Article

Comparative management of *Chilo suppressalis* (Walker) (Lepidoptera: Crambidae) by convenient pesticides and non-chemical practices in a double rice cropping system

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Abstract

The inclination of rice growers towards double cropping system in north of Iran has raised new concerns about the excessive release of broad-spectrum pesticides, particularly organophosphates, in the environment. In this study, the efficiency of three insecticides and an integrated pest management (IPM) program for management of the striped rice stem borer, *Chilo suppressalis* (Walker) (Lep: Crambidae), in double cropping system was investigated under field condition. According to the results, one accurate application of hexaflumuron EC 10% (1.5 L/ha) or diazinon EC 60% (1.5 L/ha) for each generation of the pest resulted in significant reduction in dead heart and white head damage and increase in yield performance when compared with one application of fipronil G 0.2%, diazinon G 10% and diazinon EC 60% + diazinon G 10% as well as two application of diazinon EC 60% + diazinon G 10%. Additionally, considerable effect of IPM programs (mechanical, physical, and biological practices) on suppression of pest damage and improvement of yield performance was also observed. Given the environmental problems associated with excessive application of diazinon and fipronil, hexaflumuron, as an insect growth regulator with specific mode of action, can be efficiently integrated with other non-chemical methods for successful management of *Ch. suppressalis* in double cropping systems.

Keywords dead heart; *Chilo suppressalis*; double rice cropping; integrated pest management; white head.

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1 Introduction

Rice is the dominant staple food crop in most of developing countries. Asian countries, particularly southeastern Asia and the Middle East, account for about 90% of the global rice production and consumption

(Hossain and Narciso, 2004). Despite being cultivated on small and marginal farms in lands with extreme population pressure and land use change risks, global rice production has experienced significant increase over the last half century (Hossain and Narciso, 2004; Fan et al., 2012). For example, China has experienced a 3.2-fold increase in the productivity of rice (from 2041 kg/ha to 6585 kg/ha) within 50 years (1961-2009) (Fan et al., 2012). Most of this improvement arises from increases in yield per unit area, through technological progress in irrigation and crop management, rather than from increases in the cultivated area (Hossain and Narciso, 2004; Fan et al., 2012).

Rice is the second largest staple food crop in Iran (Feizabadi, 2011). Within four decades (from 1980 to 2010), Iran has changed from the leading rice importing country (Calpe, 2006) to the eleventh rice producer of the world (Feizabadi, 2011). This improvement is, at least in part, associated with the development of technology, cultivation of high-yield varieties and establishment of double cropping systems (i.e., harvest of two crops from the same field in a given year) (Feizabadi, 2011). In this country, most of the areas under rice cultivation are located at the coastline of the Caspian Sea (north of Iran) and surrounded by the Alborz Mountains at the southern edge. In this area, Mazandaran and Guilan provinces account for about 78% of cultivation area and rice yield of the country (Daliri et al., 2009). The double rice cropping has been firmly established in rice fields of Northern Iran.

The striped stem borer, *Chilo suppressalis* (Walker) (Lep: Crambidae), is one of the most important pests of rice cultivations in Europe, Southeastern Asia and the Middle East, including Iran (Zibaee et al., 2009). Annual crop loss by *C. suppressalis* has been estimated to range from 5% to 60% depending on population densities of the pest (Chaudhary et al. 1984). Two major kinds of symptoms are produced depending on the growth stage of rice plants: At vegetative stage and before formation of panicles, the larvae cause the death of the growing points of young shoots (dead heart). At reproductive stage, the larvae feed on growing parts of the plant and leave empty whitish panicles conspicuous in the field (white head) (Lu et al., 2014).

Despite chemical insecticides have been the most common tools for management of *C. suppressalis* (Su et al., 2014), their efficiency is questionable due to the cryptic activity of larvae as well as rapid development of pesticide resistance by the pest (Li et al., 2007; Zibaee et al., 2009; Cheng et al., 2010; Yao et al., 2017). Apart from the environmental pollution and human health risks, indiscriminate use of broad-spectrum pesticides may also negatively affect natural enemies (such as egg and larval parasitoids, and predators) and underlie pest resurgence (Zhang et al., 2011). The integrated pest management (IPM) concept proposes the use of environmentally friendly pesticides in combination with other strategies (such as resistant cultivars and biological control) for management of pest populations (Ehler, 2006). In this context, monitoring and management of insecticide resistance through the use of insecticide mixtures and rotation to new classes on pesticides are essential to maintain the efficiency of pest control as well as to ensure sustainable management (Su et al., 2014; Yao et al., 2017).

Since 1990s, a variety of insecticides, including diazinon, fipronil, abamectin, methamidophos, chlorpyrifos, and triazophos have been extensively used for *C. suppressalis* control (He et al., 2007; Li et al., 2007; Yao et al., 2017), all of which are known to cause resistance problems and environmental concerns because of long-term indiscriminate use (Li et al., 2007; Zibaee et al., 2009; Yao et al., 2017). In this study, we evaluated the efficiency of two convenient insecticides, diazinon (G 10% or EC 60%) and fipronil (G 0.2%), as well as the insect growth regulator, Hexaflumuron (EC 10%), for control of *C. suppressalis* in double cropping systems of north Iran. Hexaflumuron [N-(((3,5-dichloro-4-(1,1,2,2-tetrafluroethoxy) phenyl)- amino) carbonyl) 2,6 diflurobenzamide] is a type of benzoylphenyl urea (BPUs) insecticide which interfere with molting process of immature insects by inhibiting chitin synthesis and formation of cuticle (Abo-Elghar et al., 2003). As a result, hexaflumuron has been widely used against the larval stage of Lepidoptera, Coleoptera, and Diptera as well as immature stages of termites (Doucet and Retnakaran, 2012). Given this specificity to immature stages of arthropods, chitin synthesis inhibitors are suitable candidates for use in IPM programs, as they have very low toxicity to vertebrates (human, mammals, birds, and fish) and adult beneficial insects such as predators, parasitoids and pollinators (Merzendorfer, 2013).

2 Materials and Methods

2.1 Materials

Diazinon (Granular formulation 10% and EC 60%), Fipronil (G 0.2%), and Hexaflumuron (EC 10%) were obtained from Golsam Gorgan Chemicals Company (Gorgan, Iran). Commercial packages (trichocards) of the egg parasitoid, *Trichogramma brassicae* (Hym: Trichogrammatidae) were obtained from a local producer (Gohar Sabz Mazandaran, Babol County, Iran). Delta traps and *C. suppressalis* pheromone [(Z)-9-hexadecenal, (Z)-11-hexadecenal, (Z)-13-octadecenal] were obtained from Guilan Agricultural and Natural Resources Research and Education Center (Rasht, Iran). Light traps were constructed using 100 w lamps.

2.2 Experimental design

This study was carried out in a field with an area of 4000 m^2 at Rice Research Institute of Iran located at Amol County (Mazandaran province, north Iran) (36˚29’N, 52˚29’E, 29 meters above sea level). This study was performed in a Randomized Complete Block Design with eight treatments, each containing four replicates. Therefore, the field was divided into 8 major plots (500 m^2) and each plot was sub-divided into four replicates. The experimental units were separated from each other by a distance of ~4 m and the ridges were covered by an impermeable plastic (80 cm width) to avoid water and pesticide exchange between two adjacent plots. Twenty-five-day old seedlings of Tarom Amrolahy rice cultivation were transplanted into the field on 17th August 2016 with a regular interval of 20 cm.

The plots were treated with one of the eight following options as treatment. 1) Integrated Pest management (hereafter IPM) involving the four following non-chemical practices without any insecticide application: I) Mechanical control: cut heading of seedlings before transplanting into the experimental field with the purpose of partial extermination of leaves containing *C. suppressalis* eggs and decreasing the damage of dead heart. II) Installation of light traps before the preparation of the rice paddy until the harvesting of double cropping with the purpose of monitoring the pest population and determining the exact timing of chemical control (Fig. 1a). III) Installation of pheromone-baited delta traps (containing 2 mg of the active ingredient) from 23rd May 2016 until the second harvest (20th October 2016). The traps were installed at a distance of ~90 m from each other's and the pheromone sources were replaced every 35 days. IV) Release of *T. brassicae* wasps two times in the first generation and three times in the second generation of the pest according to instructions (10 Trichocards for 500 m^2) (Osku et al., 2012). 2) Application of Hexaflumuron (EC 10%) at a rate of 1.5 L per ha in the first and second generation of *C. suppressalis*. 3) Application of diazinon (G 10%) at a rate of 20 kg per ha in the first and second generation of the pest. 4) Application of diazinon (EC 60%) at a rate of 1.5 L per ha in the first and second generation of the pest. 5) Application of diazinon (G 10%) at a rate of 20 kg per ha and diazinon (EC 60%) at a rate of 1.5 L per ha one time for each of the first and second generations of the pest. 6) Application of fipronil (G 0.2%) at a rate of 20 kg per ha in the first and second generation of the pest. 7) Application of diazinon (G 10%) at a rate of 20 kg per ha and diazinon (EC 60%) at a rate of 1.5 L per ha two times for each of the first and second generations of the pest. 8) Control without and insecticide application and other management practices. For all insecticides, the timing of spraying was determined on 6 and 14 September 2016 according to the flight peak of female moths monitored using light traps (see Fig. 1a).

2.3 Sampling

To evaluate the two major types of larval damage in each treatment (i.e. dead heart (Dh) and white head (Wh)), two sampling was carried out on 12 and 25 September 2016. For each sampling, 10 samples, each containing 4 rice bushes, were randomly collected (Majidi Shilsar and Ebadi, 2012). The percentage of dead heart and white head was estimated according to the formula of Onate (1965) as follows:

$$Dh\%\ or\ Wh\% = \frac{N_i}{N_t} \times \frac{N_I}{N_T}$$

where N_i is the number of infected bushes, N_t is the total number of samples bushes, N_I is the number of infected tillers, and N_T is the total number of tillers in infected bushes.

The performance of rice plants was also compared among treatments. For each treatment, after excluding the two marginal rows, 4 plots, each containing 40 bushes, were randomly selected. The rice panicles of the 40 bushes were threshes and the resulting grain was weighed using a digital scale with a precision of 0.01 g. After correction of dry weight using the correction table 14%, the final performance of each treatment was calculated on kg/ha basis.

The efficiency (%) of control methods based on decrease in the percentage of dead heart and white head damages was calculated using Abbott (1925) formula as follows:

Control efficiency based on Dh (%) = $\frac{\%Dh_t - \%Dh_c}{\%Dh_c}$

where Dh_t and Dh_c are the percentage of dead heart in treated and control plots, respectively.

Control efficiency based on *Wh* (%) = $\frac{\%Wh_t - \%Wh_c}{\%Wh_c}$

where Wh_t and Wh_c are the percentage of white head in treated and control plots, respectively.

2.4 Data analysis

The percentage data were transformed to $\sqrt{x + 0.5}$, then exposed to one-way Analysis of Variance (ANOVA) using SAS program (version 9.1) (SAS Institute, 2004). Significant differences among treatments were evaluated using LSD procedure of SAS at $P<0.05$.

3 Results

The population fluctuation of adult male and female *C. suppressalis*, attracted to light traps during the growing season of 2016 has been shown in Fig. 1a. As the figure shows, in the second cropping, two peaks were recorded for both male and female moths on 5 September and 12 September, which were corresponding to the second and third generations of the pest, respectively. In addition, Fig. 1b shows the average number of adult males trapped in pheromone-baited delta traps during the first and second cropping. Light traps were significantly more efficient than pheromone-baited traps in term of attraction and capture of adult male moths in second cropping (*t*-test: df=63, t=6.41, $P<0.01$). Light traps attracted an average of 7.36 and 11.81 male moths per day in the first and second cropping period, whereas the average numbers of entrapped moths in pheromone-baited traps were 6.71 and 3.64 moth per day for the first and second cropping periods, respectively (data for the first cropping are not shown).

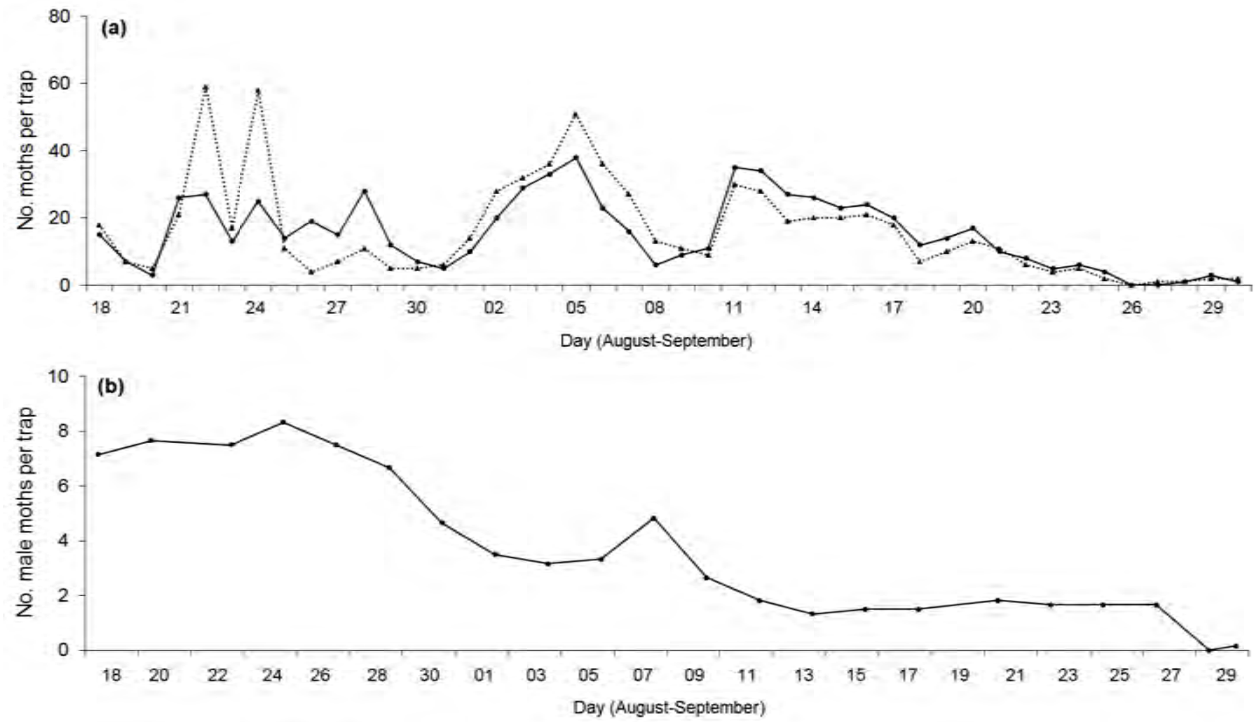

Fig. 1 The average number (n=6) of adult *Chilo suppressalis* captured by light traps (a) and pheromone-baited traps (b) during the second rice cropping period (18 August-30 September 2016).

There was significant difference in the frequency of dead heart symptoms among treatments (ANOVA: df=7, F=29.15, $P<0.001$) (Fig. 2a). Plots treated with any type of management options (insecticide or IPM) showed significantly lower frequency of dead heart when compared with control (16.06%).

Apart from control, plants treated two times with diazinon G 10% and four times with diazinon G 10% + diazinon EC 60% exhibited the significantly higher percentage of dead heart in comparison with other treatments. The least percentage of dead heart symptoms were recorded for plants treated two times with diazinon EC 60% (1.82%) and hexaflumuron EC 10% (1.90%) (Fig. 2a).

In addition, the average frequency of white head symptoms was significantly different among treatments (ANOVA: df=7, F=15.01, $P<0.001$) (Fig. 2b). The highest percentage of white head symptoms was observed in control (12.25%) followed by plots treated four times with diazinon EC 60% + diazinon G 10% (9.6%) and those treated two time with diazinon G 10% (9.59%). By contrast, plots treated two times with diazinon EC 60% (5.02%) and hexaflumuron EC 10% (5.40%) exhibited the least percentage of white head symptoms (Fig. 2b).

The efficiency of control methods in decreasing the percentage of dead heart and white head symptoms in the second cropping period has been summarized in Table 1. All control methods significantly decreased the percentage of both dead heart (ANOVA: df=7, F=34.31, $P<0.001$) and white head (ANOVA: df=7, F=22.55, $P<0.001$) damages. Unexpectedly, four applications of diazinon EC 60% + diazinon G 10% had the least effect on reducing both dead heart and white head damage by *C. suppressalis* larvae. By contrast, plants treated two times with diazinon EC 60%, hexaflumuron EC 10%, or diazinon EC 60% + diazinon G 10% suffered the least damages in terms of both dead heart and white head symptoms (Table 1).

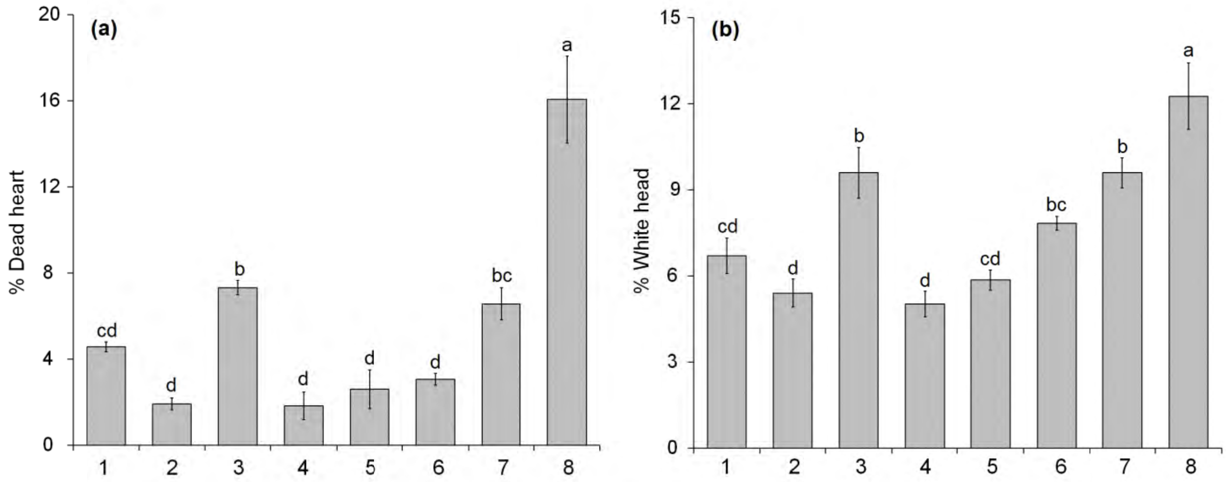

Fig. 2 The average frequency (%) of dead heart (a) and white head (b) symptoms on rice plants treated with different management options, different letters show significant differences at $P<0.05$ level (ANOVA); For ease of reference, the names of treatments have been shown by numbers 1-8 (see Table 1).

The yield performance of the second cropping was also significantly different among treatments (ANOVA: df=7, F=7.02, $P<0.001$) (Fig. 3). The least average performance was recorded for control plot (362.47 kg/ha), followed by plots treated two times with diazinon G 10% (376 kg/ha). The best performance, however, was recorded for plots treated two times with hexaflumuron EC 10% (721.95 kg/ha), followed by those treated with diazinon EC 60% (719.28 kg/ha) and IPM group (560.08 kg/ha) (Fig. 3).

Table 1 Reduction (%)* in dead heart and white head symptoms by *Chilo suppressalis* larvae in plots treated with different management options; *Dh*, dead heart, *Wh*, white head, different letters show significant differences at $P<0.05$ level (ANOVA).

Number	Treatment	No. application for each generation	% control	
			Dh	*Wh*
1	Integrated management	continuous	64.28^{ab}	41.75^{ab}
2	Hexaflumuron EC 10%	one time	84.56^{a}	54.89^{a}
3	Diazinon G 10%	one time	84.90^{a}	32.87^{bc}
4	Diazinon EC 60%	one time	85.07^{a}	57.46^{a}
5	Diazinon G 10% + EC 60%	one time	81.79^{a}	56.09^{a}
6	Fipronil G 0.2%	one time	76.87^{ab}	51.23^{ab}
7	Diazinon G 10% + EC 60%	two times	47.47^{b}	20.13^{c}
8	Control	-	0.00^{c}	0.00^{d}

*The control efficiency was calculated based on damage in control using Abbott's formula (1925).

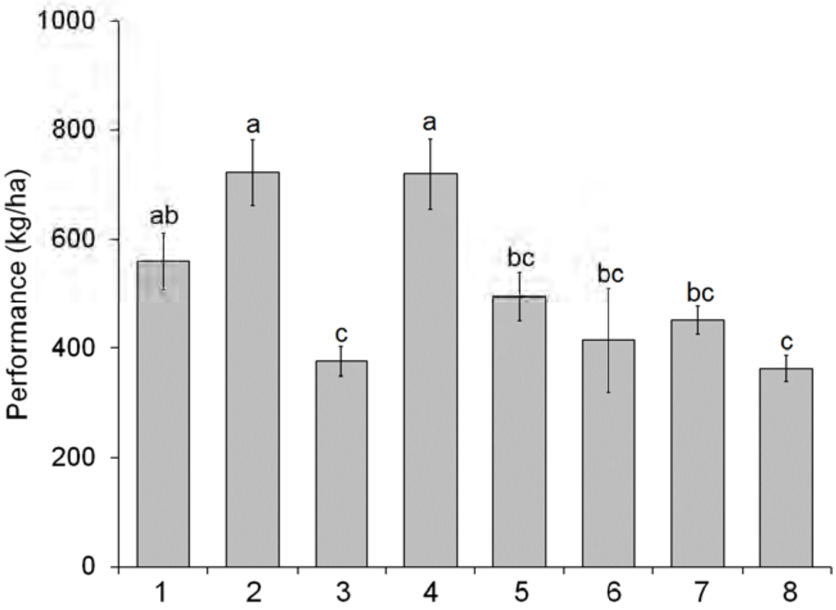

Fig. 3 The average yield performance (kg/ha) of the second rice cropping in plots treated with different management options, different letters show significant differences at $P<0.05$ level (ANOVA); For ease of reference, the names of treatments have been shown by numbers 1-8 (see Table 1).

4 Discussion

In this study, the efficiency of different insecticides as well as an integrated pest management program for control of *Ch. suppressalis* in the second rice cropping period was studied under field conditions. In recent years, double rice cropping system have received growing attention by both growers and governors in north of Iran as a mean for raising farmer's income and increasing rice production. However, besides these advantages, double rice cropping may introduce a variety of concerns such as increase in pest populations and changes in life cycle parameters of pests. As a result, significantly higher frequencies of insecticides would be required for control of pests in double cropping systems, a condition that intensifies environmental pollutions and human health risks.

Diazinon and fipronil are among the most widely used pesticides for control of *Ch. suppressalis* (Zibaee et al., 2009; Yao et al., 2017). In Iran, diazinon has been extensively used against *Ch. suppressalis* for more than 35 years. A recent study indicated that the susceptibility of the pest to diazinon is no longer similar to that of 1979, when the pesticide was used for the first time (Zibaee et al., 2009). Similar evidence exists for fipronil, where a 19.1-fold resistance has been reported to develop in several areas of China within only 10 years (Hu et al., 2010). Unfortunately, in most studies, both fipronil and diazinon have been considered as highly toxic compounds to various non-target organisms at both acute and sub-lethal doses (Elzen et al., 2000; Brunner et al., 2001; Sun et al., 2008; Cheng et al., 2010; Rogers et al., 2011). In order to manage resistance development by the pest and avoid harmful effects of broad-spectrum pesticides on non-target organisms, it is necessary to exploit more narrow-spectrum pesticides with more specificity against the target pest, make a long-term rotation in insecticide application, and include other control strategies in pest management programs.

Results of the current study indicate that a single application of diazinon EC 60% and hexaflumuron EC 10% can decrease both dead heart and white head damages by *Ch. suppressalis* more efficiently than other control methods (Fig. 2). Additionally, the best performance was obtained in plots treated with diazinon EC 60% and hexaflumuron EC 10% (Fig. 3). Unexpectedly, the control efficiency and rice performance in these groups were even higher than plots treated four times with diazinon EC 60% + diazinon G 10% (*i.e*. two times for each generation). The decreased efficiency in the latter treatment may be related to the inappropriate timing of insecticide application. According to previous studies, the most suitable time for insecticide application is when at least 50% of the first and second instar larvae are active in rice sheath and before penetrating into the stem (Chen et al., 2014). Apart from this short period, insecticides would face a reduced efficiency because of internal activity of the pest larvae, if they do not impose converse effects by negatively impacting the populations of natural enemies.

With respect to the disadvantages associated with widespread application of diazinon and fipronil against rice pests (see above), hexaflumuron may provide promising insights for efficient control and resistance management of *Ch. suppressalis*. Since its first introduction in 1983 (Sbragia et al., 1983), Hexaflumuron has been widely used against immature stages of termites, moths, beetles, and Diptera (Doucet and Retnakaran, 2012). Given the novelty and specific mode of action, hexaflumuron are very effective for control of insecticide-resistant pests. Additionally, as hexaflumuron has very low toxicity to non-target organisms including adult natural enemies (Merzendorfer, 2013), it seems an ideal option for integrated management of stem borers in rice cultivations.

Integrated Pest Management is an effective and environmentally sound approach to control pest populations (Ehler, 2006). According to our results, the use of IPM options (physical, mechanical, and biological control) resulted in significant decrease in percentage of dead heart and white head damages in comparison with control (Fig. 2 & 3). These results were comparable with or even more favorable than those obtained from application of all insecticide types and formulations. Nonetheless, the contribution of some other non-chemical methods, which were not included in this study, to pest control should be also taken in account. For example, the use of resistant rice cultivars has long been considered as a pillar of IPM programs against *C. suppressalis* (Das, 1976; Smith, 2005). Khan et al. (2005) reported that the efficiency of chemical pesticides against *Ch. suppressalis* varies significantly depending on the level of resistance in rice cultivars. Resistant cultivars have been reported to raise the economic threshold for chemical control and decrease the dose of pesticides required for satisfactory control of pests on several crops (Smith, 2005).

Mass trapping and mating disruption with sexual pheromones is another component of IPM that has been successfully used for control of a wide variety of lepidopteran pests (Stelinski et al., 2008, 2013, Trimble et al., 2004; Chen et al., 2014). The use of appropriate number of pheromone dispensers and high-quality pheromone are major determinants for control efficiency in mass trapping and mating disruption methods (Chen et al., 2014). Chen et al. (2014) claimed that the use of 40, 50, and 500 pheromone dispensers per hectare can result in more than 85% reduction of larval damage, which is comparable with application of dimethoate and deltamethrin at recommended dose (0.35 kg/ha). Pheromone traps can also be used to avoid extra application of pesticides by exactly timing of a single application against the newly emerged larvae of *Ch. suppressalis* (Chen and Klein, 2012). Light traps can also be used for attraction and capture of both male and female moths with the purpose of both mass trapping and timing of insecticide application (Zhu et al., 2007). In our study, pheromone traps attracted significantly less number of male moths in comparison with light traps. This difference may be related to inappropriate number and distance of dispensers and the low quality of pheromone source used.

5 Conclusion

Broad-spectrum pesticides, particularly organophosphates, have adverse effects on non-target organisms (Jiang et al., 2005; Fernandez, 2015). The inclination of rice growers towards double cropping system has raised new concerns about the excessive release of pesticides in the environment. In this study, hexaflumuron, an insect growth regulator with specific mode of action, was found to be effective against *Ch. suppressalis*. The efficiency was comparable with or even higher than convenient pesticides such as diazinon and fipronil. Additionally, considerable effect of IPM programs on suppression of pest damage and improvement of yield performance was observed in this study. Given the environmental problems associated with application of diazinon and fipronil, hexaflumuron can be efficiently integrated with other non-chemical methods for successful management of *Ch. suppressalis*. In this context, a variety of methods, including mass trapping and mating disruption using light traps and pheromone traps, use of resistant rice cultivars, biological control by egg parasitoids, accurate timing of insecticide application, and several cultural practices have been proposed promising (Zhu et al., 2007; Jiang et al., 2011).

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