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Effect of the rhizobacterium *Bacillus subtilis* on the development of the root-knot nematode *Meloidogyne arenaria* at different temperatures

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Abstract

The root-knot nematode Meloidogyne arenaria was cultured in a controlled environment to determine the influence of temperature on the efficiency of the rhizobacterium Bacillus subtilis. Seedlings of the second stage of the Mycobacterium arenaria were introduced to potato plants (Solanum tuberosum L.). Bacterial suspensions of two local strains of B. subtilis (A1 and B1) were applied to the plant rizosphere on the 7th, 14th, and 21st days following worm inoculation. The potato plants were cultivated in a controlled environment with temperatures ranging from 16±1 to 32±1ºC. After treating potato roots with B. subtilis (strains A1 and B1), the rate of M. arenaria development was shown to be reduced compared to untreated plant roots. Under these conditions, the bacteria inhibited the growth of Mycoplasma arenaria to the greatest extent.

Keywords: root-knot nematode, *Bacillus subtilis*, temperature influence, development, biological control

Introduction

Globally, nematodes account for about 20.6% of yield loss (Sasser, 1989: Nematode culture and sterilization). An important plant pest is the root-knot nematode (Meloidogyne sp.).According to Samaliev and Stoyanov (2008), the root-knot nematode M. arenaria was sourced from parasites that feed on field-grown vegetables and cultures that are made from individual egg masses kept on tomato plants in Bulgarian greenhouses. The use of pesticides is the major method for controlling these infections in greenhouses. One example is the (Lycopersicon esculentum Mill., cv. Velositi).26ºC. The egg masses of Myrmecophila arenaria were carefully selected by hand utilizing But sterilized needles and forceps are often needed to extract badly contaminated tomato roots using very poisonous nematicides. lower losses and raise harvests (Sikora and Fernandez, 2005).The egg masses were treated with 0.1% streptomycin sulfate to ensure their sterilization. There has been a recent uptick in the usage of sterile distilled water (SDW) for rinsing nematodes before use in research, and the idea of biological control of nematodes has been around for 45 minutes (Sawhney and Webster, 1975). The habitat of Mycobacterium arenaria J2 (Moens et al., 2004; Gowen et al., 2005).were taken from tomato roots that had been infected. One of the most significant bacterial genera, Bacillus, which may inhibit nematode invasion, can be found in galled roots containing eggs (Kloepper and Ryu, 2006). Hussey and Barker (1973) described macerating Gokte with 1.5% NaOCl in a blender. Bacillus subtilis, Bacillus cereus, and Bacillus pumilus were found to beEvery 24 hours, the hatched J2 were collected from the cotton-wool filter after the suspension had shown larvicidal action against the second stage juveniles (J2) at 26ºC. Meloidogyne incognita was used to sterilize J2 in vitro. Using tomatoes that had been washed in SDW before to the experiment, Gautam et al.(1995) found that the multiplication of M. incognita was reduced after 15 minutes of treatment with 0.1% streptomycin sulfate (Mountain, 1955). As a result of treating seeds with B. subtilis, B. thuriengiensis was

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reported as possible agents for controlling several plant parasitic nematodes, including Meloidogyne sp. (Borgonie et al., 1996; Marroquin et al., 2000; Khyami-Horani and Al-Banna, 2006). Earlier research by Mohamedova and Samaliev (2006) found that some strains of the rhizobacterium B. subtilis killed M. arenaria J2 in the lab, and later by Mohamedova and Samaliev (2010) found that at 25ºC, certain strains of B. subtilis inhibited J2 from invading tomato roots.The temperature is one of the elements that limits the action of the rhizobacteria on root-knot nematodes. There was no substantial change in the activity of B. thuringiensis var. israelensis against several plant pathogens between 19 and 33°C, according to Becker et al. (1992). Nevertheless, according to Andreoglou and Gowen (2000), the impact of Pseudomonas oryzihabitans on Globodera rostochiensis hatch and J2 is at its peak between 21 and 25°C and diminishes below 20°C.Finding out how the rhizobacterium B. subtilis affected the growth of Mycelia arenaria at various temperatures was the main goal of this experiment.

Identification and culture of bacteria
Bacillus subtilis strains B1 and A1 were grown in tryptic soy broth (TSB) at a temperature of 28ºC. In 250 ml Erlenmeyer flasks containing 100 ml of TSB, single colonies of the two strains were cultured at 27ºC for 48 hours with shaking (150 rpm) for inoculum formation. Twenty minutes were spent centrifuging the bacterial suspensions at 2800 g. In order to achieve the concentrations needed for the studies, the concentrated suspensions were diluted with sterile tap water. Using a spectrophotometer, the concentrations of bacteria were measured. Two strains of bacteria were identified using fatty acid analysis, which was performed by Microbial Identification System Inc. in Delware, USA.
Impact of two strains of Bacillus subtilis on Meloidogyne arenaria growth at different temperatures
The sterilized potato tubers (cv. Nadezda) were placed in sealed plastic pots measuring 4.5 cm in height and 10 cm in diameter. Each pot contained 200 g of soil, ideally a 3:1 loam/sand combination, and were watered at a rate of 40%.

at the same level. In 1980, Phillips et al. made some adjustments to this method. There was a glaring disparity between the two extremes (almost 0 and 100%). For two weeks, the containers were kept in a dark place at a constant temperature of 211C. After being infected with 200 J2 of Mycorrhiza arenaria, two-week-old potato plants were put in dark incubators at temperatures of 161C, 201C, 241C, 281C, and 321C. The plants were removed from their pots after 7 days, rinsed to remove dirt, and then replanted in fresh, sterile soil.

Thirteen, seven, and twenty-one days after J2 inoculation, the soil around the roots was treated with a bacterial suspension of strains A1 and B1, each containing 108 cells/ml of B. subtilis. As a control, four plants that were not treated were given 20 cc of sterile distilled water. Four plants were collected at the end of each treatment to find out how far along the nematode growth stage the plants were when the bacteria was given. The plants were returned to their incubators after each treatment. After inoculation with J2 at 161C, 51C, 42C, 33C, and 321C for 83, 51, 42, 33, and 36 days, respectively, the roots were harvested, rinsed to remove dirt, dyed with acid fuchsin (Bridge et al., 1982), chopped in a blender, and the nematode stages counted. Each treatment in the experiment was replicated four times.
Data analysis using statistical software
We used the SPSS software for all of our statistical analysis, with a significance level of P=0.05. Most of the study relied on logistic regression based on binomial data since the majority of the data were counts out of a total, such as the number of nematodes in the roots following inoculation with 200 J2. Some data,

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however, were deemed too excessive to warrant analysis (e.g.

Temperature modulated B. subtilis (strains A1 and B1)'s influence on Mycoplasma arenaria root growth and fertility. The strains' effects were amplified by increasing the temperature and the proximity of the bacterium after J2 inoculation. Strain B1 exerted the greatest suppression of nematode growth into plant roots at 28±1ºC and 7 days after J2 inoculation. Table 4 shows that only 25% of mature females had egg masses when the temperature was this high. At 24±1ºC and 32±1ºC, the strain's impact was not as noticeable. In contrast, Tables 3 and 5 show that the proportion of fertile females in treated plants was only 37.5%, whereas the proportion in untreated roots was 41.7%.
Strain B1 considerably postponed the development of egg masses in comparison to the untreated plants during 14 days of exposure to temperatures of 24±1ºC, 28±1ºC, and 32±1ºC. Tables 3, 4, and 5 show that 46.7%, 50.0%, and 63.2% of the mature females had egg masses, respectively. For all temperatures tested (24±1ºC, 28±1ºC, and 32±1ºC) up to 14 days after J2 inoculation, the number of eggs per egg mass was noticeably reduced in the potato plants treated with strain B1 compared to the untreated plants (Tables 3, 4, and 5).
Borgonie G, Claeys M, Leyns G, Arnaut G, Waele De D, and M. arenaria root growth and reproduction were both ameliorated by B. subtilis (strain A1) compared to strain B1. In: Coomans (1996). Tables 1, 2, 3, 4, and 5 show the effects of the nematicidal Bacillus thuringiensis. A far smaller proportion of females feed on nematodes that are free-living. 1. It took one day of exposure and mutant Caenorhabditis worms for light microscopic examination to reveal biological stage specificity, egg masses into roots treated with strain A1, and the presence of resistant, untreated plants. The percentages for the fundamental and applied temperatures are 24±1ºC and 28±1ºC, respectively, with 68.2% and 72.2%, as shown in Tables 3 and 4 for the field of nematology, 19, 391-398.The authors of the 1992 publication are Becker, Zgonib, Ludwig, Petric, and Rettich. The results show that M. arenaria can't grow and multiply in potato roots when infected with the B. subtilis strain (strain Factors affecting the activity of Bacillus thuringiensis var. B1). therapies involving the israeli. National Mosquito Control Association Journal The strain's impact in the bioassays was association-dependent (8, 285-289).

temperature. B1 bacterial suspension performance at its peak It was 1982 when Bridge, Page, and Jordan became authors. In situations when staining nematodes were present in the roots, an enhanced approach was seen at temperatures ranging from 24C to 28C. Rothamsted Report for 1981, Harpenden, UK, 1, 171. application, Nematology Department, when plant roots contained J2 and J3. According to Gautam A, Siddiqui I, and Mahmood A (1995), the nematicidal effect becomes apparent rapidly at 24±1ºC. The temperature integrates slowly at 20±1ºC and then at 28±1ºC and 32±1C. Meloidogyne incognita treatment has little impact on tomato yield. Investigation of B1 at a temperature of 16±1ºC. The findings corroborate those published in the Mediterranean Journal, 23, 245-272.
Gowen and Andreoglou (2000). After noting the most elevated In 1988, Gokte and Swarup published a paper. Regarding the thermodynamic effects of root-knot and cyst nematode biocides on the bacterial action of P. oryzihabitans against G. rostohiensis.... The temperature range covered by the Indian Journal is 22–26 degrees Celsius. In their 1994 publication, Hackenberg and Sikora noted in Nematology, 18, 152-153. Gowen S., Queneherve P., and Fogian R. (2005) found that the rhizobacterium Agrobacterium radiobacter decreased the development. Bananas and plantains may be infested with nematode parasites of the ment of G. pallida to a degree of up to 70% at temperatures of 20ºC and 25ºC. Egg hatching in vitro at temperatures Wallingford, UK was severely reduced by plant parasite nematodes in subtropical B. subtilis (strain A), which caused 90% mortality of M. arenria J2, and tropical agriculture, edited by Luc, M., R. Sikora, and J. Bridge. between 24 and 30 degrees Celsius, CAB International, 611-643. But it had no discernible impact

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on living organisms. Published in 1994 by Hackenberg and Sikora. The effect of temperature on both The biological control of potato cyst nematode plant roots may be hindered because bacterial colonization is delayed or because plant-health-promoting rhizobacterium cannot produce the secondary metabolites Globodera pallida. These metabolites interfere with M. arenaria at Agrobacterium radiobacter. Prevent its progression, according to the Journal of Phytopathology (142, 338). In a similar vein, Siddiqui and Mahmood (1992) 344. documented the same effect from two B. licheniformis strains. Berker KR and Hussey RS 1973. The efficacy of several techniques in killing M. incognita in vivo differed from their in vitro results. gathering several types of Meloidogyne inocula, including a novel approach. In Plant Disease Reporter, 57, 1025-1028, the writers discussed the findings using several degrees of root analysis. invasion of the pathogens.In 2006, Khyami-Horani and Al-Banna collaborated. Bacillus thuringiensis jordanica's effectiveness in combating Meloidogyne javanica, a tomato pest. The Mediterranean Phytopathogia, 45, 153–157.

Conclusion

Our research shows that at temperatures ranging from 20 to 32 degrees Celsius, the rhizobacterium B. subtilis (strain B1) is nematicidal when exposed to living organisms. Both at and above these temperatures, the strain had no discernible impact on the growth and reproduction of Mycoplasma arenaria in plant roots. Planting tomatoes and other plants in plastic greenhouses often happens in early March, when soil temperatures below 16ºC—lower than the effective ones for B. subtilis (strain B1)—allow the invasive J2 of Mycoplasma arenaria to infect plant roots. But when soil temperatures rise over 17ºC, egg laying in egg masses begins. It is recommended to use strain B1 in conjunction with lower dosages of nonfumigant nematocides in the early stages of plant development for effective control of M. arenaria. An alternate strategy for developing B. subtilis as a biological control agent may lie in selecting strains that are both adaptable and effective at lower temperatures.

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