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Article

Potassium silicate to control *Oebalus insularis* Stål in rice in Quevedo, Ecuador

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Abstract

Oebalus insularis Stål, colloquially known as the rice stink bug, causes damage to rice crops, the production losses of which can range between 30 and 65%, which is why agroecological alternatives for its control without the application of polluting synthetic chemicals are increasing. The research aimed to assess the use of beneficial microorganisms and compost with silicon for the control of *O. insularis* Stål, in *Oryza sativa* L., cultivar INIAP-14. The research was conducted in the summer-autumn 2023 in Quevedo, Ecuador, under field conditions in a clay loam soil, using six treatments consisting of a control without any application (T1), Buffago insecticide at 0.5 L ha^{-1} (T2); *Azotobacter chroococcum*, strain Ag, at 1 x 10^{9} CFU ml^{-1} (T3); liquid compost with potassium silicate (K_4SiO_4) at 5 L ha^{-1} (T4); *Metarhizium anisopliae*, strain 45, at 1.2 L ha^{-1} (T5), and a combined treatment with *A. chroococcum*, liquid compost with potassium silicate, and *M. anisopliae* (T6), in a completely randomized design. The dependent variable evaluated was the number of insects per plot before and after applying the treatments. The results show a significant variation in the insect population before and after applying the treatments, with differentiated results between treatments, indicating that the use of combined treatments and that with *Metarhizium* show effective results to be considered as an agroecological alternative in the control of *Oebalus insularis* bug.

Keywords:

Metarhizium anisopliae, *Oebalus insularis* Stål, *Oryza sativa* L., rice stink bug..

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Introduction

Rice (*Oryza sativa* L.) is the second plant species with the largest cultivated area worldwide, after wheat (*Triticum aestivum* L.), due to its high nutrient contribution, yields per hectare, and easy cooking (Edison-Zambrano *et al*., 2019). In Ecuador, rice is one of the most important and extensive crops, occupying more than a third of the country's area of transitory products, with about 340 000 ha sown annually, distributed among 75 000 agricultural production units (Mendoza-Avilés *et al*., 2019).

Among the factors that reduce agricultural yields of rice were poor agronomic management and abiotic and biotic agents; abiotic agents include high temperatures, sudden rains, and salinity, among others and in relation to the biotic ones, the damage by insectile pests stands out, and among these pests, the species *Oebalus* Stål, 1872, can be cited, which is commonly known as the ear bug or rice stink bug and can cause losses in crop yields that can range between 30 and 65% (Krinski and Amilton-Foerster, 2017), and damage caused by the suction of the aqueous content of rice grains can be visualized in the nymph and adult stages of the insect (Hajjar, 2023).

To control *O. insularis*, the most widespread method is the use of chemical insecticides with their consequent polluting effects on the environment (Rodríguez-Delgado *et al*., 2018). These harmful effects can be counteracted by using biological products, specifically with entomopathogenic bacteria and fungi, an aspect that today supports biological control as an important component of integrated pest management. Among the entomopathogenic fungi used for the control of insect pests is *Metarhizium anisopliae*, found in a wide variety of soil types, which represents its natural place of development, and in diverse climatic conditions (Zhe-Yu *et al*., 2022; Hajjar, 2023).

Another product with bioinsecticidal action is silicon (Si) (Ahmad *et al*., 2019). This element is known to improve the resistance of rice plants and other Poaceae to pest attack (Tenguri *et al*., 2023). One of the formulations of silicon as a marketable chemical fertilizer for farmers in Ecuador is in the form of liquid potassium silicate, which incorporates the beneficial effects of potassium as one of the main macroelements in plant nutrition. Similarly, in different studies, growth-promoting bacteria, such as *Azotobacter chroococcum*, have been demonstrated to act against pathogens, especially soil nematodes (Akram *et al*., 2016).

In studies related to the control of *O. insularis* Stål, in *Oryza sativa* L., cultivar INIAP-14, by the effect of beneficial microorganisms and compost with silicon, it has not been demonstrated; the hypothesis is that this type of control, when combined, will be safe to be considered an agroecological alternative in the control of *Oebalus insularis* bug. The research aimed to assess the use of beneficial microorganisms and compost with potassium silicate for the control of *Oebalus insularis* Stål in *Oryza sativa* L, cultivar INIAP-14, in Quevedo, Ecuador.

Materials and methods

The research was conducted from May to August 2022, at the 'La María' experimental farm of the State Technical University of Quevedo, located at km 7.5 of the Quevedo-El Empalme road, Mocache Cantón, province of Los Ríos, Ecuador, Located at 1° 04' 48.6" south latitude and 79° 30' 04.2" west longitude, at an altitude of 75 m. The area is characterized as humid tropical, with an average annual temperature of 25.3 °C, an average annual rainfall of 1 587.5 mm, 86% relative humidity and 994.4 h of sun per year. The soil has a flat topography, clay-loam texture and an average pH of 5.5 (INANHI, 2021).

Description of treatments

The treatments of the study were: Treatment 1 (T1). Absolute control. Application of distilled water; treatment 2 (T2). Buffago® insecticide at 0.5 L ha^{-1}; treatment 3 (T3). Liquid compost with potassium silicate (K_4SiO_4). Liquid compost at 5 L ha^{-1} and 10 ml of potassium silicate per liter of water; treatment 4 (T4). *Azotobacter chroococcum* strain Ag, 10^9 CFU ml^{-1}, at 1 ml L^{-1}; treatment 5 (T5).

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Metarhizium anisopliae, strain 45, at 1 x 10^8 conidia ml^{-1}, at a dose of 1.2 L ha^{-1}; treatment 6 (T6). *Metarhizium* + *Azotobacter* 10^9 CFU ml^{-1} + liquid compost.

Statistical analysis

The design used was a completely randomized one with six treatments and four replications. The effective size of the plots was 4 m^2 (2 m x 2 m) plus an edge area considered ineffective of 0.5 m, based on the criteria by Romero-Cortes *et al*. (2022).

Application of treatments

The chemical insecticide-based treatment was applied using a Guarany® backpack sprayer with a fan nozzle #6; the insecticide used was Buffago® in a dose of 0.5 L ha^{-1} (Ruilova-Cueva *et al*., 2022). For its part, the treatment with *A. chroococcum* strain Ag 10^9 CFU ml^{-1} used the commercial product Nemagreen®, manufactured by the company Ecovad and containing *A. chroococcum* strain Ag at 1 x 10^9 CFU ml^{-1} in liquid form; the dose used was 20 L ha^{-1}.

Inoculation was carried out by immersing the seeds for 60 min in distilled water at a dose of 10 ml^{-1} of water, then the seed was aerated in the shade for 12 h according to Llerena-Ramos *et al*. (2021). In relation to the treatment based on liquid compost with potassium silicate, 45 kg of substrate was prepared using a platform scale (Camry, TCS 300 ZE21®), consisting of 30% fresh cattle manure (13 kg), corn (*Zea mays* L.) (23 kg), 10% (5 kg) remains of the legume species (*Pueraria phaseoloides* L.) tropical kudzu, 9% soil (4 kg), and 1% ash (0.5 kg). Molasses from the industrial processing of sugarcane (*Saccharum* spp.) were added to the substrate.

The aforementioned compost was used to prepare a liquid organic fertilizer; for this purpose, 250 g of compost was weighed, mixed in a liter of water, and left to stand for 24 h in order to obtain a mass-volume ratio (m/v) of 25%, and a volume/volume ratio (v/v) of 50 ml^{-1} was determined, which represented a dose of 10 L ha^{-1} for 1 ha. For each liter of the final solution, 10 g of potassium silicate (K_4SiO_4) in liquid form was added. The compost made for this research had a pH of 7.5, organic matter of 11.5%, and contents of nitrogen (N) of 1.8%, phosphorus (P) 0.48%, potassium (K) 1.2%, calcium (Ca) 3.93%, magnesium (Mg) 0.48%, and sulfur (S) 0.25%.

The concentrations of microelements in mg L^{-1} of boron (B), zinc (Zn), copper (Cu), iron (Fe), and manganese (Mn) were 36, 96, 42, 603, and 338, respectively. It was applied with a Guarany® backpack sprayer and fan nozzle #6, which allowed us to achieve a uniform distribution on the foliage of the rice plants, and the application was made when the main stem characteristic of the tillering stage in the crop appeared.

The combination of this compost with molasses was used based on the work carried out by Álvarez-Sánchez *et al*. (2021). Treatment with *Metarhizium anisopliae* consisted of the application of the commercial product Meta 45®, (Ecofertilizin, SAC) containing spores of *Metarhizium anisopliae* strain 45 in concentrated liquid solution of 1 x 10^8 conidia ml^{-1} according to Valle-Ramírez *et al*. (2020), the foliar applications based on *M. anisopliae* used the manufacturer's recommended dose of 1.2 L ha^{-1}, being applied in the early evening hours.

Two applications were made, the first five days before the start of flowering and the other at the beginning of flowering, and a Guarany® backpack sprayer, described above, was used for the foliar applications. The treatment based on *Metarhizium* + *Azotobacter* 10^9 CFU ml^{-1} + liquid compost with potassium silicate consisted of applying *Azotobacter* to the seeds before sowing and the rest of the treatment was made by mixing the liquid compost at 10 L ha^{-1} with 10 g of potassium silicate (K_4SiO_4) plus 1.2 L ha^{-1} of *Metarhizium* and a single application was made at the beginning of the flowering stage.

Experiment management

Sowing was done manually using a tip spatula, depositing 20 seeds per hole, with sowing distances of 30 cm between rows and 25 cm between plants. The experiment was conducted according to what was cited by Ruilova-Cueva et al. (2022). To confirm the presence of the insect, the cultivated areas were monitored, preferably at the beginning of the flowering stage (5% of the flowered plot) and in surrounding areas with the presence of weeds (once/week/one month).

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An entomological net was used, and sampling was carried out both in the center and at the edges of the selected plots (10 samples per plot) following imaginary diagonals, with 10 sweeps with the entomological net in two of the four replications of each treatment. The number of bugs (nymphs and adults) captured was quantified. The collected insects were taken in plastic jars to the entomology laboratory of the National Institute of Agricultural Research (INIAP, for its acronym in Spanish) for classification, located at the Pichilingue Tropical Station, in Empalme, Mocache cantón, 5 km from the city of Quevedo, province of Los Ríos, Ecuador.

Variables evaluated and respective statistical analyses

The variables were analyzed by means of multiple comparisons with Dunnett's T3 test after applying the treatments and the number of *O. insularis* before and after these applications. For each plot (two of the four for each treatment), the number of bugs in the nymph stage and adults were counted before the application and 15 days after applying the treatments.

The similarity of the variance of the time factor (before and after applying the treatments) was verified with Mauchly's test, whereas the analysis of the effects of the time factor and its interaction with the treatments was verified through the Huynh-Feldt test. A multivariate analysis of variance was performed using Wilks's lambda statistic (Cárdenas-Castro and Arancibia-Martini, 2014). The multiple comparison between treatments was performed with Dunnett's T3 test, and the SPSS statistical package, version 26, was used for statistical analysis (Ates *et al*., 2019; IBM, 2019; Christensen, 2022).

Results

The results of the use of beneficial microorganisms and compost with potassium silicate to control *Oebalus insularis* show a probability (*p*# 0.0001) with Mauchly test (Table 1). There was an effect size of 0.836 due to the influence of the insect factor and a larger effect for the interaction of insects x treatments, with a value closer to one (0.906).

Table 1. Multivariate analysis of variance and the interaction of insect by treatments in pre- and post-application.

Effect	Significance	Partial Eta squared	Parameter without centrality		Observed power
Insects	Wilks' lambda	0	0836	91.745	1
Insects by treatments	Wilks' lambda	0	0.906	174.327	1
Mauchly sphericity	-	0	-		

Multiple comparisons between the post-applied treatments using Dunnett T3 test

The control of the pest insect *O. insularis* with the chemical insecticide statistically outperformed the other treatments, except for the combined treatment and the application of *Metarhizium*. The application based on chemical insecticide reduced the population of insects per plot by 10.3 insects compared to the control treatment. The results with *Metarhizium* do not show significant differences, showing that the chemical insecticide decreased the population by three insects and by one insect compared to the combined treatment, treatments that also corresponded to the narrowest confidence intervals (Table 2).

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Table 2. Multiple comparisons of treatment averages by the number of insects after each application.

(A) Treat	(B) Treat	*Diff in means (A-B)	Sig	Confidence interval at 95%	
				Lower limit	Upper limit
Chemical	Control	-10.25*	0	-12.46	-8.04
	Compost-K_4SiO_4	-8*	0	-10.61	-5.39
	Azotobacter	-8.75*	0.002	-12.39	-5.11
	Metarhizium	-3.25*	0.01	-5.46	-1.04
	ACM	-1.25	0.33	-3.46	0.96
Compost-K_4SiO_4	Control	-2.25	0.108	-4.97	0.47
	Chemical	8*	0	5.39	10.61
	Azotobacter	-0.75	0.989	-4.3	2.8
	Metarhizium	4.75*	0.003	2.03	7.47
	ACM	6.75*	0	4.03	9.47
Azotobacter	Control	-1.5	0.596	-5	2
	Chemical	8.75*	0.002	5.11	12.39
	Compost-K_4SiO_4	0.75	0.989	-2.8	4.3
	Metarhizium	5.5*	0.007	2	9
	ACM	7.5*	0.002	4	11
Metarhizium	Control	-7*	0	-9.47	-4.53
	Chemical	3.25*	0.01	1.04	5.46
	Compost-K_4SiO_4	-4.75*	0.003	-7.47	-2.03
	Azotobacter	-5.5*	0.007	-9	-2
	ACM	2	0.119	-4.47	4.47
ACM	Control	-9*	0	-11.47	-6.53
	Chemical	1.25	0.33	-0.96	3.46
	Compost-K_4SiO_4	-6.75*	0	-9.47	-4.03
	Azotobacter	-7.5*	0.002	-11.00	-4
Metarhizium	-2	0.119	-4.47	0.47	

* Diff= the mean difference is significant at the 0.05 level. ACM is *Azotobacter*-compost potassium silicate-*Metarhizium*. Sig= significance.

The comparison between compost-potassium silicate and the rest of the treatments showed no significant differences compared to the *Azotobacter* treatment and the control. This same treatment reached average populations of eight insects per plot compared to the chemical treatment, with confidence intervals ranging from a lower limit of 5.39 to an upper limit of 10.61 insects.

Treatment with *Azotobacter* and the application of compost-potassium silicate were the treatments that controlled the population of pest insects, although the compost-potassium silicate treatment had a tendency without significant differences to control more than *Azotobacter*. The application of the treatment with *Azotobacter* did not differ statistically from the treatment based on compost-potassium silicate and the control, but it did differ from the rest of the treatments; so these three treatments can be considered as those that had the least control over the pest.

The plants in the plots in which *Azotobacter* were applied presented on average about nine more insects compared to the chemical control. On the other hand, the *Metarhizium* treatment did not show significant differences compared to the combined treatment between *Azotobacter*-compost potassium silicate-*Metarhizium*.

The *Metarhizium*-based application showed an average control of seven fewer insects compared to the control. The combined treatment between *Azotobacter*-compost potassium silicate-*Metarhizium* did not differ significantly from the chemical control and the application of *Metarhizium*, but it did

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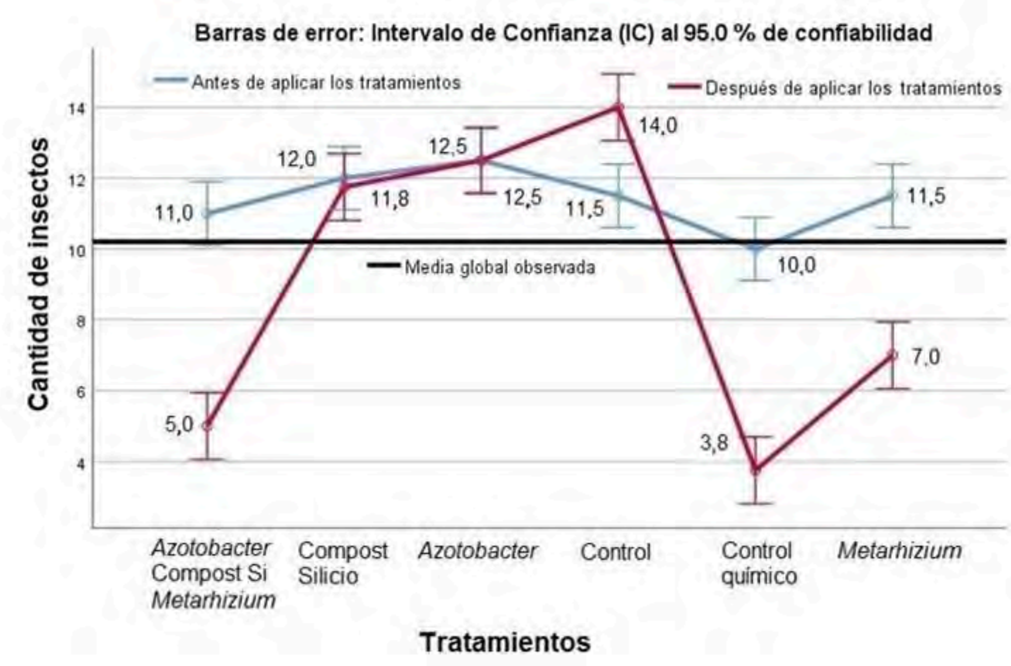
differ from the rest of the treatments. The combined treatment reduced the population per plot by nine insects compared to the control. Nonetheless, it should be noted that there were no significant differences compared to the treatment based on *Metarhizium*.

Number of *Oebalus insularis* Stål before and after applying the treatments

The average number of insects per treatments before (pre) and after being applied (post), (Figure 1), showed that the combined application of the mixture of *Azotobacter*-compost potassium silicate-*Metarhizium* managed to reduce the population of the pest from 11 insects before application to five insects, for a percentage reduction of 54.5%, and it is the second largest reduction after chemical treatment, the only treatments that reduce population levels by percentages greater than 50%. Contrary to the above, it was the treatment based on compost-potassium silicate.

The control treatment before application presented an average population of 11.5 insects in pre-application and about seven days later, the population of *O. insularis* increased by fourteen insects on average for an increase in the pest population of 17.9% (Figure 1).

Figure 1. Average number of insects in pre and post applications for each treatments. Error bars identify confidence intervals with 95% reliability.



In the chemical treatment, the pest population before being applied (pre) was 10, once the chemical product was applied (post), it fell to 3.8, equivalent to a population decrease of 62%, the treatment with the greatest population reduction in percentage terms comparing both moments. For the *Metarhizium* treatment, it decreased to seven average insects, which represents a percentage decrease of 39.1%.

Discussion

The results show a significance value (*p*) of Mauchly's test of the multivariate analysis of variance (Table 1) of less than 0.05, indicating that the sphericity hypothesis is rejected; however, Ateş *et al*. (2019) propose as an alternative solution to make to statistics provided by multivariate analysis of variance, such as Wilks' Lambda, responsible for the decision because the determination of these statistics is not affected by non-compliance with the premise of sphericity from a multivariate approach.

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In relation to the non-centrality parameter, according to Christensen (2022), when this value increases, the statistical power of the analysis increases and the probability of rejecting the nullity hypothesis decreases. Although it is widely documented that *Azotobacter chroococcum* has an important beneficial effect on plant growth and development (Sumbul *et al*., 2020; Llerena-Ramos *et al*., 2021) and its suppressive effect on pathogens (nematodes) has also been demonstrated (Akram *et al*., 2016; Edison-Zambrano *et al*., 2019; Hajjar *et al*., 2023), this research found no significant control over the pest insect *Oebalus insularis*.

Regarding the application of *Metarhizium anisopliae*, in Ecuador, some studies, such as that by McGuire and Northfield (2021) and that by Valle-Ramírez *et al*. (2020), isolated and characterized strains of *Metarhizium anisopliae* with potential for the control of *Mahanarva andigena* (Jacobi) in sugarcane (Saccharum spp.), and the present study demonstrated the bio-insecticidal effect on the insect *O. insularis*.

The mechanism of *M. anisopliae* on the insects it parasitizes is by contact, which gives it an advantage over the mechanism used by bacteria and viruses, which need to be digested to act on the insect. Contact with the insect *M. anisopliae* facilitates its infection through the cuticle due to the production of enzymes with the ability to hydrolyze the cuticle, whose compounds derived from this hydrolysis serve as nutrients for the fungus (Acuña-Jiménez *et al*., 2015).

Various studies related to silicon (Abada and Eman, 2017; Mukarram *et al*., 2022; Bhavanam and Stout, 2022) show that silicon causes silicification of cell walls and tissues and the formation of physical barriers or that it is an elicitor of plant defense pathways; nevertheless, in the results obtained with the presence of potassium silicate in the compost, it did not control the pest effectively.

The effectiveness of the combined treatment in the control of *O. insularis*, the inclusion of compost and *Azotobacter* is an important aspect to assess due to the synergy that can be established between both components in a positive way for the plant (Aslam and Ahmad, 2020; Bhavanam and Stout, 2022). In the same sense, the results achieved by the joint treatment of compost-potassium silicate-*Azotobacter*-*Metarhizium* may be associated with the joint effects of potassium silicate and the presence of *Azotobacter* as a microorganism that promotes plant growth and development (Salim and Hikal, 2019; Ranjan *et al*., 2021).

As an independent element, silicon stimulates plant development and leaf architecture and keeps the leaves and stems more erect, which reduces the detrimental lodging at the time of harvest of crops that are harvested mechanized, such as rice, the process of photosynthesis and water ratios, whereas potassium, as an essential macroelement of plant physiology, fulfills important functions in the formation of carbohydrates, such as starch in the rice grains, protein synthesis, cell division, vegetative development, and seed size and quality (Hasanuzzaman *et al*., 2018).

Silicon also favors the growth of the root system and photosynthetic pigments, regulates the opening and closing movement of stomata, the water status of plants due to its function as an osmoregulator, ionic balance and the activity of antioxidant enzymes (Ahmad *et al*., 2019), the function of which is closely related when plants are influenced by some type of stress, such as pest attacks, of the type of abiotic stress.

Conclusions

The results regarding the assessment of the use of beneficial microorganisms and compost with silicon for the control of *Oebalus insularis* Stål in *Oryza sativa* L., cultivar INIAP-14, show differentiated results between treatments, indicating that the use of combined treatments based on potassium silicate (K_4SiO_4) and that with *Metarhizium* show effective results to be considered as an agroecological alternative in the control of *Oebalus insularis*. It is recommended that the results achieved with the use of combined treatments should be expanded with future research considering variables of growth and development of rice crops.

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