

Biological effectiveness of the Bt 26 strain of *Bacillus thuringiensis* in fighting the root-knot nematode *Meloidogyne incognita*

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Plant-parasitic nematodes pose a great threat to various crops, killing them and causing damage to agriculture. The bacteria-based approach to fighting them seems particularly promising, as it also makes it possible to lower the use of chemicals and reduce the effects caused by environmental changes. The goal of this work is to study the biological effect of the bacterium *Bacillus thuringiensis* against nematodes from the genus *Meloidogyne*. Samples of cucumbers from an experimental site deliberately infected with the southern root-nematode *Meloidogyne incognita* were used as material for this work. The study was conducted in 2023–2024. Phytohelminthology and microbiology methods were used in the research. In vivo experiments and studies in greenhouses and in the field have shown that bacteria can help regulate in various ways the development of endoparasitic nematodes. In conclusion, as a result of our studies of the local bacterial strain Bt 26, which has high nematocidal activity against *M. incognita*, we determined the optimal schemes of application of the biopreparation. Application of seed encapsulation with Bt-26 strain bacteria further increased the efficiency of microbial agents. Seed treatment with Bt-26 contained the lowest galling index of 0.5. Treatment with bacteria, which initiates protective mechanisms in plants, is regarded as an optimal solution, since it is no less or even more effective than the use of chemical pesticides. Moreover, bacteria have additional positive effects on plants, such as growth stimulation, which increases their productivity.

Keywords: *Bacillus thuringiensis*; knot formation index; *Meloidogyne incognita*; parasitic nematodes of plants.

Introduction

Plant-parasitic nematodes (PPNs) are common natural parasites which can cause serious damage to crops and lead to agricultural losses (Mitiku, 2018; Sasanelli et al., 2018). PPNs account for 12% to 25% of total damage caused annually to most economically significant crops around the world, which costs more than 80 billion US dollars (Agrios, 2005; Nicol et al., 2011). Plant-parasitic nematodes also form associations with bacterial and fungal pathogens. Preventive measures can be taken to fight pathogens, which makes it possible to stabilise plant biological indicators and improve crop yields (Lamelas et al., 2020; Leoni et al., 2020).

Plant-parasitic nematodes are known as common parasites causing a sharp decrease in crop production and, as a result, serious damage to the agricultural sector. Root-nematodes from the genus *Meloidogyne* deserve special attention. Species from this genus are recorded as agents of dangerous diseases of cultivated plants in Uzbekistan, causing significant damage to the national economy. PPNs are obligate parasites, common throughout the world and parasitising almost all types of vascular plants. Nematodes from the genus *Meloidogyne* spp. are polyphagous and regarded as the most dangerous of PPNs. This genus comprises about 100 species (Sub-

botin et al., 2021), the most important of which, commonly referred to as the big four, are *M. arenaria*, *M. incognita* and *M. javanica*, as well as *M. hapla*, which is regarded as somewhat less significant. This means that new approaches are urgently required to develop efficient methods and means to control diseases caused by these nematodes in crops. These nematodes kill root epidermal cells. This results in the formation of knots on the roots, which gradually grow and connect with each other to develop into complex structures. They block root vessels and impede the flow of nutrients and moisture to the plant (Perry & Moens, 2006).

The damage caused by root-nematodes represents a more than 50% drop in plant productivity. In some cases, it may lead to the death of a plant. Controlling this parasitic nematode is very costly and requires much effort. One of the main problems in growing vegetables under glass is the low effect of measures to reduce the number of root-nematodes during the growing season (Bukhanova, 2010). The use of biological preparations against diseases caused by root-nematodes is an alternative protection of plants from pathogens.

The goal of this work is to assess the biological effect of the bacterium *Bacillus thuringiensis* against nematodes from the genus *Meloidogyne*.

Materials and methods

Samples of agricultural plants – cucumbers from an experimental site deliberately infected with the southern root-nematode *Meloidogyne incognita* were used as material for this work. The study was conducted in 2023 and 2024. To extract nematodes from the soil we used Berman's modification method and the root incubation method (Kiryanova & Krall, 1969). The nematodes were soaked and dehydrated, and temporary and permanent preparations were made using methods generally accepted in the study of plant parasites (Kiryanova & Krall, 1969). Root-nematode species were identified based on the morphology of females, sometimes males and infectious larvae. A special focus was on patterns in the perianal area of sexually mature females. The anal and vulvar area of oviparous females was used to make permanent and temporary preparations (Kazachenko & Mukhina, 2013).

The local strains of *B. thuringiensis* were cultivated in a liquid nutrient medium by placing a 5% inoculum into a liquid peptone medium in Erlenmeyer 750-millilitre flasks and incubating it at 28–30 °C in an orbital shaker at 160 rpm. The strain completely sporulated in 72–80 hours in the liquid medium, with cell sporulation ranging between 90% and 95% (Minayeva et al., 2018).

The effect of the bioagents on infected vegetables was analysed on cucumbers grown in a greenhouse. The soil for the experiment was sterilised in an LS-75-HD autoclave at a pressure of 104 kPa and a temperature of 121 °C for 40 minutes. Components of the soil for the plants and their ratio: earth, sawdust and sand – 1:1:1.

The experiments were conducted in an experimental greenhouse at the Institute of Zoology, Academy of Sciences of the Republic of Uzbekistan. The Orzu variety of cucumbers was used for the experimentation. To study the nematocidal activity of the local strains of *Bacillus thuringiensis* 26, they were diluted in the proportions of 1:100 – 1×10^7 CFU/mL and 1:200 – 5×10^6 CFU/mL, while the BioSleep preparation was used as a standard. There was also a reference sample infected with the nematode *Meloidogyne incognita*, which was not treated with any agent.

The effect of the bioagents was measured with the help of plants' biometric indicators, such as plant height, leaf blade area, weight of the plants' aboveground and underground parts, the correlation between the number of knots and the weight of the root (knot formation index) and plant yield (Slobodyaniuk, 2011).

1 g of seeds was placed in the solutions of Bt 26 and BioSleep, 5 mL each, and soaked in water for 2 hours, after which they were planted in sterile soil. Cucumbers, which developed real leaves, were replanted in 5-litre pots, one in each pot. A total of 120 seedlings were transplanted into the flower pots. One week later, the cucumbers were deliberately infected with nematodes. On the same day, the plants' biometric characteristics were recorded. To detect nematodes, their roots were stained with fuchsin (Byrd et al., 1983).

In all regions of Uzbekistan, including the Tashkent oasis and Fergana Valley, greenhouses provide favourable conditions for the development of nematodes, which feel particularly comfortable at a temperature of 26–28 °C and a humidity of about 70%. Under these conditions, the parasite is able to breed up to seven times a year.

After screening various strains of the bacterium *B. thuringiensis*, we opted for the Bt 26 strain (MN 244693.1), which we took from the collection of the Institute of Microbiology under the Academy of Sciences of the Republic of Uzbekistan. It proved highly effective against the nematode *Meloidogyne incognita* parasitising cucumbers in greenhouses. We also specified the titres, timing and frequency of use for the biopreparation of this strain.

Treating the roots of the growing plants with bacterial strains twice – shortly before planting and 3 weeks after that – proved highly effective. The use of the bacterial agents significantly reduced the number of parasites. To develop methods for the biological control of root-nematodes from the genus *Meloidogyne*, we used the Bt 26 strain in the proportion of 1:100 and 1:200 in a greenhouse to specify its nematocidal activity. A BioSleep biopreparation, diluted in the proportion of 1:100 and 1:200, was used as a standard. All data on the plants' biometric features were averaged $n = 5$, and the experiment was repeated three times.

Results

Over the five weeks of the experiment, we specified the influence of biological preparations on the plants' biometry. During this period, the plants grew significantly in height, from 52 to 253 cm. In the first week of the experiment, the plants from the reference group had the shortest stems – 52 cm, while those, whose seeds were treated with the Bt 26 bacterial strain diluted in the proportion of 1:200, were the tallest. The other three groups of plants, treated with 1:100 Bt 26 and 1:100 and 1:200 Biosleep, had stems whose height was 72, 71 and 74 cm, respectively. In the second week of the experiment, plants in all the experimental groups grew significantly in height. The tallest were the plants treated with the 1:200 Bt 26 bacterial strain, which were 182 cm in height. The plants treated with 1:100 Bt 26 and 1:100 Biosleep grew to 134 cm and 132 cm, respectively, which was 25% and 23% higher than in the reference group, where during one week the plants doubled in height to 107 cm. In the last experimental group, the height of the plants was 172 cm, which was 60% higher than in the reference group. The plants grew significantly between the third and the fifth weeks of the experiment. In the reference group, the plants grew 0.3 times, from 135 to 177 cm. The fastest growth was in the plants treated with the 1:200 Bt 26 bacterial strain. In the last 3 weeks, the plants in this group grew from 211 to 253 cm. In the last week of the experiment, the length of the plants in this group increased by 43% compared to the reference group. The plants treated with 1:100 Bt 26 and 1:100 Biosleep showed a similar growth over the three weeks. Their height increased from 162 cm to 204 cm and from 160 to 202 cm, respectively, which is 14% – Bt 26 and 15% – Biosleep higher than in the reference group (Fig. 1).

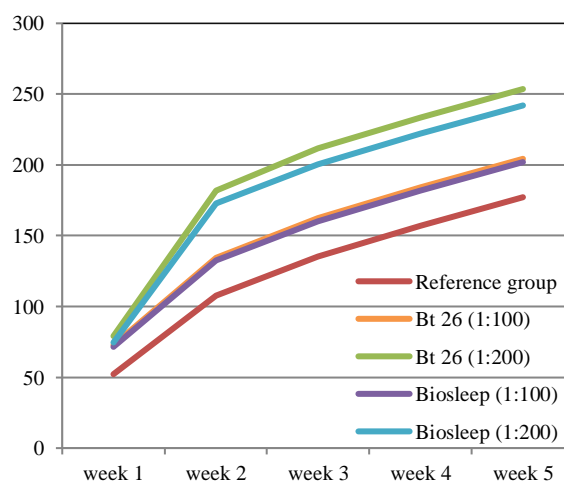


Fig. 1. Effect of Bt 26 strains on plant height (cm)

The decrease of the parasite's population allowed the plants to grow faster than in the reference group. The height increased by 15.3% when using 1:100 Bt 26, by 43.1% with the same agent diluted 1:200, by 14.0% with 1:100 Biosleep and by 36.6% with 1:200 Biosleep. 1:200 Bt 26 proved by 6.6% more effective than 1:200 Biosleep.

Studying the leaf blade area over five weeks, we observed the greatest changes in the leaf blade area in plants whose seeds were treated with the 1:200 Bt 26 strain. The leaf blade area in this group grew from 5.5 to 7.1 cm² over the mentioned period of time. In the reference group, this figure changed from 5 to 6 cm². The plants in the other groups, which were treated with Bt 26 1:100 and Biosleep 1:100 and 1:200, showed similar results in the first week of the experiment, their leaf blade areas varying between 5.2 and 5.3 cm². In the next four weeks, the values in these experimental groups grew to 6.2 and 6.5 cm². Thus, in the groups of plants whose seeds were treated with 1:100 Bt 26 and 1:200 Biosleep, the leaf blade area increased by 8.3% in the fifth week of the experiment compared with the reference plants (Fig. 2).

Treating the plants with the preparations led to an increase in the leaf blade area. In relation to the reference sample, the area increased by 8.3% with 1:100 Bt 26, by 18.3% with 1:200 Bt 26, by 3.3% with 1:100 Bio-

sleep and by 8.3% with 1:200 Biosleep. Bt 26 1:200 showed a higher efficiency than any of the Biosleep solutions by 15% and 10%, respectively.

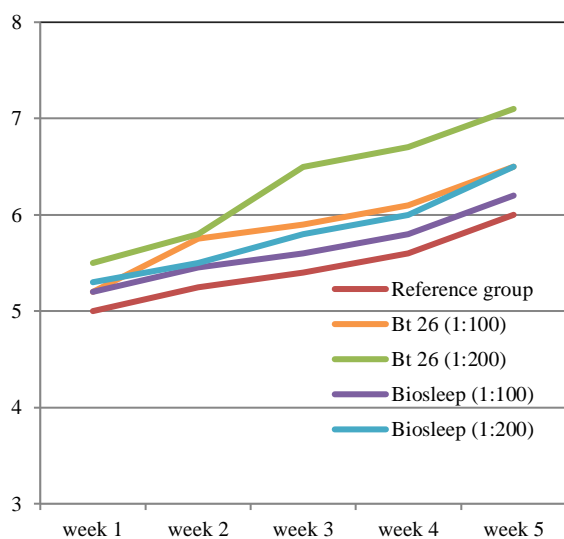


Fig. 2. Effect of Bt 26 strains on the area (cm²) of the leaf blade

During the experiment, the weight of the aboveground part of the plant changed from 18 to 97 grams. In the first week of the experiment, the lowest weight of the aboveground part was in the reference group. In four weeks, this biometric characteristic in the reference group increased to 68 g, which was the lowest value among all groups. The greatest changes in the weight of the aboveground part were observed in two experimental groups: those treated with 1:200 Bt 26 and 1:200 Biosleep. In these groups, the weight changed from 32 to 97 g and from 30 to 93 g, respectively. Therefore, the weight of the aboveground part in these groups increased by 41.0% – Bt 26 and 35% – Biosleep compared to the reference group in the fifth week of the experiment. In the other two experimental groups – those treated with 1:100 Bt 26 and 1:100 Biosleep, the changes were moderate – from 24 to 75 grams and from 27 to 78 grams, respectively (Fig. 3).

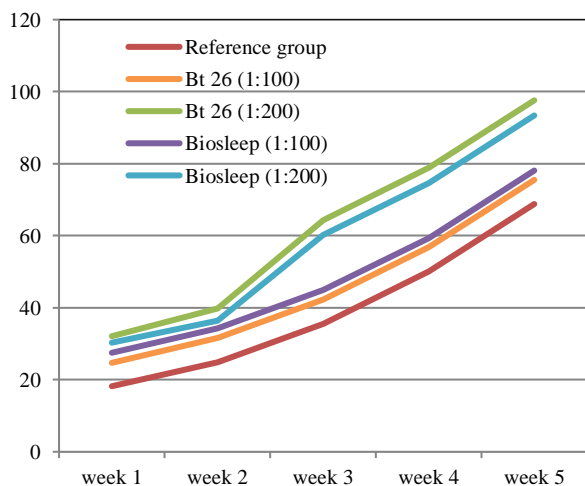


Fig. 3. Effect of Bt 26 strains on the weight (g) of the aboveground part

The decrease in the number of parasites allowed the plants to gain weight in their aboveground parts. Compared with the reference group, the weight of their aboveground parts increased by 9.73% when using 1:100 Bt 26, by 41.9% with 1:200 Bt 26, by 13.5% with 1:100 Biosleep and by 35.8% with 1:200 Biosleep. 1:200 Bt 26 proved by 6.1% more effective than 1:200 Biosleep.

Throughout the experimentation period, the weight of the underground part changed significantly. The fastest growth was recorded in the reference group, where in five weeks the weight of the underground part in-

creased from 9.0 to 34.4 grams. The groups of plants treated with 1:200 Bt 26 and 1:200 Biosleep showed similar figures, with 7 to 8 grams at the beginning of the experiment and 31 g at the end of it. The groups of plants treated with 1:100 Bt 26 and 1:100 Biosleep had almost identical weights from the first week of the experiment – 7 g to the fourth week – 25 grams. In the fifth week, however, the underground parts in these two groups had already varying weights – 27.0 g in the former group and 26.2 g in the latter (Fig. 4).

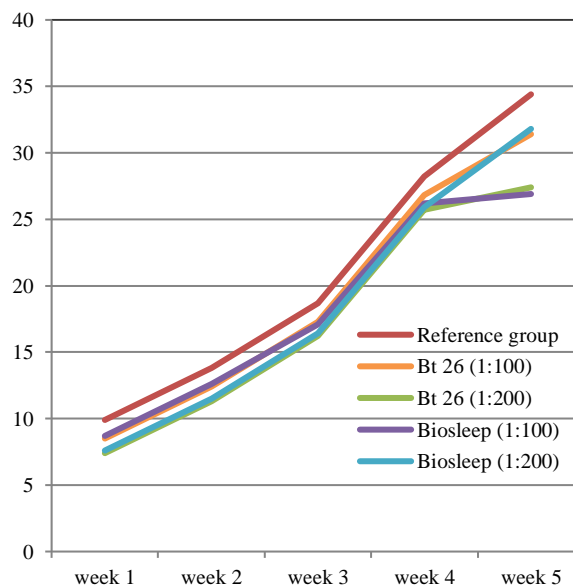


Fig. 4. Effect of Bt 26 strains on the weight (g) of the root

When plants are infected with root-nematodes, their roots become several times heavier due to the formation of knots. The experiments showed that the root weight of the plants treated with agents decreased in comparison with the reference group: Bt 26 1:100 – 19%, Bt 26 1:200 – 20%, Biosleep 1:100 – 8% and Biosleep 1:200 – 24%.

During the experiment, we found the knot formation index, which takes into account the root weight and the number of knots on it. The highest knot formation index was observed throughout the experiment in the reference group. Here, this figure changed from 4.2 to 5.9 in five weeks, which is significantly higher than in the other four experimental groups. Among the biopreparations, the best results were achieved with 1:200 Bt 26: the knot formation index in this group changed from 0.45 to 0.5 throughout the experimentation period. The results were slightly lower for Biosleep 1:200. 1:100 Bt 26 and 1:100 Biosleep showed changes in the knot formation index from 0.97 to 1.1 and from 0.95 to 2.4, respectively (Fig. 5).

Treatment with the agents led to a decrease in the knot formation index. In relation to the reference group, the knot formation index increased by 1.1 with 1:100 Bt 26, by 0.5 with 1:200 Bt 26, by 1.5 with 1:100 Biosleep and by 0.9 with 1:200 Biosleep.

The flowering period lasted from the third to the fifth week of the experiment. The largest number of flowers was produced by the reference group, where in three weeks the number of flowers increased from 6 to 11. The group of plants treated with 1:100 Biosleep showed moderate flowering growth (from 5 to 8 flowers), while in the other three groups (treated with 1:200 Bt 26 and 1:100 and 1:200 Biosleep), the flower development rate was the lowest (from 4 to 7) throughout the experimental period (Fig. 6).

We noticed that plants infected with root-nematodes had a higher number of flowers than healthy plants. The reason for this is that plants try to survive during the stress caused by pathogens. The experiments showed that the number of flowers on the plants treated with agents decreased in comparison with the reference group: Bt 26 1:100 – 11%, Bt 26 1:200 – 47.1%, Biosleep 1:100 – 21% and Biosleep 1:200 – 44.5%.

The experiment helped specify the effectiveness of Bt 26 bacterial strains and the Biosleep biopreparation on plant productivity. The lowest

productivity was recorded in the reference group, where the plants yielded 2 kg of cucumbers. In the other four experimental groups, the yield ranged from 2,690 to 2,980 grams. The highest productivity was shown by plants whose seeds were treated with the 1:200 Bt 26 bacterial strain. This group produced 2,980 grams of cucumbers, which is 49% more than in the reference group. The yield of plants treated with 1:100 Bt 26 and 1:100 Biosleep was 2,713 and 2,690 g, respectively, which is 38.5% and 34.5% higher than in the reference plants (Fig. 7).

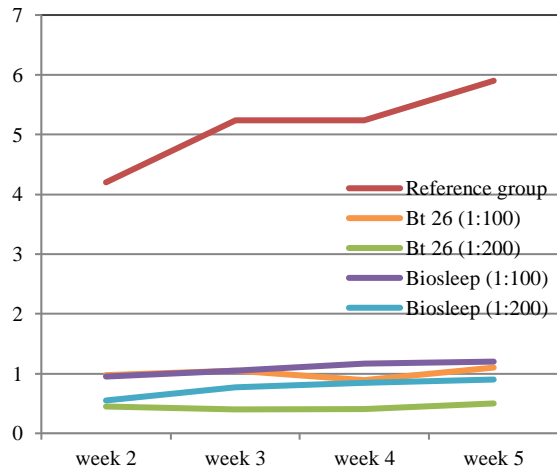


Fig. 5. Effect of Bt 26 strains on the knot formation index

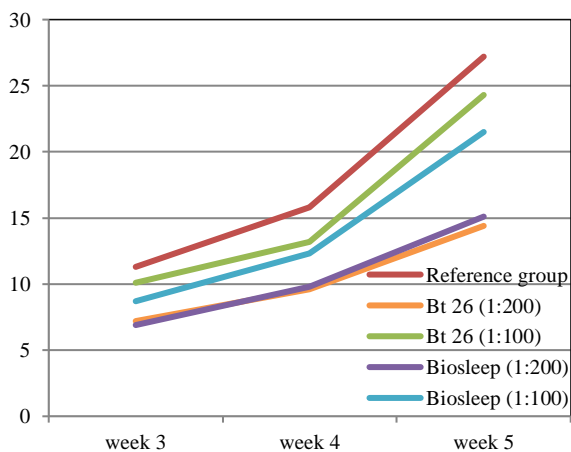


Fig. 6. Effect of Bt 26 strains on the flowering (number of flowers) of the cucumber

In the fifth variant, where the plant seeds were treated with 1:200 Biosleep, the yield was 2,860 grams, which is 43% higher than the reference group. Treatment with agents led to an increase in productivity. Compared to the reference group, the yield in the other groups increased in the following proportions: 1:100 Bt 26 – by 38.5%, 1:200 Bt 26 – 49%, 1:100 Biosleep – 34.5% and 1:200 Biosleep – 43%. Bt 26 1:100 showed a 4% higher yield than Biosleep 1:100, and Bt 26 1:200 made the plants 6% more productive in comparison with Biosleep 1:200.

Discussion

Numerous strains of *Bacillus* can suppress plant pests and pathogens and promote plant growth. Some species are pathogens of nematodes (Li et al., 2005). In addition, a number of studies report direct antagonism from other *Bacillus* spp. with regard to nematode species from the genus *Meloidogyne* parasitising plants (Dash et al., 2015; Cetintas et al., 2018; Sreegyathi et al., 2018).

A large number of bacterial biocontrol agents have been identified, which have nematicidal effect on RKN, and they are becoming a promising source of biopesticides. Among biological control agents, antagonis-

tic bacteria are promising agents against nematodes (Jayakumar, 2019). Migunova & Sasanelli (2021) provided detailed information on the antagonistic properties of various potential bacterial species, including those we used in this study against nematodes parasitising plants.

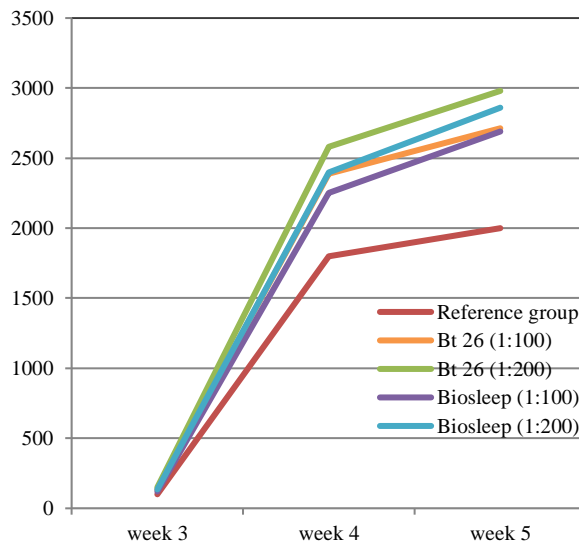


Fig. 7. Effect of Bt 26 strains on the productivity (g) of cucumber plants

Bacillus thuringiensis (Bt) is an aerobic, gram-positive, spore-forming soil bacterium, which has revolutionised pest control today. Bt is recognised worldwide as a bioinsecticide for its safety. Bt has a complex environmental profile. According to recent estimates, Bt occurs in a wide range of habitats, even those without insects, which highlights the importance of various transmission systems (Ruan et al., 2015; Aswathi et al., 2024). The Bt lifecycle consists of four distinct stages (phases). Phase I – growth, phase II – transition to sporulation, phase III – sporulation, phase IV – maturation of spores and cell lysis (Berbert-Molina et al., 2008).

Mohammed et al. (2008) have revealed the nematicidal effect of spores/crystalline proteins in ten Bt isolates, which have been studied *in vitro* against the nematode *Meloidogyne incognita*. The spore/crystalline proteins of the Bt7N, BtDen, Bt18, BtK73, BtSoto and Bt7 isolates showed the highest nematicidal activity, with mortality ranging between 86% and 100%. In addition, their nematicidal activity was evaluated *in vivo* using tomato plants as a host. The results showed that both the crude suspension and the cell-free supernatant of the Bt7N isolate reduced the number of egg masses by 78% and 77%, respectively, and the number of eggs by 84% and 76% compared with reference samples.

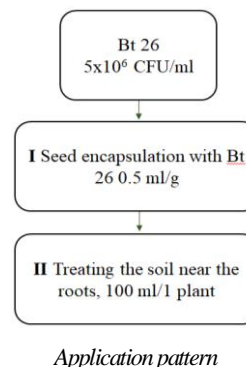


Fig. 8. Pattern for the use of Bt 26 strain

Other researchers noted an increase in the number of microorganisms in the soil when using nematode antagonist bacteria. Qiao et al. (2017) obtained similar results for *Bacillus* spp. when they used it in the rhizosphere of the tomato. However, Aballay et al. (2021) contradicts previous statements by pointing out that higher CFU formulas do not increase the degree of nematode control, maintaining that lower concentrations should be used in integrated control modules.

Our laboratory studies showed that the bacterium *Bacillus thuringiensis* has the potential to control root-nematodes in cucumbers, that is, it inhibits egg hatching and kills the larvae of *M. incognita*. The seed encapsulation by Bt 26 strain further increased the effectiveness of microbial agents. The seeds treated with Bt 26 showed the lowest knot formation index – 0.5. Below we provide a pattern for the use of Bt 26 strain, which, when diluted in the proportions of 1:100 and 1:300, gave contradictory results (Fig. 8). Both low and high concentrations proved inefficient, whereas the proportion of 1:200 had a good effect. Apparently, this was associated with various host groups, environmental factors in a particular region, and plant growing conditions (pattern).

Conclusion

In lower concentrations, the *Bacillus thuringiensis* bacteria had a higher potential for controlling root-nematodes in the cucumber in the laboratory, which means that they inhibited egg hatching and killed *M. incognita* larvae. The seed encapsulation by the Bt 26 strain diluted in the proportion of 1:200 increased the effectiveness of microbial agents. The seeds treated with Bt 26 showed the lowest knot formation index – 0.5.

The authors declare that they have no potential conflict of interest.

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