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Laboratory and Field Trials to Identify Reduced-Risk Insecticides for the Control of the Golden Twin-Spot Moth *Chrysodeixis chalcites* (Esper) (Lepidoptera: Noctuidae) in Banana Plantations

Taylan Cakmak [1,*], Estrella Hernández-Suárez [2], Mehmet Bora Kaydan [3], Denis Achiri Tange [4], Santiago Perera [5] and Ana Piedra-Buena Díaz [2,*]

[1] Department of Agricultural Biotechnology, Faculty of Agriculture, Duzce University, Duzce 81620, Turkey
[2] Department of Plant Protection, Instituto Canario de Investigaciones Agrarias, Valle de Guerra, 38270 Tenerife, Spain
[3] Department of Biotechnology Application and Research Center, Institute of Science and Technology, Çukurova University, Balcali, Adana 01330, Turkey
[4] Department of Agronomic and Applied Molecular Sciences, Faculty of Agriculture and Veterinary Medicine, University of Buea, Buea P.O. Box 63, Cameroon
[5] Servicio Técnico de Agricultura y Desarrollo Rural, Cabildo Insular de Tenerife, 38007 Tenerife, Spain
* Correspondence: taylancakmak@duzce.edu.tr (T.C.); apbuena@icia.es (A.P.-B.D.)

Abstract: The golden twin-spot moth (*Chrysodeixis chalcites*) is one of the most important pests in banana production on the Canary Islands (Spain). The efficacy of different biorational insecticides based on bioenzyme complexes (Intruder®), plant extracts: Rutaceae and Piperaceae (Avenger®), Rutaceae and Lauraceae (BioKnock®), cinnamon, citronella, and Menta (Cinamite®), Alliaceae and Solanacea (Garlitrol-Forte®), citrus (Prevam®), and neem oil (Indasol®) was assessed against *C. chalcites*. Laboratory assays included: choice (repellent effect), no choice, and contact toxicity on *C. chalcites* 2nd instar larvae. The highest repellent effect was observed with Prevam® (85.19 ± 1.7%), followed by Garlitrol® (68.44 ± 5.7%) and Intruder® (67.54 ± 4.3%). In no choice assays, Prevam® (0.92 ± 0.4%), Indasol® (0.98 ± 0.33%), and Intruder® (2.7 ± 0.33%) had the lowest leaf consumption. The contact toxicity assays showed the highest mortality with Intruder® both at 1 day and 7 days post-application (20.22 ± 2.98% and 77.77 ± 5.7%, respectively). In the screenhouse trial, the best results for *C. chalcites* larvae mortality, fruit damage, and fruit classification in quality categories 7 days after application of the bioinsecticide were obtained with Intruder®, Prevam®, and Indasol®. An economic analysis of biorational treatments was also performed. The results of this study provide successful alternatives to chemical pesticides for the control of *C. chalcites* on banana plants in the Canary Islands.

Keywords: biorational insecticides; contact toxicity; plant extracts; repellent effect

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1. Introduction

Banana (Musacea, *Musa acuminata* Colla) is the main crop of the Canary Islands and accounts for approximately 23.1% of the agricultural area and 46% of the total agricultural production (in ton) of the islands [1]. In this region, the golden twin-spot moth, *Chrysodeixis chalcites* (Esper, 1789) (Lepidoptera: Noctuidae), is responsible for annual losses of approximately 2.68 million euros [2]. The adult female lays eggs mainly on the underside of the leaves. Larvae hatch from the eggs and undergo 6 instars before the pupal stage, from where the insects emerge as adult males and females. The full cycle is usually completed within 45 days at 20 °C, while at temperatures lower than 25 °C the larvae cycle can be completed in 44 to 50 days [3]. Damage to plants is caused by larval feeding; in leaves, this reduces photosynthetic surface, which could be very detrimental for young plants, and in fruits, even if the typical feeding only affects their surface, this leads to depreciation of the product and, consequently, economic losses for banana growers [2].

The worldwide distribution of this insect includes areas between 45° N and 35° S, from southern Europe and the Mediterranean and the Middle East to southern Africa, and many countries consider this lepidopteran pest as the most serious one [4]. It feeds on more than 30 different plant species [5]: lucerne, maize, and soybean in Spain [6] and soybeans [7] and artichokes [8] in northern Italy. It has also been reported as a serious pest in Bulgaria and Turkey [9,10] where it affects tomatoes, cucumbers, and peppers. In Israel [11], it is considered the main threat for tomato production. In Sicily, it is one of the four main noctuid pests of glasshouse crops [12]. In the Netherlands [13] and Belgium [14], it is established as a permanent pest in glasshouses. In Ontario, Canada, it has been described as an invasive pest in tomato and green bean crops [15], thus representing a new potential pathway for introduction of this pest into the United States [16].

Chemical-based control measures usually involve applications of chlorpyrifos, fenamiphos, or indoxacarb, among others [17]. Pyrethroids such as cypermethrin or deltamethrin can provide control of *C. chalcites*. Effective control of *C. chalcites* using indoxacarb (an oxadiazine) on vegetable crops in open fields and plastic houses in Italy has been reported [18]. The insect growth regulator cyromazine provided good control of second- and fourth-instar larvae of *C. chalcites* in glasshouses on tomatoes, lettuce, and ornamentals when applied as a foliar spray [19]. However, synthetic insecticide treatments of a limited number of authorized insecticides during the crop cycle [20,21] would benefit the development of resistance [11,22], further reducing their effectiveness and increasing the production costs [21,23] and residues [17], thus hampering the commercialization of products that can occasionally contain pesticide residues.

The number of pesticides used in banana fields can be reduced by increasing our knowledge of the use of biorational insecticides in specific stages and/or parts of the plants. Such knowledge is especially important not only to protect the environment but also to avoid the deleterious side effects of pesticides on biological control agents. For example, *Trichogramma* species that only parasitize eggs are the most effective natural enemy of *C. chalcites* and the only one used, at present, in the Canary Islands [20]. Additionally, plant extract-based products may be useful if alternated with *Bacillus thuringiensis* application in organic banana production systems with low populations of *C. chalcites*. Different strains of *B. thuringiensis* gave full control (100% efficacy) of *C. chalcites* when sprayed on tomatoes grown under net protection or in non-heated greenhouses in Sicily, Italy [24]. In Israel, *B. thuringiensis* var. *kurstaki* is used to control *C. chalcites* [11]. On the other hand, it was demonstrated that the entomopathogenic fungus *Nosema manierae* can kill *C. chalcites* larvae in a few days [25].

Currently, integrated pest management (IPM) is a mandatory measure in all EU countries (Directive 2009/128/EC, OJEU 24-XI-2009, L309: 71–86), including Spain (RD 1311/2012, BOE-A-2012-11605), promoting more environmentally-friendly methods in pest control. Bioinsecticides are characterized by a very low toxicity to humans and other vertebrates, decomposition within a few hours after being applied, specificity for the target pest, and environmental friendliness [26]. The aim of this work was to evaluate the repellent effect, antifeeding effect, and direct toxicity of the selected products to *C. chalcites* larvae under laboratory conditions, and the associated reduction in fruit damage under screenhouse conditions.

2. Materials and Methods

2.1. Insects

The larvae used in the laboratory and screenhouse assays were obtained from a colony maintained at the Instituto Canario de Investigaciones Agrarias (ICIA) in a climatic chamber at 25 ± 1 °C, 65 ± 5% relative humidity and a photoperiod of 16:8 light(L):dark(D) h. The colony was maintained on a semi-synthetic diet based on corn flour, wheatgerm, and yeast following the methodology devised by [6]. The adults were fed ad libitum with 10% *v*/*v* honey solution. The colony was established 3 months before the trials from

natural populations originally collected on organic banana growing areas in Las Galletas (28°01′52″ N, 16°39′32″ W), Tenerife, Canary Islands, Spain.

2.2. Products Applied

The bioinsecticides were mixed in water at 20–24 °C and 7.0 pH. The selected products were Avenger®, BioKnock®, Cinamite®, Garlitrol-Forte®, Indasol®, Intruder®, Prevam®, and Ripelser® [27–31]. A description of the products, extracted from their technical sheets, is presented in Table 1.

Table 1. Characteristics and description of products used in the bioassays.

Product Name	Active Ingredient	Formulation (%*w*/*w*) *	Dose Used (mL/L) *	Manufacturer
Intruder®	Enzymatic soap	Free amino acids 6%; Active proteins complex 8%	1.2	Blue Heron, Spain
Avenger®	Plant extracts	Plant extracts and oils (Rutaceae and Piperaceae): 12.0% pH: 7.8	1.4	Blue Heron, Spain
BioKnock®	Plant extracts	Plant extracts and oils (Rutaceae and Lauraceae): 22%; pH: 8.0	2.4	Blue Heron, Spain
Cinamite®	Plant extracts	Citronella-cinnamon: 30%; Mint: 13%; pH: 7.2	2.4	Blue Heron, Spain
Ripelser®	Spicy chili	Capsicin: 15–50%; Iron sulfate: 10–25%	2	Capa Ecosystems S.L., Spain
Garlitrol Forte®	Garlic, spicy chili	Garlic oil: 25–<50%; Capsicin: 1–<2.5%	5	DAYMSA, Spain
Prevam®	Orange	Orange oil 6%	3	Oro Agri Europe S.A., Portugal
Indasol®	*Azadirachta indica*	*Azadirachta indica* (neem) oil, azadirachtin 2%	3	Canbio (Canaria de Biología Agrícola), Spain

* Upon technical sheet specifications.

The characteristics and use of the products are described by the manufacturers as follows: Avenger® and Intruder® have a hydrolytic and degrading effect on chitins, sugars, and other substances secreted by white flies, moths, thrips, and mites. BioKnock® has an insecticidal effect on white flies, mealybugs, moths, psyllids, aphids, and other insects. Ripelser® and Garlitrol® have an insecticidal effect against pests such as green mosquitos and lepidoptera. Cinamite® has an insecticidal effect against *Tetranychus urticae*, *Planococcus citri*, *Thrips tabaci*, and *Frankliniella* sp. in citruses, fruit trees, and vegetables. Prevam® causes dehydration of insect cuticles and Indasol® reduces insect feeding.

The products were prepared for bioassays by dilution in water at 20–24 °C and 7.0 pH.

2.3. Laboratory Experiment

The insecticidal efficacy of the selected products on 2nd instar larvae of *C. chalcites* was evaluated under laboratory conditions at the Instituto Canario de Investigaciones Agrarias (ICIA). The descriptions and application doses of the tested products are shown in Table 1. The type of bioassays was derived and combined from [32] and three types of bioassays were carried out:

2.3.1. Choice Assay: Repellent Effect

Two discs of 2 cm^2 from banana leaves sprayed with 0.5 mL of the test product per disc and two discs sprayed with 0.5 mL of water per disc were placed in Petri dishes (140 mm diameter). Five replicates (dishes) per treatment were conducted. The leaves were allowed to dry for 30 min, and then two 2nd instar *C. chalcites* larvae were added to each Petri dish and allowed to feed on the discs for 24 h. After 24 h, the leaf discs were scanned with a standard image scanner, and the software ImageJ v1 [33] was used to calculate the area of the leaves eaten by the larvae.

2.3.2. No Choice Assay: Damage Rate

In this case, four discs of 2 cm^2 from banana leaves sprayed with 0.5 mL of the product (or water only for the control treatment) per disc were placed in each Petri dish. Five replicates (dishes) per treatment (also five dishes for the control) were conducted. The leaves were allowed to dry for 30 min, and then two 2nd instar *C. chalcites* larvae were added to each Petri dish and left to feed on the treated leaves for 24 h. After 24 h, the leaves were scanned with a standard image scanner, and the software ImageJ v1 [33] was used to calculate the area of the leaves eaten by the larvae.

2.3.3. Contact Toxicity of Products on *C. chalcites* Larvae

To evaluate the direct effect of each product on *C. chalcites* 2nd instar larvae, one banana leaf disc with a 5 cm diameter was placed into a Petri dish and infested with 10 2nd instar *C. chalcites* larvae. Each tested product, including the control, was then sprayed on the leaf using a 1 L manual hydraulic sprayer, with five replicates (dishes) per product. After 1 h, the number of dead larvae in each disc was registered, and the surviving larvae were carefully transferred into individual disposable cups with a standard diet and kept at 25 ± 1 °C, 60% RH and a 16:8 (L:D) photoperiod. The larvae were observed at 1 day, 3 days, and 7 days post application, and the time to death (if that was the case) was recorded.

2.4. Screenhouse Experiment

2.4.1. Experimental Plot and Treatments

The trials were conducted in a mesh-built screenhouse at the Instituto Canario de Investigaciones Agrarias (ICIA), in Pajalillos (Valle de Guerra, La Laguna, Tenerife; 28°31′38.4″ N–16°23′09.4″ W, 100 m.a.s.l.), for two spring–summer seasons. The mesh-built screenhouse area was 7200 m^2. The planting density was 2400 plants per ha, with double rows: 1.67 m between the plants in the row and 5.0 m between the rows. Drip irrigation with fertilization was scheduled.

The experimental design consisted of randomized plots with four replicates per treatment. Hence, each experimental unit consisted of four banana bunches (about 60 bananas per bunch) close to harvest. The bunches were hung with a hook on a mobile metal wire and placed 2 m away from each other. Based on previous (unpublished) works, forty 2nd instar larvae of *C. chalcites* per banana bunch were used for artificial infestation. This number of larvae allows for appreciable damage to fruits to be achieved for the assay, as the number of surviving larvae usually decreases dramatically after egg hatching. This is because the small larvae sometimes fall from the plant (accidentally or by looking for another host) or die due to their cannibalistic behavior or because of predation by ants and spiders [34].

After 24 h of introducing the larvae, the selected bioinsecticides (see Section 2.2) were sprayed on banana bunches with a 2 L compressed-air hand sprayer (SOLO1 402, Sindelfingen, Germany) at the recommended commercial concentration (assuming an application volume of 1000 L/ha) and water as a control, with all of them including 0.1% (*v*/*v*) Agral wetter-sticker. The bioinsecticides were applied between 8.00 a.m. and 11.00 a.m. A data logger Omega model OM-92 was set for registering temperature and humidity inside the screenhouse during the experiment.

2.4.2. Evaluation of the Treatment Effects on Larval Survival and Fruit Damage

Survival. Larval mortality was estimated by counting the larvae 1 day before and 1 day, 3 days, and 7 days after treatments in each bunch.

Efficacy. The efficacy of the products was calculated using Henderson–Tilton's formula:

$$\text{Efficacy \%} = [1 - (n \text{ Co before treatment} \times n \text{ T after treatment} / n \text{ Co after treatment} \times n \text{ T before treatment})] \times 100$$

where n = insect population (number of larvae), T = treated, and Co = control.

Damage. One week after application of the products, banana bunches were taken off the hook and the "hands" (group of bananas within a bunch; usually, there are 10–16 hands per bunch) and "fingers" (individual bananas) were cut and separated. The number of hands and fingers (total number and number damaged by *C. chalcites* larvae feeding), and the number of larvae by bunch were counted. Hands from each bunch were classified based on *C. chalcites* damage using three EU categories of quality standards and then weighed: Category 1 corresponds to banana hands with no damage or damage smaller than 1 cm^2; Category 2 corresponds to damage areas larger than 1 cm^2 on one finger or smaller than 1 cm^2 distributed on several fingers; and Category 3 corresponds to damage areas greater than 1 cm^2 and on more than one finger. Categories 1 and 2 are marketable fruit, whereas Category 3 is not marketable.

2.4.3. Estimation of Economic Losses Caused by *C. chalcites*

To estimate the economic impact of the damage caused by *C. chalcites* on banana fruit, the incomes to be received by the farmer were calculated for each treatment, based on the prices of banana fruit in the high-price and low-price seasons and the weight of fruit in the different quality categories.

Concerning banana fruit prices, in the high-price season Categories 1 and 2 receive EUR 0.97/kg and EUR 0.81/kg on average, respectively, whereas in the low-price season, Categories 1 and 2 receive approximately EUR 0.73/kg and EUR 0.52/kg, respectively. Category 3 is considered unmarketable and has a EUR 0/kg value. It was assumed that 50% of the plants had banana bunches because the emergence of fruit is unevenly synchronized, and this value corresponds to 900 bunches per ha because the mean plantation density for banana plants in the Canary Islands is 1800 plants per ha. Hence, as the mean production per ha in the region is 50,000 kg, in our experiment 25,000 kg of production was assumed for the calculations. As a consequence, the gross income in each treatment was calculated as:

$$GI_{treatment} = 25{,}000 \times [(\text{income for C1}) + (\text{income for C2})].$$

$$GI_{treatment} = 25{,}000 \times [(\%C1 \times \text{EUR } C1_{LP,HP}) + (\%C2 \times \text{EUR } C2_{LP,HP})].$$

where $GI_{treatment}$ = gross income of the treatment; C1 = Category 1, C2 = Category 2; % = percentage of the category in the treatment; EUR = price of the category in the season; LP = low-price season, HP = high-price season.

The cost of the applied products was calculated based on a volume of prepared product of 650 L per ha (usual rate: 1300 L per ha—application to the banana bunch and plant bracts, corrected by 50% of plants with bunches) using the manufacturer's recommended dose (Table 1) and a retail price of the product that was provided by the local distribution company. The calculation was made as follows:

$$Cost_{treatment} = (EUR_{product}/U_{product}) \times D \times 650 + \text{cost worker (EUR/h} \times \text{hs application)}$$

where $Cost_{treatment}$ = cost of application of the product, $EUR_{product}$ = price of the product (per bottle, bag), $U_{product}$ = units of the product (per bottle, bag … for example: 1000 mL or 5000 g), D = dose of product in the preparation (g/L, mL/L); cost worker for each 900 bunches treatment was estimated to be EUR 150 (3 days of work at EU 50/day).

The avoided economic loss estimation for each treatment was calculated as it is explained in the following formula, based on the gross income in each treatment ($GI_{treatment}$) minus the cost of the treatment ($Cost_{treatment}$) and minus the gross income value of the control bunches ($GI_{control}$) that was calculated as for the treated bunches. The final value represents the avoided economic losses for each product.

$$\text{Avoided economic loss}_{treatment} = GI_{treatment} - Cost_{treatment} - GI_{control}$$

2.5. Data Analysis

The eaten leaf area (damage index) from the choice and no-choice experiments, cumulative mean mortality percentages in the contact toxicity experiments, damaged banana fingers, and damaged banana finger categories were subjected to one-way ANOVA, after checking the normality of the data by Kolmogorov–Smirnov ($p > 0.05$).

The analysis of the larval mortality data for the estimation of the contact toxicity of the products was performed by the Henderson–Tilton formula, and the efficacy values obtained were used in a repeated measured analysis to evaluate the mortality percentage of *C. chalcites* over time (days 1, 3, and 7). Kaplan–Meier curves were used to determine the fractions of dead larvae in the treated units relative to the control units over time. A Cox regression analysis was used to estimate the hazard ratios (HRs). All analyses were performed in SPSS v23. When significant mean differences were observed ($p < 0.05$), the means were separated using Tukey's HSD test.

3. Results

3.1. Choice Assay (% Repellence)

Damage index values in choice tests under laboratory conditions are presented in Figure 1 as percentage repellence or avoidance of consumption of the treated leaves ($F = 2.175$, $df = 7.72$, $p = 0.046$). The eaten area of leaves treated with each product was compared with that of the control leaves.

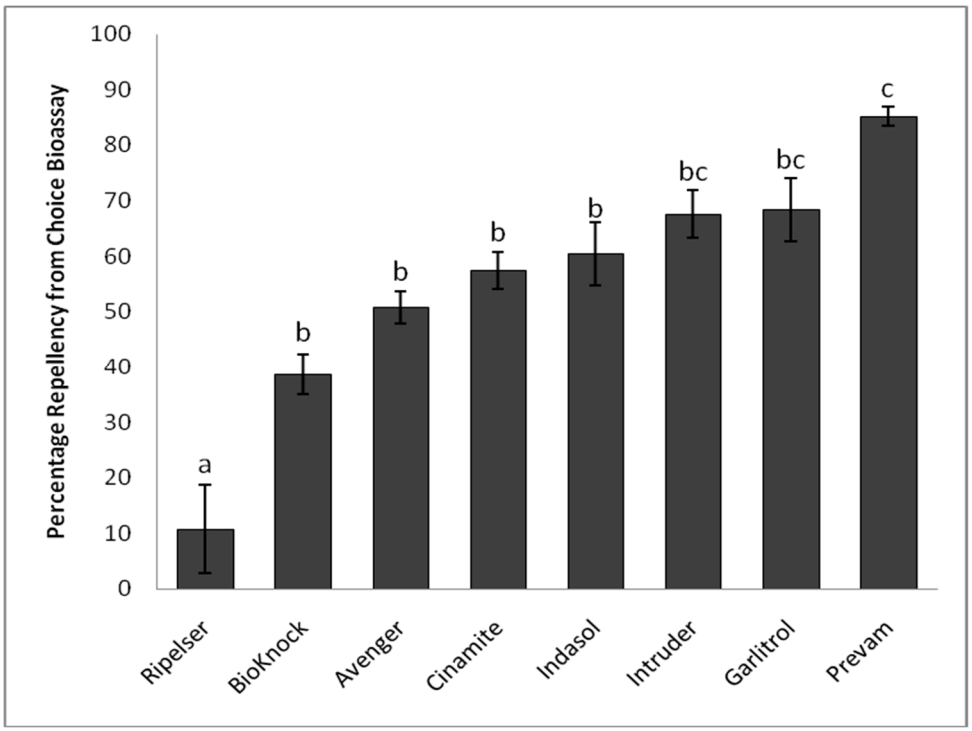


Figure 1. Repellence test of selected products on the 2nd instar larvae of *C. chalcites* under laboratory conditions (choice assay). Different letters above bars indicate a significant difference among the groups according to ANOVA followed by Tukey's HSD test ($p < 0.05$).

The results showed a first group: Prevam® (85.19 ± 1.7%) followed by Intruder® (67.54 ± 4.3%), and Garlitrol® (68.44 ± 5.7%), which significantly differed ($p < 0.05$) from the other products. The second group was composed of Indasol® (60.44 ± 5.7%), Cinamite® (57.46 ± 3.3%), Avenger® (50.79 ± 2.9%) and Bioknock® (38.64 ± 3.6%). Ripelser® (10.77 ± 7.9%) showed the lowest repellent effect on the choice assay.

3.2. Non-Choice Assay

The mean percentage of consumed area of the treated leaves (anti-feeding effect) in the non-choice test is presented in Figure 2 (F = 10.479, df = 8.36, $p < 0.0001$). The effect of each product on the treated leaves was compared with that of the control leaves.

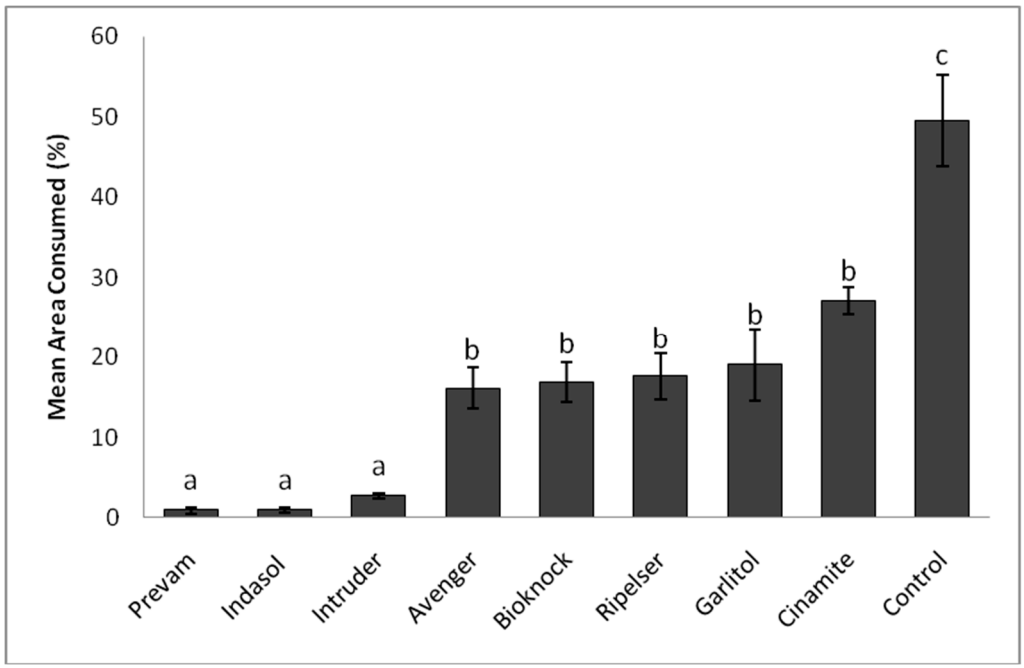


Figure 2. Mean area (%) of leaves consumed by 2nd instar larvae of *C. chalcites*). Different letters above the bars indicate a significant difference among the groups according to ANOVA followed by Tukey's HSD test ($p < 0.05$).

A significant difference ($p < 0.05$) was detected between the treatments. Prevam® (0.92 ± 0.4%), Indasol® (0.98 ± 0.33%), and Intruder® (2.7 ± 0.33%) had the lowest consumed areas 24 h post application. The leaves treated with Avenger® (16 ± 2.55%), Bioknock® (16.86 ± 2.43%), Ripelser® (17.59 ± 2.9%), Garlitrol® (19 ± 4.5%), and Cinamite® (27.06 ± 1.67%) showed higher consumption by the 2nd instar *C. chalcites* larvae than the previous group but were significatively lower than the control leaves (49.54 ± 11.47%).

3.3. Contact Toxicity of Products on C. chalcites Larvae

The contact toxicity of the treated leaves is shown in Figure 3 (Day 1 F = 4.770, df = 8.36, $p < 0.0001$; Day 3: F = 5.776, df = 8.36, $p < 0.000$; Day 7: F = 7.301, df = 8.36, $p < 0.0001$).

At 1 day post application, the highest mortality was detected in Intruder®-treated leaves (20.22 ± 2.98%), followed by Indasol® (18.44 ± 3.8%) and Bioknock®-treated leaves (10.44 ± 4.7%). Three days post application, Intruder® still had the highest contact toxicity effect (49.11 ± 4.04%), followed by Indasol® (47.33 ± 6.8%). Seven days post application, the highest mortality was detected again on Intruder® (77.77 ± 5.7%) and Indasol®-treated leaves (76 ± 9.27%), followed by Prevam® and Garlitrol®-treated leaves (58 ± 11.13% and 49.55 ± 5.5%, respectively).

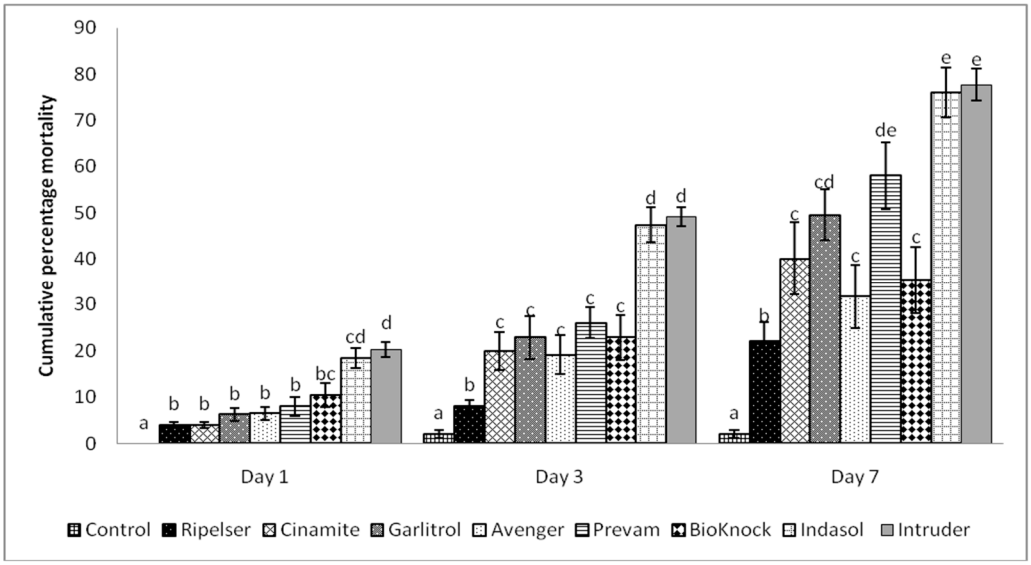


Figure 3. Contact toxicity of products on 2nd instar larvae of *C. chalcites*. Different letters above bars indicate a significant difference among the groups according to ANOVA followed by Tukey's HSD test ($p < 0.05$).

3.4. Screenhouse Experiment

The average temperature and humidity of the plots in the period of the assay were 22 °C and 72%, respectively, which are favorable conditions for *C. chalcites* development. The initial number of alive larvae (40 larvae) decreased in both the treated and control plots (see the table and figure in Supplementary Material), even before the application of the biorational products, by natural causes, as was observed in previous works [34]. In fact, *C. chalcites* scores were significantly reduced ($p < 0.05$) in all of the treated plots 3 days after spraying (1 to 23 larvae), but the total number of alive larvae after the products' application was always lower on the treated plants than on the control.

Efficacy values calculated using the Henderson–Tilton formula after 1 day, 3 days and 7 days after the application of the products are presented in Table 2.

Table 2. Henderson–Tilton efficacy values on banana bunches 1 day, 3 days, and 7 days post application [a].

Treatment	Day 1	Day 3	Day 7
Prevam®	58.92 ± 12.18 a,B	66.58 ± 10.67 a,B	100.0 ± 0.0 a,A
Cinamite®	58.63 ± 4.27 a,A	33.68 ± 10.86b c,d,A	15.59 ± 9.03 d,B
Intruder®	51.18 ± 15.14 a,b,A	21.08 ± 15.35b c,d,e,A	36.25 ± 23.75 c,d,A
Avenger®	30.80 ± 15.56 a,b,c,A	11.76 ± 7.88 d,e,A	9.88 ± 5.71 d,A
Indasol®	26.55 ± 9.20 b,c,A	46.21 ± 9.62 a,b,c,A	42.53 ± 21.47 a,b,A
Ripelser®	26.50 ± 6.59 b,c,A	6.10 ± 4.93 e,B	36.74 ± 12.74 c,d,A
Bioknock®	25.73 ± 8.94 b,c,A	27.90 ± 7.78b c,d,e,A	11.16 ± 6.47 d,A
Garlitol Forte®	24.39 ± 8.77 b,c,B	34.68 ± 8.61 b,c,d,A	65.78 ± 12.0 a,b,c,A

[a] Lowercase letters shows the differences within each day; capital letters resemble the difference between the post-application days.

At 1 day post application, three groups can be discerned: most effective, intermediately effective, and least effective products. According to the Henderson–Tilton formula, on the first day post application, the most insecticidally effective product was Prevam® (58.92 ± 12.18%), closely followed by Cinamite® (58.63 ± 4.27%). Intruder® (51.18 ± 15.14%) had an intermediate effect, and all the other products were less effective. On day 3, the most efficient product was Prevam® (66.58 ± 10.67%), followed by Indasol® (46.21 ± 9.62%). The intermediate group on the 3rd day consisted of Garlitol® (34.68 ± 8.61%), Cinamite® (33.68 ± 10.86%), Bioknock® (27.90 ± 7.78%), and Intruder®

(21.08 ± 15.35%). The least effective products on the 3rd day were Avenger® (11.76 ± 7.8%) and Ripelser® (6.10 ± 4.93%). Seven days after application, the Prevam®-treated bunches showed 100.0 ± 0% efficacy on *C. chalcites* 2nd stage instars. Indasol® (42.53 ± 21.47%), Ripelser® (36.74 ± 12.74%), and Intruder® (36.25 ± 23.75%) were in the intermediate group, while Cinamite® (15.59 ± 9.03%), Bioknock® (11.16 ± 6.47%), and Avenger® (9.88 ± 5.71%) remained in the less effective group.

In brief, all of the applied products decreased the initial populations of *C. chalcites* (number of alive larvae). However, not all of them had a significant effect on reducing the damage. The percentage of damaged fingers is presented in Figure 4.

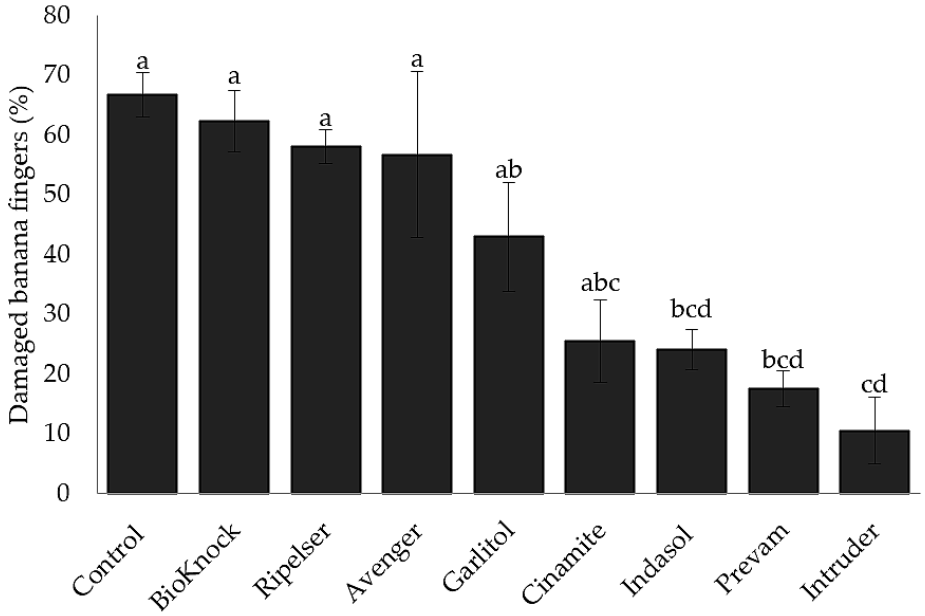


Figure 4. Percentage of damaged banana fingers per treatment. Different letters above bars indicate a significant difference among the groups according to ANOVA followed by Tukey's HSD test ($p < 0.05$).

According to these results, treatments were assigned to one of three groups: low, intermediate, and high damage. Intruder® (10.54%), Prevam® (17.63%), Indasol® (24.11%), and Cinamite® (25.61%) were in the group with the lowest damage levels (high protection), while Garlitrol® (43.00%) was in the intermediate group. Avenger® (56.17%), Ripelser® (58.17%) and Bioknock® (62.30%) were in the high damage group (more than 50% damaged fingers, low protection), with no significant differences with the control. It is noteworthy that the phytotoxic effects of the applied products were not observed.

Fruit classification by quality categories is presented in Figure 5.

Banana bunches in the control treatment were highly damaged and classified into Category 3 (56.9%) and Category 2 (33.3%). The highest percentage of fruit in Category 1 was found in the treatments with Indasol® (76.81%), followed by Intruder® (73.68%) and Prevam® (71.42%). Category 1 (the best quality) fruits decreased in the treatments with Bioknock® (36.04%), Cinamite® (25.12%), Garlitrol® (23.67%), Avenger® (18.18%), and Ripelser® (11.11%).

Category 2 fruits (intermediate quality) were predominant in the treatments with Ripelser® (77.78%), Cinamite® (64.15%), Avenger® (47.15%), Garlitrol® (31.40%), Intruder® (26.32%), Prevam® (24.75%), Indasol® (16.15%), and Bioknock® (9.17%). Bioknock® and Garlitrol® are the treatments with the most damage and have 54.79% and 44.93% fruit in Category 3 (lowest quality), respectively.

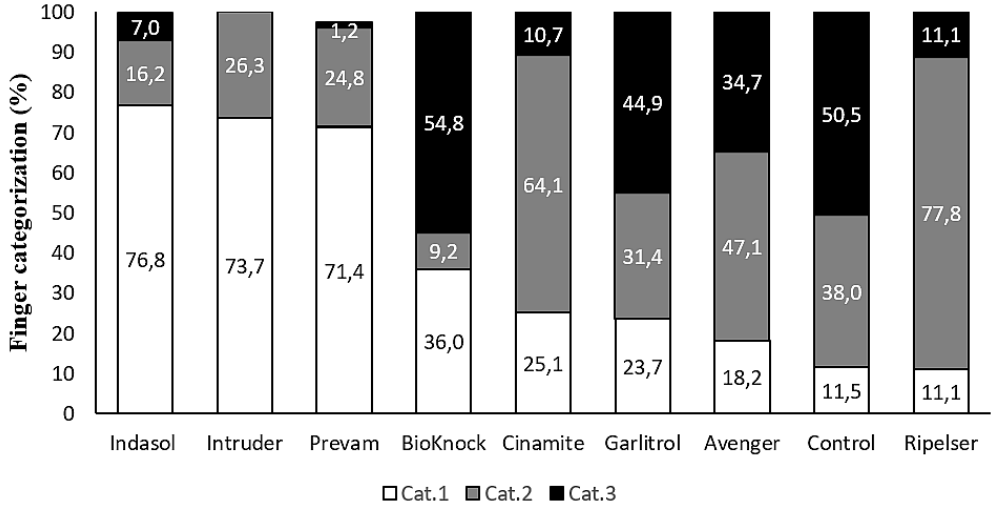


Figure 5. Damage level categorization in each treatment according to European Standards (EPPO, PP1). Cat. 1: without damage and/or the damage is <1 cm^2 at 1 finger; fulfilling European norms; Cat. 2: damage > 1 cm^2 on 1 finger, or <1 cm^2 on some fingers; Cat. 3: damage > 1 cm^2 more than 2 fingers.

3.5. Estimation of Economic Losses

The results of the estimation of avoided economic losses with respect to the untreated control and assuming that 50% of plants had banana bunches are shown in Table 3.

Table 3. Economic analysis assuming that 50% of plants had bunches in 1 ha (EUR).

	Gross Income		Cost of the Treatment *	Avoided Losses **	
	High Price	Low Price		High Price	Low Price
Intruder®	23,197.4	16,117.3	172.8	12,539.6	8905.1
Prevam®	22,331.2	16,251.7	281.6	11,564.7	8930.7
Indasol®	21,896.8	16,117.3	274.1	11,137.7	8803.8
Cinamite®	19,081.7	12,923.8	193.7	8403.1	5690.7
Ripelser®	18,444.4	12,138.9	217.5	7742.0	4881.9
Avenger®	13,956.1	9447.1	164.1	3307.1	2243.6
Garlitrol®	12,099.0	8402.2	238.5	1375.6	1124.3
BioKnock®	10,596.4	7769.3	190.7	−79.3	539.2
Control	10,485.0	7039.4	0	0.0	0.0

* Cost of the treatment = cost of the product + cost of worker. ** Avoided losses = gross income of the treatment-(cost of the treatment + gross income in the control).

Avoided losses are calculated as the value of gross income of the treatment minus the treatment cost and the gross income in the control. The highest avoided losses values were found with Intruder® (high price season: EUR 12,539.6/ha, low price season: EUR 8905.1/ha). Prevam® provided the second highest economic income (high price: EUR 11,564.7/ha, low price: EUR 8930.7/ha), followed by Indasol®, which provided EUR 11,137.7/ha in the high-price season and EUR 8803.8/ha in the low-price season. Decreasing values were obtained in this estimation with Cinamite® (high price: EUR 8403.1/ha, low price: EUR 5690.7/ha), Ripelser® (high price: EUR 7742.0/ha, low price: EUR 4881.9/ha), Avenger® (high price: EUR 3307.1/ha, low price: EUR 2243.3/ha), and Garlitrol® (high price: EUR 1375.6/ha, low price: EUR 1124.3/ha). The application of BioKnock® was found to induce economic losses, compared to the control, when banana prices are high (EUR −79.2/ha), whereas at low prices of the fruit, it avoided losing EUR 539.2/ha. Therefore, this product seems to be useful only when banana prices are low.

It must be noted that the economic benefits of using these natural products refer to 50% of plants having bunches. In the whole area, considering all the bunches, and the corresponding treatment for them, the avoided economic losses (i.e., economic benefits of applying the products) would be doubled.

In addition, it must be kept in mind that artificial infestation with *C. chalcites* was adjusted to 40 larvae per bunch to ensure fruit damage [34]. In fact, the number of larvae on the fruit decreased 12.5–87.5% in the first 24 h (see Table S1 and Figure S1 in Supplementary Materials), due to natural causes, just before the application of the biorational products. As a consequence, the real number of larvae feeding on the fruit was variable and lower than the added amount, as would be expected in a field assay. Hence, the obtained results cover different levels of infestation, resembling real conditions, which is hardly standardizable. In this context, the results of the economic analysis give an approximation of the expected benefits of the application of biorational products, and more studies are encouraged to contribute and validate our results.

4. Discussion

Our results show that Intruder®, Prevam®, and Indasol® are highly efficient insecticides against the 2nd stage larvae of *C. chalcites* based on laboratory and screenhouse experiments. These findings agree with previous works with natural products focused on azadirachtin (which is one of the main insecticidal compounds of neem oil, an ingredient of Indasol®), which led to significant reductions in reproductive parameters in adults of *Spodoptera littoralis* [35], *S. exempta* Walker (Lepidoptera: Noctuidae) [36], *Plutella xylostella* (L.) [37], and *Cosmopolites sordidus* (Germar) (Coleoptera: Curculionidae) [38]. Concerning Prevam®, its efficacy on larvae and eggs of *Tuta absoluta* (Meyrick) in semi-natural conditions was tested, with a high efficacy of this product toward all instars of *T. absoluta* being found [39].

Screenhouse experiments showed that Prevam®, Intruder®, and Indasol® reduced the number of larvae and the level of damage of banana fruit and achieved approximately 70% of the bunches classified as Category 1. The effectiveness of some of the products decreased over time (Cinamite®, Avenger®, Intruder®, and Bioknock®), increased over time (Prevam® and Garlitrol Forte®), or initially decreased and then increased (Ripelser® and Indasol®). These findings indicate different modes of action, which may be studied in the future.

Based on this assay, the application of these products doubled the fruit incomes with respect to the control. BioKnock®, Avenger®, Cinamite®, Garlitrol®, and Ripelser® had intermediate protection against *C. chalcites* larvae on banana bunches after 7 days of application and a damage level with more than 50% of bunches considered as unmarketable fruit. The values of the avoided economic losses agree with the results obtained from laboratory and screenhouse efficacy tests; the most efficient products also provided higher incomes. The results of economic analysis are just indicative since the financial losses depend on the infestation level which may be different from that of the present work.

A complete analysis of this work (laboratory assays, screenhouse experiment, and estimation of avoided economic losses) showed that Prevam®, Intruder®, and Indasol® were the three most successful products against 2nd stage larvae of *C. chalcites*, with a direct beneficial impact on economic incomes. Moreover, phytotoxic effects of these products were not observed. Thus, the results of this study indicate that the new generation of biorational insecticides would be effective against 2nd stage *C. chalcites* larvae. A second application may increase efficacy and lead to an even greater reduction in fruit damage, although application costs must be considered.

The experiments presented here allowed for a preliminary estimation of the potential of plant extract-based products, so-called biorational insecticides, for use in the integrated management of *C. chalcites* in banana crops. The products evaluated have highly biodegradable compounds from a natural origin, are not phytotoxic, and are usually harmful to neither users nor consumers [40]. Even if synthetic insecticides are effective and available, the risk of insects developing resistance, as well as their toxicity both for humans, other

vertebrates, and biological control agents, is challenging their commercialization in the medium–long term. Biorational products profiles are, in contrast, demanded by European authorities and have preference in current IPM strategies [41]. Further field experiments that study damage levels at larger scales (more units of study) and investigate different phenological stages of banana bunches and various seasons of the year are needed to provide a better understanding of these products, with the aim to strengthen IPM programs against *C. chalcites* in banana production.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy12123141/s1, Figure S1: Survival analysis of *C. chalcites* larvae 1 day before, 1 day, 3 days, 7 days after the application of (**a**) Bioknock®, (**b**) Intruder®, (**c**) Avenger®, (**d**) Cinamite®, (**e**) Garlitrol Forte®, (**f**) Ripelser®, (**g**) Prevam®, and (**h**) Indasol® (neem oil); Table S1: Minimum, mean, and maximum number of C. chalcites alive larvae observed on banana bunches at 1 day prior to application and 1, 3 days, and 7 days post application of biorational products.

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