

Effects of different pesticides on the biological control agent, *Habrobracon hebetor* Say (Hymenoptera: Braconidae): A review

Abstract

I reviewed effects of different pesticides used against target insect of *Habrobracon hebetor* Say (an idiobiont and gregarious ectoparasitoid wasp), on the demography and functional response of this important biocontrol agent in Iran. For demographic studies, the young wasps of *H. hebetor* (under 24h old) were exposed to LC₃₀ of each pesticide for 24h and then their desired features are investigated. About the functional response, sixty mated females of *H. hebetor* were treated by LC₃₀ of selected pesticides for 24h. Then, among them six wasps were selected and introduced to different densities of host larvae for 24h. In conclusion, result of review confirmed that botanical and insect growth regulator (IGRs) pesticide due to the lowest negative effects on *H. hebetor* were compatible compared with the other pesticides for their successful combination in integrated pest management (IPM) designs.

Key words: Biological control, ectoparasitoid, chemical compounds.

Introduction

Habrobracon hebetor Say (Figure 1) is a valuable natural enemy on Lepidopteran pests, especially the larvae of Noctuidae and Pyralidae moths (Rafiee-Dastjerdi et al. 2008). This important biological control agent is widely used under mass rearing and releasing methods for control of mentioned harmful larvae in different crops worldwide (Abedi et al. 2012, 2014). This ectoparasitoid wasp first paralyzes its hosts and then lays on them (Sarmadi et al. 2010). The wasps' larva feed from content of its host's body and controls their population (Hooshmandi et al. 2012, 2014). Different chemical compounds are used in agriculture and horticultural crops over the world. Naturally, they can damage to biological control agents when are exposed to them (Croft 1990; Desneux et al. 2006). To date, there are good researches about the effects of different pesticides (chemical and botanical) on demography and functional response of *H. hebetor*, which will be briefly discussed in the following. The pesticide application is one of main part in IPM and it is necessary to study different aspects of them on the beneficial insects (Jepson 1989). The right combination of these agents can provide a bright future for IPM programs. Such studies can be useful in identifying safety compounds on these useful agents; but, with appropriate toxicity on their hosts for their simultaneous usage in IPM programs. I have done this mini-review with collection of different studies on this biocontrol agent, organized them, and stated their main results. Finally, a brief discussion on them is given.

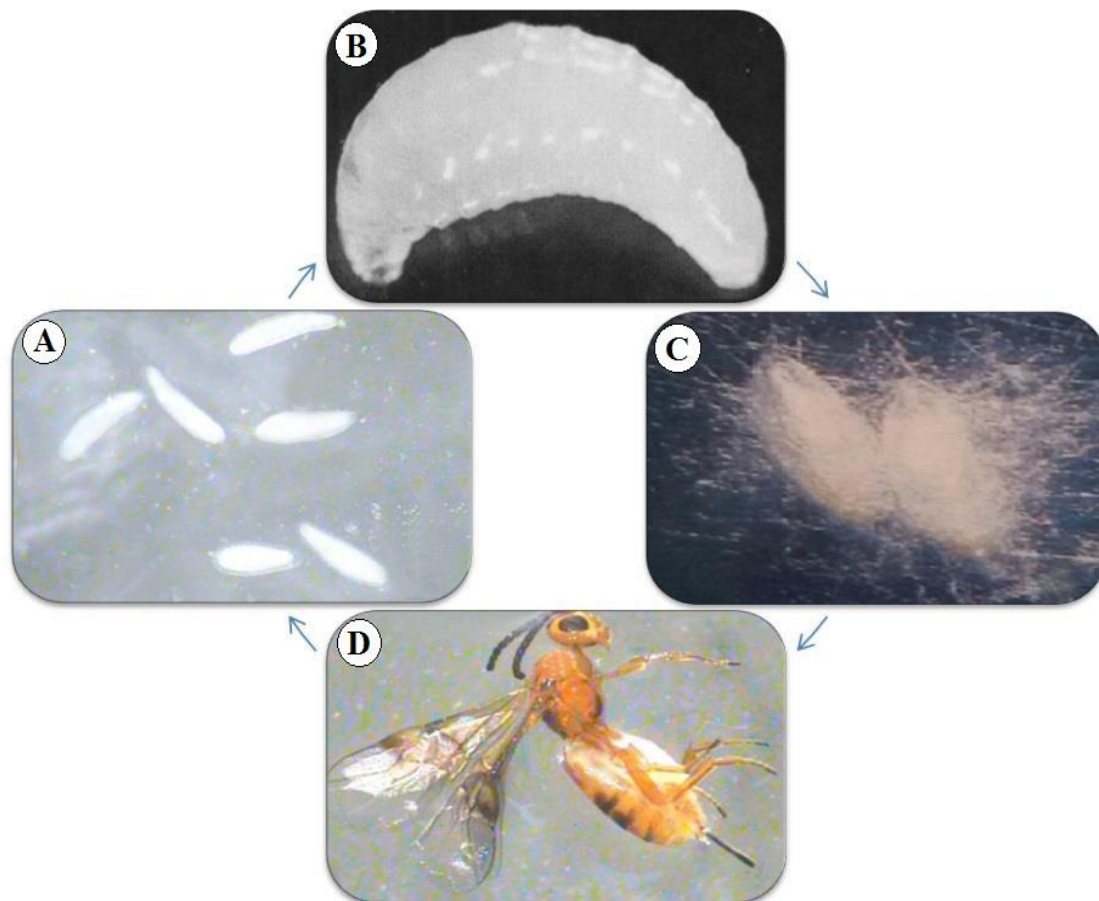


Figure 1. Different growth stages of *H. hebetor*; A: egg, B: larva, C: pupa, and D: adult wasp.

Method

Adult bioassay experiments

Initial bioassay experiments are performed with young female wasps of the parasitoid wasp by contact exposure to obtain suitable concentration range (commonly mortality rate between 20% and 80%) for final bioassays. Accordingly, toxic solutions for pesticides are prepared in one replication based on their field recommended concentration (FRC). In final bioassay experiments, series of five concentrations are prepared. In some research, suitable surfactant is used for uniform distribution of the concentrations in each pesticide treatment. The control insects are released into the Petri dishes that sprayed with distilled water. Surfaces of Petri dishes (commonly 10 cm in diameter) are sprayed with sufficient volume of each pesticide concentration and then the Petri dishes are dried under room condition. Ventilation in the Petri dishes is provided by small pores in their lids. In final bioassays, 20 young female wasps of parasitoid wasp (under 24h old) are released in each Petri dish and provided with feed source. Then, the exposure Petri dishes are transferred to $25\pm 1^{\circ}\text{C}$, $60\pm 5\%$ RH, and photoperiod of 16:8 (L: D) h. Each pesticide concentration is commonly assayed in four replications and after 24h; the number of dead wasps in each Petri dish is recorded. The bioassay analysis is done by SPSS software according to concentrations, number of total wasps, and number of dead wasps in each concentration. Accordingly, the lethal concentrations including LC_{30} , LC_{50} , and

LC₉₀ with 95% confident limit are determined by using the probit method (Rafiee-Dastjerdi et al. 2008; Abedi et al. 2012, 2014).

Demographic parameters study

In sublethal experiments, commonly one-hundred mated females are exposed to LC₃₀ of treatments in the Petri dishes. The control insects are released into the Petri dishes that are sprayed by distilled water. After 24h, twenty-five alive female are selected randomly and transferred individually to Petri dishes and are paired with untreated young males (under 24 h old). Each day, the paired wasps (twenty-five replications) are provided with feed source such as honey and seven fifth instar larvae for ovipositing of the females. The biological features of treated female wasps are recorded daily to death time of all female wasps and among their laid eggs, one-hundred eggs are randomly isolated and the numbers of produced larvae, pupae and adult wasps are recorded (Abedi et al. 2014). Finally, the data are synchronized according to the life table of Carey (1993) and use to calculate the different demographic parameters. In data analysis, jackknife method are used for all treatments and then pseudovalues are determined. In jackknife method, the data in all replications are deleted subsequently and demographic parameters are calculated for residual replications and these actions are continued to last replication (Abedi et al. 2012; Mahdavi 2013; Rafiee-Dastjerdi et al. 2008). Then, the pseudovalues are tested for normality distribution. Finally, the normal data are analyzed by one-way ANOVA and their means are compared by different statistical methods at probability level $p < 0.05$ by using SPSS or SAS softwares (Meyer et al. 1986; Maia et al. 2000).

Functional response study

In functional response, LC₃₀ of each insecticide is applied as low lethal concentration; because, this concentration previously has been recommended for the sublethal studies on different insects in IPM programs worldwide (Desneux et al. 2006). This concentration previously is obtained from bioassays analysis. According to this, surfaces of Petri dishes are sprayed with LC₃₀ of each insecticide and eighty mated emerged females of natural enemy are introduced to the Petri dishes. In the control, distilled water is used in all replications. Then, the Petri dishes are transferred to growth chamber of laboratory at 25 ± 1 °C, 60 ± 5 % RH, and a photoperiod of 16:8 (L:D)h for 24h. After this time, six alive female wasps are selected randomly and introduced individually to the Petri dishes contained densities including 2, 4, 8, 16, 32, and 64 of host larvae under mentioned conditions for 24h. Each host density is conducted in eight replications for all examined treatments. During the mentioned experiments, the wasps are supplied with the honey, and after 24 h; the numbers of parasitized (paralyzed) larvae by the treated wasps are recorded in each Petri dish. The regression models (non-linear and logistic) by using SAS software are applied for determining the functional response type and its parameters including attack rate, handling time, and theoretical maximum attack rate (Abedi et al. 2012; Rafiee-Dastjerdi et al. 2009b).

Studying and finding new methods to determine the exact effects of chemical compounds on natural enemies of plant pests is essential that effective steps must be taken in this field. Given the availability of current study methods in this field, we also used the same methods.

Discussion

Toxicity and demographic parameters

Malek-Asa and Joudaki (2016a) studied the sublethal effects of thiodicarb (larvin[®]) on the biological parameters of *H. hebetor* on the growth stages of the Mediterranean flour moth (*Ephestia kuehniella* Zeller) as its host. They found that there was a significant difference between the parasitism percentages on adult wasps exposed to sublethal concentrations of thiodicarb compared to the control. Also, the parasitism percentage of *H. hebetor* increased with decreasing the sublethal concentration of the insecticide. They showed that the parasitism percentages of wasps under thiodicarb treatment at the specified concentrations (0, 90, 86.5, 52, 39.5, and 30 mg a.i./mL) are determined as 99.33, 88.67, 90.67, 95.33, and 88.67%, respectively. According to their results, with increasing the sublethal concentrations of thiodicarb, the adult longevity was also decreased compared to the control. In another study, Malek-Asa and Joudaki (2016b) investigated the sublethal effects of trichlorofen (diptrex[®]) on the biological parameters of *H. hebetor* at different developmental stages and concluded that along with increasing the insecticide concentration, parasitism percentage decreased in the adult wasps compared to the control. Also, at concentration of 90 mg a.i./mL, the fertility of wasps increased from 31.58% to 67.94% compared to the control. Their findings showed that wasp pupa was more sensitive than larva to this insecticide.

Ebaid (2004) studied the effects of two insecticides, endosulfan and tebufenozid, on the parasitoid wasp, *Bracon brevicornis* (Wesmael). According to his results, endosulfan showed more toxicity on the adult wasp than tebufenozid through contact and digestion methods. Ebaid et al. (2017) also investigated the effects of some insecticides on the parasitoid wasp, *H. hebetor*, and found that among the studied insecticides, the lowest toxicity was related to profenofos and then runner[®] as new commercial insecticide. Also, cyfluthrin had the most adverse effects on various biological parameters such as fecundity, percentage of pupa production, and emergence of adults. Rasool Khan et al. (2005) in their studies examined the effects of insecticides from different groups on this parasitoid wasp and found that Insect Growth Regulators (IGRs) were less toxic than the other chemical insecticides on the female wasp on this parasitoid wasp. Moreover, Ahmed and Ahmad (2006) investigated the effects of two insecticides, lambda-cyhalothrin and spinosad, on *H. hebetor* under the laboratory conditions and concluded that lambda-cyhalothrin had more acute toxicity than spinosad for this parasitoid wasp.

Rafiee-Dastjerdi et al. (2008) studied the effects of diflubenzuron, hexaflumuron, profenofos, spinosad, and thiodicarb on *Helicoverpa armigera* Hübner and its parasitoid wasp, *H. hebetor*. They determined toxicity of the insecticides on *H. armigera* by artificial feeding

method as 595.05, 0.31, 3.69, 0.13, and 11.2 ppm, respectively, and by leaf disc method as >2000, 0.46, 9.55, 0.20, and 15.52 ppm, respectively. They also reported the effects of these insecticides on *H. hebetor* and LC₅₀ tested by them on female wasps was as >2000, >2000, 12.44, 15.64, and 81.04 ppm, respectively, and on male wasps was as >2000, >2000, 6.91, 11.73, and 39.40 ppm, respectively. According to their results, spinosad and hexaflumuron were less dangerous than the others due to the highest toxicity for the pest and the lowest toxicity on the parasitoid wasp, respectively. Rafiee-Dastjerdi et al. (2009a) also studied the sublethal effects of these insecticides on this parasitoid wasp, *H. hebetor*, and found that the lowest and highest gross reproductive rates (GRR) belonged to profenofos and spinosad treatments. In their study, the highest value of r_m (0.17 day⁻¹) being in the control population and among insecticides the highest value of this important parameter was in hexaflumuron treatment (0.17 day⁻¹) and the lowest being in spinosad (0.10 day⁻¹). In the study of demographic parameters, longevity of female wasps in the control population did not show a significant difference with the insecticides hexaflumuron and spinosad; but, the sex ratio was significantly affected. Rafiee-Dastjerdi et al. (2012) investigated the effects of three insecticides, imidacloprid, indoxacarb, and deltamethrin on the life table parameters of this parasitoid wasp under pupal stage treatment and found that deltamethrin and imidacloprid had the most destructive effects on the parasitoid wasp; but, the effects of indoxacarb were not obvious. Therefore, they recommended usage of indoxacarb along with release of wasps for usage in IPM programs.

Oluwafemi et al. (2009) studied the effects of biological insecticide, *Bacillus thuringiensis* Berlinger (B.T.) on *H. hebetor* for control of the Indian flour moth larvae (*Plodia interpunctella* Hübner) and found that BT and the parasitoid wasp lonely caused 41.67% and 35.35% mortality in the host larvae; but, their combination significantly increased mortality in them. Also, they found that the growth and appearance of the parasitoid wasps were sensitive to the presence of BT; but, the emergence of adult parasitoid occurred in combination less than direct treatment of parasitoid wasps with BT; However, since BT did not inhibit the growth of parasitoid wasp, they recommended the combined use of them to control of Indian moth larvae be better than their separate use. This method can be very effective in controlling this important storage pest. Sarmadi et al. (2010) investigated the effects of imidacloprid, indoxacarb, and deltamethrin on the demographic parameters of *H. hebetor* in adult treatment and concluded that the growth and reproductive parameters in the control and under insecticides treatments showed significant differences. They found that deltamethrin had more severe adverse effects on stable wasp population parameters; but, the difference between indoxacarb and imidacloprid was not significant.

Mahdavi et al. (2011) investigated the effects of carbaryl and Abamectin insecticides on *H. hebetor* and found that the intrinsic rate of growth (r_m), finite rate of growth (λ), net reproductive rate (R_0), and gross reproductive rate (GRR) were significantly affected by the insecticides. They recorded the highest and lowest r_m value in the control population and

carbaryl treatment, respectively; but, they found that the sex ratio of wasps was not affected by the insecticides treatment. The results of their studies showed that carbaryl has more destructive effects on the population of this wasp than abamectin. Mahdavi (2013) also investigated the residual toxicity of the field recommended concentrations (FRC) of imidacloprid, thiacloprid, deltamethrin, thiodicarb, carbaryl, abamectin, and spinosad on the larval stage of *H. hebetor* and showed that some insecticides were more toxic to the wasp larva than the others. He found that imidacloprid and thiacloprid had less adverse effects on the wasp and could be used as low-risk insecticides in IPM programs. Moreover, Mahdavi et al. (2013a) investigated the sensitivity of *H. hebetor* to the entomopathogenic fungi, *Beauveria bassiana* Vuillemin and *Metarizhum anisopliae* Sorokin, and found that *B. bassiana* had less lethal effects on this parasitoid wasp than any of the other isolates and none of the wasp pupa studied were not infected by the fungus. The results of their studies showed that these pathogenic fungi do not have adverse effects on the wasps and can be used in IPM programs in addition to the use of *H. hebetor*. Mahdavi et al. (2015) also investigated the effects of chlorpyrifos and spinosad insecticides on this parasitoid wasp and found that chlorpyrifos had more acute toxicity on adult and immature stages of wasps. Also, this insecticide had the most negative effects on the wasp demographic parameters and r_m value in the control population, chlorpyrifos, and spinosad treatments were determined as 0.23, 0.10, and 0.21 day^{-1} , respectively. The results showed that spinosad was less toxic to this parasitoid wasp and could be used in IPM programs along with the release of this wasp.

The lethal and sublethal effects of two commercial insecticides called as bioneem and neem guard and the insecticide cypermethrin on the demographic parameters of *H. hebetor* were studied by Abedi et al. (2014). They found the value of r_m in their treatment was as 0.143, 0.149, and 0.160 day^{-1} , respectively. Results of their studies showed that cypermethrin showed higher toxicity to the *h. hebetor* than two commercial compounds of azadirachtin. Hooshmandi et al. (2012) in their studies found that thiacloprid as a nicotinoid insecticide was slightly toxic to *h. hebetor* and could be used as a compatible insecticide with this parasitoid wasp in IPM programs. Hooshmandi et al. (2014) also studied the sublethal effects of two insect growth regulators (IGRs) including lufenuron and lufox, as well as the insecticide thiacloprid on the parasitoid wasp, *H. hebetor* and found that three insecticides decrease longevity and host search efficiency in this ectoparasitoid wasp. Faal Mohammad-Ali et al. (2014) evaluated the effects of acute toxicity of chlorpyrifos and fenpropathrin insecticides on the larval, pupal, and adult stages from *H. hebetor* under laboratory conditions and based on their results, the LC_{50} value for chlorpyrifos was 40 to 2000 times more toxic and the LC_{50} of fenpropathrin was 10 to 100 times less toxic than the FRC for this parasitoid wasp.

Jarahi and Safavi (2015) studied the sublethal effects of *M. anisopliae* on the life table parameters of the parasitoid wasp, *H. hebetor*, on chickpea caterpillar larvae (*H. armigera*) at different interval times and found that fungal infection affected the life table parameters of the parasitoid wasp and varied depending on the time of exposure. The highest and lowest r_m in

the control population and larval treatment with fungi were determined as 0.223 and 0.109 day⁻¹, respectively. Finally, their findings showed that proper time for introduction of *H. hebetor* in combination with the fungus is important for the successful management of the chickpea pod worm.

Stoianova et al. (2015) studied the effects of some biological insecticides on the parasitoid wasp, *H. hebetor*, in the laboratory and found that Spinosad was highly toxic to adult wasps while caused 100% mortality in the first day after treatment. In their study, neem-azal and dipel insecticides showed low toxicity to the parasitoid wasp and did not affect various demographic parameters such as survival, mating, fertility, and the parasitism activity. In their study, the parasitoids treated with these insecticides survived well on potato moth larvae. Tebozada et al. (2015) investigated the effects of sublethal concentrations from two nicotinoid insecticides, thiametoxam and thiacloprid, on the immature stages of parasitoid, *B. brevicornis*, and found that there was no significant difference between the effects of two compounds on the larval and pupal stages of parasitoid wasp; but, thiacloprid reduced the incidence of female wasps and greatly affected longevity of male and female wasps. therefore, they recommended the use of thiametoxam for it less harmful effects on the population of this parasitoid wasp for use in IPM programs. Shankarganesh et al. (2017) in their studies examined the effects of some insecticides on *B. brevicornis*, and found that the insecticide carbosulfan was highly toxic to this parasitoid wasp, while pymetrozine showed less toxicity on it. in addition, among three neonicotinoid insecticides, acetamiprid had the highest toxicity to wasps, followed by thiametoxam and imidacloprid, respectively. According to the results obtained by these researchers, the LC_{50} values for the insecticides were more or less as the following: carbosulfan, lambda-cyhalothrin, indoxacarb, acetamiprid, thiametoxam, imidacloprid, buprofezin, and pymetrozine. finally, they concluded that the use of selective insecticides to protect parasitoid wasps could improve the compatibility of biological and chemical control methods in IPM programs when using this parasitoid wasp.

Asadi A et al. (2018) investigated the effects of malathion on some biological parameters of *H. hebetor* and found that with increasing the toxic concentrations, percentage of wasp parasitism also decreased compared to the control. Also, production of males under the treatment of this insecticide increased compared to the control. Their research showed that the pupa was more sensitive to malathion than larva. Rezaei et al. (2018) in their studies on the lethal and sublethal effects of three insecticides, biscaya, neem-azal, and tondexir on the parasitoid wasp, *H. hebetor* found that LC_{50} values for these insecticides by contact methods were 0.18, 1.16, and 122.7 mg a.i./mL, respectively. Also, the highest life expectancy (e_x) after control was related to tondexir treatment and the highest number of fecundity being under tondexir treatment. the results of their studies showed that the use of Tondexir had the least effect on population growth parameters of this parasitoid wasp.

Matthew et al. (2018) studied the effects of direct and indirect host control by BT on different growth parameters of *H. hebetor*, one of the most important parasitoids of the rice pest,

Corcyra cephalonica Stainton in a parasitoid-host system, and found that BT intermittently affected the growth stages of the treated wasps ; but, none of them did not stop reproduction and sex ratio of the parasitoid wasps were also significantly reduced by treating the host with BT. Moreover, BT did not affect the larval and pupal stages of the parasitoid; but, the entire life cycle of the parasitoid wasp lasted under BT exposure. Such studies will help to evaluate and design appropriate strategies for IPM and will provide new ways to effectively manage storage pests such as *C. cephalonica*.

Asadi M et al. (2018) investigated lethal and sublethal effects of five commercial insecticides on the demography of parasitoid wasp, *H. hebetor* and found that that adult longevity, survival, fecundity, fertility, hatch rate, and sex ratio were negatively affected by them. Also, r_m values were obtained after treatment and were 0.27 day⁻¹ for the control versus 0.14, 0.18, 0.21, 0.19, and 0.24 day⁻¹ for the insecticides (fenvalerate, propargite, buprofezin, dayabon, and palizin), respectively. Their results showed that palizin had the lowest negative effects on the demography of *H. hebetor* and therefore was a compatible insecticide for IPM programs.

Functional response

Rafiee-Dastjerdi et al. (2009b) in studying the effects of the insecticides on the functional response of *H. hebetor* found that the functional response in the control and all insecticides treatments was type II; but, Spinosad and Hexaflumuron had the most and the least effect on the search efficiency of the wasps, respectively.

Mahdavi and Saber (2013) investigated the effects of phosphorus insecticides, Malathion and Diazinon, on the functional response of *H. hebetor* to the Mediterranean flour moth larvae (*E. kuehniella*) and found that the functional response of wasp treated by both insects and control were from type III; but, search efficiency and handling time were significantly different in the control and insecticides treatments. Also, diazinon and malathion showed the most and least adverse effects on the wasp search efficiency. They proved that Malathion is an insecticide compatible with *H. hebetor* in IPM programs. Also, Mahdavi et al. (2013b), in another study in the same field, investigated the effects of some insecticides on the functional response of the ectoparasitoid wasp, *H. hebetor*, and found that the type of functional reaction in the control and all studied insecticides was type III; but, the search efficiency in the control and abamectin treatment showed the highest value; but, chlorpyrifos and carbaryl showed the greatest effects on the handling time of wasps. Their results showed that spinosad and abamectin had less negative effects on the functional response parameters of the wasp. finally, after field studies, they found that spinosad and abamectin can be used as compatible chemical compounds with this important biological pest control agent in IPM programs.

Abedi et al. (2012) investigated the effects of three insecticides, cypermethrin, metoxyfenozide, and pyridalil on the functional response of *h. hebetor* and found that the functional response of wasps in all studied treatments and in the control population were type ii. also, in the control and cypermethrin treatment, the lowest and maximum handling time

values were also observed, respectively. They determined the highest and lowest attack rate in pyridalil and cypermethrin treatments. Faal Mohammad-Ali et al. (2015) in their studies reported that the functional response of this parasitoid wasp under treatment of chlorpyrifos and fenpropathrin insecticides was type III. They found that two mentioned insecticides had not significant negative effects on the search efficiency of wasps compared to the control; but, the handling time under their treatment increased than the control. Jarahi and Safavi (2016) investigated the effects of deltamethrin, fenvalerate, and insect growth regulators (IGRs) on the parasitism of *H. hebetor* on the Mediterranean flour moth larvae and found that the functional response in all treatments was type II; but, there was no significant difference between attack rate and handling time values in the control population compared to the mentioned insecticides.

Rostami et al. (2018) investigated the effects of neem-azal and flubendiamide on the functional response of the parasitoid, *H. hebetor*, and found that type II functional response was observed in all insecticides treatments and the control. Also, the shortest and longest handling times were observed in flobendiamide and neem-azal treatments, respectively. The highest and lowest attack rate or searching efficiency was also determined in fluobendiamide and neem-azal treatments, respectively. according to their results, flubendiamide was more compatible with this parasitoid wasp and can be used in IPM programs along with the wasp release.

Asadi et al. (2020) studied the sublethal effects of above-mentioned commercial formulations which use in chemical control of target pests of *H. hebetor*, on the functional response of this important biocontrol agent. The regression analyses indicated the functional response type II in the control, Palizin, and buprofezin and type III in fenvalerate, propargite, and dayabon. Moreover; the highest and lowest attack rates were obtained in palizin and fenvalerate treatments, respectively. In addition, the treated wasps by dayabon and fenvalerate showed the shortest and longest handling times, respectively. Accordingly, they concluded that Palizin and Dayabon due to the lowest negative effects on *H. hebetor* were compatible insecticides for combination with this biocontrol agent in IPM.

As mentioned earlier, researches about the effects of pesticides on the biological control agent, *H. hebetor* and the others, are extensive. Different researches have been studied variable aspects of pesticides on this natural enemy which some time reaching to a suitable result is complex. It can be said that feature, structure, and mode of action of selected pesticides in addition to wasp species were very effective elements about the obtained results. Although, laboratory conditions and site of experiments were also effective in this direction. As you can see, most of the studies were performed under the laboratory conditions which making a suitable decision based on them very hard; therefore field studies is need for reaching a correct decision. This subject exhibits weakness of research about this biocontrol agent. The main accepted methods IPM schedules are chemical and biocontrol methods over the world and their suitable integration is an essential component for the success of any IPM

program. In conclusion, the present mini-review indicated that different insecticides had variable effects on the ectoparasitoid wasp *H. hebetor*. Dangerous insecticides negatively affected the demographic parameters and functional response of *H. hebetor*; but, some safety compounds commonly botanicals caused the least negative effects on this parasitoid wasp compared to the control population. The present work confirmed that the botanical insecticides including had low toxic effects on this ectoparasitoid wasp compared with the chemical insecticides. This subject indicating that natural or botanical compounds were safer on the natural enemies compared than chemicals that must be considered seriously in biological control and IPM programs. Applying of effective natural enemies can change the approach to using half-dose of chemical compounds or even their complete elimination. The ultimate goal of such an issue could be to develop and implement safe control methods for plant pests, the provision of healthy human food by the production of organic products, the protection and support of natural enemies and the other desirable aspects.

Competing Interests Disclaimer

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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