# Comparative Study of Essential Oils and Insecticides on the Functional and Numerical Response of Trichogramma Pretiosum (Hymenoptera: Trichogrammatidae) in Eggs of Neoleucinodes Elegantalis (Lepidoptera: Crambidae)

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# Abstract

Reconciling chemicals and natural enemies is an attractive method for the management of *Neoleucinodes elegantalis*. This study aimed to evaluate the sublethal effects of *Origanum majorana* L. and *Copaifera officinalis* L. oils and the insecticides azadirachtin and deltamethrin on the functional and numerical response of *Trichogramma pretiosum* to different densities (2, 4, 8, 16, 32 and 64) of eggs from *N. elegantalis*. The type of functional and numerical response, search efficiency (a) and handling time (Th) were estimated by the Disc equation. Exposure to oils and insecticides influenced which type of functional response the parasitoid presented in relation to the pest, where azadirachtin had a type I response; *O. majorana*, deltamethrin and control type II and *C. officinalis* type III. Exposure to oils decreased handling time and attack rate in relation to the control; the insecticides, on the other hand, increased handling time and reduced the attack rate. For numerical response, exposure to oils and control, there was an increase in the rate of parasitized eggs in response to a greater supply of hosts. *C. officinallis* demonstrates to be more compatible when integrated with *T. pretiosum*, as it presented shorter manipulation time and higher attack rate, among the studied products.


# Introduction

*Neoleucinodes elegantalis* Guenée is an oligophagic pest that has significant economic importance in crops because it causes direct damage by severely infesting the fruits (Picanço et al. 2007, Diaz et al. 2011, EPPO 2015). Its main control tactic is chemical, with around 131 products from the most varied chemical groups being registered (pyrethroids, organophosphates, methylcarbamates, diamides, benzoylurea, neonicotinoids, oxadiazines, among others (Badji et al. 2003, AGROFIT 2019*)*.

The use of parasitoids is a viable alternative to pest control and aims to reduce the use of insecticides. Species of the genus *Trichogramma* are commonly used and parasitize lepidopteran pests in the egg stage. Its advantage lies in preventing its hosts from passing the larval stage and causing damage to the plants or fruits; in addition to having easy reproduction in alternative hosts, highly aggressive parasitism and wide geographic distribution (Witting et al. 2007, Parreira et al. 2018).

The species *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae), is among the most used parasitoids in the control of lepidopterans in several countries by inundative application in economically important agricultural crops (Parreira et al. 2018, Laurentis et al. 2019, Queiroz et al. 2020, Costa Lima et al. 2021).

One of the important behavioral aspects that must be observed when using natural enemies are their functional and numerical responses. The study of the functional response is one of the necessary resources for the adequate selection of the natural enemy for biological control programs (Menon et al. 2002, Moezipour et al. 2008).

Holling (1959, 1961, 1966) characterized three types of functional responses. In the type I response, the attack rate is constant (independent of density) until satiety is reached. In type II, the number of attacked

hosts reaches a fixed rate, and in type III, where the attack rate increases and then gradually decreases (density dependence) until it reaches an upper limit. The numerical response is commonly of key interest as it is responsible for suppressing the pest population and helping to calculate the number of parasitoids needed to regulate the estimated host population (Parween and Ahmad 2015; Kumar and Kumar 2021).

The interaction of different pest management methods, such as biological and chemical control, has been recommended in IPM (Integrated Pest Management) (Abedi et al. 2012; Asadi et al. 2018). parasitoid-host or predator-prey interactions, as in the work presented by Lima et al. (2015) where the insecticides abamectin, azadirachtin and fenpyroximate were used on *Neoseiulus baraki* (Acari: Phytoseiidae) at different densities of *Aceria guerreronis* (Acari: Eriophyidae). Results showed that attack rate was modified by abamectin and fenpyroximate, and peak consumption was reduced by abamectin. All allowed the maintenance of the predator in the field, but exposure to abamectin and fenpyroximate compromised prey consumption.

Essential oils can also cause changes in functional response parameters, for example, oils from *Allium sativum, Rosmarinus officinalis, Piper nigrum, Salvia officinalis*, and *Glycyrrhiza glabra* that were tested on the parasitoid *Habrobracon hebetor* (Hymenoptera: Braconidae) where, *P. nigrum, S. officinalis* and *G. glabra* indicated type II response, and in *A. sativum* and *R. officinalis*, type III. Furthermore, the essential oil of *R. officinalis* and the control showed the longest (0.542 h) and shortest (0.411 h) handling times, respectively. The highest (0.047 $h^{-1)}$ and lowest (0.033 $h^{-1)}$ attack rates were also recorded in the control and essential oil of *R. officinalis*, respectively, indicating changes in behavior and other parasitism activities of *H. hebetor* (Asadi et al. 2018). Thus, the present study aimed to evaluate the oils of *O. majorana* and *C. officinallis* in addition to azadirachtin and deltamethrin with *T. pretiosum*, analyzing the effects of these products on the functional and numerical response of the parasitoid.

# Materail and Methods

The present study was carried out at the Laboratory of Insect Physiology - LAFI of the Department of Animal Morphology and Physiology - DMFA of the Federal Rural University of Pernambuco - UFRPE.

The egg parasitoid *T. pretiosum* was obtained commercially from the company TopBio, Mossoró, Rio Grande do Norte, Brazil, raised in eggs of *Anagasta kuehniella* (Zeller) (Lepidoptera: Pyralidae). Obtained in the egg stage and after emergence, the parasitoids were reared in BOD laboratory conditions set at 25 ± 1°C, 60 ± 5% RH and a 12:12 h photoperiod, on *N. elegantalis* eggs as a host for activities of parasitism. Honey solution (10%) was applied as a food source for parasitoid adults.

For the investigation of essential oils and insecticides in young females of *T. pretiosum*, previously mated with an age of up to 24 hours, under eggs of *N. elegantalis*, $CL_{30}$ of *O. majorana* oils (667.90 mg $mL^{-1)}$ were used. and *C. officinalis* (111.75 mg $mL^{-1)}$ (Santana et al. 2022) and the recommended field dose for the insecticides, azadirachtin (0.6 mg/L), and deltamethrin (12.5 mg/L). The eggs were

collected and transferred to cardboard and glued with arabic gum. Subsequently, these eggs were immersed in the solutions for five seconds and left to dry at room temperature for 30 minutes. The established egg densities were 2, 4, 8, 16, 32 and 64 eggs/replication. Then, adult females of *T. pretiosum* were identified with the aid of a stereomicroscope and individualized in glass tubes without the presence of the host and immediately sealed with parafilm to prevent the adults from escaping. A drop of honey (10%) was used to feed the females. They were then transferred to the BOD adjusted to 25 ± 1°C, 60 ± 5% RH and photoperiod of 12:12 h for 24 h. Each treatment of essential oils, insecticides and the control (distilled water) were done in twenty repetitions, with each repetition consisting of one female, totaling 120 females/repetition. After this period, the females were removed and parasitism (eggs with dark coloration) was observed after 4 days with the aid of a stereomicroscope.

Disc Holling's Eq. (1959) referring to the functional response of different natural enemies was used in this study as explained below: $N_a = aT_t N_0 (1 + aT_h N_0)$. Where: $N_a$ = number of hosts attacked by *T. pretiosum*, $N_0$ = different host densities (2, 4, 8, 16, 32 and 64 eggs of *N. elegantalis)*, $T_t$ = total experiment time (24 hours), a = attack rate (host discovery area) by *T. pretiosum*, Th = handling time of *T. pretiosum* for its host. Linear regression models were applied to determine types of functional response and to estimate parasitoid attack rate and handling time under different treatments and control, respectively, using SAS software (SAS Institute 2002). The numerical response was estimated through linear regressions between the number of offspring produced and the number of hosts offered to the parasitoid. Comparison between regressions was performed using the confidence interval of the angular coefficients of the regressions.

# Results

Treatment with *C. officinallis* oil showed a type III response and azadirachtin provided a type I functional response to *T. pretiosum* parasitoids in *N. elegantalis eggs.* The essential oil of *O. majorana*, the insecticide deltamethrin and the control provided the parasitoids with a type II functional response, evidenced by the negative value of the linear coefficient and the significant probability value (Table 1).

Table 1
Holling´s Disc Equation and type of functional response of *T. pretiosum* parasiting *N. elegantalis*

| treatments | Holling's Disc Equation | $\chi^2$ | DF | *P* | Linear regression coefficient | FR² – |
|---|---|---|---|---|---|---|
| | | | | | ¹L ( *P* ) | |
| Control | $y = \frac{\exp[(-0{,}00015x^3)+(0.0147x^2)-(0.337x)-0{,}78]}{1+\exp[(-0{,}00015x^3)+(0.0147x^2)-(0.337x)-0{,}78]}$ | 922.59 | 116 | < 0.0001 | -0.337 (< 0.0001) | II |
| *O. majorana* | $y = \frac{\exp[-(0.0128x)-0{,}92]}{1+\exp[-(0.0128x)-0{,}92]}$ | 1043.33 | 117 | < 0.0001 | -0.0128 (< 0.0001) | II |
| *C. officinalis* | $y = \frac{\exp[-(0{,}0004x^2)+(0.0330x)-1{,}43]}{1+\exp[-(0{,}0004x^2)+(0.0330x)-1{,}43]}$ | 1119.42 | 117 | < 0.0001 | 0.0330 (0.0039) | III |
| Azadirachtin | $y = \frac{\exp[-(0.0159x)-3{,}86]}{1+\exp[-(0.0159x)-3{,}86]}$ | 105.37 | 118 | 0.7910 | -0.0159 (0.0564) | I |
| Deltamethrin | $y = \frac{\exp[-(0.0223x)-3{,}72]}{1+\exp[-(0.0223x)-3{,}72]}$ | 108.26 | 118 | 0.7286 | -0.0223 (0.0119) | II |

$\chi^2$ – chi square

DF – Degrees of freedom

P - Probability

FR – Functional response

The number of parasitized hosts for *C. officinalis, O. majorana* and control increased as host density increased. For the graph of average proportions observed for *C. officinalis* it initially increases and then decreases and in *O. majorana* there is a decrease as the density increases. For azadirachtin and deltamethrin the average number of parasitized eggs and the proportion of parasitized hosts showed the lowest values in all densities. (Fig. 1A and B)

The handling time was estimated at 1.344 h and attack rate at 0.00684 for the control. The essential oil of *O. majorana* provided manipulation time of 1.5588 h and attack rate of 0.0148. As for the essential oil of *C. officinalis*, the handling time was 0.3622 h and the attack rate estimated at 0.0132 (Fig. 2). As for the insecticides used, azadirachtin provided handling time of 28.7119 h and attack rate of 0.000823 and deltamethrin handling time of 48.2927 h and attack rate of 0.00124 (Fig. 2).

Regardless of exposure to oils, the number of offspring produced increased as the host supply increased. For all treatments, the number of offspring produced fitted the linear regression model, where at least 84% of the observed variations are explained by the model (Fig. 3).

# Discussion

The results of the present study showed that exposure to essential oils and insecticides differed in the type of functional response. Studies have indicated that type II and III responses are more frequent among parasitoids (Paes et al. 2018; Rostami et al. 2018; Ranjbar et al. 2018, Saber et al. 2020).

In the type I response, parasitism increases linearly with the increase in their density. The identified type II functional response suggests that *T. pretiosum* parasitoids increased their parasitism rate when host density increased, until they reached maximum reproductive capacity (Fernández-Arhex and Corley 2003; Jarrahi and Safavi 2016; Paes et al. 2018) therefore, there is a physiological reduction in the search rate over time as the eggs are successively depleted (Mills and Lacon, 2004).

*T. brassicae* (Hymenoptera: Trichogrammatidae) exposed to insecticides, fipronil and diazinon revealed a type II functional response in the control and fipronil, and type III to diazinon (Saber et al. 2020). Two botanical insecticides, Dayabon® and Palizin®, were evaluated on the functional response of *Lysiphlebus fabarum* (Hymenoptera: Braconidae) and the results revealed a type II functional response in all experiments, including the control (Jam 2018); these same botanical insecticides were also evaluated for the parasitoid *Habrobracon hebetor* (Hymenoptera: Braconidae), where Palizin® showed a type II response and Dayabon® type III (Asadi et al. 2020).

It is assumed that parasitoids that present a type III functional response have better opportunities to regulate their host than type II parasitoids (Ghorbani et al. 2019), however, the functional response is one of the characteristics considered related to parasitoid success, only the shape of the curves of the types of functional response cannot be attributed to the performance of the parasitoids in biological control, other aspects such as, time and rate of release, quality and quantity of parasitoids, climatic conditions must be considered (Rostami et al. 2018 ) .

The functional response relies on two important aspects, including search efficiency (a) and manipulation time (Th). The rate of these parameters and the type of functional response in predators and parasitoids are influenced by different factors (Farrokhi et al. 2010; Milanez et al. 2018). One is the sublethal concentrations of chemicals used to control insect pests. In this study, *O. majorana* and *C. officinallis* showed sublethal effects on *T. pretiosum* functional response parameters. Wasps exposed to the oils had longer and shorter manipulation times for *O. majorana* and *C. officinallis*, respectively, compared to the control. In the insecticides azadirachtin and deltamethrin, there was an increase in handling time and the attack rate was lower for both insecticides used, when compared to the control.

The reduction in handling time causes an increase in oviposition rates due to exposure to oils, compared to the control. Handling time includes time to find the host, oviposition, rest, and time to ingest water or sap. On the other hand, an increase in the handling time of *T. pretiosum* in the control and insecticides may be due to the prolongation of each of the items mentioned, and consequently, low rates of parasitism. (Sule et al. 2014; Jam and Saber 2018). According to Jarrahi and Safavi (2016), azadirachtin had no significant adverse effect on functional response parameters (“a” and “Th”) in the parasitoid *H. hebetor*, while deltamethrin and fenvalerate significantly decreased the handling time of *H. hebetor.*

The attack rate, which represents the host's search efficiency, in azadirachtin was the lowest in all treatments, indicating that this important parameter was reduced by the tested insecticide, which is a negative aspect and may be enough to compromise the parasitism potential, since this parameter considers the proportion of successful attacks during the search (Oliveira et al. 2006).

The shortest handling time and high search efficiency recorded for *C. officinallis* can be considered a good result. Furthermore, short handling times and high search efficiency lead to optimal foraging of parasitoids and population stability between parasitoids and hosts (Paes et al. 2018).

The functional response of parasitoids to host population density is also based on reaction to host kairomones (Reznik and Umarova 1991). In the natural environment, insects are attracted by odors, which are usually mixtures of chemicals. In these mixtures, there are compounds that are considered key components and can sometimes cause attraction (Zhu et al. 2016). As the number of eggs increases, the concentration of kairomones increases and, consequently, the percentage of females that parasitize (Reznik and Umarova 1991).

In this study, handling the eggs to separate them according to each density may have influenced the low parasitism, the presence of host body scales left on the eggs, emit volatile compounds, which influence the attractiveness and behavior of the parasitoid. The volatile compounds of substances accumulated on or close to the eggs during oviposition or to fix them to the substrate can act in the short-distance attraction (Carneiro et al. 2006).

The oils tested in sublethal concentrations may have triggered an olfactory response, helping the parasitoids in the process of locating the host, reducing handling time, thus differentiating them from other treatments.

Some plant species (e.g. tomatoes) are known to naturally release specific compounds such as sesquiterpenes, which significantly influence the attraction of *Trichogramma* species, which uses chemical cues as a search strategy to increase parasitism in eggs (Rani et al. 2017; Gontijo et al. 2019; Souza et al. 2021). *C. officinalis* and *O. majorana* oils are mainly composed of sesquiterpenes and monoterpenes, respectively (Arruda et al. 2019; Santana et al. 2022). It is possible that these chemical products combined with the egg could help localize the parasitoid to the eggs, however, such a statement would require further studies.

Additionally, in the numerical response, a population with a large availability of hosts will have a greater chance of surviving and successfully reproducing, which will lead to a population increase (Solomon 1949). Among some aspects, the increase in the rate of parasitized eggs in the treatment with the oils, as a response to the greater supply of hosts, suggests that the parasitoid has the potential for population regulation of the pest (Godfray 1994). As for exposure to insecticides, it is suggested that this parasitoid could not regulate the pest at high densities; being a feature of the type of functional response found for azadirachtin (Type I) where the rate of parasitism is kept constant regardless of host density (Chen et al. 2008).

## Conclusions

Studies on the effects of essential oils and insecticides on the functional and numerical response of *T. pretiosum* are a useful tool for predicting its success in IPM programs. Our results suggest that among the tested oils and insecticides, *C. officinalis* oil in sublethal concentrations was more promising for increasing the attack rate and reducing handling time, contributing to a better performance of *T. pretiosum*, not demonstrating adverse effects, and may be recommended for simultaneous use with the parasitoid.

## Declarations

**Conflict of interest:**

There is no conflict of interest.

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# Figures

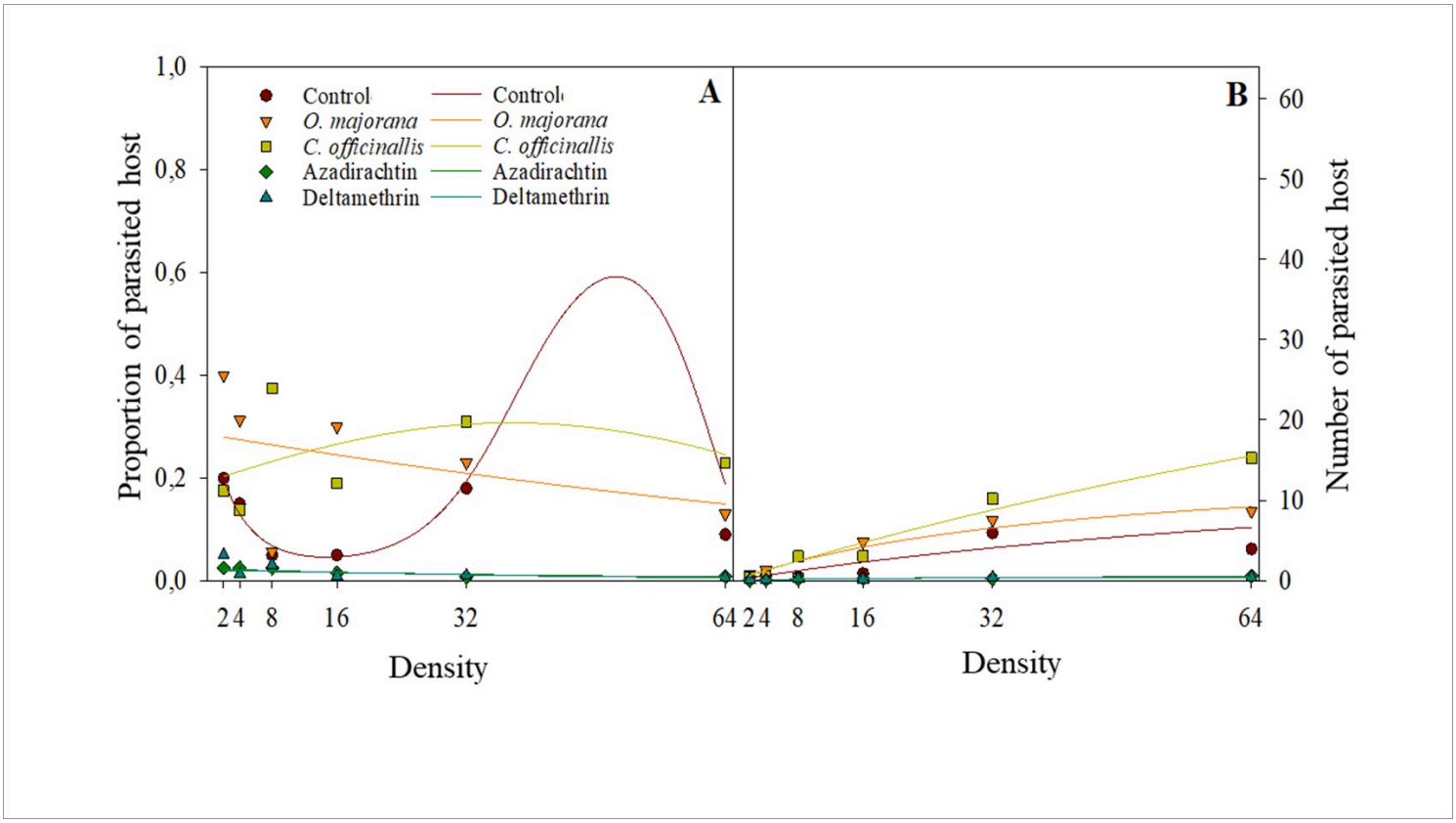
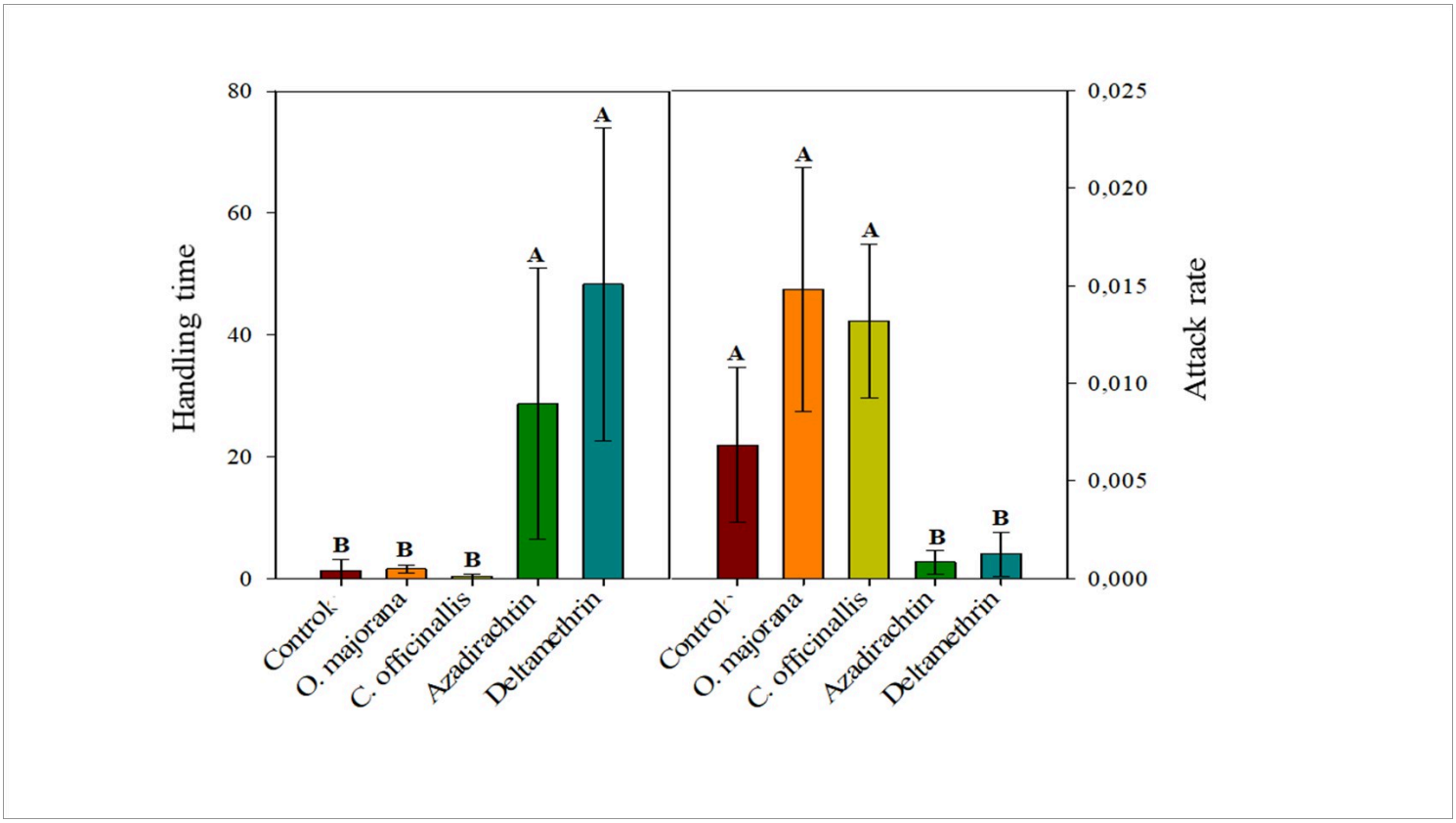


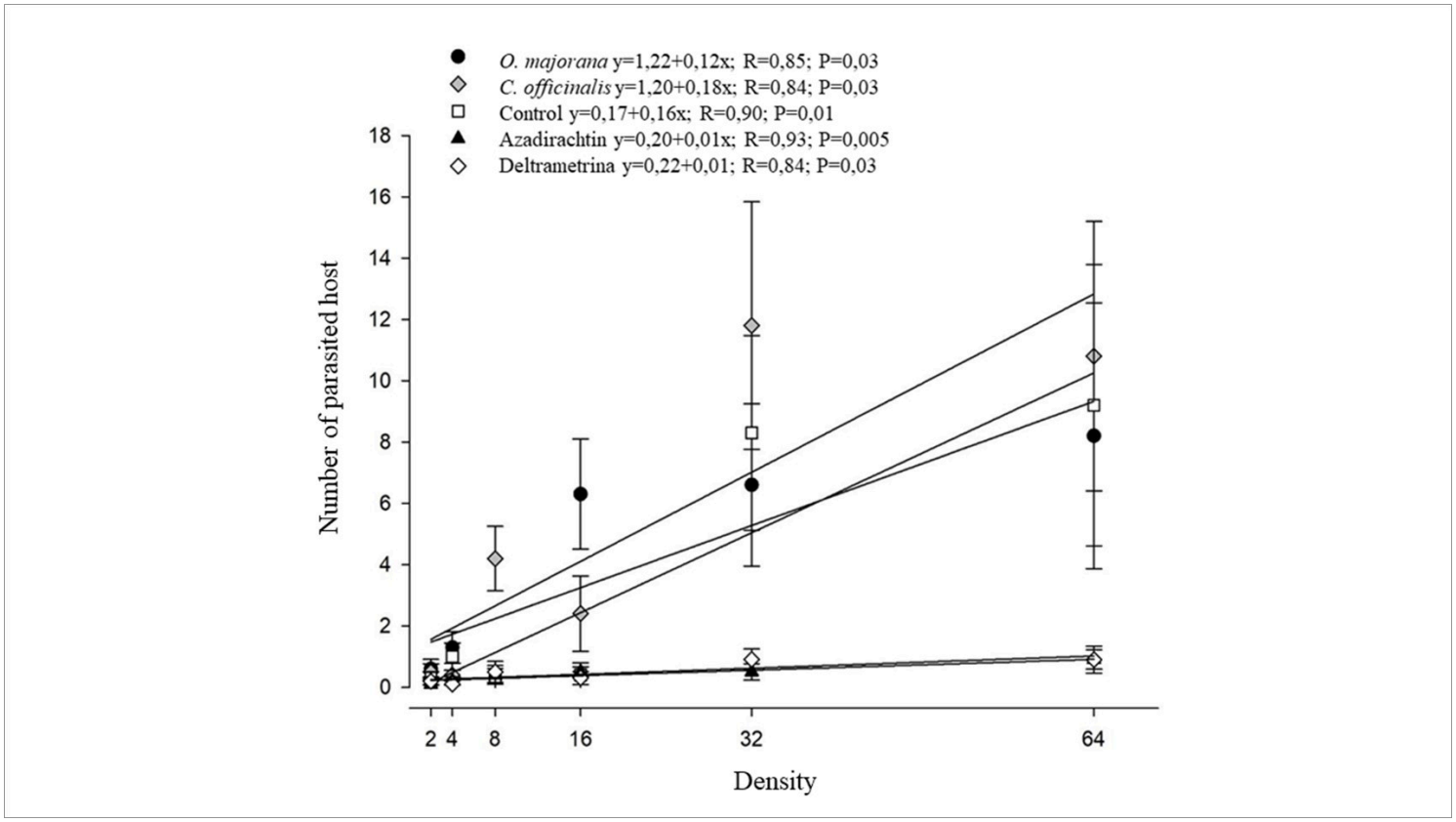

**Figure 1**

Functional response curves of *T. pretiosum* parasitizing increasing densities of *N. elegantalis eggs* after exposure to two essential oils and two insecticides (A) and proportions of parasitism of eggs by the parasitoid (B). The lines represent the values estimated by the functional response from the Holling equation




## Figure 2

Attack rate (in units of hosts parasitized by the parasitoid per unit of search time) (A) and handling time (average time for parasitism of hosts) (B)




## Figure 3

Average number of eggs parasitized by *T. pretiosum females* exposed to oils and insecticides. Relation calculated by regression analysis using a linear model