Jurnal Perlindungan Tanaman Indonesia, Vol. 30, No.1, 2026: 1−7
DOI: 10.22146/jpti.107062
Available online at http://jurnal.ugm.ac.id/jpti
ISSN 1410-1637 (print), ISSN 2548-4788 (online)

Research Article

Ultrasonication for the Control of *Aphelenchoides besseyi* in Local Rice Seed Varieties from North Sumatra

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Received May 28, 2025; revised September 22, 2025; accepted February 9, 2026

ABSTRACT

Aphelenchoides besseyi is one of the most important plant parasitic nematode in rice causing the white tip disease. Its dormant phase is seed-borne, making rice seeds a medium for the long-distance dispersal of this nematode. This study aimed to assess the potential of ultrasonic wave application as a seed treatment method for eliminating *A. besseyi* nematodes from rice seeds. The seed varieties tested were six local varieties from North Sumatra namely *Siserang*, *Sipining*, *Sipala*, *Sirias*, *Sigambiri Merah*, and *Sigambiri Putih*. The commercial rice variety *Pak Tiwi-1* was used as a comparison. Seed treatment was conducted with an ultrasonic cleaner unit at 40 kHz for 5, 10, 15, 20, 25, and 30 minutes, respectively. Ultrasonic exposure at 40 kHz for 20 and 30 minutes were able to eliminate *A. besseyi* by 100%, resulting in 0% incidence of white tip disease in seedlings. Ultrasonic seed treatment shows potential as an effective, practical, and relatively fast technique for eliminating *A. besseyi* from rice seeds.

Keywords: insidence; nematode; seed-borne; ultrasonic; white tip.

INTRODUCTION

Aphelenchoides besseyi is one of the most important plant parasitic nematodes in rice causing the white tip disease. Infected young leaves will show symptoms of chlorosis, leaf edges becoming wrinkled, leaf tips becoming white that could reach 3 to 5 cm in length. Under certain conditions, leaves become wrinkled and twisted, thus inhibiting panicle formation. Additionally, young leaves can be speckled with white spots or have chlorotic areas, leaf margins may be distorted, and wrinkled. Infected plants can also exhibit stunting, reduced tillering, and reduced grain (Tulek *et al.*, 2015). Nematodes can be dispersed by seeds and survive under anhydrobiosis conditions (Kurniawati & Supramana 2016). Nematodes can survive on seeds for 8 months to 3 years (Khan, 2015). Seeds infected with *A. besseyi* show black spots and sunspot symptoms on seed surfaces (Diana *et al.*, 2018). During the dormant phase, nematodes are on seeds and will reactivate when the seed is planteded.

The nematode *A. besseyi* has been found in almost all areas of Indonesia, including North Sumatra. *A. besseyi* was detected on commercial varieties several districts in North Sumatra, including Deli Serdang, Serdang Bedagai, Simalungun, Batubara, and Langkat (Hutauruk, 2018). In addition, this nematode was also found on local rice seeds in Samosir Regency (Sitanggang *et al.*, 2024). These nematodes were identified based on morphological, morphometric, and molecular characters.

In India, nematode elimination techniques include physical and chemical treatments, such immersing in hot water at 52−53 °C or chemical application of carbofuran. These treatments effectively reduce nematode populations by 17−37% (Pashi *et al.*, 2017). Elimination of *A. besseyi* from rice seeds in Indonesia has been reported by the use of hot water treatment and soaking in guano tea for 30 minutes. These techniques were able to reduce the population by 87% and 37%, respectively (Syarifah *et al.*, 2020).

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A common elimination technique used to eradicate *A. besseyi* is hot water treatment. This treatment requires a relatively long time while higher temperatures can reduce rice seed viability. Therefore, an effective and efficient *A. besseyi* elimination technique without reducing rice seed viability is needed. One such elimination technique is ultrasonic treatment. Ultrasonic waves are sound waves with a frequency greater than 20 kHz. These waves can break down particles attached to the surface of certain objects or materials. One widely used method is the ultrasonic cleaner. Ultrasonics are commonly used to clean medical equipment, including surgical instruments, laboratory equipment, and some optical instruments before sterilization (Safitri *et al.*, 2022).

The use of ultrasonic waves through an ultrasonic cleaner as an elimination technique for *A. fragariae* nematodes has been reported in shallot bulbs (Kurniawati, 2024). This elimination technique can eliminate up to 100% of nematodes after 60-minute ultrasonic wave applications. This treatment can eliminate *A. fragariae* nematodes without reducing the viability of shallot bulbs (Kurniawati, 2024). The use of ultrasonic waves applications to eliminate dormant nematodes, such as *A. besseyi*, in rice seeds has yet to be reported to the best of our knowledge. This study aimed to assess the potential of ultrasonic wave application as a seed treatment method for eliminating *A. besseyi* nematodes in rice seeds. Therefore, the use of ultrasonic waves is expected to effectively eliminate *A. besseyi* nematodes without reducing rice seed viability.

MATERIALS AND METHODS

Rice Seed Sampling

Sampling was carried out by sampling 5 kg of rice seed for each variety from farmers without differentiating between symptomatic and asymptomatic seeds. Rice seed were sampled from Samosir Regency (Pangururan and Palipi Districts), Simalungun Regency (Raya and Dolok Silau Districts), and Pakpak Bharat Regency (Kerajaan and Salak Districts). Local varieties obtained from several locations included varieties *Siserang* and *Sipining* from *Samosir Regency*, *Sipala* and *Sirias* from Pakpak Bharat Regency; *Sigambiri Merah* and *Sigambiri Putih* from Simalungun Regency. The commercial rice variety, *Pak Tiwi-1*, was used to compare between local and commercial varieties.

Aphelenchoides besseyi Extraction

Nematode extraction was based on the International Seed Testing Association (ISTA) standard (2014). Ten grams of rice seeds (~400 seeds) were cut from the hilum and nematodes were extracted using a modified Baermann funnel method. The rice seeds were placed on a gauze sieve in a container filled with water for nematodes to come out towards the bottom of the water. The seeds were incubated for 24 h in a dark place at room temperature (20 °C). The soaked seeds were filtered using a 400 mesh sieve. Fifteen milliliter nematode suspension was transferred to a collection bottle and labeled.

Nematode Counting

The nematode extraction results were poured into a syracus dish and counted under a stereo microscope. Five milliliter of suspensions were taken from the collection bottle. Counts were done for three replicates.

Ultrasonic Nematode Treatment

The ultrasonic treatment began by placing 10 nematodes in glass bottles and exposing them to ultrasonic waves for 5, 10, 15, 20, 25, and 30 minutes, as well as a control (no ultrasonic exposure). Each treatment had three replicates. All treatments used ultrasonic waves at a frequency of 40 kHz. Parameters observed included nematode mortality and morphology after treatments. The experiment was set as a completely randomized design with ultrasonic treatment duration as the single treatment factor. Mortality was calculated using the formula from Hooper *et al.* (2005)

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set as a completely randomized design with ultrasonic treatment duration as the single treatment factor. Mortality was calculated using the formula from Hooper *et al.* (2005) as following:

$$Percentage\ mortality = \left(\frac{Number\ of\ nematodes\ killed}{Total\ number\ of\ nematodes\ treated}\right) \times 100\%$$

Ultrasonic Seed Treatment

During ultrasonic treatments, seeds were placed in gauze and placed in an ultrasonic chamber (60W-KZ-DW). Ultrasonic treatments followed settings and durations from nematode treatments. Ultrasonic wave treatments were done on all rice varieties. Each treatment combination was replicated three times. After ultrasonic treatment, 100 rice seeds were planted in beds filled with media of soil:manure (3:1) in a greenhouse (26−31°C, RH 70-80%) under natural lighting. Observations were made for 21 days after sowing (DAS) and included germination rate, plant height, and the number of plants showing white tip disease symptoms. The experimental design for the rice seed viability test used a completely randomized factorial design. The percentage of disease incidence was calculated using the formula proposed by Cooke (2006):

$$DI = \frac{n}{N} \times 100\%$$

DI = disease incidence/occurrence; n = number of symptomatic plants; N = number of plants observed.

Data Analysis

The observed variables were analyzed using ANOVA and further testing using the Tukey was done if significant difference were detected. All test were done at 5% using SPSS program. Data that did not meet statistical assumptions were transformed using a square root transformation before analysis. The square root transformation is calculated as $\sqrt{x+0.5}$, where x is the original (untransformed) value of each observation.

RESULTS AND DISCUSSION

Population of *Aphelenchoides besseyi*

Nematode extraction from seeds of seven rice varieties resulted in presence of *A. besseyi* nematodes (Table 1). The average nematode population in local varieties ranged from 5−22 nematodes per 10 g of seed, while in commercial varieties it reached 68 nematodes per 10 g of seed.

Table 1. Population of *Aphelenchoides besseyi* on several rice varieties from North Sumatra

Regency	District	Varieties	Population (individuals per 10 g seed)
Samosir	Palipi	*Sipining*	11.4±3.5
	Pangururan	*Siserang*	22.0±5.9
Simalungun	Raya	*Sigambiri Merah*	5.0±2.1
	Dolok Silau	*Sigambiri Putih*	5.4±3.2
Pakpak Bharat	Kerajaan	*Sirias*	6.4±2.7
	Salak	*Sipala*	6.0±2.2
		Pak Tiwi-1	68.8±7.3

Aphelenchoides besseyi Mortality After Ultrasonic Treatment

Ultrasonic treatment was able to cause *A. besseyi* mortality and its duration significantly affected mortality levels. The results showed that ultrasonic treatment for 15 minutes reduced the nematodes population by approximately 70%. Meanwhile, 100% nematode mortality were observed in ultrasonic treatments of 30 minutes or longer while nematodes in controls were unaffected (Table 2) (Figure 1A and C). Nematodes after treatment showed signs of lysis starting from the cuticle and immobilize (Figure 1B and D).

Table 2. *Aphelenchoides besseyi* mortality (%) after in-vitro ultrasonic treatment

Treatment duration (min)	Final water temperature (°C)	Mortality (%)
0	-	0d
5	31	45c
10	34	54bc
15	36	70b
20	39	87ab
25	41	100a
30	43	100a

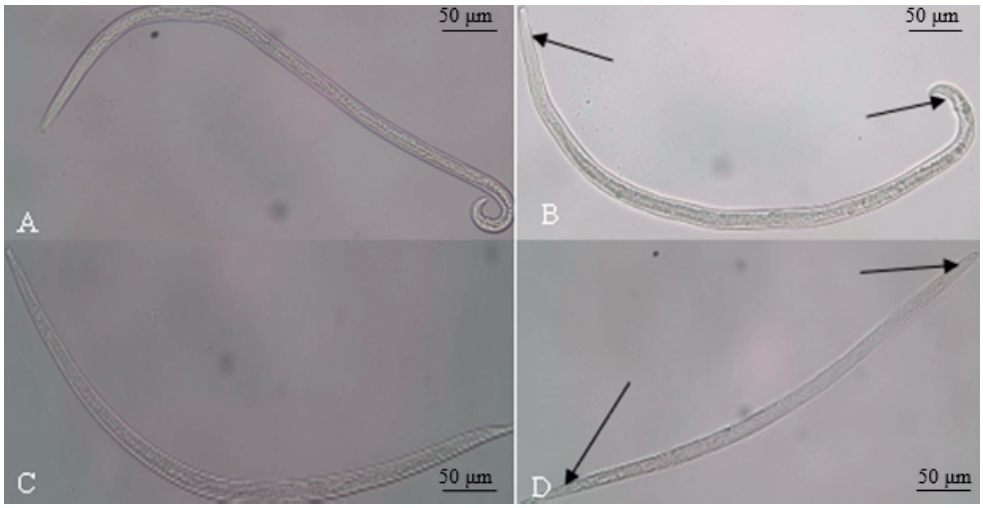


Figure 1. Morphology of *Aphelenchoides besseyi* nematodes. (A) Male nematodes before ultrasonic treatment. (B) Male nematodes after ultrasonic treatment (median bulb and spicule lysis). (C) Female nematodes before ultrasonic treatment. (D) Female nematodes after ultrasonic treatment (stylet and tail section lysis).

Table 3. Incidence of white tip disease (%) in ultrasonic treatment of rice seeds 21 days after sowing

Treatment duration (minutes)	Varieties						
	Siserang	*Sigambiri Merah*	*Sirias*	*Sipining*	*Sipala*	*Sigambiri Putih*	*Pak Tiwi-1*
0	10.2a	5.3a	5.3a	13.8a	6.2a	6.6a	26.0a
5	5.2b	0.6b	1.6b	5.5b	2.6b	2.0b	10.3b
10	1.5c	0.2b	0.2b	2.2c	0.5b	0.6b	3.3c
15	0.0c	0.0b	0.0b	0.2c	0.0b	0.2b	0.3c
20	0.0c	0.0b	0.0b	0.0c	0.0b	0.0b	0.0c
25	0.0c	0.0b	0.0b	0.0c	0.0b	0.0b	0.0c
30	0.0c	0.0b	0.0b	0.0c	0.0b	0.0b	0.0c
Variety average	2.4bc	0.8d	1.0d	3.1b	1.3cd	1.3cd	5.7a

Seed Viability, White Tip Disease Incidence after Ultrasonic Treatment of Seeds

Ultrasonic treatment also affected the disease incidence rate of rice plants (Table 3). Ultrasonic seed treatment significantly reduced the disease incidence rate of the tested varieties 21 DAS. In the control (0 min), Pak Tiwi-1 variety showed the highest disease incidence, at 31%, followed by the Siserang variety with 15% and 12% at two treatments. As treatment duration increased, disease incidence decreased respectively in all varieties. At the 10th minute, *Siserang* and *Pak Tiwi-1* still showed disease symptom, although the percentage had been reduced compared to the exposure time in the control treatment. In the 20th to 30th minute, all varieties showed 0% disease incidence, indicating that ultrasonic seed soaking effectively eliminated nematodes. In addition, the disease incidence rate and number of nematodes found in *Sigambiri Merah*, *Sigambiri Putih*, *Sirias*, and *Sipala* varieties tended to be lower than in other varieties. Plant growth capacity after ultrasonic treatment showed results that were not significantly different between control and ultrasonically treated plants (Table 4). Based on agronomic parameters, *Siserang*, *Sigambiri Merah*, and *Sigambiri Putih* varieties showed higher values than others.

Plant germination after ultrasonic treatment showed no significant differences between control plants and ultrasonically treated plants (Table 5).

Table 4. Germination of rice seeds (%) subjected to ultrasonic treatment at 14 days after sowing

Treatment duration (minutes)	Varieties						
	Siserang	*Sigambiri Merah*	*Sirias*	*Sipining*	*Sipala*	*Sigambiri Putih*	*Pak Tiwi-1*
0	99.3a	86.3a	97.0a	91.0ab	83.7a	81.7b	100.0a
5	100.0a	87.7a	94.7a	91.7ab	84.0a	82.3b	99.3a
10	99.3a	87.7a	98.0a	95.3a	85.3a	85.0ab	98.3a
15	99.3a	87.3a	98.0a	95.3a	87.7a	91.0a	99.3a
20	100.0a	86.7a	96.7a	95.3a	86.3a	85.3ab	99.0a
25	100.0a	87.3a	95.0a	91.0ab	84.3a	89.0a	97.7a
30	95.3ab	88.7a	92.7ab	90.0ab	85.0a	90.0a	98.7a
Variety average	99.0a	87.4c	96.0ab	92.8b	85.2c	86.3c	98.9a

Table 5. Rice plant height (cm) in ultrasonic treatment 21 days after sowing

Treatment duration (minutes)	Varieties						
	Siserang	*Sigambiri Merah*	*Sirias*	*Sipining*	*Sipala*	*Sigambiri Putih*	*Pak Tiwi-1*
0	27.0b	41.9a	41.5a	36.9a	38.7a	39.1a	38.3a
5	39.9a	42.3a	41.6a	37.0a	38.8a	39.5a	38.6a
10	39.9a	42.2a	41.5a	37.1a	38.7a	39.5a	38.2a
15	40.1a	42.6a	41.4a	37.2a	38.6a	39.8a	38.3a
20	40.1a	42.4a	41.5a	37.2a	38.7a	39.7a	38.4a
25	40.2a	42.3a	41.0a	37.3a	38.2a	39.5a	38.0a
30	40.2a	42.6a	40.9a	37.3a	38.1a	39.8a	37.9a
Variety average	38.2ab	42.3a	41.3a	37.1b	38.5ab	39.5ab	38.2ab

The results showed that ultrasonic treatment of rice seeds had no significant effect on germination and plant height 21 DAS.

Discussion

Nematodes were found in six local rice varieties grown in North Sumatra, although their populations were still below the economic threshold. The economic threshold for controlling *A. besseyi* is approximately 300 nematodes per 100 rice seeds (Bridge & Star, 2007). Differences in the average nematode populations among each variety may be influenced by the degree of host resistance. Additionally, humidity also affects nematode populations. *A. besseyi* exhibits normal activity at 70% humidity. In mature grains with a moisture content of 35%, the nematode enters a dormant phase, while in grains with a moisture content of 27%, nematodes may dehydrate and die (IPPC, 2016). The *Pak Tiwi-1* rice variety was used as a control variety compared to the local varieties. This variety is an inbred type of flood-tolerant rice, making it suitable for developing *A. besseyi* (Rahman *et al.*, 2018). The *Sigambiri Merah*, *Sigambiri Putih*, *Sirias*, and *Sipala* varieties are upland rice types, whereas *Siserang* and *Sipining* are lowland rice types. Differences also influenced the each variety in rice growth characteristics. Lowland rice variety tends to grow in moist conditions and tolerant against waterlogging, which are conditions that support the development of *A. besseyi*. Plant germination after ultrasonic treatment showed no significant differences between control and treated plants. Based on agronomic parameters, *Siserang*, *Sigambiri Merah*, and *Sigambiri Putih* varieties showed higher values than the other varieties. The results showed that ultrasonic treatment of rice seeds had no significant effect on germination and plant height 21 DAS.

This indicates that ultrasonic treatments used did not have negative effects on rice seed viability. Ultrasonic treatment is relatively safe for use in eliminating nematodes such as *A. besseyi*, because it does not damage the seed embryo or interfere with important physiological processes during the seedling stage.

Ultrasonic treatment for 5 to 30 minutes had no significant effects on rice plant growth. This occurs because the high temperature after ultrasonic treatment increases kinetic energy and causes protein molecules to move or vibrate very rapidly (Estiasih, 2016). This process can disrupt nematode cell integrity and therefore can be used as a control method for the organism (Zhang *et al*., 2007). The ultrasonic mechanism for eliminating the nematode *A. besseyi* in rice seeds is based on the principle of cavitation, namely the formation and implosion of microbubbles due to high-frequency ultrasonic waves (40 kHz) in a liquid medium. When seeds are immersed in water and exposed to ultrasonic waves, cavitation bubbles form. The microbursts of these bubbles generate very high local pressures and temperatures, which can damage the structure of the nematode cuticle layer.

Ultrasonic treatment can produce localized increases in surface temperature due to the rapid vibrations and cavitation effects generated during sonication. These microthermal hotspots, combined with strong mechanical shear forces, can disrupt the structural integrity of nematode tissues. As this process continues, the surrounding water temperature can also increase, creating thermal stress that further weakens the nematode's physiological stability. Consequently, both the mechanical action and the thermal effects of ultrasound can work synergistically to induce nematode cell lysis. Microscopic observations suggest possible structural alterations in the nematode cuticle after ultrasonic treatment; however, further examination with higher magnification or additional techniques is required to confirm cuticle damage. This combined effect helps explain the significant decrease in nematode viability observed after ultrasonic exposure.

Rice seed ultrasonic treatment can cause damage or lysis to the cuticle of the nematode *A. besseyi*. Nematode external structures were damaged because of the ultrasonic treatment. Some nematodes appeared lysed, indicating that the treatment caused extensive damage to the nematode's body. This effect demonstrates the potential of ultrasonics as a method for controlling nematode populations, especially when used with appropriate frequency and duration (Kurniawati, 2024).

CONCLUSION

The use of ultrasonic waves at a frequency of 40 kHz can eliminate nematode *A. besseyi* in rice seeds. The effectiveness of ultrasonic treatment in eliminating *A. besseyi* can reach up to 100% and reduce white tip disease incidence to 0% with an exposure time of 25 to 30 minutes. The use of ultrasonic treatment can eliminate *A. besseyi* without reducing the viability of rice seeds.

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