Revista de CIENCIAS AGRÍCOLAS

e-ISSN Online 2256-2273

Received: October 29 2025 Accepted: July 31 2026 Published: August 31 2026

Research article: Crop Protection

Integrated evaluation of biological and chemical control against *Globodera pallida* in potato

Evaluación integrada de las estrategias de control biológico y químico contra *Globodera pallida* en la papa

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Cite: Betancourth García, C., Salazar González, C., Vargas Rosero, R. D., Rosero Ortega, I. D., & Aux, D. (2026). Integrated evaluation of biological and chemical control against *Globodera pallida* in potato. *Revista de Ciencias Agrícolas*, *43*(2), e2308. https://doi.org/10.22267/rcia.2026432.308

ABSTRACT

The cyst nematode *Globodera pallida* poses a critical threat to potato production in Colombia, where severe infestations can cause yield losses of up to 90%. This study evaluated the efficacy of two biological products, Onix® (*Bacillus methylotrophicus*) and Alicerce® (*B. licheniformis* and *B. subtilis*), alongside a chemical alternative, Verango® (fluopyram), for the control of *G. pallida* under potato agroecological conditions. Compared with the control, Onix® reduced cyst populations by 77.6%, J2 juveniles by 88.1%, and female counts by 68.5%. Furthermore, Onix® significantly increased the first-grade tuber production, demonstrating high biological efficacy. In conclusion, Onix® (*B. methylotrophicus*) represents a sustainable and effective alternative for the integrated management of *G. pallida*, providing significant environmental benefits without compromising crop yield.

Keywords: *Bacillus methylotrophicus*, crop yield, cyst nematodes, integrated management, phytoparasitic nematodes, *Solanum tuberosum*, sustainable production

RESUMEN

El nematodo del quiste *Globodera pallida* representa una amenaza grave para la producción de papa en Colombia, donde las infestaciones severas pueden causar pérdidas de rendimiento de hasta el 90 %. Este estudio evaluó la eficacia de dos productos biológicos, Onix® (Bacillus methylotrophicus) y Alicerce® (*B. licheniformis* y *B. subtilis*), junto con una alternativa química, Verango® (fluopyram), para el control de G. pallida en condiciones agroecológicas de cultivo de papa. En comparación con el control, Onix® redujo las poblaciones de quistes en un 77,6 %, los juveniles J2 en un 88,1 % y el número de hembras en un 68,5 %. Además, Onix® aumentó significativamente la producción de tubérculos de primer grado, lo que demuestra una alta eficacia biológica. En conclusión, Onix® (*B. methylotrophicus*) representa una alternativa sostenible y eficaz para el manejo integrado de G. pallida, ya que brinda importantes beneficios ambientales sin comprometer el rendimiento del cultivo.

Palabras clave: *Bacillus methylotrophicus*, manejo integrado, nematodos de quiste, nematodos fitoparásitos, producción sostenible, rendimiento de cultivos, *Solanum tuberosum*

INTRODUCTION

Potato (*Solanum tuberosum* L.) is one of the most economically and socially important crops in Colombia. The country ranks as the third-largest producer in South America and is also a significant producer globally. According to the latest FAO (2024) report, national potato production reached 2.8 million tons from approximately 120,000 hectares, with an average yield of 23.3 tons per hectare, representing an increase compared with previous years. In Nariño, the third-largest potato-producing department after Cundinamarca and Boyacá, nearly 25,000 hectares are planted annually, primarily with the Diacol Capiro variety. Pasto, Ipiales, Pupiales, Guachucal, and Túquerres lead production because of their strong agricultural potential, accounting for about 23% of the country's total production (Fedepapa-FNFP, 2023). This crop not only stimulates the regional economy but also constitutes a fundamental pillar for food sovereignty.

Per capita potato consumption in Colombia is 63 kg/year, making potatoes an important source of carbohydrates, protein, vitamins, and minerals in the population's daily diet (MADR, 2023). Furthermore, the sector generates approximately 264,000 direct and indirect jobs, representing 7.3% of national agricultural employment and demonstrating its socioeconomic relevance (DANE, 2019).

One of the main phytosanitary challenges facing potato production is the potato cyst nematode *Globodera pallida* (Stone) Behrens, a plant-parasitic nematode capable of reducing yields by 50% to 90% under severe infestations (Contina *et al*., 2020). This specialized sedentary endoparasite has a complex biological cycle in which second-stage juveniles (J2) invade the roots and establish permanent feeding sites, causing multiple physiological alterations, including reduced water and nutrient uptake, hormonal imbalances, decreased photosynthetic activity, and changes in root architecture (FAO, 2022; Ravichandra, 2014). In Colombia, *G. pallida* was first detected in 1971 in the municipality of Cumbal by the Instituto Colombiano Agropecuario (Guerrero, 1982). Studies have shown that a density of only 0.6 cysts per gram of soil can reduce potato production by up to 48%, representing a serious threat because the nematode can persist in the soil for prolonged periods as cysts, which may remain viable for up to 20 years (Obando *et al.*, 2017). This situation was exacerbated following the ban on carbofuran in 2010, as nematode populations increased significantly in association with intensive monoculture and repeated potato cultivation, with up to three cropping cycles per year (Betancourth *et al.*, 2020).

Although chemical nematicides such as fluopyram and abamectin are registered for use, their high cost, adverse environmental impacts, and potential toxicity to farmers considerably limit their use as stand-alone management strategies (Collange *et al*., 2011). This has prompted the search for sustainable alternatives within integrated management, where biological control agents have emerged as promising options.

Among biocontrol agents, *Bacillus licheniformis* and *B. subtilis* have demonstrated efficacy through several mechanisms, including the production of metabolites with nematicidal activity, efficient rhizosphere colonization, competition for space and nutrients, induction of systemic resistance in plants, degradation of root exudates that attract nematodes, and production of enzymes that degrade the nematode cuticle (Engelbrecht *et al.,* 2018). Similarly, *B. methylotrophicus* produces volatile organic compounds with nematicidal activity and lipopeptides that compromise the integrity of nematode cell membranes, while also promoting plant growth through the synthesis of phytohormones and improved nutrient availability (Béjar Luque *et al*., 2016).

It was hypothesized that biological treatments would achieve nematode population control and yield outcomes comparable to those obtained with the chemical standard, thereby providing a sustainable alternative for the integrated management of this nematode that is compatible with the region's agroecological conditions.

The objective of this study was to evaluate the efficacy of three control agents — Onix® (*Bacillus methylotrophicus*), Alicerce® (*B. licheniformis and B. subtilis),* and Verango® (fluopyram) — against *Globodera pallida* in potato (*Solanum tuberosum* L.) under field conditions in Pasto, Nariño, by assessing their effect on cyst, juvenile (J2), and female population dynamics and on commercial tuber yield.

MATERIALS AND METHODS

Location

The study was conducted in the department of Nariño, municipality of Pasto, district of Catambuco, in the village of San Antonio de Casanare, located at coordinates 1°8'48.539"N 77°14'17.543"W. A commercial plot was used, located at an altitude of 2,800 m, with an average temperature of 12°C and annual precipitation of 923 mm (IGAC, 2025).

Area and experimental design

The experiment was established using a randomized complete block design (RCBD) with five treatments: T1 (Verango®), T2 (Alicerce®), T3 (Onix®, single application), T4 (Onix®, split application), and T5 (control), as detailed in Table 1. Each treatment was replicated four times, with the experimental area divided into four blocks. Four replications were used, consistent with comparable RCBD trials for potato cyst nematode management (Turner *et al*., 2006), given the labor-intensive nature of cyst, J2, and female extraction procedures. A formal post hoc power analysis is recommended for future trials to confirm the ability to detect smaller treatment effects. The experiment was conducted in a 600 m² field divided into 30 m² plots (5 × 6 m). One tuber was planted per planting site, with rows spaced 1.2 m apart and plants spaced 0.4 m apart within rows. Each plot contained five rows and 13 plants per row, for a total of 65 plants per experimental unit. Thus, each treatment included 260 plants (65 plants × 4 replications), resulting in a total of 1,300 experimental plants across the five treatments (260 plants × 5 treatments).

Table 1. *Description of the treatments evaluated for the control of G. pallida*

Treatment	Commercial Name	Active Ingredient	Concentration	Total Dose	Application Timing (DAP)
1	Verango®	Fluopyram	500 g/L	500 mL/ha	0 (Planting)
2	Alicerce®	*B. licheniformis + B. subtilis*	1 × 10^9 CFU/mL	1000 mL/ha	25
3	Onix® – single application	*B. methylotrophicus* GF 267	1 × 10^8 CFU/mL	1 L/ha (1 × 1 L)	25
4	Onix® – split application	*B. methylotrophicus* GF 267	1 × 10^8 CFU/mL	1 L/ha (2 × 0.5 L)*	25 and 55 (30 days after the first application)
5	Absolute control	—	—	—	—

Note. DAP: Days After Planting.

Variables evaluated

To evaluate the efficacy of the five treatments, nematode incidence was determined by quantifying cyst populations before and after the application of the nematicide product, as well as by counting J2 and immature females on two occasions, considering the life cycle of *G. pallida* and the phenological stages of the potato crop, as shown in Figure 1. Subsequently, commercial tuber yields were determined.

Figure 1. *Evaluation dates according to plant phenology*

27/05/2024	10/06/2024	05/07/2024	17/07/2024	19/08/2024	01/09/2024	21/09/2024	21/12/2024
Initial Cyst count	Planting	Treatment application	First J2 count	Second J2 count	First Female count	Second Female count	Final Cyst count
Before planting	0	25 DAP	37 DAP	70 DAP	83 DAP	103 DAP	194 DAP
Before planting	Before application	0	12 DAA	45 DAA	58 DAA	78 DAA	169 DAA

Note. DAP: Adapted from Potato Growth Stages (Illustration), by EOS Data Analytics, 2026.
DAP: Days After Planting; DAA: Days After Application.

Cyst counts

Cyst quantification was performed at two stages: an initial assessment on May 27, 2024, before planting, and a second assessment on December 21, 2024, corresponding to 169 days after treatment application. Cyst quantification was conducted both in the field and at the Laboratory of the Plant Health Research Group (GRISAV) at the University of Nariño, following a systematic sampling and analysis protocol. For each experimental unit, 1 kg of soil was collected as ten 100-g subsamples following an X-shaped sampling pattern at a depth of 20–25 cm, corresponding to the zone of root-system influence and ensuring representative sampling of the nematode population (Sañudo *et al.*, 2003). The samples were dried at room temperature for 48 h and then gently crushed with a roller to homogenize the material. For cyst extraction, the methodology described by Fenwick (1940) was followed. Briefly, 100-g subsamples of sieved soil were suspended in 1 L of water in volumetric flasks and allowed to stand for 24 h to facilitate differential sedimentation of soil particles. The supernatant was subsequently separated using a filtration system consisting of a funnel fitted with filter paper (Piedra Naranjo, 2020). The material retained on the filter paper, containing the cysts, was dried for an additional 24 h. Cysts were then quantified by direct observation under a stereomicroscope, allowing the cyst population to be counted in each sample. The identity of the nematode *G. pallida* was previously confirmed through morphological analysis of perineal patterns conducted by Professor Carlos Betancourt. These findings are consistent with those of Vallejo *et al.* (2021), who reported *G. pallida* as the only *Globodera* species recorded in Colombia.

Second-stage juvenile (J2) count

The first J2 assessment was conducted 12 days after treatment application (July 17, 2024), and the second assessment was conducted 45 days after treatment application (August 19, 2024), as shown in Figure 1. The extraction and quantification of second-stage juveniles (J2) were performed following a methodology adapted from standardized nematological techniques (Ravichandra, 2010). Soil samples (500 g) were collected from each replicate across the five treatments, resulting in a total of 20 samples. The samples were processed using a modified Baermann method, which is widely used for motile nematode extraction (Baermann, 1917). Briefly, 100 g of soil was suspended in 3 L of water and vigorously agitated to disperse the soil and facilitate the separation of large aggregates. The resulting suspension was then passed sequentially through sieves with different mesh sizes (20, 60, 200, and 325 mesh) (Ravichandra, 2010). The 200- and 325-mesh sieves were used to retain and separate J2 from other soil particles. The material retained on the 325-mesh sieve was carefully collected and transferred to an absorbent towel, after which the Baermann funnel technique was used to extract the nematodes based on their active movement (Baermann, 1917). For final quantification, five 1-mL aliquots were taken from the resulting nematode suspension, and J2 were counted by direct microscopic observation following the protocol described by Ravichandra (2010).

Immature females

Female nematodes were quantified in the root systems on September 1, 2024, 58 days after treatment application, as shown in Figure 1. For this assessment, two plants were randomly selected from each experimental plot and carefully removed from the field. Their root systems were examined, and females attached to the roots were counted in two sampled root systems per experimental unit. A second assessment was conducted on September 21, 2024, 78 days after treatment application, to determine the period with the highest number of females based on the nematode's life cycle (Betancourth *et al.*, 2021).

Yield

Yield was calculated based on the net plot area. The three central rows were harvested, excluding border plants to minimize edge effects, and yield was expressed in t ha^{-1}. Tubers were classified into three commercial categories as follows: first class (120–150 g, "Cero"), second class (70–120 g, "Comercial"), and third class (40–70 g) (ICA, 1997; ICA, 2015). In addition to the categorical classification, total yield was calculated as the sum of the three commercial categories for each experimental unit and is reported alongside the categorical yields in the Results section.

Statistical analysis

Statistical analyses and data interpretation were performed using InfoStat and R software. Data on nematode population density, second-stage juvenile (J2) and immature female counts, and crop yield were analyzed using analysis of variance (ANOVA), followed by Tukey's honestly significant difference (HSD) test at $\alpha = 0.05$. Before conducting ANOVA, the assumptions of normality of the residuals and homogeneity of variances were assessed using the Shapiro–Wilk and Levene's tests, respectively. The data met both assumptions ($p < 0.05$); therefore, parametric ANOVA was performed without data transformation.

RESULTS

Initial cyst population

The analysis of variance (ANOVA) (Table 2) revealed no significant differences among treatments, indicating a homogeneous initial distribution of the cyst population across the experimental area. This provided a reliable baseline for subsequent evaluation of treatment efficacy and confirmed the need for control measures given the high infestation levels detected.

Table 2. *Statistical analysis of all variables*

Variable	First Cyst count	First J2 count	Second J2 count	First Female count	Second Female count	Second Cyst count	Yield Cat. 1°	Yield Cat. 2°	Total Yield
SS	3466.8	14077.8	29461.7	1348.3	4934.8	42404.7	499.77	286.23	1616.08
Mean Square	866.7	3519.45	7365.45	337.08	1233.7	10601.2	112.44	71.56	404.02
F-Value	1.17	16.36	25.27	26.05	5.43	25.03	3.76	2.85	3.46
p-value	0.3731	0.0001	<0.0001	<0.0001	0.0099	<0.0001	0.0331	0.0713	0.0423

Table 3. *Tukey's mean comparison test*

Treatment	First J2 (12 DAA)	Second J2 (45 DAA)	First Female (58 DAA)	Second Female (78 DAA)	Second Cyst (169 DAA)
T1 – Verango®	13.25 a	13.25 a	11.75 a	19.50 a	39.75 a
T2 – Alicerce®	26.75 a	68.25 b	22.75 b	41.00 ab	95.50 b
T3 – Onix® (single, 1 L)	25.00 a	13.00 a	13.00 a	18.75 a	36.00 a
T4 – Onix® (split, 2×0.5 L)	9.25 a	20.50 a	18.50 ab	45.50 ab	61.75 ab
T5 – Control	82.75 b	109.75 c	34.50 c	59.50 b	160.50 c

Note. (α = 0.05) for J2, female, and final cyst counts across treatments (consolidated table; means followed by the same letter within a column are not significantly different).

First and second J2 count

At 12 DAA, the first J2 assessment revealed significant differences among treatments according to the ANOVA (Table 2; $p < 0.0001$), with mean densities ranging from 9 to 82 J2 per 5 mL of sample. Tukey's mean comparison test (Table 3) showed that the highest J2 population occurred in the control, whereas the other treatments did not differ significantly from one another. Verango®, Alicerce®, and Onix® treatments therefore resulted in significantly lower J2 densities than the control.

At 45 DAA, the second J2 assessment also revealed significant differences among treatments (Table 2; $p < 0.0001$), with mean densities ranging from 13 to 109 J2 per 5 mL of sample. Tukey's mean comparison test (Table 3) showed that the control had the highest J2 population, followed by Alicerce®, which had the second-highest population and differed significantly from the other three treatments. The remaining three treatments did not differ significantly from one another.

First and Second Female Count

The analysis of variance (Table 2) indicated significant differences among treatments for the first female count ($p < 0.0001$). Table 3 indicates that Verango® and Onix® (single application) had significantly lower female densities than the control and Alicerce®, but did not differ significantly from Onix® (split application). Onix® (split application), in turn, did not differ significantly from Alicerce®; however, both treatments differed significantly from the control. The averages ranged from 11 to 34 females per plant, with the lowest value obtained by Verango®.

For the second female count, the analysis of variance (Table 2) indicated significant differences among treatments ($p < 0.0099$). Table 3 indicates that Verango® and Onix® (single application) statistically outperform the control, but not Alicerce® and Onix® (split application), which in turn are equal to the control. The averages ranged from 18 to 59 females per plant, with the lowest value obtained by Onix® (single application).

Second Cyst Count

The ANOVA (Table 2) revealed significant differences among treatments ($p < 0.0001$). According to Tukey's mean comparison test (Table 3), Onix® (single application) and Verango® had significantly lower cyst densities than the control and Alicerce®, but did not differ significantly from Onix® (split application), which, in turn, did not differ significantly from Alicerce®. The control had the highest mean number of cysts per 100 g of soil. Mean cyst densities ranged from 36 to 160 cysts per 100 g of soil, with the lowest value recorded for Onix® (single application).

Yield

For first-class potato production, the analysis of variance (Table 2) showed significant differences among treatments ($p < 0.0331$), and the mean comparison test in Table 4 indicated that Onix® (single application) statistically outperformed the control, but the other treatments did not; however, they are equal to the control. The results showed a correspondence between the reduction in J2, female, and cyst populations generated by Onix® (single application), which allowed for a higher yield compared to the control. The production of first-class commercial Diacol Capiro potatoes using Onix® (single application) significantly exceeded the yield obtained from the control by 13 tons per hectare, corresponding to a 65% loss in the untreated plot.

Regarding the data obtained for the second-class potato harvest, the analysis of variance (Table 2) showed no significant differences among treatments ($p = 0.0713$), indicating that the nematode effect was not reflected in this variable or was equal across all treatments.

For the total yield from the sum of the three categories, the analysis of variance (Table 2) showed significant differences among treatments ($p < 0.0423$), and the mean comparison test in Table 4 indicated that Onix® (single application) statistically outperforms the control, but not Verango®, Onix® (split application), and Alicerce®, which in turn are equal to the control. The single-application Onix® treatment exceeded the control by 24 tons per hectare, corresponding to a 39% loss in the untreated plot. The overall yield results showed a pattern identical to that of first-class yield, highlighting the single-application Onix® treatment as the most effective.

Table 4. *Tukey's test for first-class yield and total yield (t/ha) (consolidated table; means followed by the same letter within a column are not significantly different)*

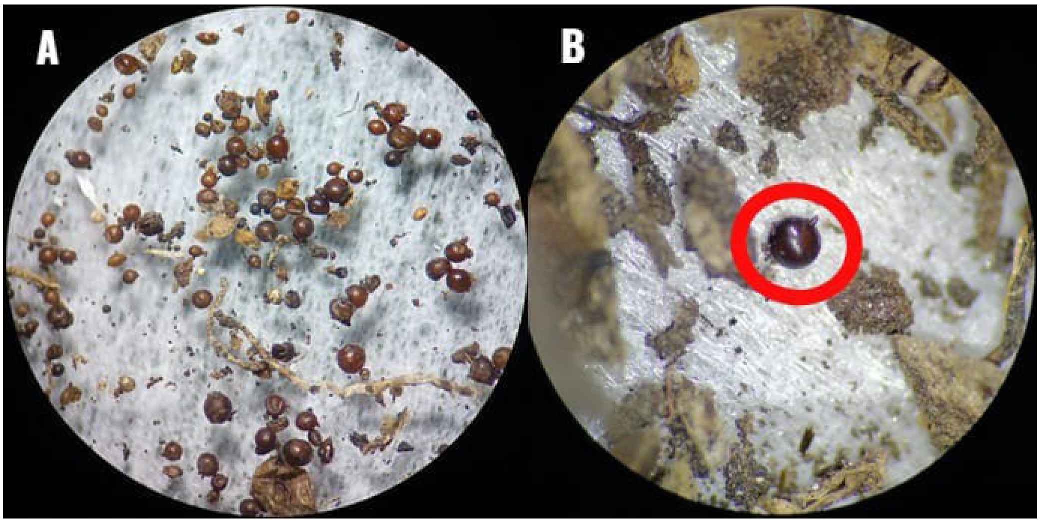
Treatment	Yield – Category 1 (t/ha)	Total Yield (t/ha)
T1 – Verango®	17.71 ab	53.93 ab
T2 – Alicerce®	12.13 ab	41.28 ab
T3 – Onix® (single, 1 L)	18.89 a	59.00 a
T4 – Onix® (split, 2×0.5 L)	17.70 ab	52.11 ab
T5 – Control	6.31 b	34.36 b

DISCUSSION

Initial cyst population

Initial *G. pallida* population homogeneity is a fundamental requirement in experimental studies evaluating the efficacy of nematode control treatments because it helps ensure that subsequent differences among treatments can be attributed to the interventions rather than to pre-existing variation in soil infestation levels. This principle is supported by studies such as that by (Turner *et al*., 2006), who reported relatively homogeneous populations of *G. pallida* before planting and emphasized the importance of establishing adequate nematode population densities when evaluating their effects on potato production. Considering the initial cyst densities detected in the experimental area, these populations substantially exceeded the reported economic injury levels for *G. pallida*. Piedra Naranjo (2020) reported that infestations exceeding 20 cysts per 100 g of soil can significantly reduce potato yield, with reductions of up to 40%, which is consistent with the high infestation levels detected in the present study. Furthermore, Orlando and Boa (2023) reported a direct relationship between initial cyst density and economic losses in commercial potato production, particularly when control measures are not implemented early in the crop cycle. These findings support the need for interventions such as those evaluated in the present study to reduce nematode populations and minimize their potential impact on potato productivity.

Figure 2. *Globodera pallida cysts under a stereoscope*



Note. Panel A: field sample showing cyst aggregation among soil debris, 2× stereoscope field of view; Panel B: 4× stereoscope field of view used for confirmatory counts.

The confirmed homogeneity in this study and the high infestation levels detected reinforce the urgency of implementing control strategies, as classified by the International Plant Protection Convention (IPPC, 2025), which classifies *G. pallida* as a quarantine pest with the potential to generate multi-million-dollar losses in agriculture.

Biocontrol mechanisms and J2 suppression

Fluopyram is a broad-spectrum nematicide that inhibits the mobility and development of J2. Studies such as that of Desaeger *et al.* (2020) have shown that fluopyram-based nematicides are highly effective in reducing nematode populations during the early stages of infection, which may explain the superior performance of this treatment in the present study, as evidenced by the significantly lower J2 density compared with the control. In contrast, the biological treatments Alicerce® (*Bacillus subtilis* + *B. licheniformis*) and Onix® (*B. methylotrophicus*) also provided effective control, which may be associated with their ability to produce antimicrobial metabolites and extracellular enzymes capable of degrading nematode structures.

The suppressive activity of these *Bacillus*-based products is best understood as the combined effect of several complementary mechanisms rather than a single mode of action. These mechanisms may include the production of lipopeptides, such as surfactins and iturins, with direct nematicidal and membrane-disrupting effects; secretion of extracellular enzymes, including chitinases and proteases, that can degrade nematode cuticles and eggshells; release of nematicidal volatile organic compounds; competitive colonization of the rhizosphere, which may limit nematode access to root-derived chemical cues; and induction of systemic resistance in the host plant (Engelbrecht *et al*., 2018; Béjar Luque *et al.*, 2016). Vasantha-Srinivasan *et al*. (2025) reported that *B. subtilis* and *B. methylotrophicus* produce lipopeptides and proteases that can affect J2 viability and reduce their ability to infect roots. Furthermore, these microorganisms can promote plant growth and induce systemic resistance, thereby contributing to integrated nematode management.

The efficacy of these biological products may also be related to their ability to colonize the rhizosphere and compete for resources with other soil microorganisms, thereby potentially limiting nematode development. Khan *et al.* (2023) reported that *Bacillus* spp. strains can reduce *G. pallida* populations by more than 50% under field conditions, which is consistent with the results in the present study. The absence of significant differences between the biological and chemical treatments suggests that both approaches can be effective in controlling *G. pallida*. However, biological products may offer additional environmental benefits because they can reduce reliance on conventional agrochemicals, making them potentially useful components of more sustainable potato production systems (Oka, 2020).

At 45 DAA, the treatment effects followed a similar trend, with Verango® and Onix® (single application) again showing the lowest J2 populations. The treatment containing *B. licheniformis* and *B. subtilis* showed intermediate control, and uncolonized J2 were observed (Figure 3). The intermediate response may be related to the time required for these bacteria to establish and express their antagonistic activity in the rhizosphere. It may also reflect variability in rhizosphere colonization, as the efficacy of microbial biocontrol agents can be influenced by interactions with indigenous soil microorganisms and environmental conditions (Migunova & Sasanelli, 2021). In addition, *B. methylotrophicus* has been reported to exhibit nematicidal activity through the production of secondary metabolites that can interfere with the nematode life cycle (Zhou *et al.*, 2024).

Figure 3. *J2 viewed under a microscope, 40x magnification*

The consistent efficacy of fluopyram and *B. methylotrophicus* in both counts underscores the importance of integrating chemical and biological approaches to managing *G. pallida*. While chemical nematicides such as fluopyram offer rapid and effective control, biological products like *B. methylotrophicus* provide a more sustainable solution compatible with environmentally friendly agricultural practices (Mateille *et al.,* 2020).

Female counts

The first assessment of female nematodes at 58 DAA showed that the Verango® and Onix® (single application) treatments resulted in significantly lower numbers of females than the control. Mean densities ranged from 11 to 34 females per plant. The efficacy of Verango® was consistent with the findings of Faske and Hurd (2015), who reported that fluopyram, the active ingredient in Verango®, exhibited high nematicidal activity by inhibiting succinate dehydrogenase in the mitochondrial respiratory chain of nematodes. The reduction to 11 females per plant represents substantial suppression compared with the population levels reported by Greco and Crozzoli (1995) in naturally infested fields. Onix® (*B. methylotrophicus*) also resulted in significantly lower female densities than the control. This effect may be associated with the ability of *B. methylotrophicus* to produce antimicrobial metabolites and enzymes capable of affecting nematode development and reproduction (Béjar Luque *et al.*, 2016; Engelbrecht *et al*., 2018). Furthermore, this microorganism has been reported to induce systemic resistance in plants, potentially contributing to broader nematode suppression.

Onix® applied as a split application (2 × 0.5 L ha^{-1}) was compared with the same total dose applied as a single application (1 L ha^{-1}). Because both treatments received the same total application rate, this comparison primarily evaluated the effect of application timing and fractionation rather than total dose. Studies such as Afridi *et al.* (2024) have shown that the establishment and persistence of *Bacillus*-based biocontrol agents in the rhizosphere can depend on the size of the initial population established early in the crop cycle, particularly before critical periods of nematode root invasion. A plausible explanation for the more consistent performance of the single application across most variables (Table 3) is that applying the full 1 L ha^{-1} rate at 25 DAP may have established a larger initial *Bacillus* population in the rhizosphere before peak J2 invasion. In contrast, dividing the application into two doses may have delayed establishment, while the second application at 55 DAP may have occurred after substantial root invasion and female establishment had already taken place, thereby limiting its additional effect. This interpretation is consistent with the capacity of *Bacillus* spp. to form endospores and persist in soil after successful establishment (Kloepper *et al.*, 2004).

In the second assessment at 78 DAA, mean female densities ranged from 18 to 59 females per plant. Onix® (single application) and Verango® were the most effective treatments, with the lowest female densities, whereas the control exhibited a high level of root infestation (Figure 4). The persistence of the single-application Onix® treatment in the second assessment may be related to the characteristics of biological control agents. As reported by Kloepper *et al.* (2004), *Bacillus* spp. can form endospores, which may enhance their survival under adverse environmental conditions and contribute to their persistence in soil.

These results suggest that Onix® (single application) may provide more sustained suppression over time than Verango®, for which female density increased between the first and second assessments. The sustained effect observed with Onix® may be associated with the ability of *B. methylotrophicus* to colonize the soil and plant roots, potentially providing longer-lasting suppression of *G. pallida* (Afridi *et al.*, 2024).

Fluopyram also resulted in a significant reduction in female density, reinforcing its potential role as an important component of integrated nematode management. However, the potential environmental impacts of chemical nematicides and the risk of resistance development associated with repeated use should be considered (Schleker *et al.,* 2022). Therefore, integrating chemical and biological nematicides may provide a more sustainable long-term approach to nematode management (Stirling, 2014).

It is important to note that Alicerce® (*B. licheniformis* + *B. subtilis*) and split-application Onix® (*B. methylotrophicus*) did not differ significantly from the control in terms of female density. This result may be related to differences in colonization timing or interactions with environmental and biological factors in the soil. Mendes *et al.* (2011) reported that the efficacy of *Bacillus*-based bionematicides can vary according to application strategy, formulation, and soil conditions. In addition, competition with indigenous soil microorganisms may limit the establishment and activity of these biological control agents.

Figure 4. *Females of G. pallida on the root system.*

Second cyst count

The final cyst assessment at 169 DAA demonstrated the effectiveness of Onix® (single application) and Verango® treatments, which resulted in the lowest cyst densities among the treatments, corresponding to reductions of 77.6% and 75.2%, respectively, relative to the control. These results are consistent with those reported by Aguilera (2016), who documented similar reductions in cyst populations following biological and chemical treatments. The present findings are also supported by previous research demonstrating the efficacy of *Bacillus* spp.-based bioinoculants and chemical nematicides against plant-parasitic nematodes. For example, Abd-Elgawad and Askary (2018) reported that *Bacillus* spp., including *B. methylotrophicus*, can produce secondary metabolites with nematicidal activity, which may contribute to their ability to suppress cyst populations in soil. Similarly, the efficacy of fluopyram has been documented in studies such as that of Desaeger *et al.* (2020), who reported significant reductions in nematode densities in soils treated with this compound.

Yield

Onix® (single application) showed a production of approximately 20 tons per hectare of first-class potatoes, significantly outperforming the control, which produced approximately 7 tons per hectare. This difference represents a 65% loss in the untreated plot. These results are consistent with previous studies indicating that *G. pallida* infestations can reduce potato crop yields by up to 80% in the absence of control measures (Whitworth *et al.,* 2018). The use of biological agents such as *B. methylotrophicus* has proven effective in controlling phytopathogenic nematodes and improving crop yields. Research by Béjar Luque *et al.* (2016) has shown that *B. methylotrophicus* can stimulate plant growth and act as a biological control agent against nematodes; these findings support the efficacy of Onix® (single application) in enhancing potato production in areas infested by *G. pallida*. Allccahuamán-Bedrillana and Ochoa (2019) reported that the application of *B. methylotrophicus* in potato crops can significantly reduce nematode populations and increase crop yield. Studies by Bonaterra *et al.* (2012) have documented that *B. methylotrophicus* produces lytic enzymes and antimicrobial metabolites that suppress nematodes such as *Meloidogyne incognita*, in addition to improving nutrient availability through phosphate solubilization, which would explain the yield increase observed with Onix® (single application). Furthermore, the 1 L/ha dose might have optimized root colonization, a critical factor for bioinoculant efficacy (Pérez-García *et al.*, 2011).

Figure 5. *Yield separated into three commercial categories*

Note. A. First grade, B. Second grade, C. Third grade.

The greater efficacy of *B. methylotrophicus* compared with fluopyram may be related to differences in the persistence and mode of action of biological and synthetic nematicides. Although the development of resistance to synthetic pesticides is an increasing concern in intensive agricultural systems (Fisher *et al.*, 2018), resistance to fluopyram was not evaluated in the present study and therefore cannot be directly attributed to the observed differences. In addition, variation in the response to Verango® may be associated with environmental factors, such as soil pH and moisture, which can influence the behavior and efficacy of fluopyram in soil (Gupta & Gajbhiye, 2002). Maldonado *et al.* (2024) evaluated the effects of Onix® on root health and highlighted the importance of managing pathogens that affect the root system because their presence can adversely affect crop yield. The present results suggest that the application of *B. methylotrophicus* in potato may contribute to improved crop performance through a combination of nematode suppression and plant growth-promoting effects, potentially resulting in higher yields and reduced yield losses.

CONCLUSIONS

The application of Onix® (*B. methylotrophicus*) with a single total dose of 1 L/ha at 25 DAP proved to be the most effective treatment for the control of *G. pallida*, significantly reducing the final cyst population (36.00 cysts/100 g for the single application) compared to the control (160.50 cysts/100 g). This reduction resulted in higher yields of first-class tubers (up to 20 t/ha) and prevented production losses of up to 65%. The results demonstrate that *B. methylotrophicus* represents a viable, effective, and sustainable alternative for the integrated management of this nematode in potato crops, especially when populations exceed established economic thresholds.

AUTHOR CONTRIBUTIONS

Conceptualization, C.B.G. and C.S.G.; Methodology, C.B.G. and C.S.G.; Formal Analysis, C.B.G., R.D.V.R. and I.D.R.O.; Investigation, C.B.G., R.D.V.R., I.D.R.O. and D.A.; Resources, C.B.G. and C.S.G.; Data Curation, R.D.V.R. and I.D.R.O.; Writing—Original Draft Preparation, C.B.G., R.D.V.R. and I.D.R.O.; Writing—Review & Editing, C.B.G., C.S.G., R.D.V.R. and I.D.R.O.; Visualization, R.D.V.R. and I.D.R.O.; Supervision, C.B.G. and C.S.G.; Project Administration, C.B.G. and C.S.G.; Funding Acquisition, C.B.G.

ACKNOWLEDGMENTS

The authors express their deepest gratitude, first and foremost, to God for the strength and guidance provided throughout the development of this research. The authors also extend their recognition to Professor Mónica Eliana Córdoba Figueroa for her valuable academic guidance and steady support throughout this work. Likewise, the authors thank the Universidad de Nariño and ADAMA Colombia for the financial and institutional support that made this study possible, as well as everyone who, directly or indirectly, contributed their time, knowledge, and willingness to the successful completion of this research.

FUNDING

This research was funded by the Universidad de Nariño and ADAMA Colombia (Soluciones para la protección de cultivos, https://www.adama.com/colombia/es).

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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