How Does Peptide D4E1 Affect Soybean Cyst Nematode Survival Rate?

to

The Ohio Academy of Science

by

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February 13, 2024

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Abstract

The purpose of this experiment was to determine the survival rate of Soybean Cyst Nematodes when exposed to peptide D4E1.The null hypothesis is that the peptide will have no effect on the survival rate. The simplified procedure includes extracting SCN from an infested soybean field and counting the number of living nematodes. There were 9 living nematodes in each sample. The extraction process was repeated 8 times so that both the experimental and control groups had 36 living nematodes. After this, the experimental group was exposed to a 200 micromolar solution of peptide D4E1. In contrast, the control group was exposed to distilled water. Both groups were exposed to their solutions for 48 hours. After the 48 hours, both groups were examined underneath a microscope to determine the number of living nematodes post exposure. 5.5% of the peptide exposed group survived, and 97.2% of the water exposed group survived. After constructing confidence intervals, the null hypothesis was rejected. Instead, the data supports the claim that peptide D4E1 decreased the survival rate of Soybean Cyst Nematodes when exposed at 200 micromolar concentration for 48 hours.

Testing Peptide D4E1 on Soybean Cyst Nematodes

Soybeans (*Glycine max*) are one of the most useful and versatile plants across the globe. They are a staple of American agriculture as well as other temperamental weathered countries. Soybeans are one of the most commonly exported crops in the United States. They can be eaten fresh, or dry, and can be used to make soy oil, or soy milk. Soybean meal is also a main ingredient in most livestock feed. Soybeans are flowering plants (angiosperm) and are also dicots (eudicot) included in the Fabacaea family (pea family). This means that they will have 2 seed leaves upon germinating, and will flower in order to reproduce. Soybeans are annual herbaceous plants that grow to be approximately 1 meter in height . When time to reproduce, they will grow small, self fertile, purple flowers from the base of leaves. The flowers produce male gametophytes (pollen) as well as female gametophytes (ovary). Since this is the case, there is not much room for genetic variation within the species. After self fertilization occurs, the flowers develop into pods that mature to contain 2-3 soybeans each and are about 10 centimeters long (Briggs, 2021). Soybeans have an allorhizic root system which contains a taproot, lateral roots, and small tertiary roots that stem off from the lateral roots (Torrion et al., 2012).

One of the biggest obstacles soybean farmers face is crop loss due to Soybean cyst nematodes (SCN) (*Heterodera glycines*). It is one of the most significant pathogens of soybeans, and can be found just about anywhere soybeans are grown. Soybean cyst nematodes are microscopic roundworms that infect the roots of soybeans, as well as other host plants (Malvick, 2018). There are 3 developmental stages of SCN, the egg, juvenile, and adult. In total, a lifecycle with these stages is from 24-30 days. Within the juvenile stage, there are 2 phases, J1, and J2. J2 is the phase of which the nematodes enter the roots and infect the soybeans (Iowa State University, 2023). J2 phase nematodes often leave cysts or galls in the roots of the plant (Iowa

State University, 2023). SCN uses a hollow stylet to pierce through the root tip, and enter the vascular tissue of the plant. Once the root is penetrated, the SCN causes the formation of specialized feeding cells for the nematodes. Up until the J2 phase, the nematodes have no gender. Once they begin to mature inside the plant, they will develop into a male or female. If it is a male, it will feed on the plant for a couple days longer before moving back into the soil leaving the plant. However, if it is a female, it stays in the roots and continues to mature. Females will molt 3 additional times before becoming adults (Nelson & Bradley, 2003). Eventually, they swell up into a yellow or white ball attached to the roots approximately 1 millimeter in diameter (Nelson & Bradley, 2003). Soon after this, they burst open. The specialized feeding cells made for the nematodes now will only supply nutrients to them instead of the plant. This is what causes stunted growth and yield loss. To reproduce, the females will lay a small amount of their eggs in the roots. However, the majority of eggs are released when the female dies and becomes a brown cyst. The eggs left inside of her are then protected by the cyst and left to hatch. Up to 6 generations of SCN can be produced in one growing season depending on maturity of the plant, weather conditions, temperature, pH levels, and other factors (Iowa State University, 2023).

In order to see if there are nematodes in the soil, or to collect them for specific experiments, many scientists use a method called extraction. Extraction is used to separate nematodes from the soil, and move them to a small water sample to put under a microscope to look at them. Many steps must be taken to make sure that this process is effective. First, soil must be obtained from infested plant roots. After this, the soil is placed on a filter material of some sort over a container filled with water. After placing the soil on the filter, the container is filled with more water so that water covers both the filter material, and most of the soil. Then, the apparatus is placed somewhere it will not be disturbed for 24-72 hours. A nematode's natural

instinct is to move downwards. When they do this in the soil being tested, they go through the filter material into the water beneath it. Once the 24-72 hours are complete, scientists then take the nematode water sample and put it into a centrifuge. Once the nematodes are centrifuged and collected on the bottom of the container, it is possible to use the water from the floor of the container to test for nematodes. It is recommended to take between .5 and 2 milliliters of the infested water and look at it under a microscope. The microscope should be set between 10 and 100X magnification with good resolution to clearly see the nematodes (Kaya & Stock, 2023).

SCN is also responsible for over 30% of yield loss nationwide every year (Malvick, 2018). There are several reasons crop loss can be so devastating. Primarily, the first 1-5 years of infestation tend to go unnoticed as above ground symptoms are not yet visible (Malvick, 2018). This means that by the time there are physical signs of illness in the plants, there has already been a significant amount of irreversible damage done by the nematodes. In addition to this, the visible symptoms of stunted growth and chlorosis can easily be misidentified as a nutrient deficiency or other diagnosis. In this case, the plants are not given the right treatment for their ailment. Thus, leading to further infestation and a waste of treatment resources.

Although soybeans are their main host, SCN has a number of other crops and weeds that it also thrives on. A sample of these host plants include: Alsike clover, Bird's foot trefoil, most types of beans, Crimson clover, Crown vetch, Lespedeza, Common and Mouse Ear chickweed, Common mullein, Henbit, Pokeweed, and Purple deadnettles (The Ohio State University Extension, 2019). Most fields, specifically those with "no till" practices, are extremely susceptible to winter weeds like Henbit and Purple deadnettles. If not treated properly, the nematodes will survive through the winter feeding on the roots of these weeds. Both Henbit and Deadnettles start to grow at the end of the soybean season from September to early November

(The Ohio State University Extension, 2019). This being the case, they must be treated for as soon after harvest as possible to prevent the nematode population from flourishing over the winter.

In 1999, it was discovered that the peptide D4E1 could inhibit fungal growth in plants and in vitro. Genetically modified tobacco plants that produced the synthetic antimicrobial peptide D4E1, ciphered by a gene subject to an enhanced cauliflower mosaic virus (Cmv) RNA promoter, were acquired by *Agrobacterium-mediated* transformation (Jaynes et al., 1999, 171). This means that the tobacco plant was genetically modified to produce a high ant of peptide D4E1 due to the gene promoter from Cmv. After the transformation, the D4E1 gene was shown by RT-PCR of the tobacco mRNA (Jaynes et al., 1999, 171). Crude protein was extracted from leaf tissue of the transformed tobacco plants. When the crude protein was applied to *Aspergillus flavus* and *Verticillium dahliae*, it reduced the amount of fungal colonies emerging from germinating conidia (an asexually produced, non-motile, spore of fungus) on each by 75% and 99% respectively (Jaynes et al., 1999, 171). Therefore, the transgenic plant crude protein proved to have higher levels of disease resistance in plants to the fungal pathogen than crude protein that was not encoded under the Cmv promoter.

Currently, there are several ways to treat SCN. One of the most popular among farmers is crop rotation. Some do this by rotating soybeans with other crops such as corn. A more effective approach however, is to rotate untreated soybeans in with those whose seeds have been treated with chemicals to be resistant to the pest, as well as non host crops (University of Minnesota Extension, 2021). There are also nematicide options available to treat SCN. Some of these include 1,3-dichloropropene, Aldicarb, and Fluopyram (Gorny & Lux, 2023). These nematicides are used by application to the plants, or the soil.

Purpose

Testing for biopesticide properties of peptide 4E1 on soybean cyst nematodes.

Null Hypothesis

Peptide D4E1 will have no effect on the survival rate of Soybean Cyst Nematodes when exposed at a 200 micromolar solution for 48 hours.

Procedure

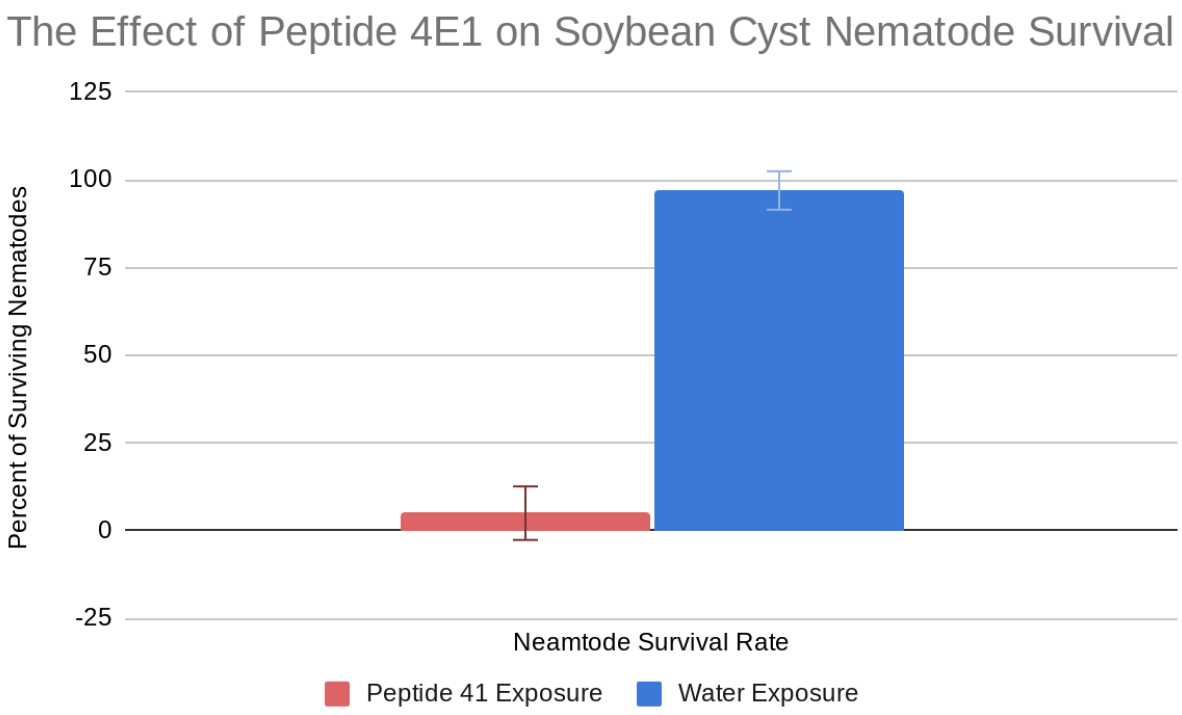
1. Retrieve Red Dead Nettles (*Lamium Purpureum*), and dead soybeans (*Glycine max*) with roots intact, from Bloom Carroll High School Land Lab.
2. Put filter paper over 6 funneled sieves.
3. Attach a tube to the end of the funnel, and clip it off with a plastic clip.
4. Fill up the funnel with water until it reaches the bottom of the sieve.
5. Remove soil from Dead Nettle and soybean roots.
6. Place filter paper over the sieve.
7. Place a small handful of soil on the filter paper.
8. Wrap edges of filter paper around soil samples.
9. Let sit for 48 hours for nematodes to move down into the water below the filter paper/sieve.
10. Remove plastic clip from tube at the bottom of the funnel and drain water into a beaker.
11. Pour water into 2.5 milliliter capped test tubes
12. Place the capped test tubes into the centrifuge and centrifuge them for 10 seconds.
13. Use a graduated pipette to remove 60 microliters of each sample from the bottom of the test tube.
14. Place 60 microliter samples on concave microscope slides.
15. Place slides under the microscope at 1000X
16. Count the number of living and dead soybean cyst nematodes
17. Use a micropipette to put samples into a test tube.
18. Place enough D4E1 peptide into the test tube so the concentration is 200 micromolar.
19. Leave the tubes to sit for 24 hours.

20. Centrifuge the tubes again for 10 seconds.
21. Place samples back onto concave slides.
22. Place slides under a microscope at 1000X.
23. Count living and dead nematodes
24. Calculate living and dead percentage of experimental group and control group respectively.
25. Compare results.

Materials

1. 1 Shovel
2. 4 Buckets
3. Soybean roots (with soil intact)
4. Purple Deadnettle roots (with soil intact)
5. Nematode extraction kit
6. Distilled water
7. Concave microscope slides
8. Microscope
9. Centrifuge
10. Capped test tubes
11. Micropipette
12. 1ml pipette
13. Peptide D4E1

Results



Group Name	Peptide or Water	Nematodes Before Exposure	Nematodes After Exposure
Control	Water	36	35
Experimental	Peptide D4E1	36	2

Conclusion

In the experiment, soybean-cyst nematodes were exposed to water, or peptide D4E1 for 48 hours. After this, the survival rate was calculated. After 48 hours, 35 of the nematodes were alive in the water solution, and only two were alive in the peptide solution. This also can be calculated to a 97.2% survival rate (control), and a 5.6% survival rate (experimental). These percentages were displayed in a bar graph with the confidence intervals. The null hypothesis was that the peptide would have no effect on the survival rate of soybean cyst nematodes. Due to the collected data and displayed confidence intervals, this hypothesis was rejected at the 1% significance level. After evaluating the confidence intervals, it can instead be deduced that peptide D4E1 at 200 micromolar concentration decreases the survival rate of soybean cyst nematodes over a period of 48 hours. A two proportion Z-test was also performed. The results are as follows:

p_1 = proportion of all nematodes that survive when exposed to peptide

p_2= proportion of all nematodes that survive when exposed to water

H_0: $p_1=p_2$

H_a: $p_1<p_2$

$Z = -7.78$

$P < 0.0001$

These results are highly applicable to agricultural and environmental fields. Soybean cyst nematodes are an extremely detrimental pest that accounts for over 1.5 billion dollars lost annually. This leaves many farmers looking for solutions that will not cause more problems for their soybean crops. Several solutions have been developed such as genetically modified soybean varieties and pesticides. However, there are no biopesticides on the market that are effective in killing soybean cyst nematodes. If this experiment were to be further researched and tested on a larger scale, it could be considered as another avenue of treatment for the American soybean industry.

If this experiment were to be repeated, it would be so with a few alterations. To start, the experiment should be done in the warmer months. Because of the soil temperature being below 50 degrees Fahrenheit, most of the nematodes were dormant and extremely hard to locate. This led to the test samples being extremely small. If this is not an option, nematodes should be obtained a different way so that it is less difficult to find large amounts of nematodes. The larger the test group, the smaller the standard error, therefore making the confidence interval more narrow. In addition to this, one could consider testing the amount of living nematodes at different times of exposure. Ex: 12 hours, 24 hours, 36 hours, 48 hours. This way, it would be able to be determined around what time the peptide is effective.

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Acknowledgments

Done in collaboration with Dr. Jesse Jaynes and Dr. Gregory C. Bernard , Tuskegee University, College of Ag. Envr.& Nutr. Sciences / AgriTREK

I would like to deeply thank my professors at Tuskegee University, Dr. Gregory Bernard and Dr. Jessie Jaynes for their help in making this project possible.