Jurnal Kultivasi Vol. 24 (1) April 2025
ISSN: 1412-4718, eISSN: 2581-138x

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# Evaluation of thermotherapy on potato tubers to control tuber-borne nematodes, *Meloidogyne* spp.

**Abstract.** Root knot nematodes (*Meloidogyne* spp.) are significant plant-parasitic nematodes frequently transmitted through potato tubers and are a major factor contributing to the decline in both crop quality and yield. This study aims to evaluate the effectiveness of thermotherapy for eliminating *Meloidogyne* spp. from potato tubers and its impact on sprouting viability. Thermotherapy was conducted by immersing potato tubers and second-stage juveniles (J2) of *Meloidogyne* spp. in water at temperatures of 50 °C, 52.5 °C, 55 °C, and 60 °C for durations ranging from 5 to 75 minutes, depending on the treatment. Parameters observed included the mortality rate *of Meloidogyne* spp. J2 and the growth viability of potato seeds. The results showed that thermotherapy applied to second-stage juveniles (J2) of *Meloidogyne* spp. at 50 °C for 20 minutes resulted in complete (100%) nematode mortality. Similarly, the application of thermotherapy to potato tubers at 50 °C for 40 minutes did not significantly affect seed viability. During this treatment, the internal temperature of the tubers, measured at a depth of 1.5 cm, reached the target temperature of 50 °C at the 20-minute mark and was maintained until the 40th minute, ensuring an effective thermal exposure. These results indicate that thermotherapy at 50 °C for 40 minutes represents a safe and effective method for the elimination of *Meloidogyne* spp. in potato tubers. This approach offers a practical and promising strategy to enhance seed health and minimize the risk of nematode dissemination in potato cultivation systems.

**Keywords**: Heat treatment · *Meloidogyne* spp. · Plant disease control · Potato seeds · Root-knot nematodes

Submitted: 21 December 2024, Accepted: 30 April 2025, Published: 30 April 2025
DOI: https://doi.org/10.24198/kultivasi.v24i1.62705

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Kurniawan W, Supramana, Munif A, Giyanto. 2025. Evaluation of thermotherapy on potato tubers to control tuber-borne nematodes, *Meloidogyne* spp. Jurnal Kultivasi, 24 (1): 104-112.

## Introduction

Potatoes (*Solanum tuberosum* L.) are among the most economically valuable and play an important role in global food security (Mishra *et al*., 2024). However, potato production in many countries, including Indonesia, faces several challenges, one of which is infestation by root-knot *Meloidogyne* spp (Moens et al., 2009; Wesemael et al., 2014; Aprilyani et al., 2015; Utami et al., 2017). These nematodes attack plant roots, inducing gall formation that disrupts the process of water and nutrient absorption by plants, thereby reducing crop yields. The prevalence of *Meloidogyne* spp. is increasing due to its spread through infected potato tubers, particularly in areas with tropical and subtropical climates (Jones et al., 2013).

One of the primary pathways for the spread of *Meloidogyne* spp. is through infested potato tubers. Consequently, managing these nematodes during the post-harvest stage is essential to prevent spread to other production areas (Holajjer et al., 2020; Singh et al., 2020; Nirula et al., 1967; Jatala et al., 1982; Gerič Stare et al., 2022). Various control methods have been developed to suppress populations of these nematodes, including the use of chemical nematicides (Desaeger et al., 2020; Becker et al., 2021; Grabau & Liu, 2021), crop rotation (Win et al., 2016; Mathebula et al., 2024), and resistant cultivars (Chiuta et al., 2021; Iwanaga et al., 2022; Pinheiro et al., 2020; Bali et al., 2021), as well as physical and biological control methods. However, the application of chemical nematicides often raises concerns related to environmental and human health (Pathak et al., 2022), while the effectiveness of crop rotation and resistant cultivars remains limited.

Thermotherapy is a physical control approach that has been applied to various crops for the elimination of pathogens without leaving harmful residues. This method has been used globally for over a century as an environmentally friendly, pre-planting disease management strategy. Among its applications is the control of plant-parasitic nematodes in various planting materials, such as bananas, rice, oranges, vegetable bulbs, and ornamental plants (Lopes et al., 2019). Thermotherapy involves the application of specific temperature via heat, cold, or irradiation to infected plant material, including potato tubers. Heat can be in the form of dry or wet heat. Hot Water Treatment (HWT), which involves soaking planting material in hot water, is a common thermotherapy technique aimed at eradicating nematodes while preserving the viability of the tubers or bulbs (Lopes et al., 2019; Knoetze, 2020; Gu et al., 2022).

Several studies demonstrated that exposing nematodes to elevated temperatures over a specific duration can significantly reduce their populations (Knoetze, 2020; Gu et al., 2022). Hot water immersion techniques have been employed to control various nematode species. For instance, Koen (1969) reported that HWT at 0 °C for 45 to 60 minutes effectively eliminated all developmental stages of *Pratylenchus* spp. in potato tubers, without adversely affecting shoot emergence. In addition to potatoes, this technique has also been applied to other commodities such as garlic (Ahmadi et al., 2019), yam, sweet potato, taro, ginger (Sikora et al., 2018), stroberry (Khanal et al. 2020) and *Syngonium podophyllum* (Lim et al. 2024).

Previous studies have demonstrated that thermotherapy within specific temperature and time ranges can effectively control nematodes in vitro without adversely affecting the quality of bulbs or other plant materials. For instance, temperatures of 45–51 °C for 20–30 minutes have been effective against *Ditylenchus destructor* in garlic (Ahmadi et al., 2019), 50–55 °C for 40 minutes against *Scutellonema bradys* in yam bulbs (Coyne et al., 2010), and a range of 50–55 °C for 10 minutes for *Meloidogyne javanica* as well as 45 °C for 3 hours for *Radopholus similis* on ginger rhizomes (Sikora et al., 2018).

However, information regarding the application of thermotherapy for the control of *Meloidogyne* spp. carried by potato tubers remains limited. The success of thermotherapy is influenced by multiple factors, including treatment temperature and duration, nematode species and developmental stage, the host plant's heat tolerance, and environmental conditions during treatment. Therefore, the application of thermotherapy in potato requires further investigation to establish effective and safe protocols.

This study aims to evaluate the effectiveness of thermotherapy for controlling *Meloidogyne* spp. in potato tubers and to determine the optimal temperature and duration that minimize adverse effects on tuber sprouting viability. It is anticipated that the findings will contribute to the development of sustainable

ISSN: 1412-4718, eISSN: 2581-138x

and applicable nematode management strategies at the farmer level, particularly in potato production systems.

## Materials and Methods

**Preparation of potato seed tubers and nematode suspension.** Healthy tubers of the Granola G3 variety were used in this study. These tubers were obtained from certified seed producers in Pangalengan, West Bandung Regency, Indonesia. Tubers were selected for uniformity in size (approximately 50 g per tuber; medium size: 31–60 g and storage age (2–3 months), with sprout lengths of approximately 0.5 cm. Root-knot nematode (*Meloidogyne* spp.) suspensions were prepared by extracting nematodes naturally infected potato tubers using the Baermann funnel technique for 48 hours. 15 live and active nematodes (J2) were transferred into a 1.5 mL Eppendorf tube containing 1 mL of distilled water. In addition, healthy potato tubers of uniform size (50–60 g) were prepared for internal temperature measurement tests. These tubers were used to determine the internal temperature at depths of 1.5–2.0 cm from the surface during hot water treatment, using a digital thermometer.

**Thermotherapy Procedure for Potato Sprouting Viability and Nematode Mortality.** The treatment window, defined as the critical combination of temperature and exposure time, is essential for optimizing thermotherapy in order to preserve potato seed viability and eliminate nematodes. Determining the correct treatment window is crucial because excessive heat exposure can damage tuber physiological quality as seed, whereas insufficient heat exposure may fail to eliminate pathogens effectively. For seed tuber evaluation, healthy potato tubers were first soaked in water at 20–25 °C for 30 minutes to equilibrate initial tuber temperature and reduce thermal shock during treatment, then placed into a holding container. Tubers were subsequently subjected to water bath treatments at temperatures of 50 °C (15–75 minutes), 52.5 °C (15–75 minutes), 55 °C (5–60 minutes), and 60 °C (5–20 minutes). After thermotherapy, tubers were hydrocooled in 20–25 °C water for 10 minutes, air-dried, and transferred into culture boxes maintained at 16–20 °C. Observations on bud whiteness, the number of new buds, and bud size were recorded from day 2 to day 28 post-treatment. Internal tuber temperature at a depth of 1.5 cm was monitored using a digital thermometer.

The treatment window also determines the critical temperature and duration of thermotherapy for nematode mortality. To assess nematode response, an Eppendorf tube containing 1 mL of distilled water and 15 live, active nematodes (10 second-stage juveniles (J2) and 5 adults) was first equilibrated by immersion in water at 20–25 °C. This pre-equilibration step helps standardize the initial conditions before exposure to heat treatments. Subsequently, the tubes were immersed in a water bath pre-set to target temperatures of 50 °C (5–75 minutes), 52.5 °C (5–60 minutes), 55 °C (5–60 minutes), and 60 °C (5–15 minutes). The untreated nematode suspension, maintained at room temperature, served as a negative control. This approach ensures that the observed nematode mortality can be attributed solely to the thermal exposure rather than handling or environmental fluctuations. Observations were made 1 day after hot water treatment by counting the number of dead nematodes. Calculation of nematode mortality with the formula:

$$M=n/N\times100\%$$

Information:
M : Percentage of nematode mortality (%)
n : Number of dead nematodes (tails)
N : Total number of test nematodes (tail)

## Results and Discussion

**Symptoms of potato tubers infected with *Meloidogyne* spp.** The results of the observation of disease symptoms in potato tubers and the identification of the cause are presented in Figure 1. Symptoms observed in potato tubers in the field show early indications of root-knot nematode infection (*Meloidogyne* spp.), characterized by uneven tuber surfaces, root-knot or lumps, non-uniform shape of tubers, and the appearance of premature shoots in some tubers. These physical deformations not only reduce the commercial value of the tubers but also indicate internal physiological disruptions caused by nematode infestation. The symptoms are visually similar to the typical signs of *Meloidogyne* spp. Infestation as reported in various literature (Moens et al., 2009; Wesemael et al., 2014).

To ascertain the type of nematode responsible for these symptoms, morphological identification of the nematodes isolated from tuber tissue and soil around the roots was performed. Microscopic observations revealed juvenile stages (J1, J2, J3, J4) and female morphological characters that matched the description of *Meloidogyne* spp., such as a vermiform-shaped body in J2, a pointed tail, and rounded females with a distinctive perineal pattern. These diagnostic features, consistent with established identification keys (Jones et al., 2013), strengthen the confirmation that the nematodes involved were *Meloidogyne* spp. The alignment between field symptoms and morphological identification results strengthens the suspicion that the nematode that infested potato tubers in this study was *Meloidogyne* spp. This confirmation is important because epidemiologically, the spread of these nematodes can occur using infested tubers as seeds, so it has great potential in spreading to other production areas (Singh et al., 2020).


**Figure 1. Symptoms of potato tubers infected with *Meloidogyne* spp. and the results of staining of infected parts (a) symptomatic potato tuber incisions show damage to the inside of the tubers, (b) *Meloidogyne* spp. eggs, (c) adult female of *Meloidogyne* spp., (d). juvenile 2 of *Meloidogyne* spp.**

Infection by *Meloidogyne* spp. induces physiological disturbances in plants through the formation of giant cells within root tissues, thereby disrupting nutrient and water uptake processes. These pathological changes directly affect tuber development, impacting tuber size, shape, and seed viability (Wesemael et al., 2014; Holajjer et al., 2020). The confirmation of *Meloidogyne* spp. as the causal agent thus provides a critical foundation for the development of effective control strategies, particularly in the postharvest phase, such as thermotherapy treatments aimed at preventing the dissemination of nematodes through seed tubers).

**Effects of Thermotherapy on Potato Seeds.** Thermotherapy significantly affected potato seed viability, as reflected in shoot number, shoot length, and growth capacity percentage. Overall, increasing the temperature and extending the treatment duration led to a marked decline in seed viability (Table 1).

The viability of potato seeds at 50 °C remained relatively high up to 40 minutes of treatment, with a germination rate of 97.30% and an average bud length of 4.4 cm. However, a significant decline in viability was observed after 45 minutes of exposure, where the germination rate dropped sharply to 53.32%. These results indicate that the physiological heat tolerance threshold of potato seeds at 50 °C lies between 40 and 45 minutes. Measurements of internal tuber temperature at a depth of 1.5 cm showed that temperatures ≥50 °C were reached after 20 minutes and maintained until the end of the 75-minute treatment period, suggesting uniform heat penetration throughout the internal tissues. Following the observations at 50 °C, further evaluation at higher temperatures was conducted to determine the impact of increased thermal exposure on seed viability and to identify the threshold beyond which irreversible physiological damage occurs.

ISSN: 1412-4718, eISSN: 2581-138x

**Table 1. Effect of thermotherapy on potato seeds 30 days after treatment (DAT)**

| Types of Treatment | | Number of Buds | Bud Length (cm) | viability (%) | Temperature of potato tubers at a depth of 1.5 cm (°C) | Duration of temperature ≥ 50 °C (minute) |
|---|---|---|---|---|---|---|
| Temperature (°C) | Duration (minute) | | | | | |
| 50 | control (0) | 4.40 ± 0.55 b | 2.96 ± 0.55 a | 100.00 | 25.60 | - |
| | 15 | 4.40 ± 0.55 b | 2.82 ± 0.55 b | 97.64 | 48.50 | - |
| | 20 | 4.40 ± 0.55 a | 2.80 ± 0.55 b | 97.30 | 49.60 | - |
| | 30 | 4.40 ± 0.55 a | 2.90 ± 0.55 a | 98.99 | 50.20 | 10 |
| | 40 | 4.40 ± 0.55 a | 2.80 ± 0.55 a | 97.30 | 50.30 | 20 |
| | 45 | 3.80 ± 0.45 a | 0.60 ± 0.55 a | 53.32 | 50.30 | 25 |
| | 60 | 3.40 ± 0.55 a | 0.36 ± 0.45 a | 44.72 | 50.60 | 40 |
| | 75 | 2.40 ± 0.55 a | 0.40 ± 0.55 a | 34.03 | 50.30 | 55 |
| 52.5 | control (0) | 4.40 ± 0.55 a | 2.96 ± 0.05 a | 100.00 | 25.60 | - |
| | 15 | 4.40 ± 0.55 a | 2.68 ± 0.55 a | 95.27 | 50.70 | - |
| | 20 | 4.40 ± 0.55 a | 2.42 ± 0.55 a | 90.88 | 50.70 | 10 |
| | 25 | 4.40 ± 0.55 a | 2.42 ± 0.55 a | 90.88 | 50.70 | 15 |
| | 30 | 4.00 ± 0.71 a | 0.80 ± 0.55 a | 58.97 | 50.70 | 20 |
| | 40 | 1.00 ± 0.00 b | 0.32 ± 0.71 a | 16.77 | 51.30 | 30 |
| | 45 | 1.00 ± 0.71 b | 0.16 ± 0.00 b | 14.07 | 51.50 | 35 |
| | 60 | 0.00 ± 0.00 c | 0.00 ± 0.71 b | 0.00 | 52.50 | 50 |
| | 75 | 0.00 ± 0.00 c | 0.00 ± 0.00 c | 0.00 | 52.50 | 65 |
| 55 | Control (0) | 4.40 ± 0.55 a | 2.96 ± 0.05 a | 100.00 | 25.60 | - |
| | 5 | 4.40 ± 0.55 a | 2.76 ± 0.55 a | 96.62 | 43.60 | - |
| | 10 | 4.00 ± 0.00 ab | 2.44 ± 0.55 a | 86.67 | 49.80 | - |
| | 15 | 3.60 ± 0.55 bc | 2.34 ± 0.00 ab | 80.44 | 52.90 | 5 |
| | 20 | 3.40 ± 0.55 c | 1.50 ± 0.55 bc | 63.97 | 53.90 | 10 |
| | 25 | 1.60 ± 0.55 d | 0.54 ± 0.55 c | 27.30 | 54.50 | 15 |
| | 30 | 0.00 ± 0.00 e | 0.00 ± 0.55 d | 0.00 | 54.80 | 20 |
| | 45 | 0.00 ± 0.00 e | 0.00 ± 0.00 e | 0.00 | 55.00 | 35 |
| | 60 | 0.00 ± 0.00 e | 0.00 ± 0.00 e | 0.00 | 55.10 | 50 |
| 60 | Control (0) | 4.40 ± 0.55 a | 2.96 ± 0.05 a | 100.00 | 25.60 | - |
| | 5 | 4.60 ± 0.55 a | 3.16 ± 0.55 a | 105.65 | 44.80 | - |
| | 10 | 3.60 ± 0.55 b | 1.32 ± 0.55 a | 63.21 | 53.00 | - |
| | 15 | 0.00 ± 0.00 c | 0.00 ± 0.55 b | 0.00 | 56.80 | 5 |
| | 20 | 0.00 ± 0.00 c | 0.00 ± 0.00 c | 0.00 | 59.03 | 10 |

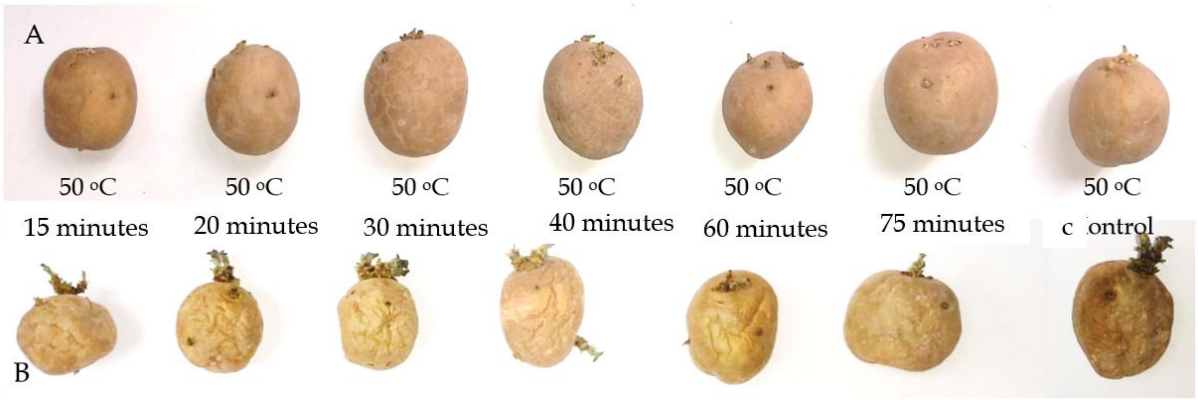


**Figure 2. Potato tubers on 50 °C thermotherapy treatment (a) before treatment, (b) 30 days after treatment**

Treatment at 52.5 °C resulted in a more rapid decline in seed viability, with a significant reduction observed at 30 minutes (58.97%) and complete loss of viability at 60 minutes (0%). These results indicate that a 2.5 °C increase from 50 °C significantly accelerates damage to the meristematic tissues of the shoots. This decline in viability is consistent with previous studies, which have shown that exposure to elevated temperatures induces protein denaturation and

physiological disruptions in seed tissues, ultimately impairing their growth potential (Tang et al., 2018; Singh et al., 2020). Exposure to elevated temperatures can lead to significant physiological damage in plant tissues, including the disruption of cellular membrane integrity, denaturation of essential enzymes, and the collapse of mitochondrial function, which impairs energy production required for cell division and growth (Park et al, 2024; Wahid et al., 2007). Additionally, high temperatures can cause oxidative stress through excessive reactive oxygen species (ROS) production, leading to lipid peroxidation and DNA damage, further compromising seed viability and regenerative capacity.

Exposure to temperatures of 55 °C and 60 °C resulted in a substantial and accelerated reduction in potato seed viability. At 55 °C, viability declined to 0% after 30 minutes, while at 60 °C, complete loss was recorded within only 15 minutes of treatment. Early physiological damage was evident through the absence and shortening of emerging buds prior to total mortality. The rapid attainment of internal tuber temperatures ≥50 °C within 10–15 minutes at these higher external temperatures likely contributed to accelerated tissue degradation. Consistent with previous findings, excessive thermal exposure compromises meristematic and vascular tissues, disrupts mitochondrial integrity, and induces protein denaturation, thereby impairing cellular respiration and division processes (Tang et al., 2018; Wahid et al., 2007; Singh et al., 2020). Although a brief exposure at 60 °C for 5 minutes slightly enhanced sprouting relative to the control, the effect was insufficient for achieving complete nematode elimination. Collectively, these findings suggest that thermotherapy at 50 °C for 40 minutes represents a critical threshold, ensuring effective internal heating (≥50 °C for ≥20 minutes) necessary for nematode control while maintaining seed viability above 95% (Holajjer et al., 2020; Shurtleff & Averre, 2000).

**Effect of Thermotherapy on Juvenile Mortality of *Meloidogyne* spp.** The results of the thermotherapy treatments showed a significant effect on the mortality of second-stage juveniles (J2) of *Meloidogyne* spp. at 24 hours post-treatment, as presented in Table 2. Increased temperature and length of treatment duration consistently increase nematode mortality rates. J2 mortality reaches 100% in almost all temperature and duration combinations above the 50 °C temperature threshold.

**Table 2. Effect of thermotherapy on mortality of J2 *Meloidogyne* spp. at 24 hours after treatment (HAT)**

| Types of Treatment | | Mortality (%) | | | |
|---|---|---|---|---|---|
| Temperature (°C) | Duration (minute) | | | | |
| 50 | control | 0.00 | ± | 0.00 | a |
| | 5 | 60.00 | ± | 0.71 | b |
| | 10 | 78.67 | ± | 0.84 | c |
| | 15 | 98.67 | ± | 0.45 | d |
| | 20 | 100.00 | ± | 0.00 | d |
| | 30 | 100.00 | ± | 0.00 | d |
| | 40 | 100.00 | ± | 0.00 | d |
| | 45 | 100.00 | ± | 0.00 | d |
| | 60 | 100.00 | ± | 0.00 | d |
| | 75 | 100.00 | ± | 0.00 | d |
| 52.5 | control | 0.00 | ± | 0.00 | a |
| | 5 | 76.00 | ± | 1.14 | b |
| | 10 | 86.67 | ± | 1.00 | c |
| | 15 | 100.00 | ± | 0.00 | d |
| | 20 | 100.00 | ± | 0.00 | d |
| | 25 | 100.00 | ± | 0.00 | d |
| | 30 | 100.00 | ± | 0.00 | d |
| | 40 | 100.00 | ± | 0.00 | d |
| | 45 | 100.00 | ± | 0.00 | d |
| | 60 | 100.00 | ± | 0.00 | d |
| | 75 | 100.00 | ± | 0.00 | d |
| 55 | control | 0.00 | ± | 0.00 | a |
| | 5 | 89.33 | ± | 0.89 | b |
| | 10 | 100.00 | ± | 0.00 | c |
| | 15 | 100.00 | ± | 0.00 | c |
| | 20 | 100.00 | ± | 0.00 | c |
| | 25 | 100.00 | ± | 0.00 | c |
| | 30 | 100.00 | ± | 0.00 | c |
| | 45 | 100.00 | ± | 0.00 | c |
| | 60 | 100.00 | ± | 0.00 | c |
| 60 | control | 0.00 | ± | 0.00 | a |
| | 5 | 100.00 | ± | 0.00 | b |
| | 10 | 100.00 | ± | 0.00 | b |
| | 15 | 100.00 | ± | 0.00 | b |

At 50 °C, the J2 mortality rate increases with the length of the treatment time. Initial mortality of 60% occurred after 5 minutes and increased to 78.67% at 10 minutes. Mortality reached 98.67% at 15 minutes and 100% after 20 minutes of treatment. This suggests that J2 *Meloidogyne* spp. has a low heat tolerance threshold, and a temperature of 50 °C for at least 20 minutes is enough to kill all J2 individuals in the sample. Treatment at 52.5 °C showed higher effectiveness, with a mortality of 76% in just 5 minutes, and reached 100% at a duration of 15 minutes. Similarly, at 55 °C, mortality reached

89.33% within 5 minutes and 100% after 10 minutes. Meanwhile, a temperature of 60 °C killed 100% of J2 in just 5 minutes, suggesting that high temperatures significantly accelerate physiological damage to nematodes, possibly through protein denaturation and disruption of cellular metabolism (Perry & Moens, 2024).

The effectiveness of thermotherapy in eliminating nematodes supports previous findings indicating that temperatures between 50–55 °C for a specific duration are effective in inactivating the eggs and juvenile stages of the nematode *Meloidogyne* spp. (Sikora & Fernandez, 2018; Holajjer et al., 2020). In the context of nematode management in potato tubers, thermotherapy treatment can be applied as an environmentally friendly, non-chemical alternative method to prevent the spread of nematodes through infected tuber seeds. However, it is important to note that effective high-temperature treatment in killing nematodes can also potentially impair the viability of potato tubers, as shown in previous data. Therefore, selecting an appropriate combination of temperature and duration must balance the efficacy of nematode elimination with the suitability of the tubers for use as seed. In this study, treatment at 50 °C for 20–40 minutes maintained tuber sprouting capacity above 95% and resulted in internal temperatures reaching 50 °C at a depth of 1.5 cm. This temperature–time range effectively caused 100% mortality of second-stage juveniles (J2) at 50 °C for 20 minutes (Table 2).

## Conclusion

The application of thermotherapy at 50 °C for 20 minutes effectively eliminated second-stage juveniles (J2) of *Meloidogyne* spp., achieving complete (100%) mortality. Moreover, treating potato tubers at 50 °C for 40 minutes did not significantly affect sprouting viability, as the internal temperature at a depth of 1.5 cm reached and sustained the target temperature during the treatment period. These findings demonstrate that thermotherapy at 50 °C for 40 minutes is a safe and effective method for the elimination of *Meloidogyne* spp. in potato tubers. Therefore, this approach can serve as a practical and reliable strategy to improve seed health and reduce the risk of nematode transmission in potato production.

## Acknowledgments

The authors express their gratitude to the Indonesian Education Scholarship (BPI) for partially funding the global research project titled "*Identification of Parasitic Nematodes Carried by Potato Tubers at the Java Island Potato Center: Identification, Diversity, and Control*."

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