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Effects of Cymbopogon Winterianus and Ocimum Basilicum against the Stored Phaseolus Vulgaris Bean Pest, Acanthoscelides Obtectus †

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† Presented at the 1st International Electronic Conference on Plant Science, 1–15 December 2020. Available online: https://iecps2020.sciforum.net/.

Published: 1 December 2020

Abstract: *Acanthoscelides obtectus* Say (Coleoptera: Chrysomelidae: Bruchinae), is an insect pest that attacks wild and cultivated common beans, *Phaseolus vulgaris* L. To control insect plagues, it is a priority to choose compounds with different modes of action, with greater selectivity and less persistence. There is an increasing demand in the search for new active substances and natural plant products for pest control for the reduction of adverse effects on human health and environment. Therefore, this investigation evaluated under laboratory conditions, three doses (24, 60 and 120 μL) of *Cymbopogon winterianuss* and *Ocimum basilicum* essential oils over bean seeds placed in Petri dish in which *A. obtectus* insects were added before. Treatments of bean seeds with different doses of essential oils provided different survival on *A. obtectus* adults. The essential oils affected the development of *A. obtectus* insects since the greatest doses applied on beans decreased the emergence of the bean weevil. Also, reduced the number of exit holes of insects by damaged beans and the vean weight los, from 2,987% in control treatment, to 1,014% and 1,221% with the dose 120 μL of *C. winterianuss* and *O. basilicum*, respectively. The ability of both doses of *C. winterianuss* and *O. basilicum* to reduce their longevity, their subsequent emergence from insects, and protect the bean seeds, make these essential oils a suitable tool for the control of adults of this insect pest in small storages.

Keywords: bean pest; essential oils; insecticidal properties; development, emergence; bean damage

1. Introduction

Phaseolus vulgaris L. is one of the most important legume crops worldwide. This crop can be affected by varius pest, both in fields and in storage. *Acanthoscelides obtectus* Say (Coleoptera: Chrysomelidae: Bruchinae), is one of those pest that begin its infection in the field continuing the damage during storage, where it causes the greatest losses, to the point of producing the loss of whole crops within a few months [1–3].

So far, the control of this pest has been carried out using synthetic insecticides such as phosphine, pyrethroids, organophosphates, etc. However, these products are highly toxic to human health and the environmental. They are also prone to induce the devolpment of resistances by the insect, therefore complicating its control. As a result, current research focuses on the development of more selective compounds, with low environmental residue and differents modes of action as a more sustainable alternative [4,5]. Essencial olis have appeared as one of these alternatives to the use of synthetic chemical products, and their use as insecticides has been increasing lately. Various essential oils have been used as potential fumigants against stored grain insect pests like *Callosobruchus maculatus* Fabricius (Coleoptera: Chrysomelidae: Bruchinae), *Sitophilus oryzae* L. (Coleoptera: Curculionidae), *A. obctetus* or *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae) [6–9]. Therefore, natural plant products are a worldwide alternative for the control of mites and insect pests, including *A. obtectus* [10,11].

Cymbopogon winterianuss Jowitt ex Bor, Poaceae (citronella), is highly appreciated in perfumery and aromatherapy, and posseses antiseptic, antibacterial, antifungal and digestive properties. It has shown repellency and substantial control capacity against many species from different insect orders, as for example, *Spodoptera exigua* Hübner (Lepidoptera: Noctuidae) [12], *Anopheles gambiae* Giles (Diptera: Culicidae) [13] and *C. maculatus* [14].

Ocimum basilicum L., Lamiaceae (basil), commonly used for its medicinal and aromatic characteristic, it is also known for its antioxidant and antimicrobial properties. It has been tested for its insecticidal effects and for the control on several pests of both stored products and crops [15]. Various researches have shown its ability to control pests such as *C. maculatus* [16], *A. obtectus* [17], *Sithopilus zeamais* Motschulsky (Coleoptera: Curculionidae) [18], or *Rhyzoperta dominica* Fabricius (Coleoptera: Bostrichidae) [19].

The present research has studied the insecticidal potential of *C. winterianus* and *O. basilicum* essential oils against *A. obtectus* on stored beans by non-fumigant applications.

2. Material and Methods

2.1. Insects Rearing

The original population of *A. obtectus* adults was collected in different bean storage facilities. The insects were reared in glass jars (150 mm in diameter and 250 mm high) with common bean (*P. vulgaris*) seeds and covered with cloth, allowing the gas exchange. Every three days all *A. obtectus* adults were removed from the jar in order to maintain a population of young adults (1 to 3-day old) ready to use in the experiments. Before and after the treatments, *A. obtectus* adults were kept in a chamber with controlled temperature (24 ± 1° C), humidity (60 ± 5%), and a photoperiod of 16 h of light (luminous intensity of 1000 lux) and 8 h of darkness.

2.2. Essential Oils

O. basilicum and *C. winterianus* essential oils obtained by hydrodestillation were purchased from Naissance *(Neath, Wales)*. Volatile components of essential oils were identified by gas chromatography (GC) according to the information provided by the company described above. Analysis for the main compounds are depicted in Table 1.

Table 1. Composition of *O. basilicum* and *C. winterianus* essentials oils obtained with GC analysis.

Essential Oil	Compounds (%)	Total (%)	Essential Oil	Compounds (%)	Total (%)
O. basilicum	Citral (0.70) Citronellol (11.50) Citronellal (34.00) Geraniol (22.00) Limonene (3.50)	71.70	*C. winterianus*	Estragol (74.00) Linalool (17.80)	91.80

2.3. Design of Experiments

2.3.1. Experiment 1: Essential Oil Effects on *A. obtectus* Adults

This bioassay was conducted to determine the dose-dependent toxicity of *C. winterianuss* and *O. basilicum* essential oils against *A. obtectus* adults. For the application, a Potter Tower (Burkard Scientific Limited, Po Box 55 Uxbridge, Middx UB8 2RT, UK) [20] of manual loading coupled to an air compressor was used. The total volume used in each spray was 1 mL, applied on Petri dishes (90 mm in diameter) over 40 *P. vulgaris* beans at 40 kPa. Three doses (24, 60 and 120 μL/petri dish) of *C. winterianuss* and *O. basilicum* oils were diluted in ethanol and five replicates were performed for each of them. A treatment with ethanol (without oil) was used as a control. After application of the treatments, twenty unsexed 1 to 3-day old *A. obtectus* were placed in the Petri dish with the beans. The Petri dishes were kept in a chamber with controlled temperature (24 ± 1° C), humidity (60 ± 5%), and a photoperiod of 16 h of light (luminous intensity of 1000 lux) and 8 h of darkness. In the covers of Petri dishes, 8 holes (8 mm^2) were made to avoid the vapour accumulation effect from the treatments. Daily monitoring was carried out during the following 32 days after the application of each dose of essential oil, counting the mortality of *A. obtectus* adults.

2.3.2. Experiment 2: Essential Oil Effects on *P. vulgaris* Beens Damaged by *A. obtectus*

From day 32 after the treatment application on the beans, the number of *A. obtectus* emerged from the beans (first generation) was recorded. Likewise, from day 48, the number of damaged beans (with at least one hole), the number of holes per bean, and the weight loss of the damaged beans were also recorded in this experiment.

2.4. Statistical Analysis

Experiment 1. Survival data of *A. obtectus* insects were submitted to the Kaplan-Meier estimator and the functions obtained from each treatment were compared by the log-rank test (Mantel-Cox) ($p < 0.05$) using IBM SPSS Statistics for Windows, version 26.0. (IBM Corp.: Armonk, NY, USA).

Experiment 2. A randomly completed experiment Generalized Linear Model (GLM) procedure, with three doses for each essential oil and five replicates, was subjected to ANOVA (data means were normally distributed and presented homocedasticity). Differences ($p < 0.05$) in number of damaged beans, number of holes per bean and bean weight loss were examined by mean comparisons using the Least Significant Difference (LSD) test using the same software.

3. Results

3.1. Effects of Beans Sprayed with Essential Oils on the Survival of A. obtectus Insects (Experiment 1)

Table 2 shows that the type of treatment with essential oil does influence in the survival of the insects (log-rank $\chi2$ = 103.071; df = 6693; $p < 0.001$). Insects that were subjected to 120 μL of *C. winterianus* and *O. basilicum* essential oils had a significantly lower survival than insect that were subjected to lower doses, as 91.00 and 83.00% of the evaluated insects died with *C.winterianus* and *O. basilicum* essential oils, respectively. The insects subjected to 120 μL of *O. basilicum* oil had an estimate of 16.01 days of life, significantly lower than that obtained in the rest of doses and the control (22.68 days). If we compare the same doses of both essential oils, only insects subjected to the 120 μL of *O. basilicum* had a significantly lower survival than the insects that were subjected to the 120 μL dose of *C. winterianus* (17.89 days) (Table 2).

Table 2. Survival of *A.obtectus* insects exposed during 15 days on Petri dishes to beans sprayed with different doses of *C. winterianus* and *O. basilicum* essential oils and controls.

Treatments	Insects			Mean *	Median	Overall Comparisons **		
	Alive	Dead	Dead (%)	Estimate ± SE	Estimate	X^2	df	*p*
Control	76	24	24.00	22.68 ± 0.48 e [a]	23.00			
C. winterianus (24 μL)	42	58	58.00	20.23 ± 0.41 dA [b]	20.00	0.571	(1,198)	0.450
O. basilicum (24 μL)	41	59	59.00	20.56 ± 0.52 dA	20.00			
C. winterianus (60 μL)	27	73	73.00	19.55 ± 0.41 cA	20.00	0.021	(1,198)	0.884
O. basilicum (60 μL)	40	60	60.00	19.12 ± 0.53 bcA	19.00			
C. winterianus (120 μL)	9	91	91.00	17.89 ± 1.06 bB	17.00	7.591	(1,198)	0.006
O. basilicum (120 μL)	17	83	83.00	16.01 ± 0.31 aA	16.00			

* Test of equality of survival distributions for all treatments (Log Rank; Mantel-Cox) (Chi-Square (X^2) = 103.071; df = 6,693; $p < 0.001$); ** Test of equality of survival distributions between treatments (Log Rank; Mantel-Cox); [a] Different lowercase letters indicate significant differences among different dose of essential oils and control; ($p < 0.05$); [b] Different capital letters indicate significant differences between essential oils within same dose; ($p < 0.05$).

3.2. Beans Damaged by A. obtectus Insects (Experiment 2)

Applications of different doses of *C. winterianuss* and *O. basilicum* over beans reduced *A. obtectus* attack, reducing significantly the number of holes per bean affected by the insects after treatment with *C. winterianus* and *O. basilicum* essential oils (Table 3) To highlight, the beans treated with the highest doses (120 μL) had a significantly (F = 2.745; df = 6.28; $p = 0.032$) lower number of holes per bean than the 24 μL doses (2.14 and 3.83 holes per bean with *C. winterianus* and *O. basilicum,* respectively) and control (3.66 holes per bean). Beans incubated with *A. obtectus*, which were previously treated with different doses of *C. winterianus* and *O. basilicum* essential oil, showed weight losses ranging between 1.01% (120 μL dose of *C. winterianus*) and 2.10% (24 μL dose of *O. basilicum*), while beans under control treatment had a weight loss (2.98%) significantly higher (F = 4.547; df = 6.28; $p = 0.002$) than the rest of doses evaluated (Table 3).

Table 3. Number of damaged beans (mean ± SE), number of holes per bean (mean ± SE) and bean weight loss (% ± SE) caused by *A. obtectus* adults emerged from 40 g of beans treated in Petri dishes with different doses of *C. winterianus* and *O. basilicum* essential oils.

Essential Oil	Dose (μL)	Number of Damaged Beans (with at least One Hole)	Number of Holes Per Bean Damaged	Bean Weight Loss (%)
--	Control	4.60 ± 0.98 a [1]	3.66± 0.88 ab [1]	2.98 ± 0.3 a [1]
C. winterianus	24	5.60 ± 0.40 a	2.14 ± 0.19 bc	2.00 ± 0.31 bc
	60	4.60 ± 0.40 a	2.61 ± 0.23 abc	1.76 ± 0.36 bcd
	120	5.00 ± 1.14 a	1.91 ± 0.16 c	1.01 ± 0.09 d
O. basilicum	24	3.60 ± 0.75 a	3.83 ± 0.87 a	2.10 ± 0.31 b
	60	6.00 ± 1.26 a	1.98 ± 0.46 c	1.91 ± 0.44 bc
	120	5.20 ± 1.06 a	1.68 ± 0.22 c	1.22 ± 0.13 cd
F		0.726	2.745	4.547
df		6.28	6.28	6.28
p		0.633	0.032	0.002

[1] Different lowercase letters indicate significant differences among beans treated with different doses of essential oils and control; LSD test at 0.05.

4. Discussion

The essential oils do not constitute a threat to the environment or to human health, thus they have been portrayed as a possible alternative to synthetic insecticides [21]. The essential oils are volatile hydrocarbon mixtures, which have a variety of functional groups [22]. These oils are derived from secondary plant metabolism and play a key role in their development through the defense of the plant against microorganisms and herbivores [22]. Many authors [23–25] have described the essential oils to be composed by substances of various chemical groups, such us terpenes (monoterpenes and sesquiterpenes), phenylpropanoids, alcohols, esters, aldehydes, ketones, among others. The biological activity of essential oils depends not only on their chemical composition, but also on the concentration and proportion of these substances [23–25].

These essential oils exhibited similar patterns for insecticidal activity over *A. obtectus* when sprayed directly over beans in Petri dishes using different doses, being the highest insecticidal activities obtained in the first 15 days for the 120 μL doses of both essential oils. The greater insecticidal activity of both oils did not exceed 15 days after application. This translated into a lower survival of the insects, observed in the higher doses of the oils, which reduced the survival of the insects to approximately 17 and 16 days of life with *C. winterianus* and *O. basilicum.* In the same range of days, this effect is well described by Ilboudo et al. [26] for several other essential oils. Loss of activity for essential oils are normally due to degradation of the active compounds. In this respect, essential oils containing more hydrogenated compounds are more susceptible to oxidation [8]. Various studies with essential oils obtained from species of the genus *Ocimum* spp. showed good results regarding their insecticidal effect against insect pests that attack grains, producing a lower survival of the insects. Kéita et al. [7] evaluated (by fumigation) the effect of *O. basilicum* and *O. gratissimum* for the control of *C. maculatus* and obtained 80 and 70% mortality of the insect population evaluated with 25 μL. Rozman et al. [27] reported toxicity against *T. castaneum*, *Rhyzopertha dominica* (Coleoptera: Bostrichidae), and *S. oryzae* in fumigation with Linalool, one of the main components of basil essential oil. Linalool was highly effective against *R. dominica*, and caused 100% mortality at the lowest tested concentration (0.1 mL/720 mL of volume). Ogendo et al. [28] obtained a 98, 99, and 100% mortality against *R. dominica*, *Oryzaephilus surinamensis* L. (Coleoptera: Silvanidae), and *Callosobruchus chinensis* L. (Coleoptera: Chrysomelidae), using 1 μL of *O. gratissimum* essential oil per litre of air.

Other studies have shown diverse activities and effects of *C. winterianus*, such us insect repellency [29], larvicidal effect for certain mosquito species [30], and insecticidal activity against *Frankliniella schultzei* Trybom (Thysanoptera: Thripidae) and *Myzus persicae* Sulzer (Homoptera: Aphididae) [31]. Citronella oil caused also repellency on *C. maculatus* adults [14], and feed deterrence and larval mortality on *S. frugiperda* Walker (Lepidoptera: Noctuidae) [32].

C. winterianus and *O. basilicum* essential oils exhibited a significant reductions on number of damaged beans produced by *A. obtectus* when greater doses were applied over the beans. In addition, the high doses (120 μL) of *C. winterianus* and *O. basilicum* oils generated lower bean weight loss in the damaged beans, showing the toxic activity of this essential oil through its Linalool component. This reduction in the severity of the attack shown by *A. obtectus* towards treated beans has also been observed by Rodríguez-González et al. [33–35] using biological control agents, bacterial proteins [36], and essential oils [37].

5. Conclusions

O. basilicum and *C. winterianus* essential oils affected the development of *A. obtectus* insects since the greatest doses applied on beans decreased the emergence of the bean weevil. Also, they reduced the number of exit holes of insects per damaged bean and the bean weight loss, from 2.987% in control treatment, to 1.014% and 1.221% with 120 μL of *C. winterianus* and *O. basilicum*, respectively. The ability of both doses of *C. winterianus* and *O. basilicum* to reduce the insect longevity, their subsequent emergence from beans, and protect the bean seeds, make these essential oils a suitable tool for the control of adults of this insect pest in small storages.

Author Contributions: A.R.-G., O.G.-L. and S.M.-P. designed the experiment. G.C.-H., S.D-S.-H. and S.A.-G. conducted the experiments. A.R.-G., A.L. and P.A.C. performed statistical analysis. A.R.-G. and P.A.C. prepared the manuscript. All authors have read and agreed to the published version of the manuscript.

Acknowledgments: This work was funded by the Ministry of Science, Innovation and Universities (Government of Spain), according to the Resolution of 27 July 2018, (BOE No. 184, of 31 July) through the grant awarded to Álvaro Rodríguez González (PTA2017–14403-I).

Conflicts of Interest: The authors declare no conflict of interest.

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