Detection and identification of *Aphelenchoides fragariae* nematodes on shallot bulbs in Bogor, West Java, Brebes Central Java, and Nganjuk, East Java

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Abstract. Shallot is one of the most important horticultural commodities in Indonesia. *Aphelenchoides fragariae* is the major parasitic nematode of shallots and is listed as a quarantine pest with limited distribution. The nematode was detected on shallot bulbs at several traditional markets in Bogor but has not been reported present on shallot plantation. This research aims to detect and identify *A. fragariae* on shallot bulbs. Shallot bulbs were sampled from Bogor (West Java), Brebes (Central Java), and Nganjuk (East Java). Morphological identification was carried out by following the standard taxonomic for the *Aphelenchoides* genus (*Aphelenchoidea*). Morphometric indications were based on de Man's formula. The result showed that *A. fragariae* was found on shallot bulbs from Bogor, Brebes, and Nganjuk. Female of *A. fragariae* has a slender body, and off-set lip characteristic distinguishes it from other nematodes. The stylet was invisible, but the metacorpus (median bulb) was visible and full rounded, Tail elongate conoid with a single mucro. Morphometric: female, n = 20, L = 312.2 – 550.5, a = 20.0 – 33.9, b = 7.0 – 12.0, c = 3.9 – 15.2, V = 34.4 – 84.4, T = 28.8 – 121.3, and stylet 6.8 -12.0. Male, n = 20, L = 263.6 – 550.5, a = 21.1 – 37.8, b = 7.0 – 14.4, c = 8.4 – 17.1, T = 28.8 – 121.3, and stylet 7.6 -12.5.

1. Introduction
Shallot is one of the necessary horticultural commodities both economically and in nutritional content [1]. In Indonesia, shallot need is very high, with an average weekly consumption of 52.89 g / capita [2]. Shallot production in 2018 is 1 503 438 tons. Nematodes are worm-like microfauna with an average body length of 300 - 1000 μm. Several species of nematodes are reported to be important parasites of shallots, one of them is *Aphelenchoides fragariae*. These nematodes are classified as Quarantine Pests category A2, based on the Indonesian Minister of Agriculture Regulation Number 31/Permentan/ KR.010/7/2018 dated July 20, 2018. *A. fragariae* has been reported in limited areas in West Java, Banten, and DKI Jakarta on sambiloto plants with symptoms of brown or black leaf spots that are bordered by leaf veins and followed by leaf fall [3]. In addition to sambiloto, *A. fragariae* is also found in shallots bulbs on the market TU Kemang Bogor [4]. In the United States, symptoms caused by *A. fragariae* in strawberries include abnormal plant growth with
stunting and deformation of buds, leaves, and flowers accompanied by blotches or leaf blight bordered by leaf veins [5]. In Indonesia, there is no report of *A. fragariae* in horticultural commodities in the field. However, it has been detected in shallot bulbs in several traditional markets in the Bogor area [4]. This study aims to detect and identify *Aphelenchoides* species in shallot bulbs from production centres in Brebes, Central Java, and Nganjuk, East Java-based on morphological and morphometric characters.

2. Methods

The research was conducted at the Plant Nematology Laboratory, Department of Plant Protection, Faculty of Agriculture, IPB.

2.1. Sampling

Shallot samples were taken at TU Market Bogor (West Java) (shallot bulbs) and shallot plantation consisting of bulbs and plants in Brebes, Central Java, and Nganjuk, East Java. Sampling was carried out purposively, such as selecting samples based on specific criteria for symptoms of *A. fragariae*, such as shallot bulbs, which are low quality, dirty and dull. Samples of shallots obtained were stored in a sample box for further processing.

2.2. Extraction of nematode

Extraction of the nematode was carried out using a mist chamber method [6] and a 20°C cold water immersion. Shallots infected nematodes were excised into small pieces and placed on a coarse sieve, then put on a funnel. At the bottom of the funnel is placed plastic cups to collect the suspension of nematodes. Bulb samples were incubated for four days (96 hours) in a dark room at 20° C. Suspension of nematode that collected in plastic cups filtered using a 100 mesh and 400 mesh sieve then stored in a collection bottle. Furthermore, the nematode extraction was also carried out with cold water immersion at 20°C for 3 hours.

2.3. Nematode slide preparation

Permanent nematode slides were prepared according to the Ryss method [7]. Preparation of fixative nematode using fixative solution FA 4:1 (formalin 4% and acetic acid 1%). The composition of 4:1 FA consists of formalin (10.8 formaldehyde), 1 ml of acetic acid, and distilled water up to 100 ml. Fixation solution FA 4:1 is added with 1000 μl nematode suspension in collection tube afterwards incubated for 1 hour in ± 85 °C water bath and let stand for 2 hours. Glycerol is added to nematode in three-time dilutions. In the glass object that has been printed with a paraffin ring, add three drops of distilled water, two drops of glycerol, and ± 150 μl of nematode suspension and store for 12 hours. Nematodes are picked and transferred to a glass slide that has been dripped with 20 μl of glycerol. Slides containing nematodes are covered with a cover glass and sealed using clear nail polish. After that, the nematode slides are given an identity to facilitate storage and observation. Nematode slides were observed under a compound microscope with a magnification of 100 - 400 times. A total of 20 slides were prepared for identification purposes. The slide contains 3-5 female and male nematodes.

2.4. Identification of nematode

Observations were made mainly on the key morphological characters of female *Aphelenchoides fragariae*, which are body shape, body length and width, lip region, stylet, stylet length, metacorpus, vulva position, and tail tip. Specific morphological characters for male observed are body shape, spicules, tail tip, and the presence of males in a population. The existence of males in a population is one factor that can support the determination of nematode species [8]. Identification of morphometric characters was carried out based on de Man's formula/indices, which are body length (L), vulva position (V), tail, stylet length, and ratio values.
Morphometric measurements were carried out using an OLYMPUS BX51 binocular microscope that has been calibrated using a micrometre.

3. Results and discussion

3.1. Results

Locations of shallots sample are in Bogor, West Java, Brebes, Central Java, and Nganjuk, East Java. Brebes and Nganjuk are shallots producing centres. For more detail in figure 2. *A. fragariae* was found on the bulbs sampled from shallot fields in Larangan, Brebes. Infected shallots show several symptoms, such as smaller size, curl leaves, and faintness, for more detail in figure 1. In Nganjuk, *A. fragariae* was found on shallot leaves.

A low population of *A. fragariae* was found in Nganjuk and Brebes. On the other hand, a high population of *A. fragariae* was found on shallot bulbs marketed in Bogor. *A. fragariae* lives an optimum at 18 °C, while the soil temperature in Larangan District, Brebes 29°C - 31°C and air temperature 31°C with relative humidity 65%. Soil temperature in Nganjuk 28°C - 32°C.

Figure 1. Shallot field: a. Brebes, b. Nganjuk.

Figure 2. Symptoms of infection *A. fragariae* on Shallot crop field; a-b: Brebes, Central Java, c-d: Nganjuk, East Java (personal documentation).
3.1.1. **Morphology A. fragariae**
The female body of *A. fragariae* is worm-like, slender with lip region protrude, and almost continuous with body contour. The stylet of *A. fragariae* is slender, small, and thin (figure 3). Posterior oesophagus overlapping intestine dorsolaterally. Metacorpus (*median bulb*) is visible with well developed and fully rounded. Vulva appears to be split and slightly protruding. Relatively long post vulval uterine sac (PUS) taking more than half of the vulva-anus distance. The tail looks like a sharp cone, elongated and pointed like blunt spines. Males *A. fragariae* have a slimmer body than females with rose-thorn shape spicules. Tail elongate conoid with a single mucro at the tail tip the male tail arcuate from 45° to 90° when relaxed (figure 4).

![Figure 3. Aphelenchoides fragariae female.](image)

![Figure 4. Aphelenchoides fragariae male.](image)

3.1.2. **Morphometry A. fragariae**
Based on de Man's formula, the measurements of *A. fragariae* are as follows: Female: $n = 20$, body length ($L$) 312.2 - 550.5 (385.7 ± 119.1), stylet 6.8 - 12.0 (8.8 ± 7.4), tail length ($T$) 28.8 - 121.3 (64.4 ± 46.3), distance from anterior to vulva ($V$) 34.4 - 84.4 (70.34 ± 25.0). Value ration in females obtained: $a = 20.0 -$
33.9 (25.9 ± 7.0), b = 7.0 - 12.0 (9.2 ± 2.5), b ' = 3.2 - 7.2 (4.5 ± 2.0), c = 3.9 - 15.2 (7.0 ± 5.7) c ' = 2.5 - 17.2 (8.8 ± 2.6) (Table 1).

Male : n = 20, body length (L) = 263.6 - 550.6 (399.87 ± 63.53), stylet = 7.6 - 12.5 (9.31 ± 1.4), tail length (T) = 19.9 - 56.5 (34.9 ± 9.5), spicules = 8.9 - 22.6 (15.88 ± 31.1), testis length = 141.6 - 256.3 (206.5 ± 31.1). Value ratio in males were obtained: a = 21.1 - 37.8 (27.8 ± 4.6), b = 7.0 - 14.4 (9.7 ± 1.9), b ' = 4.5 - 6.6 (4.5 ± 0.8), c = 8.4 - 17.1 (12.0 ± 2.6) , c ' = 1.8 - 5.2 (3.49 ± 0.9) (table 1)

Table 1. Comparison of the morphometric characters of Aphelenchoides fragariae from shallot bulbs with literature.

| Morphological Characters | Helianthus tuberosus and Weigela subsessilis | Fern | Shallot |
|--------------------------|------------------------------------------|------|--------|
| L (μm)                   | (n = 7 ♀) 620 – 895                       | (n = 25 ♀) 525-685✈ | (n = 20 ♀) 263.6 - 550.5 |
|                          | (n = 7 ♂) 480 – 623                       | (n = 24 ♂) 435 - 562 | (n = 20 ♂) 263.6 - 550.6 |
| TD                       | TD                                      | (597 ± 43)✈ | (493 ± 37) |
| a                        | -                                       | 385.7 ± 119.1 | 399.87 ± 63.53 |
| b                        | -                                       | 25.9 ± 7.0   | 27.8 ± 4.66 |
| b'                       | -                                       | 7.0 - 12     | 7.0 - 14.4  |
| c                        | -                                       | 141.6 - 256.3 | 206.5 ± 31.1 |
| c'                       | -                                       | 34.9 ± 9.5   | 34.9 ± 9.5  |
| Stylet                   | -                                       | 8 - 11       | 6.8 - 12.0  |
| Tail (μm)                | -                                       | 9            | 9.31 ± 1.4  |
| % vulva                  | -                                       | 8.8 ± 2.6    | 19.9 - 56.5 |
| Spicules                 | -                                       | 16.9 - 19    | 8.9 - 22.6  |
| Testis                   | -                                       | 204 – 289    | 141.6 - 154.3 |

3.2. Discussion
Symptoms of disease by A. fragariae nematodes on shallots in Brebes are not clear. This is due to the presence of more major pests and other diseases. In a low population, A. fragariae attacks often do not cause symptoms [9]. A. fragariae infected shallots are smaller in size and are underdeveloped or incompletely
filled. Infection caused by *A. fragariae* causes leaf spot, leaf discoloration, brownish leaf tissue, which can be necrotic and cause leaf death in *Helianthus tuberosus* and *Weigela subsessilis* [10].

In Indonesia, *A. fragariae* is found in sambiloto leaf [3]. Sambiloto leaves are indigenous host *A. fragariae* and not introduced from outside. Sambiloto infected *A. fragariae* infects specific symptoms: necrotic spots that expand then turn brown or black and sometimes purplish, limited by leaf vein [3]. On further attacks, infected leaves will fall, and the plants grow to languish. Apart from sambiloto, the host plants of *A. fragariae* include babadotan (*Ageratum conyzoides*), pulus Hayam (*Acalypha lanceolata*), calincing (*Oxalis sepium*), *Borreria* sp., *Lindernia* (*Lindernia sp.*), and fern (*Pleocnemia* sp.), which were found around the planting of sambiloto. Symptoms that appear slightly but show chlorotic symptoms of yellow to necrotic brown bordered by leaf vein. The leaves of the infected weeds by *A. fragariae* were extracted, and the babadotan leaf samples were the highest population of leaf blight nematodes (22,320 nematodes per sick plant. This suggests that babadotan is an excellent alternative host for *A. fragariae* [3].

*A. fragariae* can survive without a host for no more than three months and survive a little longer in plant parts buried in the soil. In Sambiloto nurseries with soil media containing leaf pieces infected with leaf blight, symptoms will appear for about 14 days starting from the lowest leaves close to the soil surface. Favourable conditions such as humid weather can support the development of *A. fragariae*. Sources of transmission of leaf blight in sambiloto can come from organic manure and sick leaves found in nurseries [3].

*A. fragariae* development is influenced by temperature and relative humidity. Life cycle *A. fragariae* 10-11 days at 18°C. On the 4th day, the eggs hatch, and the juveniles mature in 6-7 days; about 32 eggs are laid by a single female [11]. According to [12], the highest population of *A. fragariae* was taken from soil temperature in the polyhouse -0.5 °C to 0 °C. Polyhouse may provide a more favourable microclimate for the life cycle of *A. fragariae*. Jagdale and Grewal also report that *A. fragariae* could tolerate temperatures as low as −80°C. Furthermore, RH influenced nematode migration from soil to leaves. The higher numbers of live *A. fragariae* on leaves and petioles at 100% than at 90% RH, suggesting that free moisture is required for the survival and movement of nematodes. Wallace [13] reported that wet weather conditions or thin films of water were necessary to move and migrate *A. ritzemabosi* to leaves of chrysanthemum.

Morphometric measurements, when associated with the literature [10, 14], vary widely. These varying results can be caused by several factors: environmental factors in which nematodes are located and host diversity factor [15]. Furthermore, *Betula pendula* and *Anemone hupehensis* indicate that morphometric differences, especially in male body length. Males isolated from *B. pendula* had a longer body length [16]. The *Ditylenchus destructor* Thorne, 1945 survey, observed that parameters such as the body width and length, pharynx, and stylet length are dependent on the host plant [17]. Similar results were obtained by El-Sherif [18] concerning *A. sacchari* and *A. rutgersi*. *A. fragariae* was found in bulb and shallots in Brebes and Nganjuk with a characteristic large median bulb, full, male tail with spicules like a rose thorn, and a single mucro. The morphometric results vary but are still in accordance with the literature [10, 14]. According to [4], *A. fragariae* was found in shallots bulbs on the market TU Kemang, Bogor.

4. Conclusion
*Aphelenchoides fragariae* was detected from shallot bulbs sampled from a market in Bogor-West Java, field samples of shallot bulbs from Brebes, Central Java, and field shallot leaves from Nganjuk, East Java. The nematodes were identified based on the morphological and morphometric characters.

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