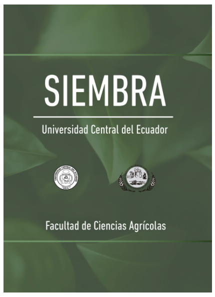
Entomopathogenic bacteria and fungi in the management of Brevicoryne brassicae (Homoptera: Aphididae) in cabbage crops

Bacterias y hongos entomopatógenos en el manejo de Brevivoryne brassicae (Homoptera: Aphididae) en los cyltivos de la col



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Abstract

The use of fungi and bacteria with pathogenic capacity towards insects is considered a sustainable agroecological alternative for efficient pest control, as they are microorganisms of easy production in industrial scale, formulation and use. Therefore, the objective was to evaluate four entomopathogenic formulations in the control of *Brevicoryne brassicae* L., a key pest of cabbage (*Brassica oleracea* L.), in a randomized complete block design with 4 treatments, plus an absolute control and 4 replicates. The variables evaluated were the number of dead and live aphids per plant and the number of infested plants. The commercial formulations Biosafe, BesT-K, Metarrizo and Yurak containing strains of *Bacillus subtilis*, *Bacillus thuringiensis* var kurstaki (*Btk*), *Metarhizium anisopliae* and *Bauveria bassiana* respectively, are the ones that were used by spray application to the crop in two seasons: planting, season August-December 2019 and August-December 2021. Among the results, *M. anisopliae* was recorded with up to 91.11 % efficiency, followed by *B. bassiana* with 89.50 % and Bs with 79.38 % in the reduction of populations under field conditions. In the laboratory, Btk and *M. anisopliae* reached 100 % mortality in a period of seven days, so it is concluded that in the medium term, entomopathogenic fungi in the field are more efficient in aphid control; however, in controlled conditions *Btk* is also efficient.

Keywords: entomopathogens, efficacy, mortality, aphids, biopesticides

Resumen

El uso de hongos y bacterias con capacidad patogénica hacia los insectos se considera una alternativa agroecológica sostenible de lucha eficiente contra las plagas por ser microorganismos de fácil producción en escala industrial, formulación y uso; entonces, el objetivo ha sido evaluar cuatro formulados entomopatógenos en el control de *Brevicoryne brassicae* L, plaga clave de la col (*Brassica oleracea* L.), en diseño de bloques completos al azar de 4 tratamientos, sumado un testigo absoluto y 4 réplicas, siendo las variables evaluadas la cantidad de pulgones muertos y vivos por planta y cantidad de plantas infestadas. Los formulados comerciales

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Biosafe, BesT-K, Metarrizo y Yurak que contenían cepas de *Bacillus subtilis*, *Bacillus thuringiensis* var kurstaki (Btk), *Metarhizium anisopliae* y *Bauveria bassiana*, respectivamente, son los que fueron utilizados mediante la aplicación por aspersión al cultivo en dos épocas de siembra, temporada agosto-diciembre 2019 y agosto-diciembre 2021. Entre los resultados se registró al *M. anisopliae* con hasta 91,11 % de eficiencia, seguida por *B. bassiana* con 89,50 % y Bs con 79,38 % en la reducción de las poblaciones en condiciones de campo; en laboratorio el *Btk* y *M. anisopliae* alcanzaron el 100 % de mortalidad en un lapso de siete días, por lo que se concluye que a mediano plazo los hongos entomopatógenos en campo resultan ser más eficientes en el control del pulgón, sin embargo, en condiciones controladas el *Btk* también es eficiente.

Palabras clave: entomopatógenos, eficacia, mortalidad, pulgón, bioplaguicidas

1. Introduction

Aphids of the species *Brevicoryne brassicae* L are specialists in the attack of cruciferous plants worldwide (Valverde Cadillo et al., 2021). Aphids cause stress to the plant and, as a consequence, economic losses due to low production. They use their mouthparts -modified into highly specialized stipes-, to pierce and feed on phloem and inject saliva into plant tissue (Broekgaarden et al., 2008). Aphid feeding can cause chlorosis and leaf rolling in the crop. The coils serve as refuges for colonies and new progeny; additionally, in the process of feeding, aphids become transmitters of viral diseases (Askar, 2021). Once infestations are initiated, they invade crops and new surrounding areas in a short time, this is caused by a type of winged female called virginopada (Kahan et al., 2002; Villacide & Masciocchi, 2014).

It is common to use broad spectrum pesticides for Aphids control, such as organophosphates, peritrates, and carbamates (Dubrovsky Berensztein et al., 2017); however, their use causes serious socio-environmental conflicts (Islam et al., 2021), as most of them are insecticides with prolonged residual leaching effect, which generates damage in the biogeochemical cycle (Jin et al., 2019; Pohare et al., 2021). As various authors highlight insects have the ability to develop genetic resistance; this, restricts the prolonged use of the same active ingredient (Datta et al., 2021; Zhang et al., 2020). The search for new control alternatives is necessary (Kim et al., 2017; Neuwirthová et al., 2019), including the use of entomopathogenic microorganisms (Lacey, 2017) that are usually natural enemies of insects, with high control efficiency and without harm to the environment and human health (Islam et al., 2021; Khan et al., 2012).

Different microorganisms, including fungi, bacteria, and entomopathogenic viruses can be efficiently applied in pest management (Chen et al., 2021; Kaczmarek & Boguś, 2021; Chakrabarty et al., 2022). Several fungal genera, including *Beauveria*, exhibit high pathogenicity due to the production of toxins capable of killing the insect (Rao & Narladkar, 2018; Yari Briones et al., 2021), the genus *Metarhizium* affects more than three hundred species of the insecta class (Gómez Pereira & Mendoza Mora, 2004), as well as *Bacillus thuringiensis* serovar kurstaki, which proves to be highly effective in reducing the population density of numerous pests (Daquila et al., 2021; Lentini et al., 2020; Mannu et al., 2020; Straw & Forster, 2022). Although the efficiency of these biocontrollers on pests differs depending on strains, isolation site, incubation time and dosage (Eidy et al., 2016); however, their use results in a sustainable alternative for integrated management (Silva et al., 2021).

There are previous studies on the use of entomopathogens in aphid control more broadly at the international level (Boni et al., 2021; Ek-Ramos et al., 2021; Gebreyohans et al., 2021; Jaber & Araj, 2018; Mukherjee et al., 2020). In Peru, information concerning this subject is scarce, this could be due to the little information at the national level and to the lack of knowledge of farmers about the effectiveness of microorganisms in pest management. No reports have been found on the success of aphid management with the use of entomopathogens, even more so under the environmental conditions of the inter-Andean valleys of Peru. Although studies have described the effects of fungi and bacteria on the larval stages of Lepidoptera, no management trials on aphids have been reported. Given their importance as an alternative to the use of chemical insecticides and within the context of environmental care and farmers' health (Arias, 2021), this paper studies the entomopathogenic potential of four formulations on *B. brassicae*, pest of cabbage (*B. oleracea* L.) both at field and laboratory levels using experimental designs.

2. Materials and Methods

2.1. Study location

The research was conducted in the plots of the Centro de Investigación Olerícola Frutícola, of the Universidad Nacional Hermilio Valdizán, located on the left bank of the Huallaga River at 1,947 m a.s.l., 09°58'12" south latitude and 76°15'08" west longitude, and the laboratories of the plant health area of the same university, during the August-December 2019 and August-December 2021 seasons.

Plots infested with *B. brassicae* under a randomized complete block design (RCBD) with 4 treatments, plus a blank control and 4 replicates, were studied. The population was considered to be the total number of aphids on 1,920 plants of Globe Master cabbage variety, and the sample was the total number of aphids on 24 plants of each experimental unit.

2.2. Treatments under study

The treatments were based on the commercially available formulations BioSafe, BesT-K, Metarrizo and Yurak containing strains of the entomopathogens *Bacillus subtilis*, *Bacillus thuringiensis* var kurstaki (Btk), *Metarhizium anisopliae* and *Beauveria bassiana*, respectively (from the mycological bank of the company PBA-Peru), evaluated by spray application to the crop (Table 1).

Table 1. Treatments.

Treatments	Applied Dose
Bacillus subtilis	4 mL l^{-1} of water
Bacillus thuringiensis var kurstaki (Btk)	4 mL l^{-1} of water
Metarhizium anisopliae	4 mL l^{-1} of water
Bauveria bassiana	4 g l^{-1} of water
Control	No application

In the field the unit of analysis was a cabbage plant, and in the laboratory it was a plate containing 100 adult individuals.

2.3. Water analysis

Prior to the dosing of the treatments, the water to be used was analyzed to measure the pH, taking into consideration that the ideal range is comprised between 5.5 and 7, and that the mineral compounds in the water should be less than 150 mg l^{-1}. Different pH values can, in fact, alter the germination of entomopathogen spores (Enriquez Vara, 2021).

According to the results obtained, the pH was 6.93, so it was not necessary to use water corrector and the presence of carbonates in the water was low, being favorable for its use. It was considered convenient to collect water and expose it to the sun for 48 hours for the elimination of chlorides, and sedimentation of the particles before applying it in the field.

2.4. Dosage and field application

The entomopathogen was added to a five-liter bucket of water (individually for each formulation) until a homogeneous mixture was obtained; in the case of fungi, it was necessary to add agricultural oil (Ec-oil) at a dose of 20 ml per 20 l of water, and then the entomopathogenic broth was left to stand for six hours. The oils protect the fungal spores from inclement weather (Gómez Ramírez et al., 2014).

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2.5. Frequency of applications

Entomopathogenic fungi are generally applied to crops by foliar spraying, or soil incorporation (Quesada-Moraga et al., 2006; Russo et al., 2015). In this study the applications of the entomopathogenic broth were carried out with the help of a 20-liter manual spray backpack, it should be noted that for each treatment a special backpack was used. The spraying was carried out in the afternoon at sunset (five to six o'clock in the afternoon), every 7 days for three consecutive times, the applications began when a considerable percentage of aphids was detected in the field.

2.6. Application of treatments under laboratory conditions

The procedures of Al-alawi and Obeidat (2014) and Gómez Ramírez et al. (2014) were used with some modifications:

- Cabbage leaves were collected from the control plot as an aphid food source; they were disinfected for 10 seconds with NaCIO 0.5% and rinsed immediately after for three consecutive times using distilled water and dried on sterile paper towels. Additionally, a total of 2,000 adult aphids were collected from the untreated plot, occupying 100 individuals per 50 ml sterile plate, in 5 treatments, and 4 replicates.
- From the Biosafe, BesT-K, Metarrizo and Yurak formulations, which contained strains of the entomopathogens, the biocontrol broth was prepared individually in 60 ml of purified water, adding the entomopathogen in a range of 0.12 g, as appropriate; in the case of the fungi it was necessary to incorporate 0.06 ml of agricultural oil. Then, the flasks containing the entomopathogen were labeled as *B. subtilis*, Btk, *M. anisopliae*, *B. bassiana* and control, this ready solution was left to stand for six hours, then they were applied on the plates according to the treatments under study, then kept in the laboratory at 24±2 °C, 65±10 % RH and 16:8 h. photoperiod for five to seven days.
- The replacement of cabbage leaves as food for the aphid was done daily, taking advantage of the moment for the evaluations and cleaning of the plates, at the same time during the seven consecutive days. Efficiency was also recorded on the same days, counting the aphids according to their state (alive or dead); the dead insect was considered to be an aphid with no movement, no response to the puncture and with the presence of fungal structures on the body of the individual.

2.7. Efficacy Analysis

Field and laboratory mortality were expressed in percentages of efficacy, given by the formulas of Henderson and Tilton (1955) and Abbott (1925), respectively, and then subjected to analysis of variance (ANOVA) and multiple comparisons through Fisher's LSD test, using the Infostat statistic (Di Rienzo et al., 2020).

3. Results and Discussion

3.1. Incidence of B. brassicae in post-application field

The incidence record prior to the applications fluctuated between 26 and 28 aphids per plant, and was statistically homogeneous throughout the experimental field ($p > 0.01$). At 7 days post-application, the percentage efficiency of treatment Bs was 52.31 % with an average population of 16.25 aphids per plant, while in treatment Btk, M. anisopliae, B. bassiana and the control (control group) populations increased to an average of 35.50, 30.75, 62.75, and 37.75 aphids plant^{-1}, respectively. At 15 days post-application, in the Bs treatment, the efficiency increased to 67.29 %, followed by the M. anisopliae treatment with 84.68 %.

Within 30 days of evaluation all treatments were effective in controlling the pest, being the Btk the one obtaining best results with 88.67 %, followed by M. anisopliae with 84.68 %, Bs with 75.17 % and *B. bassiana* with 71.01 % (Table 2), with populations ranging from 4.45 to 11.25 individuals per plant ($p < 0.01$), with a marked difference in the control treatment, where more than 40 aphids plant^{-1} were registered. The incidence of the pest, prior to the applications, was homogeneous throughout the experimental field, and then declined in the days following the application of the treatments, obtaining the highest reduction percentage with Btk, followed by *M. anisopliae*, *B. subtilis* and *B. bassiana*. Similar results of *B. bassiana* are reported by Al-alawi and Obeidat (2014) when studying the efficiency of three isolates in controlling the aphid *Myzus persicae* under laboratory and greenhouse conditions (41.3 % and 46.5 % efficiency, respectively). The findings of Peña et al. (2000) with biopreparations of *Verticillium lecanii*, *Paecilomyces fumosoroseus* and *B. bassiana* at plot level in the control of the aphid *Toxoptera citricidus* Kirkaldy, *V. lecanii* also recorded 76 % and 78 % of technical effectiveness, being surpassed only by *P. fumosoroseus* with 85 % and *B. bassiana* 83 %, respectively. Ríos et al. (2020) showed 84 % effectiveness with *B. bassiana* in the control of *B. brassicae* in 23 days, while *B. bassiana* + *M. anisopliae* had 79 % efficiency in the first six days and *M. anisopliae* registered 88 % in 23 days post-application.

Table 2. Efficacy (%) of the entomopathogens in the control of *B. brassicae* in the field.

Treatments	Previous	Aphids day^{-1} + entomopathogen efficiency (%)*					
	0 days	7 days		15 days		30 days	
B. subtilis	25.50 a	16.25 a	52.31%	15.50 b	67.29%	9.75 b	75.17%
Btk	25.50 a	35.50 d		31.00 c		4.45 a	88.67%
M. anisopliae	26.50 a	30.75 c		6.25 a	84.68%	6.25 a	84.68%
B. bassiana	25.25 a	62.75 e		29.25 c		11.25 b	71.07%
Control	28.25 a	37.75 d		52.50 d		43.50 d	
C, V %	5.41	4.86		7.56		10.04	
E, E	0.71	0.84		1.02		0.75	

* Means with the same letter in column with no statistical difference (LSD *a* = 0.05).

3.2. Efficacy of entomopathogens in the control of B. brassicae under laboratory conditions

Aphid mortality occurred from the second day post application for all treatments (Figure 1) with a gradual decrease of individuals until reaching a maximum of 0.00 live aphids plate^{-1} on days five and seven post treatment.

3.3. Decrease of live individuals per plate

Prior to application, the number of live aphids was 100 individuals per plate, populations were homogeneous for all treatments. On the first post-application day, the number of live aphids per plate-1 was less than 93 in all treatments, on the second day the population was reduced to 85 to 49, and then declined rapidly to an average of 53 to 37 on the third day, the following day with declines of 40 to 15 individuals. Up to this day there were no statistical differences ($p > 0.01$) between treatments. On the fifth and seventh days, the populations of live individuals decreased to 0.00 in some treatments, with notorious statistical differences ($p < 0.01$), being the lowest averages in the entomopathogens M. anisopliae and Btk (Table 3).

Mortality occurred with a gradual decrease until reaching 0.00 live aphids plate^{-1} on days five and seven post-treatment, with the lowest averages for the entomopathogens *M. anisopliae* and Btk. This result corroborates the reports of Ek-Ramos et al. (2021) with the population decrease of *M. persicae* at field level on bell pepper plants after applying *B. bassiana* (strain 7R) and *M. brunneum* (strain metaBr1) at 1 *10 7 or 1 * 10 8 conidia ml^{-1}, with significant differences in populations between fungi efficiency and the control ($p < 0.001$), where *M. brunneum* reduced populations to zero in a period of nine days. Entomopathogenic fungi are decisive in the control of aphids in crops, avoiding the need to apply chemical products (Núñez Seoane, 2021). It should be noted that in the reduction of populations, entomopathogens normally exhibit an initially slow and terminally abrupt mode of action (Baverstock et al., 2006), and it is known that after penetration of the fungus into the host body a dimorphic transition occurs causing the formation of hyphae with dispersal potential, in this

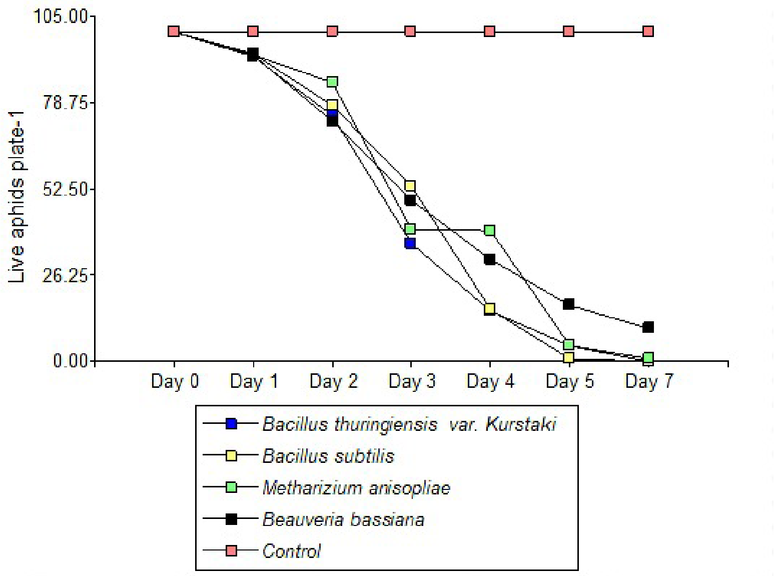

Figure 1. The lines represent the decrease in aphid population levels according to treatments, over time, live aphids plate^{-1}.

Table 3. Aphids mortality day^{-1} treatment^{-1}.

Treatments	Previous	Live aphids/treatment (mortality)*.					
	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 7
Btk	100.00	92.67 a	75.00 a	35.67 a	15.33 a	5.00ab	0.00 a
M. anisopliae	100.00	93.67 a	78.00 a	53.33 a	15.67 a	0.67 a	0.00 a
B. bassiana	100.00	93.00 a	85.00ab	40.33 a	40.00 a	4.67ab	0.67 a
B. subtilis	100.00	93.33 a	73.00 a	49.00 a	31.00 a	17.00 b	10.00 b
Control	100.00	100.00 b	100.00b	100.00 b	100.00 b	100.00 c	100.00 b
C, V %		3.08	10.04	34.03	59.44	31.36	35.36

* Means with the same letter in column with no statistical difference (LSD *a* = 0.05).

process they secrete toxins and enzymes that fulfill the function of inhibiting the metabolic process of insects, causing death (Bergamo et al., 2019; Moraes et al.,1998). Cell death also occurs when toxins and enzymes are released by *M. anisopliae* (Mwamburi, 2021).

3.4. Percentage of treatment efficacy

Efficacy evaluations were recorded up to seven days post-application, since the formulations caused high mortality at this level. The entomopathogens with 100% efficacy in aphid mortality were Btk and M. anisopliae (Table 4) outperforming the other treatments, followed by *B. bassiana* with 99.33% mortality.

Regarding the efficacy percentage of the treatments, the entomopathogens with high efficacy in aphid mortality were Btk and M. anisopliae, followed by *B. bassiana*. Similar mortality percentages were reported by Gebreyohans et al. (2021) in their studies on the management of *B. brassicae* using selected entomopathogenic fungi and insecticides, where *B. bassiana* recorded adult mortality between 67 % and 100 % after six days of incubation, and between 56 % and 83 % with *M. anisopliae* after five days of incubation. Similarly, Murerwa et al. (2014) record up to 41 % adult mortality with *M. anisopliae* at a concentration of 1 × 107 after 6 days of treatment. It is good to indicate that the efficacy of bio-controllers on aphids differs depending on strains, isolation site, incubation time and dosage (Eidy et al., 2016; Litwin et al., 2020). Becerra Verdín (2010) in evaluating

Table 4. Efficacy of the treatments (%).

Treatments	Efficacy (%)*						
	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 7
Btk	0.00	7.33	25.00	64.33	84.67	95.00	100.00
M. anisopliae	0.00	6.33	22.00	46.67	84.31	99.33	100.00
B. bassiana	0.00	7.00	15.00	59.67	60.00	95.33	99.33
B. subtilis	0.00	6.67	27.00	51.00	69.00	83.00	90.00

** % efficacy= [1 - (Td / Cd)] x 100*

commercial formulations and native strains of *M. anisopliae* and *B. bassiana* on aphid mortality on cucurbits, recorded up to 63 % efficacy in five days for both fungal species. Clifton et al. (2018) found that application of *B. bassiana* and *M. brunneum* to soybean seeds converts them to endophytes and these can affect interactions with the soybean aphid (*Aphis glycines* Matsumura), in this case *B. bassiana* was the most frequent.

4. Conclusions

The results show the effectiveness of the four entomopathogens in aphid control both at field and laboratory levels. The treatments showed a gradual increase in the mortality time of the insects. In the field with an efficiency of 71.07 to 88.67 % within 30 days and in the laboratory, causing maximum mortality in 5 to 7 days and an efficiency of 90 to 100 %.

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Authors' contributions

- Agustina Valverde-Rodríguez: conceptualization, data curation, software, writing - revision & editing.
- Antonio Cornejo y Maldonado: investigation, methodology, validation.
- Nalda Miguel Villanueva: formal analysis, writing - original draft.
- Miltao Edelio Campos Albornoz**:** data curation, project administration.

Ethical implications

Ethics approval Not applicable.

Conflict of interest

The authors declare that they have no affiliation with any organization with a direct or indirect financial interest that could have appeared to influence the work reported.

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