







# Combining host plant resistance and botanical insecticide for the management of *Zabrotes subfasciatus* (Boheman) (Coleoptera, Chrysomelidae, Bruchinae) in common bean

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**ABSTRACT:** The Mexican bean weevil *Zabrotes subfasciatus* (Boheman) (Coleoptera, Chrysomelidae, Bruchinae) has become one of the main pests of common bean (*Phaseolus vulgaris* L.) (Fabaceae) worldwide. The association of resistant bean varieties with botanical insecticides has a great potential for controlling the pest, but it has been little studied so far. Therefore, the present study aimed at selecting a botanical insecticide and evaluating its combined effect with an arcelin-bearing common bean resistant genotype against *Z. subfasciatus*. We evaluated three botanical insecticides, the rotenone-based Roteline® and the neem-based NeemSeto® and NeemPro®, on the development of the insect. NeemPro® was the most effective, presenting ovicidal effect and prolonging egg-to-adult period, being selected for the following bioassay. Then we evaluated the effect of NeemPro® combined with the resistant common bean genotype IAC 818 (RAZ-59) on some biological parameters of the pest. The most severe effects on *Z. subfasciatus* were caused by the resistant genotype. However, significant effects in some parameters of the pest were also verified for the botanical insecticide and for its combination with this resistance type/trait in the conditions of the experiment.

**Key words:** *Phaseolus vulgaris*, neem, rotenone, Mexican bean weevil, seed beetle.

## INTRODUCTION

The Mexican bean weevil *Zabrotes subfasciatus* (Boheman) (Coleoptera, Chrysomelidae, Bruchinae) has disseminated worldwide (CABI 2022) and became a major pest of bean crops, mainly of common bean (*Phaseolus vulgaris* L.) (Fabaceae) in Central and South America (Guzzo et al. 2018, Quintela et al. 2020). Adult females attach their eggs to the seed tegument. Newly hatched larvae enter the seed and spend all developmental time inside it, consuming and destroying seed tissue. Larval feeding behavior, together with fecal contamination, causes quantitative and qualitative losses (Soares et al. 2015) that can reach 13% (Quintela et al. 2020).

The most used control method for seed beetles in stored beans is still by either insecticide spraying onto the grains or fumigation (Upadhyay and Ahmad 2011, Yamane 2013), which is favored by the size of the seeds and the gaps between them (Hill 2002). However, chemical insecticides and fumigants can cause some environmental pollution and be hazardous to health (Yamane 2013), among other problems, so that the use of more environmentally safe methods should be always encouraged. Furthermore, from the perspective of integrated pest management (IPM), it is always necessary to develop grain protection methods or systems using lesser chemical insecticides or fumigants (Upadhyay and Ahmad 2011, Yamane 2013).



Host plant resistance is a pest control method that causes less or no environmental disturbance and pollution, does not leave residue on foodstuffs, does not require specific knowledge from the user, offers continuous action against pests, and is consistent with the philosophy of IPM (Norris et al. 2003, Vendramim and Guzzo 2009, 2012, Baldin et al. 2019). Plant resistance can be highly effective when used as the only control method, depending on the crop and the pest (Vendramim et al. 2019). However, it is considered that greater efficiency is generally obtained when it is associated with other control methods (Vendramim and Castiglioni-Rosales 2019).

Botanical insecticides are known by posing little threat to the environment or to human health (Isman 2006) and by frequently containing a mixture of several active principles which makes it harder for target species to evolve resistance (Guzzo et al. 2023). Four major types of botanical products are used for insect control nowadays, including neem (Indian neem tree *Azadirachta indica* A. Juss.) and rotenone (extracted from plants of the genus *Derris*, *Lonchocarpus* and *Tephrosia*) (Isman 2006).

Bean resistance (Moraes et al. 2011, Guzzo et al. 2015, 2018, Eduardo et al. 2016) and botanical insecticides (Oliveira and Vendramim 1999, Oliveira et al. 1999, 2007, Barbosa et al. 2002) have been largely studied for individual use against *Z. subfasciatus* in Brazil, with the identification of several genotypes resistant to the pest. The combination of plant resistance with botanical insecticides has been studied to control seed beetles (Coleoptera, Chrysomelidae, Bruchinae) in bean species worldwide (Lale and Mustapha 2000, Law-Ogbomo 2007, Tabadkani et al. 2017, Barbosa et al. 2020), with promising results from the economic and environmental perspectives, but little has been researched in relation to *Z. subfasciatus* (Luz et al. 2017).

The effect of the interactions between plant resistance and chemical insecticides can be either independent, synergistic, or even antagonistic, due to the possibility of enhancement or reduction of insect susceptibility to one of the methods, caused by the other. So, the effective utilization of pest management methods within a crop production system requires the nature of the interaction to be understood prior to incorporation in an integrated pest management, in order to prevent costly and counterproductive pest management strategies from being recommended and adopted (Quisenberry and Schotzko 1994).

Thus, this research aimed at selecting a botanical insecticide effective against *Z. subfasciatus* and evaluating its effect in association with an arcelin-bearing resistant *P. vulgaris* genotype on the pest.

## MATERIALS AND METHODS

### Insects, bean genotypes, and botanical products

Individuals of *Z. subfasciatus* used in the bioassays were obtained from a stock colony maintained for several generations on the susceptible cultivar Bolinha, under ambient conditions.

Bean accessions IAC 818 (RAZ-59) and IAC 853 (Bolinha CB) were obtained from the Bean Germplasm Bank of the Instituto Agronômico de Campinas (IAC), Campinas (SP), Brazil, and selected as resistant and susceptible, respectively, to *Z. subfasciatus* (Guzzo et al. 2015, 2018). Dried grains were kept in a freezer at 0°C before the use, to prevent degradation and avoid previous infestation by any insect.

Botanical insecticides evaluated were Roteline® (EC, rotenone 2,000 ppm), based on rotenone, and NeemPro® (EC, azadirachtin A 10,000 ppm) and NeemSeto® (EC, azadirachtin A and B, nimbin and salannin 2,389 ppm), both based on neem.

### Selection of botanical insecticides

Grains of the susceptible genotype Bolinha CB were infested with adults of *Z. subfasciatus* (unknown and non-standardized quantity) for one day, for oviposition. Adults were then removed, and, 24 hours later, samples of 20 grains were immersed in solutions of one of the products under test, all at the concentration of 1%, in distilled water. As a control, only distilled water was used. The samples were then dried under air flow and placed in circular plastic boxes under laboratory conditions, for subsequent evaluations. After 10 days of oviposition (enough time for larvae to emerge), viable and non-viable eggs present in the grains of each treatment were quantified in order to assess ovicidal effect of the products. The number of emerged adults was also counted daily, and the average duration of the egg-to-adult period for each treatment was calculated.

## Combination of resistant genotype with botanical insecticide

The neem-based botanical insecticide NeemPro® (considered the most effective, based on the results of the screening) was selected for this bioassay in combination with the resistant bean genotype IAC 818.

Grains of IAC 818 (resistant) and IAC 853 (susceptible) were infested with adult individuals of *Z. subfasciatus* for one day, for oviposition, and the adults were then removed. After 24 hours of removal, samples of 20 grains of each genotype under test were immersed in NeemPro® solution at a concentration of 1%, in distilled water, or only in distilled water (control). The samples were then dried under air flow and packed in circular plastic boxes under laboratory conditions. After 10 days of oviposition (enough time for larvae to emerge), the viable and non-viable eggs present in the grains of each treatment were quantified. The emergence of adults was also monitored daily. We counted the number of adults emerged and calculated the average duration of the egg-to-adult period for each treatment. Adult males and females were also weighed.

## Experimental design and statistical analysis

The selection of botanical insecticides followed the completely randomized experimental design with four treatments (three insecticides + control) and 15 replications each. The averages were submitted to analysis of variance (ANOVA) and compared by the Tukey's test at 5% significance. The efficacy of botanical insecticides was also corrected according to Abbott (1925).

The essay on the combination of resistant genotype with botanical insecticide also followed the completely randomized experimental design with four treatments (combinations of two genotypes with insecticide or control) and 15 replications each. The averages were submitted to two-way ANOVA for evaluating the effect of the two factors (bean genotype and insecticide) and their interaction on *Z. subfasciatus*, at 5% significance. For both tests, it was used the software GENES (Cruz 2013, 2016).

## RESULTS

### Selection of botanical insecticides

Neem-based products caused reductions of 47.58% (NeemPro®) and 38.49% (NeemSeto®) in the viability of *Z. subfasciatus* eggs, both differing from the control (30.93%) and from each other. Roteline® caused intermediate ovicidal effect on *Z. subfasciatus* (36.73%), not differing statistically from that observed in the control neither from NeemSeto® ( $F_{3, 56} = 17.82$ ) (Table 1). The percentage of insects that emerged in relation to viable eggs varied between 79.95 and 83.96%, but without statistical differences ( $F_{3, 56} = 1.19$ ), indicating that none of the products used caused larval/pupal mortality in *Z. subfasciatus*. When viability was considered in relation to the total number of eggs, only NeemPro® differed from the control (57.24 and 43.93%, respectively), reflecting the higher viability reduction caused to eggs, accumulated in this index. NeemSeto® and Roteline® reduced egg-to-adult viability rates in 48.37 and 49.46%, respectively ( $F_{3, 56} = 10.78$ ).

**Table 1.** Raw average ± standard error and corrected<sup>1</sup> percentage of viable eggs and adults of *Zabrotes subfasciatus* emerged from *Phaseolus vulgaris*, after grain immersion in botanical insecticides\*.

Treatment	Viable eggs (%)	Adults emerged (%) in relation to	
		Total of eggs	Viable eggs
Control	69.07 ± 1.19 a (100) <sup>1</sup>	56.07 ± 1.64 a (100) <sup>1</sup>	81.00 ± 1.32 a (100) <sup>1</sup>
Roteline®	63.27 ± 1.51 ab (91.60) <sup>1</sup>	50.54 ± 1.54 a (90.14) <sup>1</sup>	79.95 ± 1.95 a (98.70) <sup>1</sup>
NeemSeto®	61.51 ± 1.14 b (89.05) <sup>1</sup>	51.63 ± 1.29 a (92.08) <sup>1</sup>	83.96 ± 1.51 a (103.65) <sup>1</sup>
NeemPro®	52.42 ± 2.39 c (75.89) <sup>1</sup>	42.76 ± 2.15 b (76.26) <sup>1</sup>	81.49 ± 1.35 a (101.16) <sup>1</sup>

\*Means followed by the same letter in the same column are not significantly different by Tukey's test ( $P \leq 0.05$ ); <sup>1</sup>corrected according to Abbott (1925).

The duration of egg-to-adult period of *Z. subfasciatus*, regardless of the sex, was prolonged by two products tested (Table 2). The longest development period was caused by NeemPro® (35.66 days), followed by Roteline® (35.43 days), which did not differ from each other nor from NeemSeto® (35.33 days), but differed from the control (34.82 days), which did not differ from NeemSeto® either ( $F_{3, 56} = 5.66$ ). Considering only males, the result was similar, with NeemPro® and Roteline® prolonging this duration (35.51 and 35.44 days, respectively) compared to the control (34.92 days), and NeemSeto® providing an intermediate value (35.28 days), not differing from all other treatments ( $F_{3, 56} = 2.98$ ). For females, duration caused by NeemPro®, Roteline®, and NeemSeto® (35.83, 35.41 and 35.39 days, respectively) did not differ from each other, but from the control (34.71 days) ( $F_{3, 56} = 6.53$ ). By analyzing the results together, NeemPro® was the most effective product against *Z. subfasciatus* under our experimental conditions, being selected for the next test, together with the resistant genotype of *P. vulgaris* IAC 818.

**Table 2.** Average  $\pm$  standard error of the duration of egg-to-adult period of *Zabrotes subfasciatus* in *Phaseolus vulgaris*, after grain immersion in botanical insecticides\*.

Treatment	Duration (days)		
	Males	Females	Mean
NeemPro®	35.51 $\pm$ 0.14 a	35.83 $\pm$ 0.18 a	35.66 $\pm$ 0.12 a
Roteline®	35.44 $\pm$ 0.08 a	35.41 $\pm$ 0.19 a	35.43 $\pm$ 0.13 a
NeemSeto®	35.28 $\pm$ 0.17 ab	35.39 $\pm$ 0.18 a	35.33 $\pm$ 0.16 ab
Control	34.92 $\pm$ 0.20 b	34.71 $\pm$ 0.18 b	34.82 $\pm$ 0.18 b

\*Means followed by the same letter in the same column are not significantly different by Tukey's test ( $P \leq 0.05$ ).

## Combination of resistant genotype with botanical insecticide

The lowest viability of eggs was observed in the resistant genotype (57.05%), followed by the resistant genotype + neem (59.65%), the genotype susceptible + neem (71.06%) and the susceptible genotype (73.16%), which provided the highest viability (Table 3). The percentage of emerged insects was also lower in the resistant genotype in relation to total eggs and viable eggs (13.64 and 24.78%, respectively) and in the resistant genotype + neem (16.09 and 27.83%, respectively), and higher in the susceptible genotype (66.55 and 91.1%, respectively) and the susceptible genotype + neem (66.16 and 93%, respectively). The results of the two-way ANOVA indicated that the three parameters were significantly affected by genotype, rather than by insecticide or interaction (Table 4).

**Table 3.** Average  $\pm$  standard error percentage of viable eggs and adults of *Zabrotes subfasciatus* emerged from *Phaseolus vulgaris* resistant and susceptible genotypes, treated or not with neem based botanical insecticide.

Treatment <sup>1</sup>	Viable eggs (%)	Adults emerged (%) in relation to	
		Total of eggs	Viable eggs
Susceptible	73.16 $\pm$ 1.04	66.55 $\pm$ 1.90	91.10 $\pm$ 2.54
Susceptible + neem	71.06 $\pm$ 2.11	66.16 $\pm$ 2.28	93.00 $\pm$ 1.42
Resistant + neem	59.65 $\pm$ 2.27	16.09 $\pm$ 1.74	27.83 $\pm$ 3.31
Resistant	57.05 $\pm$ 2.29	13.64 $\pm$ 1.66	24.78 $\pm$ 3.69

<sup>1</sup>Susceptible = IAC 853 (Bolinha CB); resistant = IAC 818 (RAZ-59); neem = NeemPro®.

The duration of the development period of *Z. subfasciatus* males was affected by the resistant variety, regardless of the insecticide or the interaction between them (Table 4). The values observed in the resistant genotype (37.45 days) and in the resistant genotype + neem (35.88 days) were higher than those found in the susceptible genotype (27.35 days) and in the susceptible genotype + neem (27.27 days) (Table 5). For females, the development period was higher in the resistant genotype (38.14 days), followed by the resistant genotype + neem (34.75 days), the susceptible genotype (27.69 days) and susceptible genotype + neem (27.66 days) (Table 5), and was influenced by the three sources of variation (genotype, insecticide and interaction) (Table 4). This same pattern was repeated for the average duration regardless of the sex of the individuals (Table 5), with the longest duration of the development period obtained in the resistant genotype (37.68 days), followed by the resistant genotype + neem (35.23 days), by the susceptible genotype (27.51 days) and the susceptible genotype + neem (27.50 days), being also influenced by genotype, insecticide and its interaction (Table 4).

**Table 4.** Two-way analysis of variance of parameters of *Zabrotes subfasciatus* from *Phaseolus vulgaris* resistant and susceptible genotypes, treated or not with neem based botanical insecticide.

Source of variation	df	SS	MS	F-value	P-value
<b>Viable eggs</b>					
Genotype	1	2,841.9421	2,841.9421	475540	0.0000**
Insecticide	1	0.9543	0.9543	0.0160	0.8998 <sup>ns</sup>
Interaction	1	82.5061	82.5061	1.3806	0.2449 <sup>ns</sup>
Residual	56	3,346.6958	59.7624		
<b>Adults emerged in relation to total eggs</b>					
Genotype	1	39,765.7834	39,765.7834	723.6742	0.0000**
Insecticide	1	15.9258	15.9258	0.2898	0.5924 <sup>ns</sup>
Interaction	1	30.0930	30.0930	0.5476	0.4623 <sup>ns</sup>
Residual	56	3,077.1910	54.9498		
<b>Adults emerged in relation to viable eggs</b>					
Genotype	1	64,843.0012	64,843.0012	523.4468	0.0000**
Insecticide	1	91.5966	91.5966	0.7394	0.3935 <sup>ns</sup>
Interaction	1	5.0092	5.0092	0.0404	0.8413 <sup>ns</sup>
Residual	56	6,937.1099	123.8770		
<b>Duration of males' egg-to-adult period</b>					
Genotype	1	1,313.0740	1,313.0740	170.1553	0.0000**
Insecticide	1	10.2514	10.2514	1.3284	0.2539 <sup>ns</sup>
Interaction	1	8.3834	8.3834	1.0864	0.3018 <sup>ns</sup>
Residual	56	432.1471	7.7169		
<b>Duration of females' egg-to-adult period</b>					
Genotype	1	1,152.8654	1,152.8654	278.3645	0.0000**
Insecticide	1	43.8702	43.8702	10.5927	0.0019**
Interaction	1	42.2938	42.2938	10.2120	0.0023**
Residual	56	231.9278	4.1416		
<b>Duration of mean egg-to-adult period</b>					
Genotype	1	1,231.6673	1,231.6673	351.6335	0.0000**
Insecticide	1	24.1338	24.1338	6.8901	0.0112*
Interaction	1	22.0843	22.0843	6.3049	0.0150*
Residual	56	196.1513	3.5027		
<b>Males' adult weight</b>					
Genotype	1	4.9836	4.9836	2.4596	0.0000**
Insecticide	1	5.2005	5.2005	2.5667	0.6144 <sup>ns</sup>
Interaction	1	3.0142	3.0142	1.4876	0.7012 <sup>ns</sup>
Residual	56	1.1347	2.0262		
<b>Females' adult weight</b>					
Genotype	1	6.4774	6.4774	1.2261	0.0000**
Insecticide	1	2.9041	2.9041	5.4970	0.0226*
Interaction	1	1.6914	1.6914	3.2016	0.0790 <sup>ns</sup>
Residual	56	2.9586	5.2831		

\*Significant ( $P < 0.05$ ); \*\*significant ( $P < 0.01$ ); <sup>ns</sup>not significant ( $P > 0.05$ ); df: degrees of freedom; SS: sum of squares; MS: mean square.

**Table 5.** Average  $\pm$  standard error of the duration of egg-to-adult period of *Zabrotes subfasciatus* in *Phaseolus vulgaris* resistant and susceptible genotypes, treated or not with neem based botanical insecticide.

Treatment <sup>1</sup>	Duration (days)		
	Males	Females	Mean
Resistant	37.45 $\pm$ 0.85	38.14 $\pm$ 0.73	37.68 $\pm$ 0.58
Resistant + neem	35.88 $\pm$ 1.12	34.75 $\pm$ 0.74	35.23 $\pm$ 0.76
Susceptible	27.35 $\pm$ 0.18	27.69 $\pm$ 0.10	27.51 $\pm$ 0.12
Susceptible + neem	27.27 $\pm$ 0.18	27.66 $\pm$ 0.15	27.50 $\pm$ 0.16

<sup>1</sup>Resistant = IAC 818 (RAZ-59); susceptible = IAC 853 (Bolinha CB); neem = NeemPro<sup>®</sup>.

The weight of the emerged adult males of *Z. subfasciatus* (Table 6) was 1.61 mg in the resistant genotype and also in the resistant genotype + neem, 1.78 mg in the susceptible genotype + neem, and 1.81 mg in the susceptible genotype (Table 6), being influenced only by genotype, rather than by the other sources of variation (Table 4). For females, genotype, and insecticide, but not the interaction, were found to affect the adult weight (Table 4), which was lesser in the resistant genotype (2.21 mg), followed by the resistant genotype + neem (2.45 mg), the susceptible genotype (2.97 mg), and the susceptible genotype + neem (3 mg) (Table 6). As there is an appreciable difference in size and weight between adult males and females of *Z. subfasciatus*, the average weight regardless of the sex was not analyzed because it would not effectively represent the average weight of an adult individual.

**Table 6.** Average  $\pm$  standard error weight of *Zabrotes subfasciatus* males and females emerged from *Phaseolus vulgaris* resistant and susceptible genotypes, treated or not with neem based botanical insecticide.

Treatment <sup>1</sup>	Adult weight (mg)		
	Males	Females	Mean
Susceptible + neem	1.78 $\pm$ 0.02	3.00 $\pm$ 0.03	2.44 $\pm$ 0.04
Susceptible	1.81 $\pm$ 0.02	2.97 $\pm$ 0.02	2.34 $\pm$ 0.03
Resistant + neem	1.61 $\pm$ 0.06	2.45 $\pm$ 0.09	2.07 $\pm$ 0.09
Resistant	1.61 $\pm$ 0.04	2.21 $\pm$ 0.07	1.93 $\pm$ 0.08

<sup>1</sup>Susceptible = IAC 853 (Bolinha CB); resistant = IAC 818 (RAZ-59); neem = NeemPro<sup>®</sup>.

## DISCUSSION

In the present work, NeemPro<sup>®</sup> and NeemSeto<sup>®</sup> reduced *Z. subfasciatus* egg viability, but not Roteline<sup>®</sup>. Roteline<sup>®</sup> is a formulated product based on rotenone, an isoflavonoid that blocks the electron transport chain, preventing the production of energy by mitochondria and leading insects to death. However, rotenone acts by ingestion (Isman 2006, Guzzo et al. 2023) and needs to be consumed by the insect, which justifies we have not observed ovicidal effect of Roteline<sup>®</sup> on *Z. subfasciatus*. NeemPro<sup>®</sup> and NeemSeto<sup>®</sup> are both based on neem, whose main active ingredient is the triterpene azadirachtin. This substance blocks the synthesis and release of ecdysteroids by the prothoracic gland, impairing the ecdysis during insect development [Mordue (Luntz) and Nisbet 2000, Isman 2006, Martinez 2011, Guzzo et al. 2023], and acts mainly by ingestion, but also by contact (Martinez 2011, Guzzo et al. 2023). It may have been responsible for the lower viability of *Z. subfasciatus* eggs caused by the neem-based products. The difference observed between the effects of the two products based on neem is probably associated with the difference in azadirachtin concentration, which was 10,000 ppm in NeemPro<sup>®</sup> and 2,389 ppm in NeemSeto<sup>®</sup>.

It was also found that none of the products reduced viability of the larval-pupal period. Only NeemPro<sup>®</sup> was able to reduce development viability when the egg period was also considered, which reflects the effect that this product had on the eggs. It means that, once emerged and penetrated the grain, larvae were protected from the products. However, a sublethal effect of the products was observed, mainly NeemPro<sup>®</sup> and Roteline<sup>®</sup>, which slightly but significantly prolonged the duration of egg-to-adult period. Azadirachtin is recognized by its phagodeterrent or antifeedant effect [Mordue (Luntz) and Nisbet 2000, Isman 2006, Martinez 2011, Guzzo et al. 2023], an effect that can prolong the development period of insects.

However, it is not known whether the substance is capable of overcoming the bean integument, because the translocation of azadirachtin depends on the species and the structure of the plant in which it is applied. Despite this, once treated with azadirachtin, insects can reduce food consumption even after exposure has ceased (Martinez 2011). Thus, the larvae of *Z. subfasciatus* could have been affected before they had even penetrated the bean grains. Differences between the effects of the two neem-based products may be related to the azadirachtin concentration again.

Some works have shown the effect of neem on adults of *Z. subfasciatus* (Oliveira and Vendramim 1999, Oliveira et al. 1999, Barbosa et al. 2002), but the literature regarding the effect of this botanical insecticide on the immature stage of the insect is scarce, as well as the effect of rotenone on it. Barbosa et al. (2002) found that neem oil was effective in protecting *P. vulgaris* against infestation by *Z. subfasciatus*, but Paranhos et al. (2005) verified that neem oil is not effective in controlling *Z. subfasciatus* in beans in an advanced stage of infestation.

When the bean genotypes were combined with the neem-based insecticide, the adverse effects on egg viability and on egg-to-adult period viability of *Z. subfasciatus* were caused by the resistant genotype, regardless of the insecticide or the combination between them. It is noteworthy that the combination resistant genotype + neem, which can be considered an extreme condition for the insect, caused a less severe effect on females than the resistant genotype alone. This difference can be explained by the type of resistance of genotype IAC 818. Arcelin, a resistance factor present in this genotype, not only acts as a food deterrent or antinutrient, but also has toxic effect on *Z. subfasciatus* (Barbosa et al. 2000a, 2000b, Paes et al. 2000, Mazzone and Vendramim 2002, Guzzo et al. 2015). Thus, the food deterrence of azadirachtin would lead to less food consumption and, consequently, less intake of toxic bean protein arcelin by individuals of *Z. subfasciatus*.

Most studies on the combined use of bean resistance with botanical insecticides are addressed to another bruchid species, *Callosobruchus maculatus* (F.) (Coleoptera, Chrysomelidae, Bruchinae). Regarding this pest, significant interaction on at least one behavioral or biological parameter has been shown between resistant genotypes of *Vigna unguiculata* (L.) Walp (Fabaceae) and oil of neem seeds (Lale and Mustapha 2000), essential oil of *Vitex agnus castus* L. (Verbenaceae) (Castro 2013)<sup>5</sup>, or the constituents of essential oils eugenol and geraniol (Barbosa et al. 2020); and between *Vigna radiata* (L.) (Fabaceae) and the essential oil of *Echinophora platyloba* DC. (Umbelliferae) (Tabadkani et al. 2017).

With regards to *Z. subfasciatus*, Barbosa et al. (2002) also evaluated the effect of neem oil and genotypes with and without arcelin on the protection of common bean against this pest, but no results about the combined effect of these different forms of control were presented. Luz et al. (2017) evaluated the combination of bean resistance with botanical insecticide against *Z. subfasciatus*. The results of a series of bioassays with *P. vulgaris*, *V. unguiculata* and *Vicia faba* L. (Fabaceae) genotypes possessing variable degrees of antibiosis and a neem-based formulation (as well as a synthetic insecticide) suggest a synergistic effect of insecticides combined to genotypes possessing moderate and high level of resistance. At the present work, we did not find additive or synergistic effect of the association between neem seed oil and the arcelin-containing resistant bean genotype IAC 818 in the control of *Z. subfasciatus*. However, it is possible that other types of formulations of the same insecticides yield better results.

## CONCLUSION

Under our experimental conditions, we found that the most effective botanical insecticide was the neem-based formulation NeemPro<sup>®</sup>, which significantly affected *Z. subfasciatus* egg viability; percentage of adults emerged in relation to the total of eggs; and duration of egg-to-adult period of males, females and mean (males + females). When NeemPro<sup>®</sup> was combined with the highly resistant arcelin-bearing *P. vulgaris* genotype IAC 818 (RAZ-59) against *Z. subfasciatus*, the only significant interaction was observed on duration of egg-to-adult period in females and mean.

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## AUTHORS' CONTRIBUTION

**Conceptualization:** Guzzo, E. C., Vendramim, J. D. and Lourenção, A. L.; **Investigation:** Guzzo, E. C. and Corrêa, O. M. B.; **Analyses:** Guzzo, E. C. and Vendramim, J. D.; **Writing – Original Draft:** Guzzo, E. C. and Vendramim, J. D.; **Writing – Review and Editing:** Guzzo, E. C., Vendramim, J. D. and Lourenção, A. L.

## DATA AVAILABILITY STATEMENT

All dataset were generated and analyzed in the current study.

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