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Extracts of *Azadirachta indica*, *Tagetes erecta* and *Jatropha curcas* resin control the attack of *Carmenta foraseminis* on *Theobroma cacao* fruits

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Abstract: The aim of this work was to determine the effect of *Azadirachta indica* and *Tagetes erecta* leaf extract, as well as *Jatropha curcas* resin extract on the control of *Carmenta foraseminis* larvae in *Theobroma cacao* fruits. The study was conducted using a complete random block design (CRBD) with 6 treatments (T1:0%, T2:10%, T3:20%, T4:30%, T5:40% and T6:50%), 3 replicates and 10 fruits per experimental unit. The variables evaluated were: number of hatched larvae-NHL and number of attacked fruits. Extracts from *Azadirachta indica*, *Tagetes erecta* leaves and *Jatropha curcas* resin at concentrations greater than 40% were efficient in the control of *Carmenta foraseminis* in *Theobroma cacao* fruits in the fruiting phase. Further studies should be carry out to determine the concentration of secondary metabolites in *Azadirachta indica*, *Tagetes erecta* leaves and *Jatropha curcas* resin at different maturation stages for a more efficient use in the control of the insect pest.

Keywords: larvae, biocidal plant, leaves, residual effect.

Extratos de *Azadirachta indica*, *Tagetes erecta* e resina de *Jatropha curcas* controlam o ataque de *Carmenta foraseminis* em frutos de *Theobroma cacao*

Resumo: O objetivo deste trabalho foi determinar o efeito dos extratos de folhas de *Azadirachta indica* e *Tagetes erecta*, bem como da resina de *Jatropha curcas* no controle de larvas de *Carmenta foraseminis* em frutos de *Theobroma cacao*. O estudo

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foi conduzido em delineamento, em blocos casualizados (DBC), com 6 tratamentos (T1:0%, T2:10%, T3:20%, T4:30%, T5:40% e T6:50%), 3 repetições e 10 frutos por unidade experimental. As variáveis avaliadas foram: número de larvas eclodidas-NLE, e número de frutos atacados. Os extratos foliares de *Azadirachta indica*, *Tagetes erecta* e resina de *Jatropha curcas*, em concentrações superiores a 40% foram eficientes no controle de *Carmenta foraseminis* no cultivo de *Theobroma cacao*, na fase de frutificação. Recomenda-se a realização de novos estudos para a determinação da concentração de metabólitos secundários em folhas de *Azadirachta indica*, *Tagetes erecta* e na resina de *Jatropha curcas*, em diferentes estágios de maturação, para utilização mais eficiente no controle do inseto-praga.

Termos para indexação: larvas, planta biocida, folhas, efeito residual.

Introduction

Cocoa (*Theobroma cacao*) is a plant that belongs to the Malvaceae family and grows in tropical climates of Africa, Oceania, the Caribbean and Latin America. In Peru, it is grown in 16 of the 24 regions of the country. In 2020, production was 152 thousand tonnes, which represented increase of 6.94% compared to 2019. The regions where the highest production was observed were San Martín with 10.87%, Junín with 7.73%, Ucayali with 27.44%, Huánuco with 7.40% and Cajamarca 24.05%. In 2020, 67 thousand tonnes were exported to the Netherlands (11 thousand tons), Belgium (10 thousand tons), the United States of America (10 thousand tons), Indonesia (9 thousand tons) and Malaysia (4 thousand tons). For this reason, the International Cocoa Organization (ICCO) considers Peru to be the second world's organic cocoa producer (INEI, 2021).

However, cocoa cultivation in the Peruvian Amazon presents problems with pests and diseases that are the causes of low production performance. Delgado et al. (2017) studied pests in Peru and determined that cocoa plantations had 62% infestation. In the same sense, Andres et al. (2018) estimated that the effects of pests and diseases caused loss of up to 40% of global productivity. Among the most limiting pests for *Theobroma cacao* cultivation, *Carmenta foraseminis* stands out.

The insect deposits its eggs on the surface of fruits; after hatching, larvae penetrate the bark to the placenta, atrophying the grains and finally feeding on seeds (Navarro et al., 2004). The pest causes severe damage, reducing fruit productivity and quality, which results in production losses between 10% and 100% (CARABALÍ et al., 2018).

To reduce the attack of *Carmenta foraseminis*, the National Agrarian Health Service (SENASA, 2016) recommends cultural control through the collection and destruction of infested fruits in order to interrupt the life cycle of the insect, since they may have eggs, larvae and pupae. On the other hand, traps and attractants can also be used (CARABALÍ-MUÑOZ et al., 2021).

Bartolome (2018) reports that chemical products can be used; however, this practice is not recommended because chemicals decrease the population of pollinators. Likewise, they can harm the quality of honey due to the presence of residues from insecticides (SILVA et al., 2007). Furthermore, the excessive use of chemicals generates soil deterioration and serious consequences for the environment and the health of farmers and consumers (LÓPEZ, 2019). In addition, increasingly resistant organisms may emerge; therefore, it is a concern for researchers and farmers. In this sense, it is urgent to investigate safer control alternatives to reduce the environmental impacts produced by traditional agricultural production models.

The use of plant extracts has demonstrated efficiency in the control of pests of economic importance. In this sense, there is a growing interest in their use as an alternative to replace synthetic chemical pesticides (FLORES-VILLEGAS et al., 2019). Roark (1947) reported about 1200 species with this potential and more recently, Salazar (1997) indicated that 2000 species are known for their insecticidal potential. Costa et al. (2004) pointed out that, in the middle of the 20th century, these substances were widely used to control insects, mainly in tropical countries.

Thus, it is evident that certain substances of plant origin are presented as promising alternatives to control insects in crops because they act synergistically, presenting insecticidal, attractant, and repellent characteristics, among others, to be used in integrated pest management (NAVARRO-SILVA et al., 2009). Iannacone et al. (2017); Guerra-Arévalo et al. (2018) reported that in Peru, there are more than 300 plants with insecticidal properties, among them, *Azadirachta indica, Jatropha curcas* and *Tagetes erecta* L.

Azadirachta indica belongs to the meliaceae family, its active ingredient is isazaridactin, and has pesticide and repellent effect on pest insects (ISEA et al., 2013).

Jatropha curcas belongs to the euphorbiaceae family. Its leaves, bark and stem contain different compounds such as flavonoids apigenin and its glycosides vitexin and isovitexin, sterols stigmasterol, saponins, steroids, tannins, glycosides and alkaloids (IGBINOSA et al., 2009).

Tagetes erecta L. has secondary metabolites (flavonoids, terpenoids and tannins) in extracts from leaves and flowers; therefore, it is attributed antibacterial activity to combat diseases in animals and humans caused by pathogenic bacteria (CAMACHO-CAMPOS et al., 2019). Therefore, the aim of this work was to determine the effect of extracts from *Azadirachta indica* and *Tagetes erecta* leaves and *Jatropha curcas* resin on the control of *Carmenta foraseminis* larvae in *Theobroma cacao* fruits.

Materials and methods

Study area location

The study was carried out on plots of organic cocoa producers, between the months of February and May 2022 in the district of Tabalosos, region of San Martín. It is located at UTM coordinates of 9294470N and 0318853E, and from 562 to 640 meters above sea level. The area is characterized by having humid tropical climate with rainfall, average annual temperature and relative humidity of 1200 mm, 26ºC and 77%, respectively, with two seasons: the dry season between June and September and the rainy season from October to May (SENAMHI, 2021).

Preparation of extracts

The preparation of extracts followed methodology proposed by Sánchez-Choy et al. (2015). In this sense, whole, healthy and disease-free leaves were collected from 6-year-old *Azadirachta indica* and 3-month-old *Tagetes erecta* plants from the IIAP forest nursery located in Bello Horizonte. Subsequently, leaves were washed with running water, dried, weighed and placed in an oven at 76 °C for 48 hours until constant weight was reached. Dried leaves were cut and ground in a Wiley type mill until obtaining fine powder of 0.01 mm. Then, the stock solution was prepared by diluting 100 g of powder in 1 L of distilled water.

Subsequently, the solution was boiled for 20 minutes in order to extract active compounds from *Azadirachta indica* and *Tagetes erecta* leaves. Finally, the extract was filtered through nylon screen and stored in tightly closed bottles in dark environment to avoid degradation and loss of extract quality. *Jatropha curcas* resin was collected between 5 and 8 a.m. from branches and leaves located in the crown of 4-year-old plants from the IIAP forest nursery located in Bello Horizonte. To prepare the concentrations of each species, *Azadirachta indica* and *Tagetes erecta* extract and *Jatropha curcas* crude resin were used.

Experimental design

The study was conducted in three experiments under a completely random block design (CRBD), with 6 treatments (T1:0%, T2:10%, T3:20%, T4:30%, T5:40% and T6: 50%) of *Azadirachta indica* (neem) and *Tagetes erecta* (rosa sisa) extract and *Jatropha curcas* resin (white pine nut), all distributed in three replicates and with 10 *Theobroma cacao* fruits per experimental plot.

Test setup

Treatments were arranged in the *Theobroma cacao* plantation at a distance of 10 meters each in order to avoid the edge effect; then, fruits were cleaned with wet cloth and paper towel, and then protected with mallin® mesh to avoid the oviposition of *Carmenta foraseminis* butterflies and thereby achieve better experimental control. Subsequently, in the afternoon (03:00 p.m.), treatments were applied for 9 consecutive days, and in the first 3 days, they were applied with mesh to ensure the residual effect, and in the last 6 days, treatments were applied without mesh, so that fruits are exposed to the attack of the pest and also to make evaluations easier. The application of treatments was manual carried out using fumigating backpack sprayer.

Evaluated features

The number of hatched larvae (NHL) and the number of attacked fruits (NAF) was evaluated. Evaluations were manually carried out during the six evaluation days. For a better evaluation of characteristics, magnifying glass was used, and evaluations were carried out in environment with high luminosity.

Data analysis

Data were tabulated in Microsoft Excel and submitted to assumptions of homogeneity of variances and data normality using the Bartlett and Shapiro Wilk methods, respectively. Being normal and homogeneous, data were submitted to analysis of variance using the F test at 5% probability and the means of data were analyzed by polynomial regression ($p \leq 0.05$) using the Infostat software (Di-rienzo et al., 2016).

Results and discussion

According to the analysis of variance in the three trials, it was determined that the different *Azadirachta indica*, *Jatropha curcas* and *Tagetes erecta* extract concentrations caused significant statistical differences ($p \leq 0.05$) on the number of hatched larvae and number of *Theobroma cacao* fruits attacked by *Carmenta foraseminis* during the six days of evaluation.

In this sense, Figures 1 and 2 show that the hatching of larvae and the number of attacked fruits presented linear and quadratic trend due to the effect of different *Azadirachta indica* concentrations during the six days of evaluation. On the other hand, it was observed that, in *Theobroma cacao* fruits not treated with the extract, higher number of hatched larvae and attacked fruits was observed; however, concentration of 50% was more efficient in controlling the pest.

In general, it was observed that, from the third day of monitoring, the number of hatched larvae increased in all treatments except for the 50% concentration, since it caused constant effect. Therefore, it could be concluded that the residual power of the *Azadirachta indica* biocidal extract decreased more emphatically at lower concentrations; however, at higher concentrations, the residual power remained longer (Figure 1 and 2).

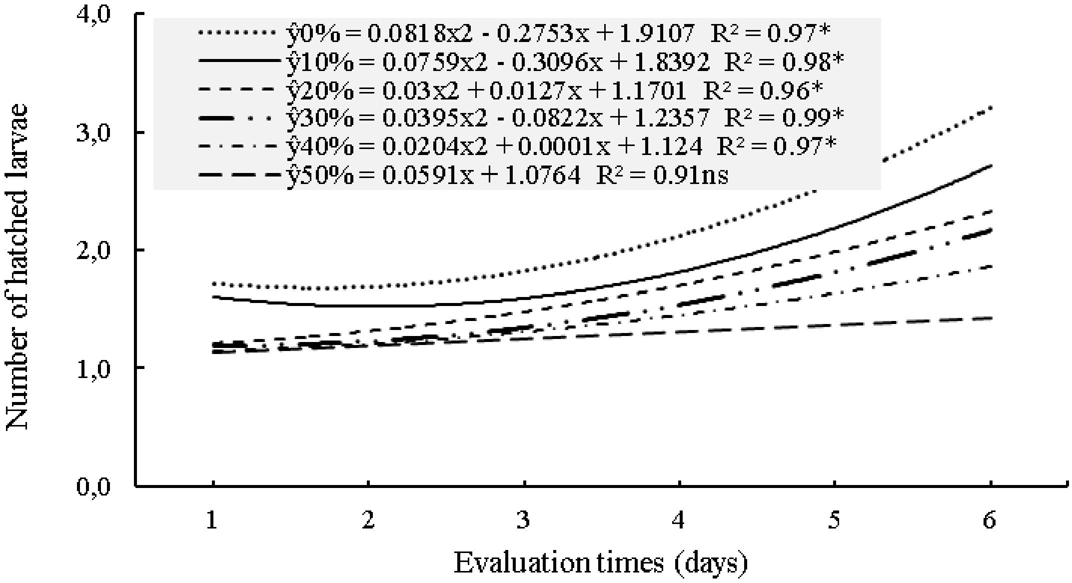

Figure 1. Number of *Carmenta foraseminis* larvae hatched on *Theobroma cacao* fruits due to the effect of different *Azadirachta indica extract* concentrations during the six days of evaluation.

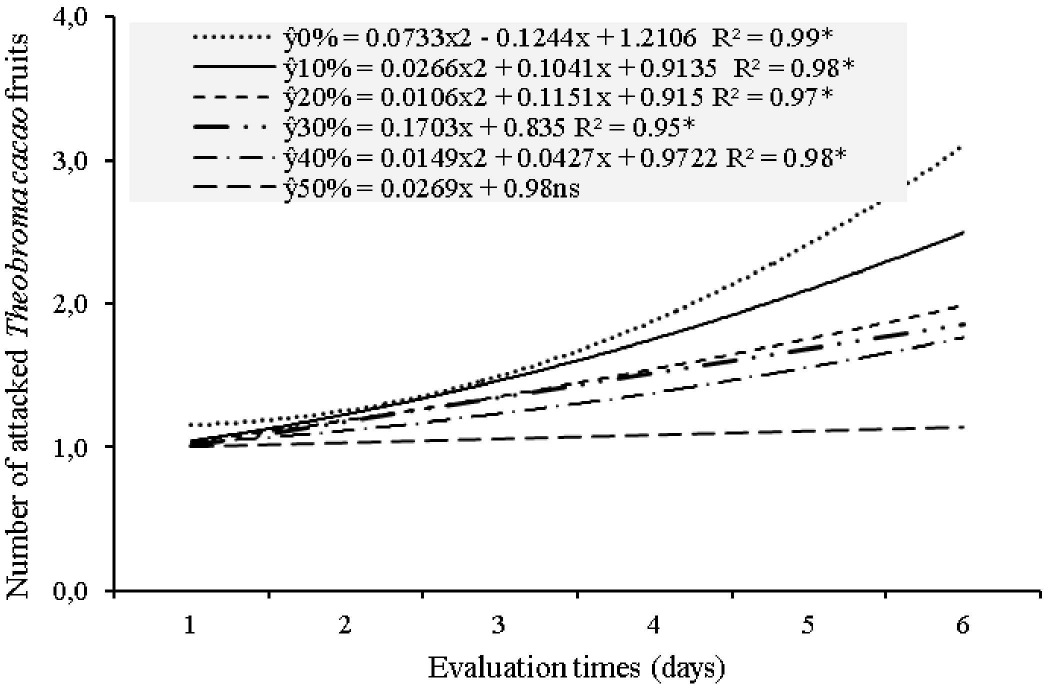

Figure 2. Number of fruits attacked by *Carmenta foraseminis* due to different *Azadirachta indica* concentrations in the six days of evaluation.

Similar results were obtained by Carvalho et al. (2008) working on the control of *Brevicoryne brassicae* and *Myzus persicae*. In the same way, Vieira and Peres (2017) used *Azadirachta indica* leaf extract and controlled the attack of *Brevicoryne brassicae* in broccoli. In the same sense, Santos et al. (2022) managed to control the oviposition of *Oligonychus punicae* with the use of different products based on *Azadirachta indica* in eucalyptus mini-gardens.

The insecticidal action of *Azadirachta indica* is due to azadirachtin, which is the main toxic substance present in the plant. It acts by inhibiting the biosynthesis of chitin, deformation in pupae and adults, changes in the attraction of insects to pheromones, sterilization and inhibition of oviposition, affecting the reproduction of insects, causing them to lay fewer and less fertile eggs, and also causing mortality in the different stages of development of pest insects (DELGADO et al., 2012).

Therefore, this study demonstrated that the use of *Azadirachta indica* extract at concentrations greater than 40% is an alternative for the control of *Carmenta foraseminis*, demonstrated its toxic effect, lower cost, biodegradability, and absence of side effects compared to traditional agrochemicals (RODRIGUES et al., 2017).

Despite the broad biocidal effect, extracts are photosensitive and thermolabile, which makes their effectiveness in the field limited; therefore, requiring greater applications at intervals of 5 to 7 days, which increases production and application costs. In this sense, it is recommended to search for alternatives to increase the residual effect when the pest infestation is persistent and the damage is severe (Aguiar-Menezes, 2005).

Otherwise, intensive use can stabilize the compound in the environment, increasing the residual toxic effect on beneficial organisms such as bees (ROUBIK et al., 2018).

Regarding the biocidal effect of *Jatropha curcas*, Figures 3 and 4 showed that the *Jatropha curcas* extract concentration of 50% was presented as the best option to control the hatching of larvae and the attack of *Carmenta foraseminis* on *Theobroma cocoa* fruits. Likewise, it had constant effect during the six days of evaluation, observing one hatched larva and one attacked fruit. On the other hand, extract concentrations lower than 0% and 10% were not efficient, since from the second day onwards, increase in hatched larvae and attacked fruits was observed, with values of 2.3 and 1.8, respectively.

This result is due to the loss of residual power of the extract due to its low concentration. On the other hand, higher concentrations presented better residual effect during the evaluation period; however, the effect is lost over time, making more applications necessary, which makes further investigations to determine the cost-benefit necessary (Figure 3 and 4).

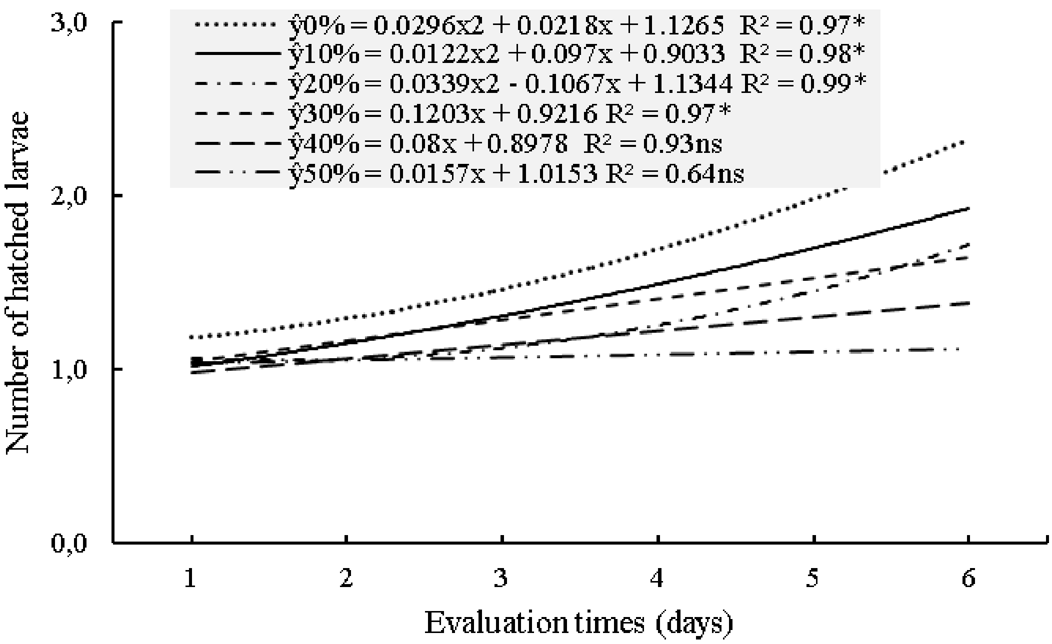

Figure 3. Number of *Carmenta foraseminis* larvae hatched in *Theobroma cacao* fruits due to the effect of different *Jatropha curcas* extract concentrations in the six days of evaluation.

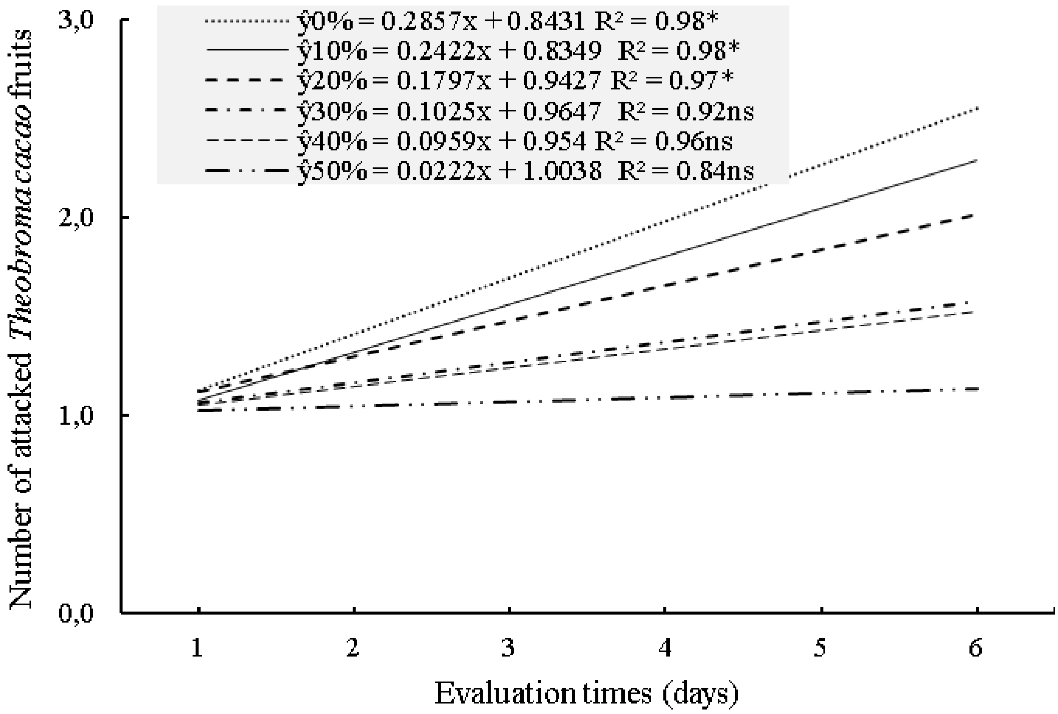

Figure 4. Number of *Carmenta foraseminis* larval attacks due to the effect of different *Jatropha curcas* extract concentrations in the six days of evaluation.

Similar results were determined by Guerra-Arévalo et al. (2018) and Guerra-Arévalo et al. (2022) working on the control of *Hypsipyla grandella* larvae in the cultivation of *Swietenia macrophylla* in monoculture and in agroforestry systems. The authors determined that *Jatropha curcas* concentrations greater than 30% were efficient in controlling the pest.

Likewise, Holtz et al. (2016) determined that all *Jatropha curcas* organs have potential in the control of *Planococcus citri* in coffee culture. Holtz et al. (2022) reported that the extract from *Jatropha curcas* leaves control infestation by *Myzus Persicae* and determined that the age or maturity of leaves influence the insecticidal activity due to the greater or lesser concentration of secondary metabolites. Further studies should be carried out to find the harvest point of leaves to obtain better results in the control of *Carmenta foraseminis* and thereby reduce concentration and increase efficiency.

The toxicity of *Jatropha curcas* is attributed to the action of ribosome-inactivating proteins (RIPs), and when ingested, they cause the death of cells in the gastrointestinal tract (AUDI et al. 2005). In this study, *Carmenta foraseminis* larvae were possibly affected, both by ingestion through the digestive system and by contact, through the respiratory tract.

In this regard, Isman (2006) indicates that the action by contact is faster than by ingestion because it depends on the digestion process for incorporation and action in the vital systems of the pest. On the other hand, the contact action possibly acts on the central nervous system of the insect, preventing the transmission of nervous impulses due to the inhibition of the acetyl cholinesterase enzyme or due to disturbances in acetylcholine and in Na^+ and K^+ channels. At the same time, they can affect cell respiration by preventing electron transport and/or inhibiting ATP synthesis (KATHRINA; ANTONIO, 2004)

In relation to the biocidal effect of *Tagetes erecta*, Figures 5 and 6 show that all extract concentrations caused significant and non-significant increasing linear trend for the number of hatched larvae and *Theobroma cacao* fruits attacked by *Carmenta foraseminis*. It should be highlighted that, as in previous experiments, the 50% concentration had constant effect on the characteristics evaluated during the six days of evaluation.

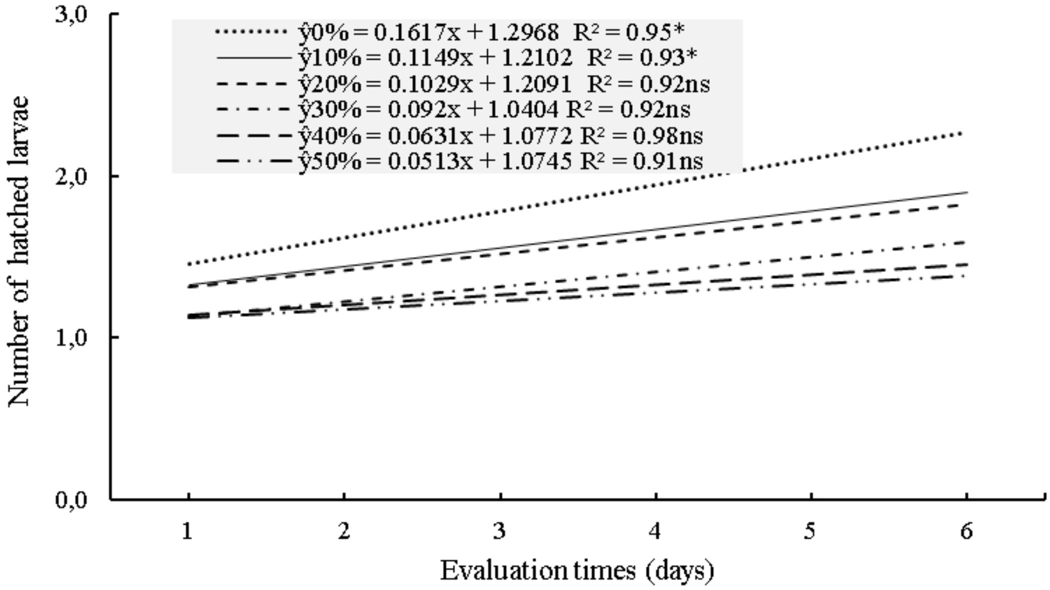

Figure 5. Effect of different *Tagetes erecta* extract concentrations on the number of hatched *Carmenta foraseminis* larvae in *Theobroma cacao* fruits during the six days of evaluation.

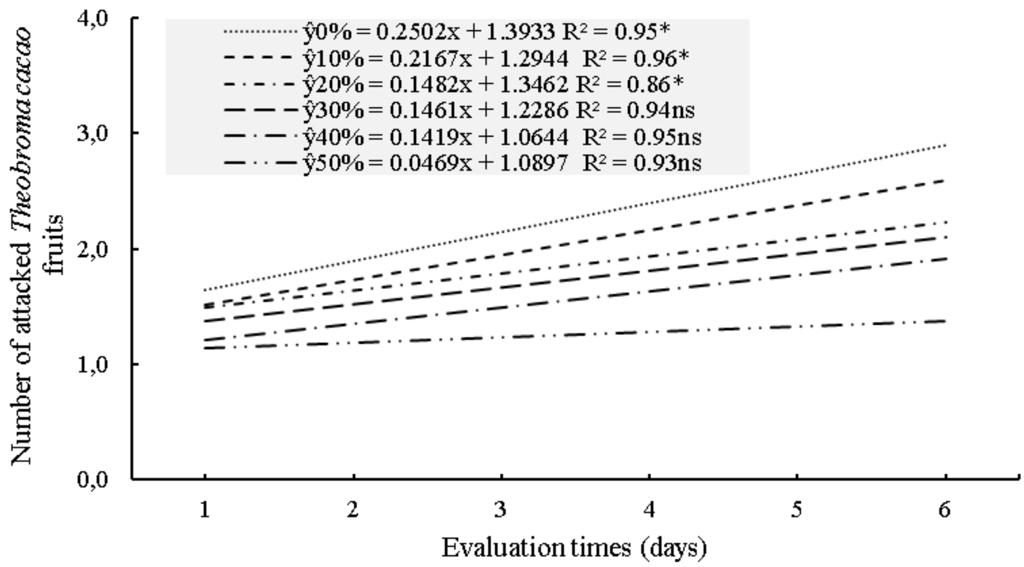

Figure 6. Number of *Theobroma cacao* fruits attacked by *Carmenta foraseminis* due to the effect of different *Tagetes erecta* concentrations at various evaluation times.

On the other hand, *Theobroma cacao* fruits not treated with *Tagetes erecta* extract were more attacked and showed greater number of hatched larvae; similar effect was observed at concentrations of 10%, 20% and 30%. Therefore, for efficient control of *Carmenta foraseminis* in cocoa crops in the fruiting phase, it is necessary to apply *Tagetes erecta* extract concentrations between 40% and 50% (Figures 5 and 6).

Similar results were determined by Batista-Balcarcel et al. (2021) in the control of aphids using *Tagetes erecta* concentrate in lettuce cultivation.

Similarly, Santos (2019) reported that the extracts and essential oils of *Tagetes erecta* and *Tagetes patula* presented insecticidal potential on insect *Sitophilus zeamais* and significantly affected the larval viability of *Spodoptera frugiperda*.

The biocidal effect of *Tagetes erecta* extract is due to the presence of secondary metabolites such as phenols, thiophenes, flavonoids and coumarins, which are hydroxylated compounds that act as anti-feeding agents, and coumarins inhibit the growth of fungi and are toxic to nematodes, mites and insects (PADMA et al., 1997).

However, Ocampo et al. (2007) reported that biopreparations decompose within a week and the repellent effect lasts 3 days, so their application must be constant.

Conclusions

Extracts from *Azadirachta indica* and *Tagetes erecta* leaves and *Jatropha curcas* resin at concentrations greater than 40% were efficient in the control of *Carmenta foraseminis* in *Theobroma cacao* crops in the fruiting phase.

Further studies should be carried out to determine the concentration of secondary metabolites in *Azadirachta indica* and *Tagetes erecta* leaves and *Jatropha curcas* resin in different maturation stages to be more efficient in the control of the insect pest.

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