

Optimizing the Storage of Entomopathogenic Nematodes: Interaction of Media Types and Temperature Regimes on Viability and Virulence

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ABSTRAK

Nematoda entomopatogenik (EPN) merupakan agen biologis vital dalam pengelolaan hama terpadu, namun menjaga kualitasnya selama penyimpanan masih menjadi tantangan teknis. Tujuan: Studi ini bertujuan untuk mengevaluasi kemanjuran berbagai media penyimpanan dan rezim suhu dalam menjaga viabilitas dan virulensi EPN. Metode: Desain faktorial acak lengkap digunakan untuk menguji tiga media penyimpanan (natrium alginat aktif, spons poliuretan pasif, dan kontrol air) di bawah dua kondisi suhu (15°C dan 27°C). Hasil: Studi ini mengungkapkan bahwa media air pada 15°C (F0T1) mempertahankan viabilitas tertinggi, sedangkan natrium alginat pada 15°C (FIT1) menunjukkan retensi virulensi yang lebih unggul pada minggu keempat. Kesimpulan: Penyimpanan suhu rendah sangat penting untuk kelangsungan hidup EPN, sementara formulasi media aktif seperti natrium alginat menawarkan keuntungan signifikan dalam menjaga patogenisitas nematoda untuk durasi yang lebih lama dibandingkan dengan media pasif.

Kata kunci: *Nematoda entomopatogenik, penyimpanan EPN, virulensi, viabilitas*

ABSTRACT

Entomopathogenic nematodes (EPN) are a vital biological agent in integrated pest management, yet maintaining their quality during storage remains a technical challenge. Objective: This study aimed to evaluate the efficacy of various storage media and temperature regimes in preserving the viability and virulence of EPN. Methods: A completely randomized factorial design was employed to test three storage media (active sodium alginate, passive polyurethane sponge, and water control) under two temperature conditions (15°C and 27°C). Results: The study revealed that water media at 15°C (F0T1) maintained the highest viability, whereas sodium alginate at 15°C (FIT1) demonstrated superior retention of virulence by the fourth week. Conclusion: Low-temperature storage is critical for EPN survival, while active media formulations like sodium alginate offer significant advantages in preserving nematode pathogenicity for longer durations compared to passive media.

Keywords: *Entomopathogenic nematodes, EPN storage, Virulence, Viability*

INTRODUCTION

Entomopathogenic nematodes (EPN) are widely recognized as effective organisms in integrated pest management for sustainable agriculture. They offer distinct advantages, including the ability to eliminate insect pests rapidly with low resistance rates, while ensuring safety for humans and the environment (Sunarto & Natasasmita, 2014). In their infective juvenile (IJ) phase, EPNs engage in a mutualistic relationship with symbiotic bacteria to decompose the host insect's body, facilitating their lifecycle (Shahina et al., 2004). Consequently, EPNs hold significant potential as a sustainable alternative to synthetic pesticides, which are often associated with adverse environmental and health impacts (Arif, 2015).

Generally, two EPN genera are utilized: *Steinernema* sp. and *Heterorhabditis* sp. These genera exhibit distinct characteristics regarding control targets, symptoms, and specific symbiotic bacteria (Satria et al., 2018). Mass production can be achieved through *in vivo* methods for small-scale needs or *in vitro* methods for large-scale industrial production (Shapiro et al., 2012). However, a critical challenge in distribution is maintaining EPN viability and virulence during storage (Poinar & Grewal, 2012).

EPN survival is heavily influenced by abiotic factors such as temperature, humidity, and soil structure (Devi, 2024). Temperature control is particularly vital, as optimal conditions slow metabolic processes and extend shelf life (San-Blast, 2013). To address this, various storage media have been developed, initially focusing on cost-effective materials (Grewal, 1998). These media are categorized into passive (inert) and active media (Kagimu et al., 2017). Passive media, such as

sponges or clay, provide a temporary environment without restricting movement, whereas active media, such as sodium alginate, inhibit metabolism to induce anhydrobiosis (Grewal & Peters, 2015). This study aims to evaluate the interaction between these storage media and temperature regimes to optimize EPN viability and virulence.

RESEARCH METHODOLOGY

Location and Time

The research on “The Effect of Storage Media on the Viability and Virulence of Insect Pathogenic Nematodes” was conducted from October 2022 to June 2023. This activity was located at the Agrotechnology Laboratory, Faculty of Agriculture, University of Jember.

Tools and Materials

The tools used were Petri dishes, jars, filter paper, micropipettes, hand counters, microscopes, plastic wrap, tweezers, beakers, syringes, sieves, plastic seals, refrigerators, plastic containers, counting dishes, orbital shakers, magnetic stirrers, magnetic bars, documentation tools, and writing instruments. The materials used were EPN suspension solutions isolated from Wiwongso, Jember Regency, aquadest, sodium alginate ($C_6H_8O_6$), $CaCl_2$, tween, sodium citrate, and polyurethane sponges.

Research Design

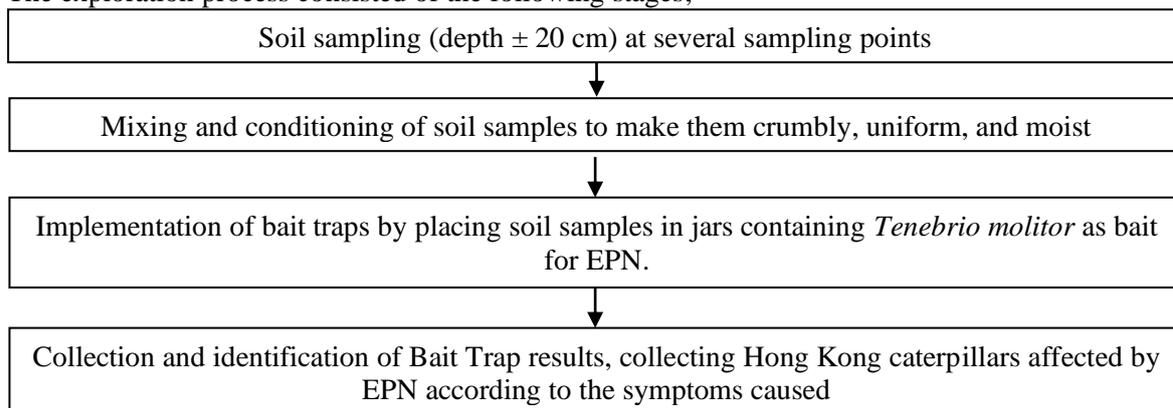
This study used a completely randomized factorial design with two factors: the type of EPN storage media and the storage environment. The first factor, storage media, consisted of three treatments, namely sodium alginate media (F1), PU sponge media (F2), and water media as a control (F0). The second factor, storage environment, consisted of two treatments, namely a refrigerator storage environment with a temperature of $\pm 15^\circ C$ (T1) and a storage environment with a room temperature of $\pm 27^\circ C$ (T2). Each treatment was repeated five times. Observations in this study were conducted every week from week 1, week 2, week 3 to week 4 of storage, by observing the survival rate of EPN by reviewing the nematode population density in various treatments, followed by testing the virulence of the nematodes. The combination of the two factors resulted in 6 treatment combinations, namely F0T1, F0T2, F1T1, F1T2, F2T1, and F2T2, where each treatment combination had 5 replicates, resulting in 30 experimental units.

Research Procedure

This research was conducted in several stages, namely;

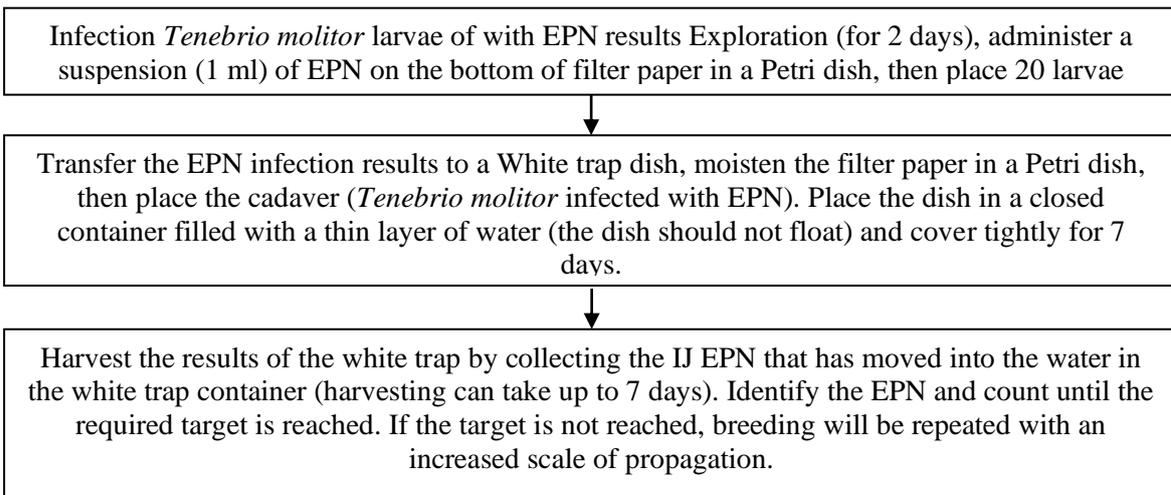
1. Exploration of Entomopathogenic Nematodes

This stage was carried out to obtain EPN found in designated agricultural areas. The exploration was conducted in various sugarcane plantation locations in Mumbulsari District, Jember Regency. The exploration process consisted of the following stages;



2. Propagation of Entomopathogenic Nematodes

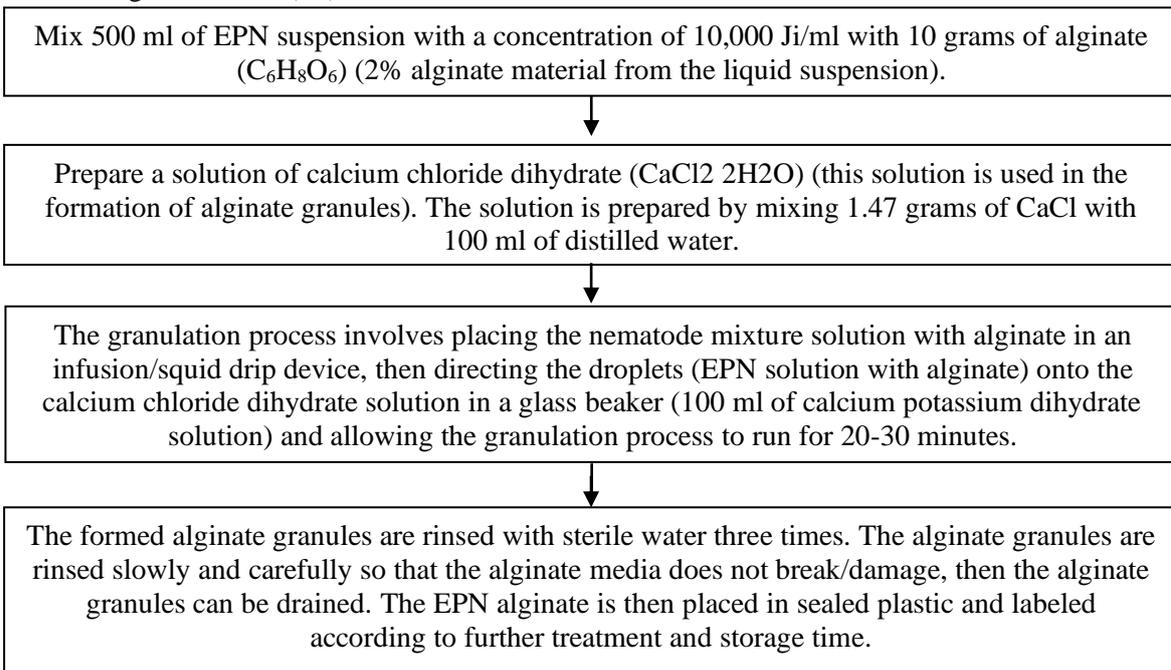
EPN propagation in this study was carried out in vivo, namely by utilizing animal bodies as hosts for propagation using *Tenebrio molitor* larvae. The initial source of EPN, which was identified through exploration, was obtained using the White trap method for ± 7 days to obtain new EPN cultures to be bred again until the target population required for research (10,000 IJ/ml with a specified volume) was reached. The stages of this propagation were as follows;



3. EPN storage using various types of storage media

The EPN storage media used in this study consisted of three types of storage media, namely sodium alginate media, PU sponge media, and water media (control). EPN propagation results that have reached the target density of 10,000 IJ/ml will be stored in several storage media, with the following stages for each media;

a. Alginate media (F1)



b. PU sponge media (F2)

Cut the sponge into 2 cm x 2 cm x 2 cm cubes, wash them with liquid soap, rinse them thoroughly, and air dry them.

Prepare the stored IJ suspension and ensure that it does not settle by using a magnetic stirrer. Take 2 ml of solution (the result of a separate bag test with reference to the research by Touray et al. (2020) for each cube with a spuid, then slowly add it to each PU sponge cube.

Store PU sponges filled with EPN in sealed plastic bags and label them according to further treatment and storage time.

c. Water media (F0)

Prepare a water suspension container (a container with a flat bottom surface) and wash it thoroughly.

Prepare the stored IJ suspension and prevent it from settling by using a magnetic stirrer, then pour 100 ml of the IJ suspension into a flat container (500 ml) to create a layer of water. The stored IJ suspension should not exceed 1 cm.

Store flat containers filled with EPN and labeled according to further treatment and storage time.

4. Media Testing and Experimental Variables

a. Viability Test

Viability testing is an effort to ensure that the nematodes are alive, which can be indicated by the movement of the nematodes or the growth portur shown by the nematodes. The nematodes will first be dissolved from the storage media that has been stored using the EPN media dissolution method in accordance with the storage media used.

- For sodium alginate media, use sodium citrate as a solvent by taking 1 gram of sodium alginate media and dissolving it in 9 ml of 0.5M sodium citrate solution to which 0.1% Tween has been added. The resulting solution is 10 ml, from which 1 ml can be taken to calculate the nematode population density,
- In the sponge storage media, EPN can be dissolved in 8 ml of plain water by squeezing 3-5 times to produce approximately 10 ml of EPN liquid. From this liquid, 1 ml is taken to calculate the concentration of the EPN population in it..
- For water storage media (control), a standard calculation was performed by taking 1 ml of liquid and then performing the calculation (dilution was performed so that it could be calculated properly). Nematodes were counted by density (juveniles/ml) by taking 1 ml of nematode solution in counting dishes. According to Haryani (2014), the total population was calculated using the following formula:

$$\text{Population (P)} = \frac{\text{Total volume of EPN media solution (ml)}}{\text{Sample density volume (ml)}} \times \text{EPN density (juveniles/ml)}$$

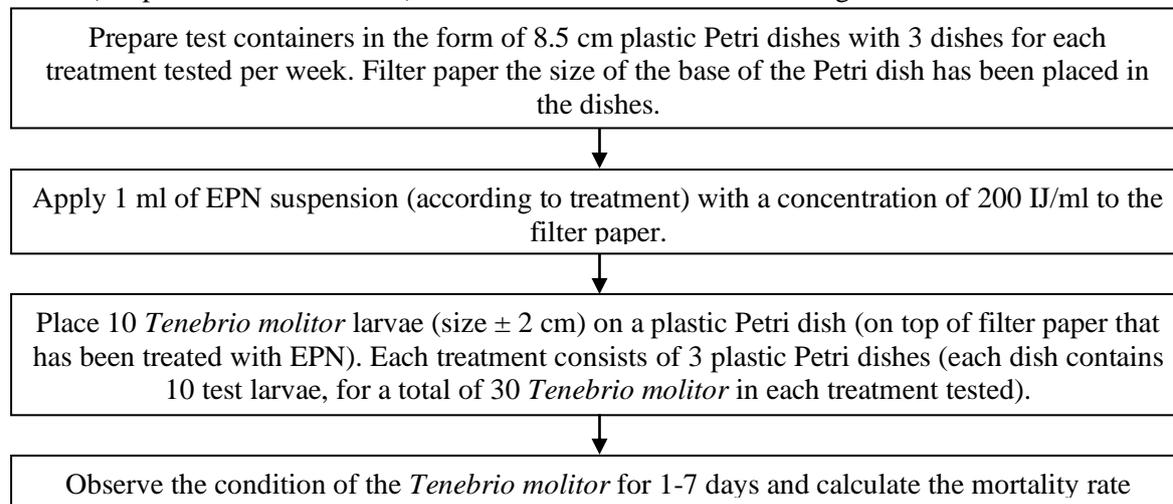
Description: EPN density is determined by taking density samples and counting them in a rodec/counting dish.

The nematodes counted are those that are actively moving or forming J or S shapes, and the population count results obtained are used in calculating the viability of the treatment (Satria et al., 2018), in the form of:

$$\text{Viability (\%)} = \frac{\text{Population density of surviving EPN on the treatment medium}}{\text{Total population added at the beginning of media preparation}} \times 100\%$$

b. Virulence Test

This virulence test examines the mortality caused by EPN from various treatments (F0T1, F0T2, F1T1, F1T2, F2T1, and F2T2) on test insects in the form of *Tenebrio molitor* larvae. This virulence test was conducted weekly using EPN liquid obtained from dissolving EPN storage media (temperature combinations) in the first to fourth weeks. The stages are as follows;



To calculate the percentage of virulence for each treatment, the following formula can be used (Indra, 2013):

$$\text{Virulence (\%)} = \frac{\text{Number of dead larvae}}{\text{Total larvae used}} \times 100$$

Data Analysis

The data obtained from testing the variables was analyzed using Analysis of Variance (ANOVA). If the variance analysis results showed a significant F-value, it was followed by Duncan's multiple range test (DMRT) at 5%.

RESULT AND DISCUSSION

Viability of Entomopathogenic Nematodes

Table 4.1 Summary of F-count Values from Analysis of Variance on Viability Variables

No	Observation Time	Storage	F-value Storage	Interaction
		temperature (T)	Media (F)	T x F
1	Viability week 1	108,068 **	18,114 **	4,624 *
2	Viability week 2	450,854 **	13,820 **	16,437 **
3	Viability week 3	669,348 **	13,858 **	24,341 **
4	Viability week 4	365,595 **	5,472 *	13,979 **

Note: * significantly different (1% level), ** highly significantly different (5% level), ^{ns} not significantly different

Table 4.2 DMRT post hoc test table storage media with storage temperature against EPN viability week 2 (a), week 3 (b), dan week 4 (c)

Treatment	Average viability value (JI/ml)	Notation	Treatment	Average viability value (JI/ml)	Notation
F1 T1	89,99 %	a	F0 T1	86,70 %	a
F0 T1	88,00 %	a	F1 T1	84,39 %	a
F2 T1	60,30 %	b	F2 T1	60,00 %	b
F1 T2	25,59 %	c	F1 T2	23,14 %	c
F2 T2	16,71 %	c	F2 T2	16,71 %	c
F0 T2	1,49 %	d	F0 T2	1,10 %	d

a. b.

Treatment	Average viability value (JI/ml)	Notation
F1 T1	82,90 %	a
F0 T1	77,17 %	a
F2 T1	57,60 %	b
F1 T2	21,35 %	c
F2 T2	15,65 %	c
F0 T2	0,99 %	d

c.

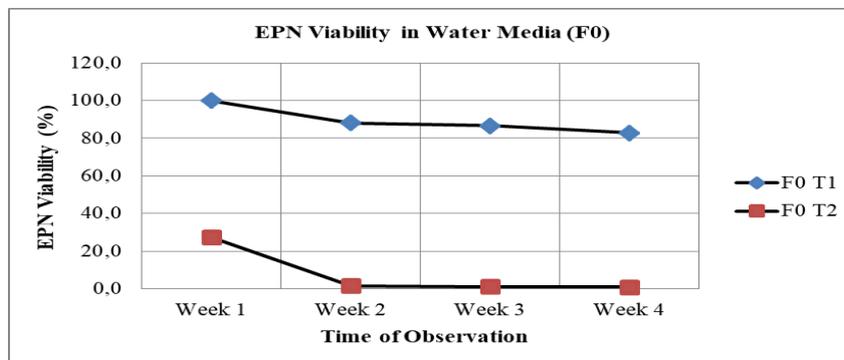


Figure 1. Graph of viability variable data in the F0/control treatment (F0T1 and F0T2) during four weeks of observation.

1. Viability EPN in Water Media Treatment /F0

The study demonstrated that the water control treatment at 15°C (F0T1) yielded the highest viability. This aligns with findings by Nagachandrabose (2022), who reported that water media at lower temperatures (5°C) maintained higher viability compared to 25°C. Sodium alginate media (F1) showed stability across temperatures. Notably, at room temperature (T2), alginate performed significantly better than passive media, supported by Maru et al. (2016), who found alginate superior to sponge and liquid media during one-month storage.

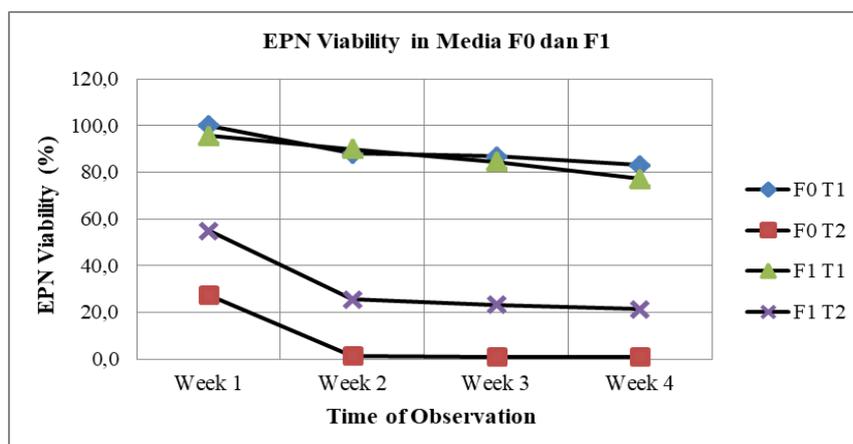


Figure 2. Graph of viability variable data for treatment F0 and F1 during four weeks of observation

2. Viability EPN in Alginate media treatment/F1

The high viability of alginate at low temperatures (F1T1) confirms that temperature is a dominant factor, as observed by Umamaheswari et al. (2006) . Conversely, nematode emergence from alginate capsules at room temperature (F1T2) was observed, a phenomenon attributed to capsule softening in warmer conditions, as described by Chen and Glazer (2004).



Figure 3. Condition of alginate media in room temperature storage (F1T2) for 2 weeks of storage

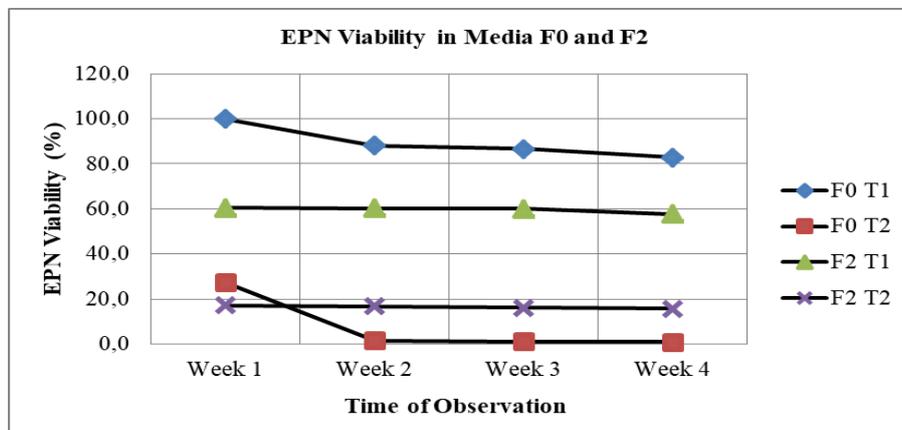


Figure 4. Graph of viability variable data in treatments F0 and F2 during four weeks of observation

3. Viability EPN in PU Sponge Media Treatment /F3

Sponge media (F2) at room temperature (T2) proved superior to the water control (F0T2). This is consistent with Touray et al. (2020) and Divya et al. (2011), who found that sponge media provided better viability than water at 27°C due to its ability to mimic a natural soil habitat. Andal et al. (2010) also noted that sponges facilitate oxygen access and reduce stress, a finding corroborated by Javed et al. (2022) . Furthermore, the stability of sponge media aligns with Guo et al. (2017), who observed no significant decline until 30 days at 25°C. However, sponge media performed better at lower temperatures (F2T1) than at room temperature, supporting Maru et al. (2016) .

The superiority of sponge media at room temperature/T2 ($\pm 27^{\circ}\text{C}$) compared to control media indicates that storage media has an effect, even though it is passive, providing a living environment without limiting EPN. However, in longer storage periods at room temperature, it has a significant impact on maintaining EPN survival compared to water media (control/F0). The significant effect of sponge at storage temperature T2 compared to water media (control/F0) is indicated by the nature of the sponge, which provides an artificial habitat for EPN that more closely resembles its natural habitat, where inert (passive) storage media, one of which is sponge, still allows EPN to obtain oxygen and space for nematode movement while reducing stress on EPN (Andaló et al., 2010). The superiority of sponge storage media over water media (control) was also observed in the study by Javed et al. (2022), which compared the viability of EPN stored in sponge media, soil media, and water media.

The viability data for treatments F2T1 and F2T2 shown in (Figure 4) indicate that from the first week of observation to the last week, the sponge media provided values that were not significantly different or more stable. This condition is similar to that found in the study by Guo et al. (2017), which also tested the viability of sponge media stored at 15°C and 25°C. Both treatments showed that there was no significant decline after 20 days of storage. A significant decline occurred after 30 days of storage in the sponge media treatment stored at 25°C, but not in the sponge media treatment stored at 15°C. Sponge media (F2) in different storage environments showed significantly different viability results in the first to fourth weeks of storage. The F2T1 treatment gave better results than the F2T2 treatment, indicating that sponge media stored in a refrigerator was better at maintaining EPN viability than room temperature (T2). The superior results of F2T1 compared to F2T2 were also found in the study by Maru et al. (2016), which showed that during 1 month of storage, sponge media at 10°C provided higher viability than sponge media at room temperature. The storage environment affects the viability of EPN stored in sponge media (F2).

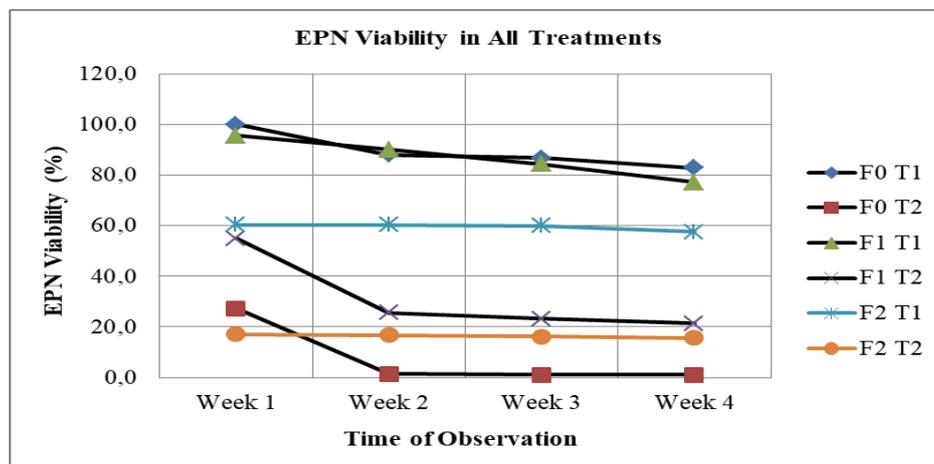


Figure 5. Graph of viability variable data for all treatments during the four weeks of observation

The EPN viability results produced by each treatment showed that water media (control) at a lower temperature (F0T1) gave the best results compared to other treatments. This is in line with research conducted by Strauch et al. (2000), which showed that liquid media (aerated) stored at 15°C provided higher viability results compared to sponge media, bentonite media, and attapulgite media. Although this study used liquid media without aeration assistance, it produced better viability results (cold condition T1) as in the study by Strauch et al. (2000). This indicates that the liquid media storage method applied in this study, considering the thickness of the stored EPN liquid with an IJ suspension height of less than 1 cm, can be considered a good EPN storage technique with liquid media without the aid of an aeration device. The F0 (water) treatment media in the graph table shows that at storage temperature T1 ($\pm 15^\circ\text{C}$), it produced the highest viability compared to other treatments observed in weeks 1, 2, and 4, although it was not significantly different from the F1T1 treatment. The superiority of the water media, especially in week 1, can be attributed to the different temperature conditions of the media, where water is believed to be able to conduct heat quickly and evenly compared to other media. This assumption is also reviewed from the thermal conductivity values produced by the media. Thermal conductivity is the ability of a material to conduct heat using the units $\text{J/s.m.}^\circ\text{C}$ or $\text{W/m.}^\circ\text{K}$. The higher the value, the higher the ability to conduct heat. Water has a thermal conductivity value of 0.6 W/mK , alginate has a value of $0.06 \text{ W/m}\cdot\text{K}$ (De la Cruz et al., 2022), while PU sponge has a value of $0.0446 \text{ W/m}\cdot\text{K}$ (Soloveva et al., 2022). The thermal conductivity value shows that water is able to conduct heat better than alginate and PU sponge. The faster heat transfer from the F0T1 treatment is strongly suspected to make this treatment better at conditioning EPN more quickly in better storage conditions (at a temperature of $\pm 15^\circ\text{C}$).

The trend from the EPN viability data in various treatments shows a decrease in EPN viability from the first observation to the last observation. The decrease in viability over time is closely related to the death of the nematodes themselves. This death is caused by various factors, both external factors from the EPN storage media environment and internal factors from the EPN

itself. Nematodes in the infectious juvenile stage will leave their host's body to find a new host in order to maintain their life cycle. When EPN is stored (infective juvenile) in JI storage media, there will be no food (organic material) obtained from the environment, and the survival of EPN stored in the media depends largely on the food reserves available in the body. As the storage time of EPN increases, the available food reserves will decrease (Askary & Ahmad, 2020). The energy reserves available in the EPN body are in the form of fat, protein, and carbohydrates. Fat itself is one of the main energy reserve components for EPN (Lewis, 1995). In line with the observations made by Badawy (2010) regarding the significant decrease in fat content in EPN, especially in the *S. riobrave* species stored for 40 days, from 46% to 18%, more than 60% of the fat was utilized within 40 days.

The viability trend also shows that treatments with storage temperature T1, such as F0T1, F1T1, and F2T1, produce better results than treatments with storage temperature T2, such as F0T2, F1T2, and F2T2. The lower temperatures in F0T1, F1T1, and F2T1 provide an advantage in maintaining better food reserves. Lower temperatures are also found to reduce the potential growth of contaminants that are harmful to nematodes (Strauch et al., 2000). Warmer temperatures, as seen in treatments F0T2, F1T2, and F2T2, are known to have a negative impact on EPN viability because the warm temperatures cause the energy reserves available in the EPN's body to decrease more quickly (Georgis and Kaya, 1998).

Virulence of Entomopathogenic Nematodes

Virulence testing can be performed with sufficient quantities of EPN. For the technical aspects of this virulence test, the remaining EPN from the viability test is used. Virulence testing on the F0T2 treatment could not be performed in the first week, and on the F2T2 treatment, it could not be performed from the second week due to the lack of the required quantity of EPN, which is 200 JI/ml (3 ml for each treatment repetition). Tests conducted in the first to fourth weeks showed that the media tested for virulence tended to decrease in EPN virulence from the initial test to the final test. The trend of decreasing virulence over time in the media was similar to the virulence tests conducted by Nagachandrabose (2022), where there was a decrease in virulence in Alginate and Sponge media.

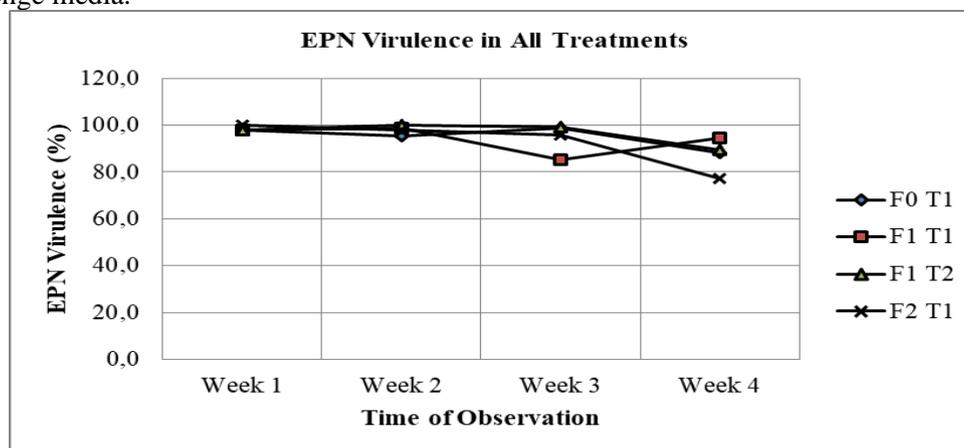


Figure 6. Graph of virulence variable data for all treatments during four weeks of observation

1. Virulence EPN in Alginate Media Treatment /F1

The results of alginate media virulence observations in week 1 for both F1T1 and F1T2 treatments showed identical virulence results, producing a virulence percentage value of 98%. This suggests that storing alginate media in different environments, namely T1 (refrigerator at $\pm 15^{\circ}\text{C}$) and T2 (room temperature at $\pm 27^{\circ}\text{C}$), did not affect the virulence of EPN during one week of storage. These identical virulence results suggest that during one week of alginate media storage in different storage environments, there was no significant impact on the virulence of stored EPN. In the second week of observation, alginate media F1T2 produced the best results, followed by F1T1. Alginate media produced the best virulence results compared to other media in the second week, although the difference was not significant. In the third week, it was found that the two alginate media treatments differed, with the F1T2 treatment yielding the highest virulence results compared

to other treatments, while F1T1 yielded the lowest virulence results compared to other treatments. It was found that from week 1 to week 4, the virulence values were fluctuating, and in week 2, the highest virulence values were obtained from treatments F1T1 and F1T2. This virulence condition also occurred in a study related to the effect of temperature on virulence in EPN *Steinernema feltiae* by Butuner et al. (2023), which showed data on storage at 15°C and 25°C, where in week 2 of testing there was an increasing trend in virulence compared to week 1, but after week 3 there was a decrease. The virulence data for treatments F1T1 and F1T2 showed that in week 2 of storage, the highest virulence values were produced.

In the final week of observation/fourth week, it was found that alginate media in storage environments T1 and T2 provided superior virulence values compared to control media F0T1 and sponge media F2T1. In the fourth week of observation, it was found that the alginate media F1T1 (refrigerator $\pm 15^{\circ}\text{C}$) provided a higher percentage than the alginate media F1T2 (room temperature $\pm 27^{\circ}\text{C}$). This superior condition of the alginate media in the T1 environment (refrigerator $\pm 15^{\circ}\text{C}$) is similar to the results of the study by Kagimu & Malan (2019), which showed that EPN (*Steinernema jeffreyense*) stored in alginate media kept at 14°C resulted in a higher mortality rate in *G. mellonella* insect larvae compared to alginate media stored at room temperature (24°C).

2. Virulence EPN in PU Sponge Media Treatment/F2

The F2T1 sponge storage media (refrigerator $\pm 15^{\circ}\text{C}$) is known to show complete virulence test results in weeks 1 to 4. The F2T1 treatment test data showed a continuous downward trend, starting with the first week of storage, where the F2T1 treatment produced the highest virulence results compared to other treatments. The second week showed a decrease in virulence, but it was still better than the control treatment (water). In week 3, F2T1 showed a decline but was better than F1T1, and the most significant decline occurred in week 4, with the F2T1 treatment showing the lowest virulence value of 77% compared to other treatments (F1T1, F1T2, and F0T1). The percentage value of 77% in this sponge media was the lowest percentage value in this study, and this percentage value was the largest decrease (down 18.67%) from the previous week (week 3 = 96%). The pattern/trend of decreasing virulence values is in line with the research by Nagachandrabose (2022), where the virulence values of the Polyurethane Sponge treatment showed a downward trend from week 1 to week 4.

3. Virulence EPN In Water Media Treatment/F0

The virulence ability of the F0 media (control/water) can only be demonstrated by the F0T1 media (water) in observations from the first to the fourth week. The F0T1 treatment is known to produce virulence values with a fluctuating pattern. The first week showed values similar to the two alginate treatments, then in the second week there was a decrease in virulence. In the third week, this media provided an increase in virulence values and also became the highest virulence value in the F0T1 treatment, then in the fourth week there was a decrease in EPN virulence values. This decrease was known to be quite high (-10.67% from the third week) in the F0 media (control/water), although not as large as the decrease that occurred in the F2T1 treatment that also occurred in the fourth week. The significant decrease observed in the fourth week of this study can be linked to the findings of Divya et al. (2011), which showed a decrease in virulence in the fourth week of water media storage, where week 4 saw a 10% decrease from the previous week, while weeks 1, 2, and 3 showed relatively stable results.

Virulence testing conducted over a period of 4 weeks showed that each treatment tended to have lower virulence results in the fourth week compared to the first week (initial) of testing. In the initial week of testing, treatment F2T1 gave the highest result of 100%, followed by treatments F1T1, F1T2 and F0T1 with a result of 98%. In week 2 of testing, treatment F1T2 produced the best result of 100%, followed by treatments F1T1 and F2T1, with the lowest result in treatment F0T1 at 95.3%. The alginate media treatments F1T1 and F1T2 produced the best results in week 2 of testing. In week 3, treatment F1T2 gave the best result of 99.33%, followed by treatments F0T1 and F2T1, and the lowest result was obtained from treatment F1T1 at 85.3%. Treatment F0T1 gave the best result in the week 3 test. In the final week of observation (week 4), the alginate treatments, namely F1T1 and F1T2, produced higher virulence results than the control treatment, namely F0T1, and the lowest results were found in the F2T1 treatment. During the 4 weeks of observation, it was found that each treatment had a different pattern of results. with treatments F0T1, F1T1, and

F1T2 showing fluctuating results, while treatment F2T1 showed a continuous decline over time. During the 4-week storage period, alginate storage media stored in a refrigerator (F1T1) showed better virulence than other treatments.

CONCLUSION

This study concludes that temperature is the dominant factor in EPN storage, with 15°C consistently providing superior results over ambient temperatures. While water media is sufficient for maintaining viability in the short term, sodium alginate serves as the most effective medium for preserving EPN virulence over longer periods. These findings imply that for commercial applications requiring extended shelf-life and high pathogenicity, active encapsulation combined with cold storage is the optimal strategy. Future research should focus on extending the storage duration beyond four weeks and exploring novel active media formulations.

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