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Three Releasing Doses of *Trichogramma* Sp. (Hymenoptera: Trichogrammatidae) with *Bacillus Thuringiensis* Kurstaki in Controlling of *Batrachedra Amydraula* Meyrick Eggs on Date Palm Trees

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Summary

The efficiency of two *Trichogramma* species *Trichogramma brassicae* and *T. embryophagum* in combination with bacterial suspension of *Bacillus thuringiensis* in controlling of the lesser date moth, *Batrachedra amydraula* Meyricke. Three release doses (50, 100 and 200 parasitoids/tree) for each species were examined in farm and the parasitism rate, adult's emergence, the number of females and adult longevity of *T. brassicae* and *T. embryophagum* were studied. The results showed that both Trichogramma species were significantly efficient, especially at higher dose (200 parasitoids/tree), in their parasitism activity on *B. amydraula* eggs. However high reduction in infestation was achieved by *B. thuringiensis* with *T. embryophagum* (70.1%) at concentration of 10^6 cell/ml *B. thuringiensis*. In addition, no significant differences was obtain between *T. brassicae* and *T. embryophagum* in mortality and adult emergence rates when they were treated with/without *B. thuringiensis* reared in the *B. amydraula*. However, the longevity of *T. embryophagum* was significantly more than *T. brassicae*. This is the first study to investigate *T. embryophagum* for parasitizing the eggs of *B. amydraula* and results of present study suggested that *T. embryophagum* with *B. thuringiensis* could be more effect for biocontrol of *B. amydraula*.

Keywords: *Batrachedra amydraula*, *Bacillus thuringiensis*, Date palm, Natural enemies, *Trichogramma* species, control parameters.

1.Introduction

Date palm (*Pheonix dactylifera* L.) is a very important economic crop in different countries (Abass and Maziel, 2019). Several factors Such as pests, weather conditions and diseases affected the plant health. The lesser date moth *Batrachedra amydraula* Meyricke

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(Cosmopteridae: Batrachedridae) in Iraq is a dangerous pest of date palm (Downson and Aten, 1962; Levi Zada *et al.* 2011; Haldhar *et al.* 2017). The harmful phase of the pest is the larva feeding inside the fruit and larvae spin webs around flowers and later infest the fruit and may damage three or four fruits during its lifetime.

Using pesticides have been influenced the relation between date palm pests and there natural enemies, wherefore the Integrate Pest Management programs become more convoluted when different pests increased (Latifian, 2001; Latifian and Zaerae, 2009; Latifian , 2012). Wasps of the genus *Trichogramma* (Hymenoptera: Trichogrammatidae) are among the most commonly used groups of natural enemies because they are relatively easy to culture and are among the most important egg parasitoids of lepidoptera agricultural pests, *Trichogramma* wasps have been commercially applied as mass releases in over 32 million hectares against more than 30 key pests worldwide (Wajnberg and Hassan 1994; Smith 1996; Ayvaz and Karaborklu 2008; Pizzol *et al.* 2010, 2012).

Otherwise *T. brassicae* Bezdenko is used widespread for the control of lepidoptera pests in another studies on different plants, Alsaedi et al. (2016) have suggested that there will be good chances and new choices for putting *T. brassicae*, *T. evanescens* and *T. embryophagum* within style of valuable assets of any of the Integrate Pest Management strategies against *Tuta bsoluta* in order to reduce the use of chemicals. Furthermore, the characteristics of both host species and the host plant on which the pest acts effect on host selection by parasitoids (Chau and Mackauer, 2001; El-Wakeil ,2007; Desneux and Ramirez-Romero, 2009). Female parasites usually accept host eggs of the same size or larger than their host. The size of the original host affects the rates of natural host intrusion in the field (Nurindah *et al.* 1999). Otherwise, the performance of *Trichogramma* releases depend on the biological characteristics of the parasitoid species, strains used and on their interactions with a specific pest-plant system (Tabone *et al.* 2010; Andrade *et al.* 2011; Yuan *et al.* 2012). The biological control programs is critical when *Trichogramma* generations developing within a crop can play a pivotal role (Mills, 2010). Meanwhile, the bacterial bio-insecticide, *Bacillus thuringiensis* kurstaki (Btk) is considered as one of most powerful biocides and widespread to combat larvae of lepidopteron species (Lacey, 2001).

In the current study, the efficiency of three doses of these parasitoids in the control of *B. amydraula* in date palm was investigated in Basrah orchards. Our main goal was to determine

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how *T. brassicae* and *T. embryophagum* exploit the egg distribution of *B. amydraula* on date palm and this is the first study for use *T. brassicae* and *T. embryophagum* in control *B. amydraula* on date palm.

2.Materials and Methods

2.1.Production of the host

Insects were collected from the University of Tehran, Department of Plant Protection, Ecology and Behavior Lab (Karaj, Iran). The parasitoid, *Trichgramma brassicae* and *T. embryophagum* were mass-produced on eggs of the Mediterranean flour moth, *Ephestia kuehniella* (Lepidoptera: Pyralidae). The insect was reared in the laboratory on wheat flour in a climate chamber (25±2°C, 70% RH, L16:D8). Moths were collected daily and were confined in oviposition cages (40cm long and 18 cm in diameter) made of metal body covered with wire screen, collected eggs were used for reification and /or rearing of the parasitoid. Only mated 2–4 days old wasps were used in the experiments.

2.2.Rearing of the parasitoid

Eggs of *E. kuehniella* were glued onto cardboard cards, 6x4 cm, each one contained 50, 100 and 200 eggs. Rearing of *T. brassicae* and *T. embryophagum* conducted in glass jars 21cm high and 10 cm in diameter covered with muslin cloth kept in position by means of rubber bands. Six cards of newly deposited eggs (one day old) were confined in each glass jar with two cards containing parasitized eggs witch give rise to parasitoid adults within 24 hours. Rearing of *E. kuehniella* , *T. brassicae* and *T. embryophagum* took place at a temperature of 25±2°C, 70% RH, L16:D8.

2.3.Releasing of the parasitoid

Each card containing parasitized eggs (50, 100 and 200 eggs). A piece of cardboard 6 x 8 cm was bound to make an envelope like which was clipped with a double piece of thread by means of a stapler. This envelope protects the parasitized eggs from predators as well as from sun radiation. Each releasing envelope contained one small piece of cardboard (50, 100 and 200 eggs) having three different ages of parasitized eggs so that the parasitoids emerged in three

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waves at three – day intervals. Releasing took place before sun set to avoid sun heat (El-Dakroury et al., 2002).

2.4.Formulation of *Bacillus thuringiensis* (Bt)

The Bt formulation used in the experiments was *Bacillus thuringiensis* sbspecies kurstaki (10^6 cell/g) WP, Biolep, Biorun Company, Iran, serial dilution of Bt was prepared using 0.5g wet table powder and dissolved in 1000ml of water.

2.5.Field Trials

Studies were conducted in Basrah during 2018. Orchards of date palm variety Halawi represented about 5Km were used. Releasing of parasitoids was carried out during 2018 in the beginning of March. Three releasing envelopes (50, 100 and 200 eggs) were hanged on palm tree branches (2meters high).

Samples size was 10 strands / one date palm taken at random from ten replicates. In any assessment fruit having alive larvae of *B. amydraula* or dropped fruit but having the webbing silk and or faces in their places were recorded.

2.6.Statistical analysis

The parameter characteristics were provided as mean ± standard error (SE). The parameter characteristics among two *Trichogramma* species were compared using one way analysis of variance (ANOVA). Comparisons between the mean of treatments were done using the Duncan's test ($P< 0.05$). Statistical analysis was performed using the software SPSS Statistics for Windows Version 21 (SPSS 2012), and for infestation figures and reduction % was calculated based on Abbott (1925).

3.Results and Discussion

The results of releasing three densities of either *Trichogramma brassicae* or *T. embryophagum* for control of *B. amydraula* have been summarized in Fig. 1 revealing that the highest control percentage (58%) of lesser date moth, *B. amydraula* infestation was achieved by release of parasitoids at rate of 200 adult per date palm tree. The results also differ significantly with that achieved (33%) by the release of parasitoids at rate of 100 parasitoids/palm tree. While,

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releasing parasitoid at the rate of 50 parasitoids/palm tree resulted in 23.5% control efficacy. In otherwise the activity rate of *T. brassicae* were 47.5, 26.4, and 17.1% when 200, 100, and 50 adult females per date palm were released in the orchard; respectively. These values of the parasitism rate of *T. embryophagum* were significantly higher than the respective parasitisim rate of *T. brassicae*, which were the densities of 200, 100, 50 parasitoid per plant (Fig. 1).

Alrubeai *et al.* (2014) released of *T. evanescens* at rate of 1000 parasitoids/palm tree were a good option to control the lesser date moth as alternative method to chemical control. Mohammad *et al.*(2011) illustrated that infestation levels of lesser date moth in Al-Anbar Governorate, Iraq, were reduced significantly after two weeks of releasing 100, 200 and 300 *T. evanescens* or *T. principium*/palm tree, with higher efficiency for *T. evanescens* and two parasitoids were effective in reducing the infestation by this pest on date fruit during both hababook and chemri stages for all release rates.

The results in Fig. 2 obtained, Elucidated ratio of mortality in which *T. embryophagum* which was low (36.05 and 36.17%) using the following respective doses of 200 and 50 parasitoids/plant respectively. Similar results were obtained releasing *T. brassicae* 40 and 41.66% using the following respective doses of 200 and 50 parasitoids /plant. Evidently, the ratio of adults emergence and number of female of *T. embryophagum* in all the releases were significantly higher than in *T. brassicae* (Figs. 3 and 4). Although, the mortality of *T. embryophagum* was constantly lower than *T. brassicae*, the difference between the mortality of these two parasitic wasps was only significant when 200 adult females per plant were released in the cage (Fig. 2). Additionally, the rate of adult emergence and the proportion of females of *T. embryophagum* were constantly higher than *T. brassicae*. However, their differences were only significant in the density of 200 and 100 adult female per plant, respectively (Figs. 3 and 4).

Gameel *et al*. (2014) showed the reduction increased of *B. amydraula* after the successive parasitoid releasing to reach the high level (75.06%) by releasing of *T. evanescens* for one time by the rate of ten releasing 20,000 parasitized eggs/4200m^2 induced a remarkable reduction in the infestation levels with the greater date moth and the lesser date.

The reduction of *B. amydraula* on date trees treated with *T. brassicae* or *T. embryophagum* as well as *B. thuringiensis* (Bt) suspension presented in Fig. 5. Significant reduction was

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observed in the *B. amydraula* on date treated with *T. embryophagum* in combination with *B. thuringiensis* (F= 54.103; df= 4; $P \leq 0.001$). In fourth treatment (T4), an average of the reduction 39.2 of *B. amydraula* were recorded 70.1.

Mohammad *et al.* (2011) illustrated that infestation levels of lesser date moth in Al-Anbar Governorate, Iraq, were reduced significantly after two weeks of the release of 300 *Trichogramma evanescens* or *T. principium*/palm tree, with higher efficiency for *T. evanescens.*

Finally, it could be useful recommended that, the release of egg parasitoid *T. brassicae* or *T. embryophagum* for one time by the rate about of 200 egg/tree gave a good results to control *B. amydraula*. Promising data were obtained and hoping carrying out more work to assure these results. This study may ensure the importance of the utilization of *Trichogramma* parasitoid to control pests in date fruits to obtain a good bio-product.

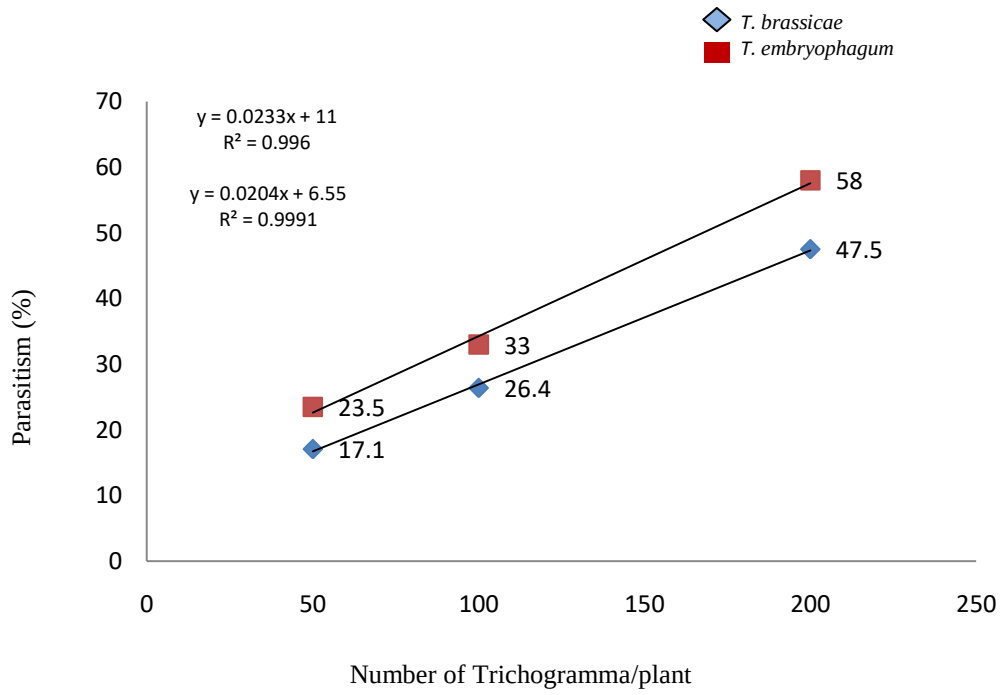

Figure 1: The parasitism according to releasing doses of *Trichogramma* sp. on the *B. amydraula* eggs.

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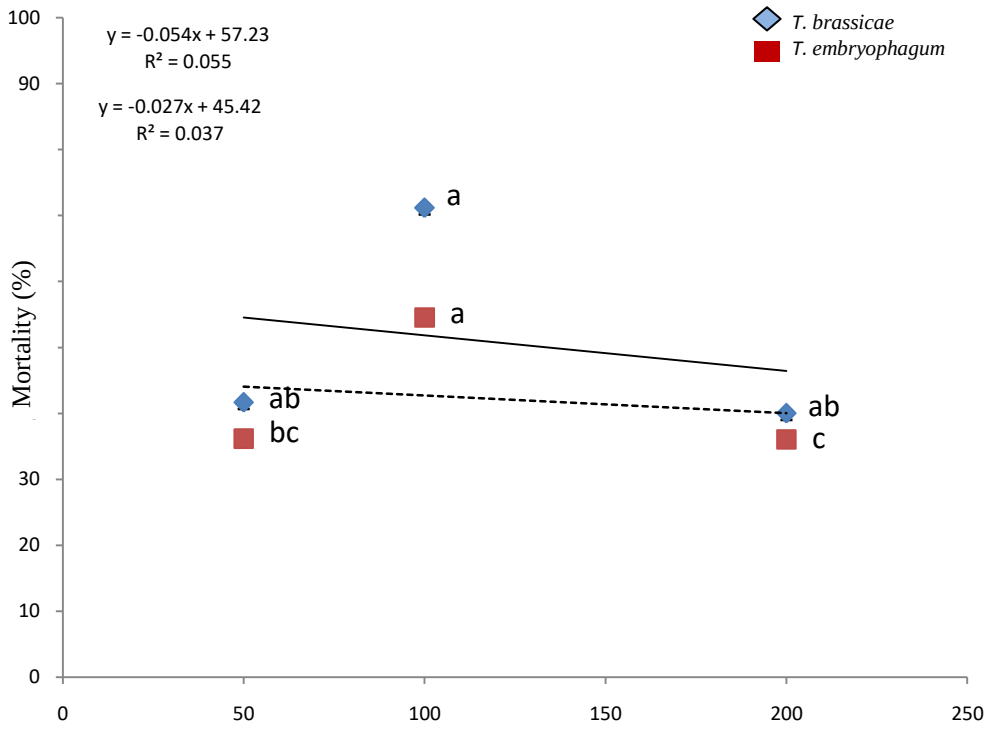

Figure 2: The mortality according to releasing doses of *Trichogramma* sp. on the *B. amydraula* eggs.

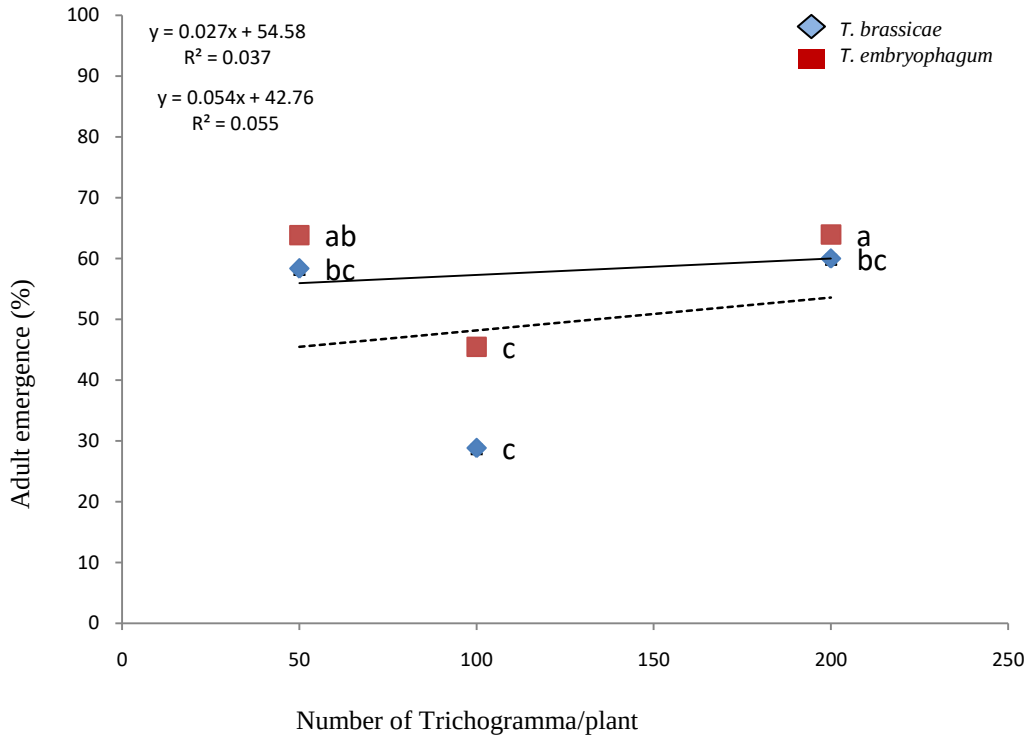

Figure 3: The Adult emergence according to releasing doses of *Trichogramma* sp. on the *B. amydraula* eggs.

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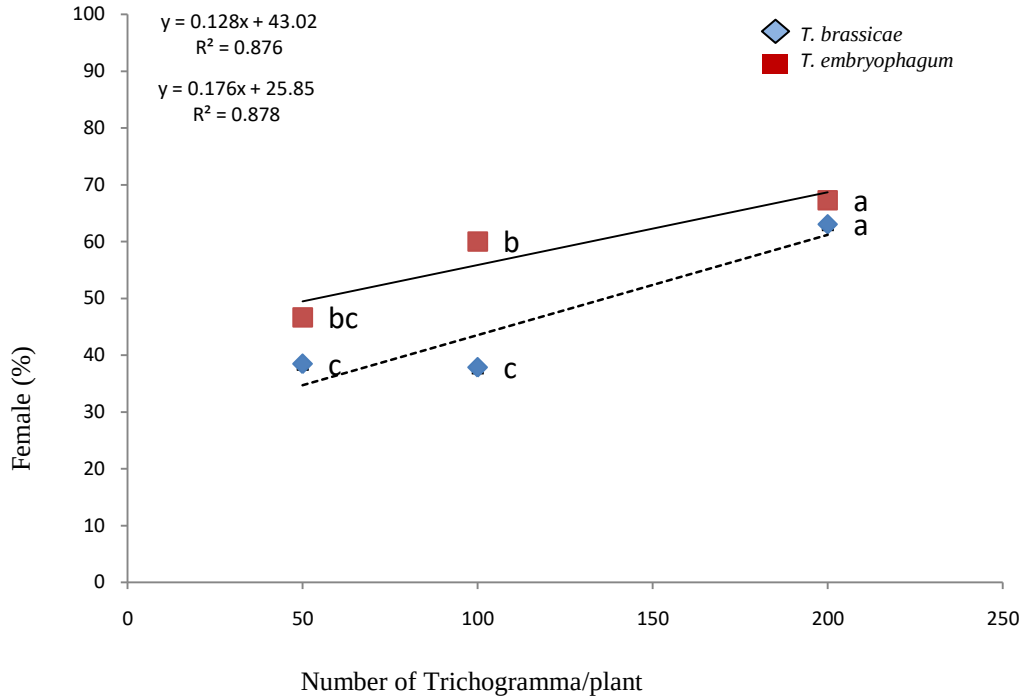

Figure 4: The number of female according to releasing doses of *Trichogramma* sp. on the *B. amydraula* eggs.

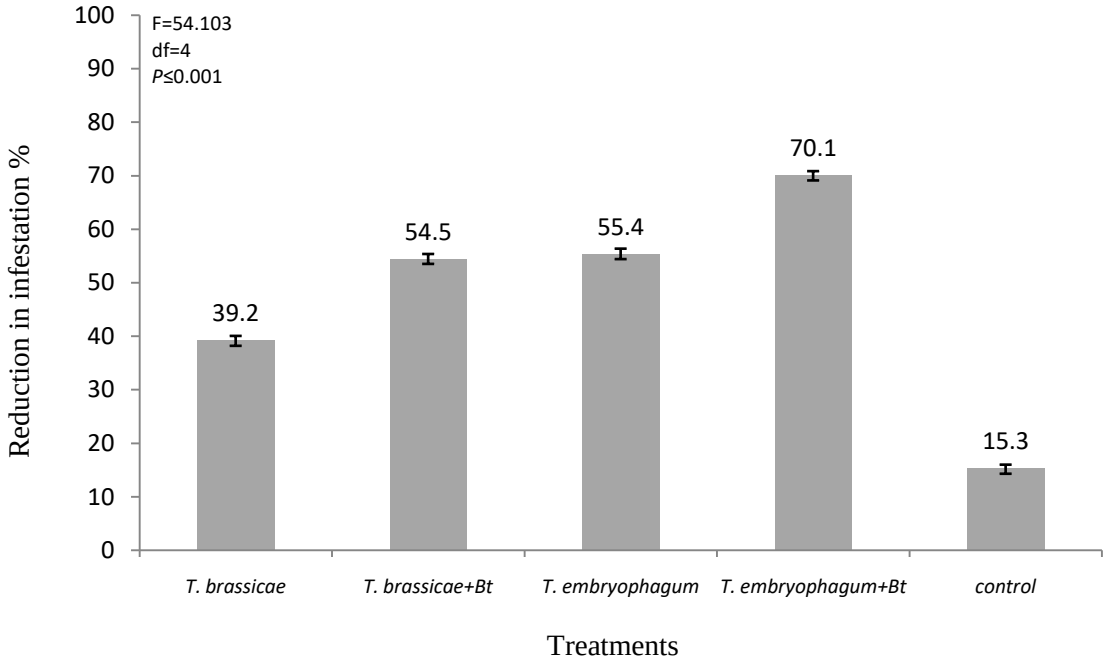

Figure 5: Mean reduction in infestation of *B. amydraula* mines per tree according to release *T. brassicae* and *T. embryophagum* with/without *Bt* in comparison to the control.

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4.Conclusion

Altogether, our results favour the use of locally-available species of *Trichogramma* wasps, for a safe and effective inclusion in Integrated pest management (IPM) programs against *B. amydraula*, Although, future studies are needed to optimize parasitoid release rates, as well as complementarities with other management procedures such as predator release, pheromone traps and insecticides, for desirable results. Our findings provide new insight for putting these parasitoids, particularly *T. embryophagum*, within the sort of valuable assets of any of the integrated pest management (IPM) strategies against *B. amydraula* in order to reduce the use of chemicals.

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