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Aqueous botanical extracts, via different extraction methods, for control of the diamondback moth, Plutella xylostella (Lepidoptera: Plutellidae)

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Abstract

Diamondback moth, *Plutella xylostella* (Linnaeus) (Lepidoptera: Plutellidae), is a vegetable pest of the genus *Brassica* worldwide. The development of new, safer bio insecticides with less negative impacts on human health, flora, fauna, and specific to the target is needed to combat this pest, particularly in small-scale organic agriculture. In this sense, the efficiency of 7 plant species in the form of aqueous botanical extracts was evaluated regarding the bio insecticide effect, using three extraction methods (orbital agitation, decoction, and infusion). There was a difference between the treatments, highlighting the decoction of *Couroupita guianensis*, which presented the highest efficiency (39%) for second-instar larvae of *P. xylostella*, followed by infusions of *Codiaeum variegatum* and *Ruta graveolens*, both with a 29% efficiency rate. The 3 extraction methods were statistically different, with decoction and orbital agitation presenting the best results.

Introduction

Plutella xylostella (Linnaeus, 1758) (Lepidoptera: Plutellidae), known as the diamondback moth, is one of the main insect pests worldwide, a status achieved as a result of the high feeding rates of the larvae causing significant damage in brassicas such as cabbage, *Brassica oleracea* L. var. *capitata*, which, in addition to the reduction in leaf area, also depreciates the marketable product, causing economic losses, sometimes quite high (Endersby and Morgan 1991; Hamilton et al. 2005; Furlong et al. 2013; Peres et al. 2017; Yusoff et al. 2021). In an attempt to control it, many horticulturists resort to insecticide applications, a fact that increases production costs, in addition to carrying risks of contamination of applicators, consumers, pollinating insects and the environment (Damalas and Eleftherohorinos, 2011; Benbrook et al. 2021).

In general, losses in yield and quality of crops caused by pests are aggravated in family and organic crops, since, especially family producers, they are financially more sensitive to fluctuations in production costs, with organic agriculture not allowing the use of chemical molecules of synthetic origin for pest control (Seufert et al. 2012; Benbrook et al. 2021). Add to this the fact that the consumer market demand for products produced sustainably, at lower costs, and that meet quality criteria, is growing in several countries in the Americas and Europe (Theunissen, 1997; Tavares et al. 2021). Thus, attention to pests, especially *P. xylostella*, in organic agriculture within Integrated Pest Management (IPM) programs has been directed towards using aqueous plant extracts, botanical insecticides, entomopathogenic fungi, bio-insecticides based on *Bacillus thuringiensis* Berliner, mineral oils, sulfocalcium broth, physical barriers and biological control with predators and parasitoids (Endersby and Morgan 1991; Ribeiro et al. 2012; Benbrook et al. 2021).

An updated review shows that 95 plant species have their extracts with insecticidal activity, which can be used as "bio-insecticides", with *Azadirachta indica* A. Juss. (Meliaceae), *Capsicum annuum* L. (Solanaceae), *Nicotiana tabacum* L. (Solanaceae), and *Tagetes erecta* L. (Asteraceae) were the most

researched, and extraction by maceration with dry leaf material was the extraction method preferred by most authors. (Tavares et al. 2021).

Botanical insecticides can be prepared from spontaneously growing plants, available locally, and which may even offer new mechanisms of action, presenting an interesting cost/benefit ratio and with ecologically correct alternatives in relation to synthetic agrochemicals, preserving the pollinator population, as well as ecosystem diversity (Kudom et al. 2011; Amoabeng et al. 2013; Deletre et al. 2013; Ladhari et al. 2013; Amoabeng et al. 2014; Couto et al. 2016; Adamou et al. 2022). These substances, when in contact with insects, can inhibit feeding, interfere with the synthesis of ecdysis (ecdysonium), cause deformations in pupae, kill immature individuals and adults, in addition to reducing the fecundity and longevity of many species (Schmutterer 1988; Mordue and Blackwell 1993).

Commercial formulations of botanical insecticides are usually in oil, however, the feasibility of using these insecticides particularly in organic agriculture requires simpler methods of obtaining, such as infusion and decoction, with the use of solvents that are also more accessible, such as water. Few studies have compared these forms of extracting insecticide compounds from plants to control *P. xylostella* (Peres et al. 2017; Barbosa et al. 2020; Silva et al. 2021; Santos et al. 2022).

Therefore, the objectives of this research were: (1) to evaluate whether the proposed extraction methodologies are efficient in obtaining bioactive compounds with insecticidal properties; and (2) evaluate the mortality second-instar larvae of *P. xylostella* caused by aqueous extracts of seven species of plants found in the Southeast Region of Brazil, namely: *Codiaeum variegatum* (L.) Rumph. ex A.Juss. (Euphorbiaceae), *Couroupita guianensis* Aubl. (Lecythidaceae), *Ruta graveolens* L. (Rutaceae), *Momordica charantia* L. (Cucurbitaceae), *Melissa officinalis* L. (Lamiaceae), *Mentha arvensis* L. (Lamiaceae), and *Salvia rosmarinus* L. (Lamiaceae) obtained by decoction, infusion, and orbital agitation.

Materials and Methods

Insect Rearing

The insects used came from a population of *P. xylostella* maintained at the Laboratory of Biology and Insect Rearing (LBIR) of the Faculty of Agricultural and Veterinary Sciences (FCAV), São Paulo State University (UNESP), Jaboticabal, SP, Brazil, for about of 1 year (with more than 30 generations), under controlled conditions of temperature (25 ± 2°C), relative humidity (60 ± 10%) and photoperiod (12L:12D).

In the maintenance rearing, the adult insects were fed with a 10% honey solution, being kept in cylindrical transparent plastic cages (15 cm in diameter x 15 cm in height), using disks of leaves as substrate for egg position (8 cm in diameter) of butter green (*Brassica oleracea* var. *acephala*) from organic cultivation, placed on moistened filter paper, supported by disposable plastic cups placed at the base of the cages in an inverted way. After egg position, the leaf discs containing the eggs were placed in disposable Petri dishes (15 cm in diameter x 1.5 cm in height), where they remained until the larvae hatched. Next, the

newly hatched larvae were transferred to plastic containers (27 cm long x 17 cm wide x 8 cm high), where they remained until they reached the pupal stage. The larvae were fed with leaves of the aforementioned cultivar, which were previously treated with a 5% sodium hypochlorite solution, washed in running water, and dried with a paper towel after treatment. This procedure was repeated daily, with the adults obtained returning to the copulation and egg position cages, restarting the insect cycle. The bioassays were performed with second-instar larvae taken from the maintenance rearing.

Collection of plants to produce aqueous extracts

Leaves of seven plant species were collected during the morning in the municipality of Jaboticabal, SP, Brazil, with collections authorized by the respective owners of the selected areas. Some species were collected in a public environment (FCAV/UNESP Farm), without the need for prior authorization. The samples of each one of the leaves of the different species were deposited in the JABOTI Herbarium (JABU) of the São Paulo State University - FCAV/UNESP, accredited by the Brazilian Herbarium Network of the Botanical Society of Brazil (SBB), with the following registration numbers: *Codiaeum variegatum* (L.) Rumph. ex A.Juss. (Euphorbiaceae) (JABU 157), *Couroupita guianensis* Aubl. (Lecythidaceae) (JABU 158), *Ruta graveolens* L. (Rutaceae) (JABU 159), *Momordica charantia* L. (Cucurbitaceae) (JABU 160), *Melissa officinalis* L. (Lamiaceae) (JABU 161), *Mentha arvensis* L. (Lamiaceae) (JABU 162), and *Salvia rosmarinus* L. (Lamiaceae) (JABU 163)

Extraction methods

Decoction

Leaves of the seven species were weighed on an analytical scale, totaling 50 grams of each. Then each sample was immersed in 333 ml of deionized water in a pan over medium heat for 10 minutes. After cooling, the extracts were strained through voile fabric, with 50 ml of each one placed in amber glass vials and stored in refrigeration at 10°C for 24 hours, thus obtaining aqueous extracts in the concentration (w/v) of 15%. After 24 hours, the extracts were used for application to the second-instar larvae of *P. xylostella*.

Infusion

For the infusion process, the leaves were dried in an oven with forced air circulation for three days at a maximum temperature of 40°C, except for *C. variegatum*, which the leaves were dried for 5 days (at the same temperature), due to the texture of the leaves. They were then crushed in a blender until obtaining a fine powder. Aqueous extracts of these materials were prepared by infusion, with 20g of each plant immersed in 133 ml boiling deionized water (100°C). After 10 minutes, the extracts were strained through voile fabric, with 50 ml of each one placed in amber glass bottles and stored in refrigeration at 10°C for 24 h. Concentration and application were similar to Decoction.

Orbital agitation

For the orbital agitation methodology, 37.5g leaves of each species were immersed in 250 ml deionized water in an Erlenmeyer flask, sealed with a Parafilm membrane, and placed in a Shaker incubator at 20°C for 48 hours, with agitation at 79.8 rpm. After 48 hours, the extracts were strained through voile fabric and placed in 50 ml amber glass bottles, and then cooled at 10°C for 24 h. Concentration and application were similar to Decoction.

Toxicity of extracts for second-instar larvae of P. xylostella.

The effects of the aqueous extracts of the seven plant species, obtained by the three extraction methods, as well as the control (Table 1), were evaluated in second-instar larvae of *P. xylostella*, with bioassays carried out in Petri dishes (9 cm in diameter x 1.5 cm in height) which received discs of moistened filter paper at the base and a disc of leaf (*B. oleraceae* var. *acephala*, cv. 'couve-manteiga') measuring 8 cm in diameter with the abaxial part face up and 20 larvae. The plates thus assembled were treated with the different extracts using a Potter's Tower (Burkard Manufacturing, Rickmansworth, Herts, UK) calibrated at 34.5 kPa for the application of 2 ml of solution. After application, the abaxial part of the leaves was placed downwards, and the plates were sealed with a Parafilm membrane ®, with the control treatment prepared using the same procedure and applying only deionized water. After being treated, the plates were kept under controlled conditions of temperature (25 ± 2°C), relative humidity (60 ± 10%), and photoperiod (12L:12D).

Table 1
Treatments used in bioassays to assess toxicity in second-instar larvae of *Plutella xylostella.*

Plant species and extraction methods for aqueous extracts		
Decoction	**Infusion**	**Orbital agitation**
T_1: *Melissa officinalis* decoction	T_8: *Melissa officinalis* infusion	T_{15}: *Melissa officinalis* orbital agitation
T_2: *Salvia rosmarinus* decoction	T_9: *Salvia rosmarinus* infusion	T_{16}: *Salvia rosmarinus* orbital agitation
T_3: *Ruta graveolens* decoction	T_{10}: *Ruta graveolens* infusion	T_{17}: *Ruta graveolens* orbital agitation
T_4: *Codiaeum variegatum* decoction	T_{11}: *Codiaeum variegatum* infusion	T_{18}: *Codiaeum variegatum* orbital agitation
T_5: *Mentha arvensis* decoction	T_{12}: *Mentha arvensis* infusion	T_{19}: *Mentha arvensis* orbital agitation
T_6: *Couroupita guianensis* decoction	T_{13}: *Couroupita guianensis* infusion	T_{20}: *Couroupita guianensis* orbital agitation
T_7: *Momordica charantia* decoction	T_{14}: *Momordica charantia* infusion	T_{21}: *Momordica charantia* orbital agitation
Control treatment (T_0) with deionized water.		

To determine the toxicity of the aqueous botanical extracts, the larvae with the different treatments and repetitions were monitored daily until the fourth day after application, observing the number of dead individuals, as well as replacing the cabbage discs with new untreated ones, after checking the larvae' feeding, to guarantee that there was contamination via food. Each experimental unit consisted of 5 replications, each containing 20 second-instar larvae of *P. xylostella*, totaling 100 larvae for each treatment.

Statistical analysis

The experiment was carried out in a completely randomized design and in a 7x3 factorial scheme (aqueous botanical extracts x extraction methods), with the data submitted to Multiple Sample Comparison, statistical analysis that is designed to compare two or more independent samples of variable data. The tests "Multiple Range Test (Tukey HSD)" and "Kruskal-Wallis Test" were performed to determine whether or not there are significant differences between the means and medians of the mortality data of the treatments ($\alpha = 0.05$), using “StatGraphics Centurion Software XIX” (StatGraphics

2009). To calculate the efficiency of the treatments, the mortality results were calculated using the formula proposed by Abbott (1925).

Results

Toxicity of aqueous botanical extracts obtained by different extraction methods for second-instar larvae of Plutella xylostella

The tested aqueous botanical extracts of the seven plant species showed toxicity to second-instar larvae of *P. xylostella*, with significance among them and with emphasis on *R. graveolens* ($H = 13.20$; $df = 6$; $p = 0.0398$), with T_6 (decoction of *C. guianensis*) standing out among treatments ($H = 63.99$; $df = 20$; $p = 0.0000$) (Fig. 1), and standing out among the methods of extraction, namely: orbital agitation and decoction ($H = 7.03$; $df = 2$; $p = 0.0296$), in relation to the mortality of second-instar larvae of *P. xylostella*, it was observed that the treatments of extracts of *C. variegatum and R. graveolens*, prepared by infusion, and of *C. guianensis*, prepared by decoction, showed significant toxicity for the second-instar larvae, with efficiencies of 29.07%, 29.07%, and 39.53%, respectively, after 96 hours of application (Fig. 2).

Because the application was made only on the abaxial surface of the leaves, as this is the preferential place for feeding, the second-instar larvae of *P. xylostella* subjected to the aqueous botanical extracts (Table 1) showed reduced feeding on the abaxial surface of the cabbage leaf disk in relation to the control, 24 hours after the treatments. The extract of *C. guianensis* obtained by infusion was the one that caused the lowest percentage of larvae mortality, only 9.75%, being statistically similar to the control and treatments T_1 (*M. officinalis* decoction), T_4 (*C. variegatum* decoction), T_7 (*M. charantia* decoction), T_8 (*M. officinalis* infusion), T_9 (*S. rosmarinus* infusion), T_{12} (*M. arvensis* infusion), T_{13} (*C. guianensis* infusion), T_{14} (*M. charantia* infusion), T_{18} (orbital agitation of *C. variegatum*), and T_{20} (orbital agitation of *C. guianensis*), which caused mortalities ranging from 9.75–19.25% (Fig. 1, Table 2).

Table 2
Mortality percentages of second-instar larvae of *Plutella xylostella* caused by aqueous botanical extracts obtained with different extraction methods.

	Mortality (%)			
Aqueous botanical extracts	Decoction	Infusion	Orbital agitation	Kruskal-Wallis test*
Melissa officinalis	12.00 ± 1.67	16.25 ± 2.92	21.50 ± 1.95	(H = 9.06; df = 2; p = 0.0107)
Salvia rosmarinus	21.75 ± 3.80	17.75 ± 2.77	21.00 ± 2.50	(H = 0.78; df = 2; p = 0.6764)
Ruta graveolens	23.75 ± 2.50	25.50 ± 3.40	21.00 ± 2.52	(H = 1.12; df = 2; p = 0.5697)
Codiaeum variegatum	17.75 ± 2.52	27.25 ± 3.06	18.75 ± 2.94	(H = 6.51; df = 2; p = 0.0384)
Mentha arvensis	23.50 ± 1.09	17.00 ± 2.40	23.75 ± 2.34	(H = 5.95; df = 2; p = 0.0510)
Couroupita guianensis	31.25 ± 3.83	9.75 ± 1.71	19.25 ± 2.95	(H = 18.46; df = 2; p = 0.0000)
Momordica charantia	19.75 ± 2.41	12.00 ± 2.09	22.00 ± 2.21	(H = 9.39; df = 2; p = 0.0091)
Kruskal-Wallis test	(H = 22.89; df = 6; p = 0.0008)	(H = 26.17; df = 6; p = 0.0002)	(H = 2.64; df = 6; p = 0.8522)	
* Test for medians				

A statistically significant difference was also observed for four extracts according to the extraction method, namely: *M. officinalis* (H = 9.06; df = 2; p = 0.0107), *C. variegatum* (H = 6.51 ; df = 2; p = 0.0384), *C. guianensis* (H = 18.46; df = 2; p = 0.0000) and *M. charantia* (H = 9.39; df = 2; p = 0 .0091), likewise, for each extraction method, there is a significant difference for the decoction and infusion methods (Table 2, Fig. 3).

The results also showed that the aqueous botanical extracts obtained by orbital agitation and decoction caused mortality in the first 24 hours, with values of between 7 and 10%, while the infusions showed less efficiency (4%) in the same period. The mortality rate of larvae fed aqueous botanical extracts by orbital agitation, decoction, and infusion after application, had decreasing trends from 48 to 72 hours, that is, during this time the extracts reduced or stopped in the mortality values of larvae, while at 24 to 48 hours and from 72 to 96 hours they presented the opposite result, where the extracts increased larval mortality with the passage of observation time (Fig. 2).

Discussion

The mortalities observed with the extracts of *C. variegatum* and *R. graveolens* prepared by infusion and *C. guianensis* by decoction, although higher than the other treatments and the control, are still not equivalent to treatments with other botanical insecticides, chemical, and biological insecticides; part of this difference may be related to the size of the plant leaf, or to the method of extracting the metabolite from the plant, where the solvent used, water, was not able to extract the insecticide compounds, mainly due to its polarity, some chemical compounds are more effective when applied by direct contact at the insect stage, while others produce toxicity through ingestion of these substances (Gosselin 1976). In addition, the existing natural barrier in the cuticle of the insect stage evaluated in the tests can help to prevent/impede the absorption of toxic products and reduce the insecticidal effect (Alecio 2012; Silva et al., 2021). This finding can be explained considering that botanical insecticides have different chemical compositions and modes of action, that is, some compounds can act immediately on insects while others may take longer (Borden et al. 2018).

Extracts and essential oils of *C. variegatum* have numerous properties of interest, among which antimicrobial, anti-inflammatory, healing, and insecticide stand out (Njoya et al. 2021), with some reports confirming the insecticidal potential for Sitophilus *zeamais* (Motschulsky) (Coleoptera: Curculionidae) (Lawal et al. 2018) and for mosquitoes of the genus *Culex* (Diptera: Culicidae) (Awosolu et al. 2018). One of the major constituents extracted from *C. variegatum* leaves is Taraxerol, a pentacyclic triterpenoid with insecticidal activity described by Santhanam et al. (2014) and Johnson and Singh (2017), although the observed action on *P. xylostella* may also be the result of the synergistic action of the extracted constituents (Njoya et al. 2021). In the case of Taraxerol, the mode of action of the substance in insects has not yet been described, requiring more specific studies. Although Johnson and Singh (2017) reported behavioral changes, changes in total protein metabolism, total free amino acids and glycogen in *Culex quinquefasciatus* Say larvae (Diptera: Culicidae). The results of this work demonstrate that extraction by infusion was more efficient than by decoction and orbital agitation ($H = 6.51$; $df = 2$; $p = 0.0384$) for *C. variegatum* (Fig. 3, Table 2), thus being, as the infusion method goes through the process of drying in an oven and grinding, the active compounds with insecticidal potential would be extracted in greater quantities due to the concentration of the compounds in the dry particles and expansion of the contact surface (smaller particles) for the action of the solvent, in this case water.

Corroborating the results of this study, the aqueous extract of *R. graveolens* also has an insecticidal effect, and repellent potential (Hana et al. 2020; Wang et al. 2022), while for *P. xylostella*, Song et al. (2022) found that the application of essential oil from *R. graveolens* promoted high rates of growth inhibition in third-instar larvae. A major component present in aqueous extracts of *R. graveolens*, the Trans-anethole, compound that has proven insecticidal activity (Cruz et al. 2017; Dias et al. 2019; Hana et al. 2020). According to López et al. (2010), Trans-anethole causes changes in different biological aspects of insects, such as, for example, the inhibition of acetylcholinesterase, which may be an explanation of its effectiveness on *P. xylostella* larvae observed in this study.

The highest mortality observed in the present study was obtained from the aqueous extract of *C. guianensis* leaves prepared by decoction ($H = 6.51$; $df = 2$; $p = 0.0384$), and researchers such as Raghavendra et al. (2017) reported insecticidal activity of leaf extracts of this species, with mortality of up to 100% of the second-instar larvae of *Aedes aegypti* (Linnaeus) (Diptera:Culicidae) with concentrations greater than 1mg/ml. In pests of agricultural interest, for nymphs and adults of *Bemisia tabaci* (Gennadius) (Hemiptera:Aleyrodidae) with aqueous extract of *C. guianensis*, there are reports of mortalities of 29.2% and 35.34%, respectively, (Yadav and Mendhulkar 2015).

Treatments with aqueous botanical extracts and with different types of extraction did not alter the larval duration of *P. xylostella*, and some researchers such as Peres et al. (2017) found that aqueous extracts of the leaves of *Alibertia intermedia* (Mart.) (Rubiaceae) and *A. sessilis* (Vell.) K. Schum. (Rubiaceae) negatively affected the development of *P. xylostella* in all phases of its life cycle, prolonging the larval period and causing larval mortality of 43.59% and 50.2%, respectively. In addition, aqueous extracts by infusion and maceration of *Psychotria capillacea* (Müll. Arg.) Standl. (Rubiaceae), *Psychotria. deflexa* DC (Rubiaceae), and *Psychotria leiocarpa* Cham and Schltdl (Rubiaceae) showed no toxicity to third-instar larvae of *P. xylostella* (Silva et al. 2021).

Some aqueous botanical extracts obtained by maceration with dry leaf material of *Schinus terebinthifolius* Raddi (Anacardiaceae), *Ludwigia tomentosa* (Cambess.) H. Hara (Onagraceae), *L. longifolia* (DC.) H. Hara (Onagraceae), *L. sericea* (Cambess.) H. Hara (Onagraceae), and *L. nervosa* (Poir.) H. Hara (Onagraceae) demonstrated insecticidal potential against newly emerged larvae of *P. xylostella*, with mortalities ranging from 14–38% (Ferreira et al. 2020).

Recently researchers have shown that aqueous ethanolic extract made by leaf maceration and seed oil of *Triadica sebifera* (L.) (Euphorbiaceae), as well as their combinations, can be recommended as anti-food to reduce damage caused by *P. xylostella*, and safely for predators/parasitoids under field conditions (Dolma and Reddy 2022). In the same sense, the aqueous botanical extract by maceration, at a concentration of 10% (w/v), of *L. tomentosa*, resulted in a reduction of approximately 81% in the feeding of *P. xylostella* larvae in relation to the control (Ferreira et al. 2022).

Approximately 57% (12 treatments in Table 1) of the aqueous botanical extracts evaluated in this research caused mortality in second-instar larvae of *P. xylostella*. However, the possibility of the existence of phytotoxicity caused by the extracts in plants of the genus *Brassica* must also be evaluated, and, after these evaluations, the treatments T_6 (Decoction of *C. guianensis*), T_{10} (Infusion of *R. graveolens*), and T_{11} (*C. variegatum* infusion) may be recommended for a next stage of applications, first in a greenhouse, for the management of *P. xylostella* larvae in *Brassica* spp., including testing mixtures with adjuvants to improve plant coverage, not least because Brassicaceae have very waxy leaves.

Thus, new studies must be conducted before incorporating the referred extracts as an IPM strategy for the management of *P. xylostella*, and these results can optimize an integrated management to control *P. xylostella*, particularly in organic gardens and family horticulture.

Declarations

The authors declare no conflict of interest.

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Figures

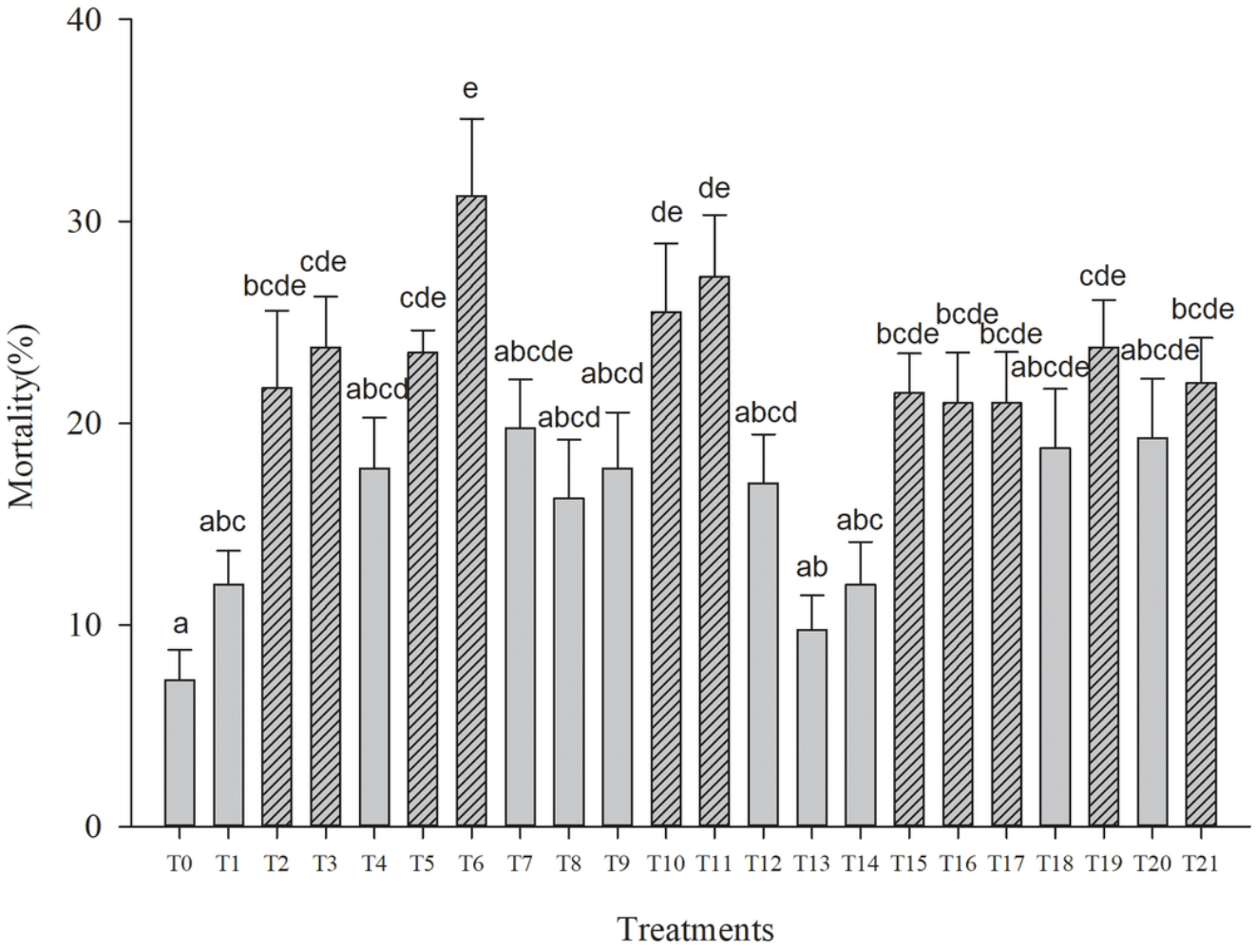

Figure 1

Mortality percentage of second-instar larvae *Plutella xylostella* caused by aqueous botanical extracts obtained by the three extraction methods. Treatments: T_0 = deionized water (control); T_1 = *M. officinalis* decoction ; T_2 = *S. rosmarinus* decoction ; T_3 = *R. graveolens* decoction ; T_4 = *C. variegatum* decoction ; T_5 = *M. arvensis* decoction *;* T_6 = *C. guianensis* decoction ; T_7 = *M. charantia* decoction ; T_8 = *M. officinalis* infusion ; T_9 = *S. rosmarinus* infusion ; T_{10} = *R. graveolens* infusion ; T_{11} = *C. variegatum* infusion ; T_{12} = *M. arvensis* infusion ; T_{13} = *C. guianensis* infusion ; T_{14} = *M. charantia* infusion ; T_{15} = Orbital agitation of *M. officinalis*; T_{16} = Orbital agitation of *S. rosmarinus* ; T_{17} = Orbital agitation of *R. graveolens* ; T_{18} = Orbital agitation of *C. variegatum* ; T_{19} = Orbital agitation of *M. arvensis* ; T_{20} = Orbital agitation of *C. guianensis* ; T_{21} = Orbital agitation of *M. charantia* . Different letters in the treatment columns indicate statistically significant differences at the 95.0% confidence level by Tukey's (HSD) method. Furthermore, 5

homogeneous groups are identified in the treatments of which there were no statistically significant differences within them. Columns of the same color as the control (T_0) are statistically equal to it.

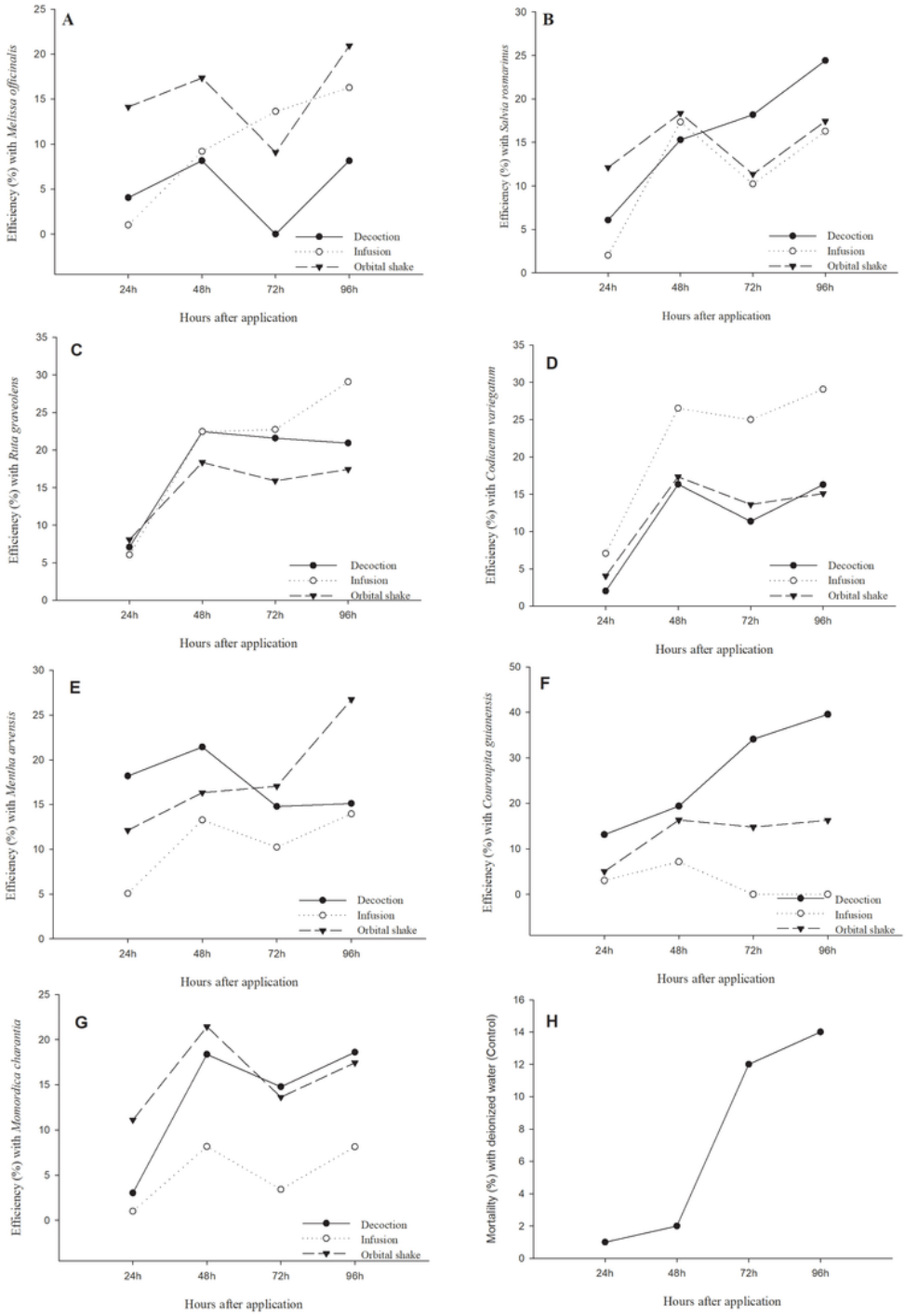

Figure 2

Mortality percentage of second-instar larvae of *Plutella xylostella* caused by aqueous botanical extracts obtained with different extraction methods, calculated by the formula proposed by Abbott (1925), in the

96 hours of evaluation. **A** - Efficiency with *M. officinalis*; **B** - Efficiency with *S. rosmarinus*; **C** - Efficiency with *R. graveolens*; **D** - Efficiency with *C. variegatum*; **E** - Efficiency with *M. arvensis*; **F**- Efficiency with *C. guianensis*; **G** - Efficiency with *M. charantia*; and **H**- Control mortality.

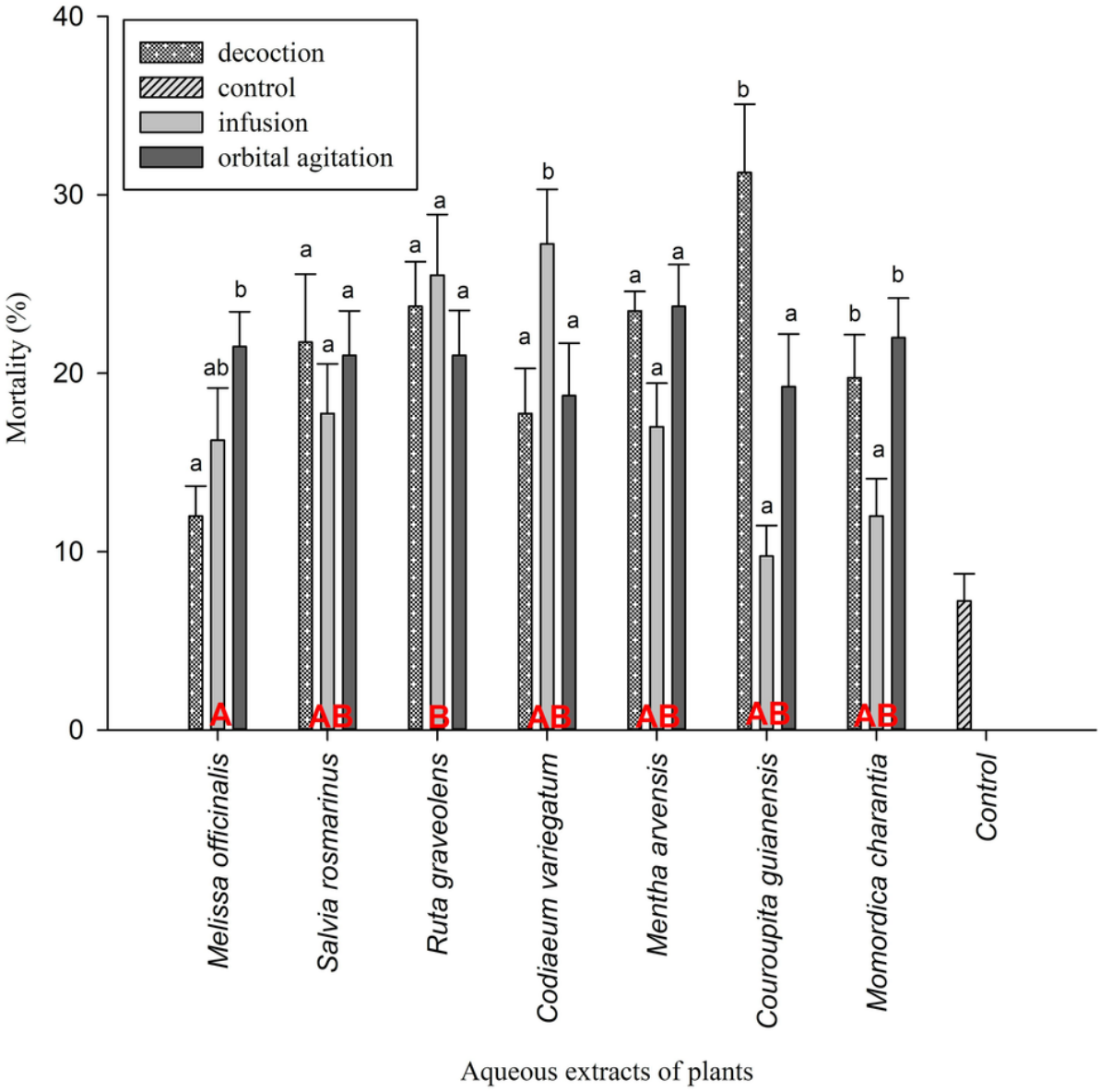

Figure 3

Mortality percentage of second-instar larvae of *Plutella xylostella* caused by aqueous botanical extracts obtained with different extraction methods. Different letters in columns of aqueous botanical extracts by extraction method indicate statistically significant differences at the 95.0% confidence level by Tukey's (HSD) method. Capital letters different from the red color indicate a significant difference between the extracts regardless of the extraction method; there was a significant difference only between *M. officinalis* and *R. graveolens* ($H = 13.02$; $df = 6$; $p = 0.0398$).