1). Plots treated with *M. brunneum* had significantly lower stem damage compared to other treatments ($F_{9,70} = 2.24$, $P < 0.05$). In the second week, we found no significant differences among the untreated, *B. bassiana*, *S. carpocapsae*, and *S. kraussei* treated plots ($F_{3,28} = 0.77$, $P > 0.05$). There were no significant differences among plots treated with *M. brunneum*, *S. carpocapsae*, *S. kraussei*, *S. feltiae*, *H. bacteriophora*, Dimilin, and Aza-direct ($F_{6,49} = 0.61$, $P > 0.05$). The plots treated with *B. bassiana* had significant lower stem damage compared to other

treatments except for plots treated with *S. feltiae* and Aza-direct ($F_{9,70} = 2.24, P < 0.05$). Stem damage in the third week was not significantly different among the untreated control, water spray, and treatments with *B. bassiana*, *S. kraussei*, *H. bacteriophora*, or Dimilin ($F_{5,42} = 0.59, P > 0.05$). No significant differences were found among treatments with *M. brunneum*, *S. carpocapsae*, or *S. feltiae* ($F_{2,21} = 0.12, P > 0.05$). These three treatments differed significantly to the untreated control, water spray, and *B. bassiana* ($F_{5,42} = 2.35, P < 0.05$), but did not differ significantly from treatments with *S. kraussei*, *H. bacteriophora*, and Dimilin ($F_{5,42} = 0.24, P > 0.05$). Plots treated with *B. bassiana* had significant lower stem damage compared to other treatments in the third week after spray ($F_{9,70} = 2.34, P < 0.05$). In the fourth week, there were no significant differences between the untreated and water sprayed plots ($F_{1,14} = 0.58, P > 0.05$). Both the untreated control and water spray treatment had significant higher stem damage than treatments with *B. bassiana*, *M. brunneum*, *S. carpocapsae*, *S. kraussei*, and *S. feltiae* ($F_{6,49} = 3.46, P < 0.05$). No significant differences were found among water spray, *B. bassiana*, *M. brunneum*, *S. carpocapsae*, *S. kraussei*, and *S. feltiae* ($F_{5,42} = 1.15, P > 0.05$). No stem damage was found in treatments with *H. bacteriophora*, Dimilin and Aza-direct (Figure 1).

3.2 Number of larvae

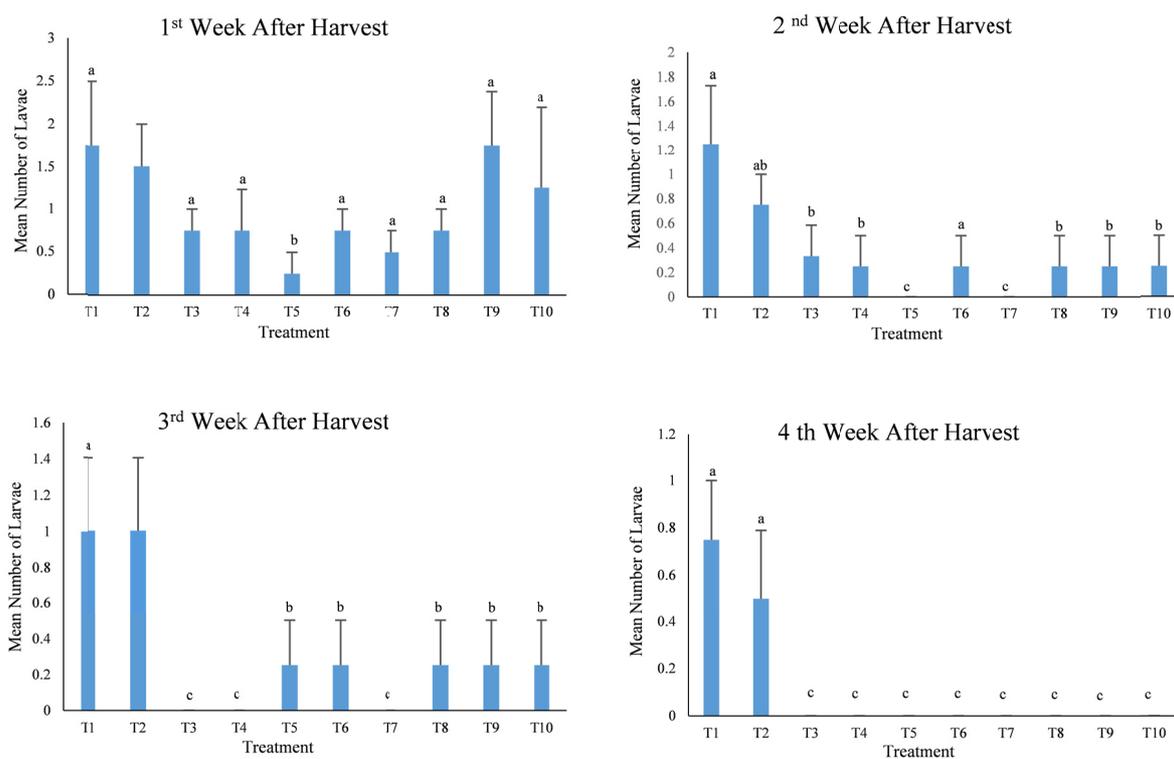


Figure 2. Number of *Cephus cinctus* larvae per stem at different times after treatment with different agents (mean \pm SEM). Different letters indicate significant differences (Two-way ANOVA, LSD test, $\alpha = 0.05$). T1 = control (no treatment); T2 = control (water spray); T3 = *Beauveria bassiana*; T4 = *Metarhizium brunneum*; T5 = *Steinernema carpocapsae*; T6 = *Steinernema kraussei*; T7 = *Steinernema feltiae*; T8 = *Heterorhabditis bacteriophora*; T9 = Dimilin; T10 = Neem (Aza-direct) Data were recorded on 08/28/2013, 09/5/13, 09/12/13, 09/23/13

In the first week after treatment, all other treatments had significantly more larvae than plots treated with *S. carpocapsae* ($F_{9,70} = 2.05, P < 0.05$) (Figure 2). No significant differences were found among the untreated control, water spray, *B. bassiana*, *M. brunneum*, *S. kraussei*, *S. feltiae*, *H. bacteriophora*, Dimilin, and Aza-direct ($F_{8,63} = 1.11, P > 0.05$). In the second week, the untreated plots showed significantly higher number of larvae than other treatments, except for water sprayed plots ($F_{9,70} = 3.04, P < 0.05$). There was no significant difference between the untreated control and water spray ($F_{1,14} = 0.77, P > 0.05$). Plots treated with *B. bassiana*, *M. brunneum*, *S. kraussei*, *H. bacteriophora*, Dimilin, and Aza-direct did not differ significantly in number of larvae ($F_{5,42} = 1.68, P > 0.05$). Plots treated with *S. carpocapsae* and *S. feltiae* had significantly fewer larvae than other treatments ($F_{9,$

$_{70} = 2.45, P < 0.05$) (Figure 2). In the third week, the untreated and water sprayed plots had significantly more larvae than other treatments ($F_{9,70} = 2.34, P < 0.05$). There were no significant differences in the number of larvae found among treatments with *S. carpocapsae*, *S. kraussei*, *H. bacteriophora*, Dimilin and Aza-direct ($F_{4,35} = 0.94, P > 0.05$). Plots treated with *B. bassiana*, *M. brunneum*, and *S. feltiae* had significantly fewer larvae than other treatments ($F_{9,70} = 2.23, P < 0.05$). In the fourth week, only the untreated and water sprayed plots had significantly more larvae than other treatments ($F_{9,70} = 4.39, P < 0.05$); the water spray treatment did not have a significantly different effect from the untreated control ($F_{1,14} = 0.00, P > 0.05$). No larvae were found in plots treated with *B. bassiana*, *M. brunneum*, *S. carpocapsae*, *S. kraussei*, *S. feltiae*, *H. bacteriophora*, Dimilin and Aza-direct.

3.3 Effect on Yield

There were no significant differences in wheat yield between the control plots and plots treated with water ($F_{1,14} = 0.15, P > 0.05$). There were no significant difference in yield among plots treated with *B. bassiana*, *M. brunneum*, *S. carpocapsae*, *S. kraussei*, *S. feltiae*, *H. bacteriophora*, Dimilin, and Aza-direct. However, plots treated with these agents produced significantly higher yield than either the untreated control or the water spray ($F_{9,70} = 2.27, P < 0.05$) (Figure 3).

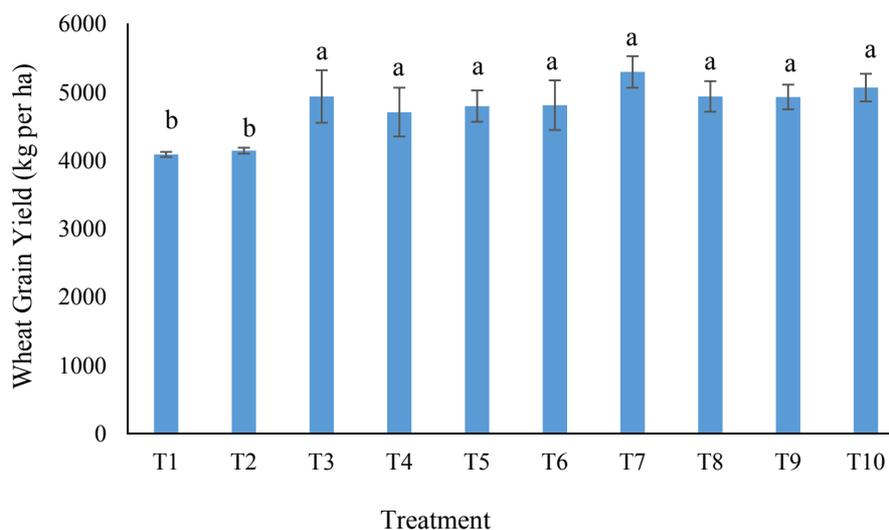


Figure 3. Wheat yield production in treatments with different agents (mean \pm SEM). Different letters indicate significant differences (Two-way ANOVA, LSD test, $\alpha=0.05$). T1 = control (no treatment); T2 = control (water spray); T3 = *Beauveria bassiana*; T4=*Metarhizium brunneum*; T5 = *Steinernema carpocapsae*; T6 = *Steinernema kraussei*; T7 = *Steinernema feltiae*; T8 = *Heterorhabditis bacteriophora*; T9 = Dimilin; T10 = Neem (Aza-direct)

4. Discussion

Currently, the management of *C. cinctus* is often limited to the use of solid-stemmed resistant cultivars. Chemical pesticides are either ineffective or more costly than the economic yield return (Knodel et al., 2009). The development of biological control agents against this pest is an important alternative approach for the management of *C. cinctus*. In the current study, treatments with the entomopathogenic fungi and nematodes resulted in higher yield production and better control of *C. cinctus* compared to the untreated control and water spray.

Results of our study showed that the fungi *B. bassiana* (Mycotrol) and *M. brunneum* (Met 52) effectively reduced wheat stem damage caused by *C. cinctus* larvae compared to the untreated plots or water spray only (Figure 1). *Beauveria bassiana* has been found to control many insects (Bhadauria et al., 2013). Also, *M. brunneum* has been documented to infect more than 200 pest species (Robert & Leger, 2004). However, to our knowledge, no work has been done to evaluate the effectiveness of these entomopathogenic fungi against *C. cinctus*. Both *B. bassiana* and *M. brunneum* kill insects via infection following spore contact with spray droplets or treated surfaces, or by consuming plant tissue treated with the fungus. After contact, spores germinate and hyphae infect the insect (EPA, 2011). Infected insects die in 3-5 days, and the cadavers may serve as source of spores for secondary spread of the fungal entomopathogen. In addition, adult insects may spread the fungus through mating (Long et al., 2000).

In our study, we found that the effectiveness of the two fungal entomopathogens started to take effect 21 to 28 days after application, in terms of reducing number of cut stems, which occurred at the third week for *M. brunneum* (Met52) and the fourth week for *B. bassiana* (Mycotrol) (Figure 2). Being consistent with stem cuts, the plots treated with the two fungi showed significantly lower number of *C. cinctus* larvae by the third and fourth weeks after wheat harvest (Figure 2).

Entomopathogenic nematodes have many attributes of effective biological control agents (Kaya & Gaugler, 1993; Grewal et al., 2005; Koppenhöfer, 2007). In our current study, *S. carpocapsae* (Millenium[®]), *S. kraussei* (Nemasys[®] L), *S. feltiae* (Nemasys[®]), and *H. bacteriophora* (Nemasys[®] G) appeared to be effective in reducing the damage caused by *C. cinctus* larvae and increased the yields compared to the untreated control and treatment with water spray alone (Figure 3). Georgis et al. (1991) also reported that Steinernematid and Heterorhabditid nematodes significantly suppressed pest populations such as *Popilla japonica* Newman (Coleoptera: Scarabaeidae), *Scapteriscus vicinus* Scudder (Orthoptera: Grylotalpidae), *Otiiorhynchus sulcatus* Fabricius (Coleoptera: Curculionidae), *Delia radicum* Linnaeus (Diptera: Anthomyiidae), and *Diabrotica virgifera viergifera* LeConte (Coleoptera: Chrysomelidae) in the field. These entomopathogenic nematodes were also proven to have no adverse effect to nontarget arthropods especially when used for short-term control of insect pests (Georgis et al., 1991). All entomopathogenic nematodes used in our experiment were equally effective in reducing the stem damage within three weeks after spray (Figure 1). However, by 28 days after application, *H. bacteriophora* (Nemasys[®] G) showed significantly higher efficacy in reducing stem cut damage compared to other nematode species (Figure 1). Different from our study, Kamali et al. (2013) reported that *S. carpocapsae* had higher virulence and better ability to locate larvae of *Dacus ciliatus* Loew (Diptera: Tephritidae) within infected fruits. Temperature thresholds for survival and infectivity vary with nematode species, their native habitat, and center of origin (Kaya, 1990). The average temperatures in the first 2 and 3 weeks after treatment were 29.3°C and 21.7 °C, respectively. The optimal temperature for *S. feltiae* to be infective ranges from 20 to 30°C, whereas some heterorhabditids can infect host from 7 to 35 °C, and *S. carpocapsae* fails to cause infection when temperature drops below 10 °C (Kaya, 1990; Georgis et al., 2006; Lacey et al., 2006). However, this may also vary with host species. Different host insects might have different cues and characteristics under different temperatures which means the temperature can affect host recognition by nematodes and also the host itself can produce less cues depending on temperatures (Chen et al., 2003). By the fourth week after harvest, we found no sawfly larvae in wheat stubbles in any nematode treatment, indicating that these nematodes effectively controlled the insect within 28 days after harvest.

Our study also showed that Dimilin was effective in controlling the damage caused by *C. cinctus* larvae compared to the treatment with water spray only and the untreated control (Figures 1, 2 & 3). Diflubenzuron (Dimilin) kills insects by interfering with chitin synthesis and disrupting insect growth. Dimilin has been shown to be effective in controlling larvae of *Anopheles gambiae* Giles (Diptera: Culicidae) (Msangi et al., 2011), *Culex quinquefasciatus* Say (Diptera: Culicidae) (Msangi et al., 2011), *Musca domestica* Linnaeus (Diptera: Muscidae) (Batra et al., 2005; Le Menach et al., 2007; WHO, 1984).

In addition, treatment with neem extracts showed significantly lower stem damage and fewer *C. cinctus* larvae compared to the untreated control and treatment with water spray alone (Figures 1, 2). Neem extracts are reported to affect over six hundred species of pests (Sivasakthi et al., 2013). Reddy and Guerrero (2000) suggested that neem had the potential to be used as a good alternative to conventional insecticides in IPM programs. In the current study, neem effectively suppressed *C. cinctus* populations (Figure 2).

Overall, all bio-rational agents used in the current study demonstrated good potential for controlling the *C. cinctus*. Among them, *B. bassiana* (Mycotrol), *M. brunneum* (Met 52), and *S. feltiae* (Numasys) were effective in killing the larvae within a shorter period of time compared to other agents. Our study indicated that these biopesticides may serve as alternative methods for controlling *C. cinctus* and may be incorporated into IPM programs, to mitigate the environmental pressure from the use of conventional insecticides.

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