# ARTIGOS

# Inoculation methods, aggressiveness of isolates and resistance of peach palm progenies to *Phytophthora palmivora*

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## ABSTRACT

Fuzitani, E.J.; Santos, A.F.; Damatto Junior, E.R.; Nomura, E.S.; Kalil Filho, A.N. Inoculation methods, aggressiveness of isolates and resistance of peach palm progenies to *Phytophthora palmivora*. ***Summa Phytopathologica,*** v.44, n.3, p.213-217, 2018.

Stem base rot (SBR) caused by *Phytophthora palmivora* is the main disease affecting peach palm in the nursery and in the field. This study aimed to: a) determine the most appropriate method for *P. palmivora* inoculation in peach palm; b) assess the aggressiveness of *P. palmivora* isolates; c) evaluate the resistance of peach palm progenies to *P. palmivora*. Experiments were carried out at "Embrapa Florestas" from March 2011 to March 2012. A descriptive rating scale was developed to assess SBR severity in peach palm. The disease severity was evaluated 7, 14, 21 and 28 days after inoculation. The conclusions were: a) injection at the stem base was the most effective method for *P. palmivora* inoculation; b) SA-5, SA-9, SA-10, SA-11, SA-14, SA-15, SA-16, SA-30 and SA-31 were the most aggressive *P. palmivora* isolates; and c) peach palm progenies T2, T4, T7, T9 and T15 showed resistance to *P. palmivora*.

**Keywords**: *Bactris gasipaes*, palm heart, phytopathology.

## RESUMO

Fuzitani, E.J.; Santos, A.F.; Damatto Junior, E.R.; Nomura, E.S.; Kalil Filho, A.N.Métodos de inoculação, agressividade de isolados e resistência de progênies de pupunheira à *Phytophthora palmivora*. ***Summa Phytopathologica,*** v.44, n.3, p.213-217, 2018.

Podridão da Base do Estipe (PBE) causada por *Phytophthora palmivora* é a principal doença da pupunheira no viveiro e no campo. O objetivo deste trabalho foi: a) determinar o método mais apropriado de inoculação de *P. palmivora* em pupunheira; b) avaliar a agressividade de isolados de *P. palmivora*; c) avaliar a resistência de progênies de pupunheira à *P. palmivora*. Os experimentos foram conduzidos na Embrapa Florestas no período de março de 2011 a março de 2012. Foi desenvolvida uma escala descritiva de notas para avaliar a severidade da PBE da pupunheira. A severidade da doença foi avaliada aos 7, 14, 21 e 28 dias após a inoculação. Concluiu-se que: a) O método de injeção na base do estipe foi o mais efetivo para a inoculação de *P. palmivora*; b) Os isolados de *P. palmivora*: SA-5, SA-9, SA-10, SA-11, SA-14, SA-15, SA-16, SA-30 e SA-31 foram os mais agressivos; e c) As progênies de pupunheiras T2, T4, T7, T9 e T15 mostraram resistência à *P. palmivora*.

**Palavras-chave**: *Bactris gasipaes*, palmito, fitopatologia

Peach palm (*Bactris gasipaes* Kunth. var. *gasipaes* Henderson) is a forest species native to the tropical regions of the Americas (Honduras, Bolivia, Guiana, Ecuador, Mexico and some Caribbean islands). In Brazil, peach palm is native to the Amazon basin covering the states of Acre, Amazonas, Pará, Maranhão, Amapá, Roraima, Rondônia and northern Mato Grosso (11). Peach palm plants have high growth rate and are renowned for developing offshoots; they are grown extensively as a perennial crop in São Paulo State (1), especially in the Ribeira Valley region (2). The peach palm has a large number of advantages like early yield, since its first harvest occurs within two years, and continued production, due to tiller formation, while for other palm species the first harvest can occur only after five years (10).

The expansion of peach palm cultivation for palm heart production, the lack of information and the inadequate crop management has furthered the occurrence of several plant problems both in the nurseries and in the field (15). Stem base rot (SBR), caused by *Phytophthora palmivora,* frequently attacks young and old peach palm plants and is initially characterized by wilting and yellowing of the first open leaf and flag leaf (5). Then, the leaves undergo necrosis and drying, which cause the clump to die. Darkening of the internal tissues, generalized rotting and longitudinal and transversal cuts along the peach palm stem are observed (5). In nurseries, the disease is favored due to an excess of humidity from irrigation water, while in the field, the pathogen can be favored due to an excess of humidity from the soil (16).

There is scarce information about this pathosystem: peach palm x *P. palmivora.* Thus, the aims of the current study were: a) to determine the most appropriate method for inoculating *P. palmivora* in peach palm seedlings; b) to evaluate the aggressiveness of *P. palmivora* isolates in peach palm; and c) to evaluate the resistance of different peach palm progenies to *P. palmivora*.

## MATERIAL AND METHODS

The experiment was carried out at the laboratory of "Embrapa Florestas" and its greenhouse in Colombo, Paraná State, Brazil. Eight-month-old peach palm plants, 30 cm high, showing 3-4 pairs of leaves, were grown in black polyethylene bags (11 cm high and 7 cm wide) containing commercial substrate. The plants were kept in a protected environment irrigated according to their needs, at average temperature of 26°C.

*P. palmivora* isolates were obtained from the Forest Pathology Laboratory Collection – "Embrapa Florestas" and were kept in glasses containing penicillin and mineral oil, preserved at 18°C. To obtain the zoospore suspension, pure *P. palmivora* cultures were subcultured to carrot agar medium (CA) and incubated in BOD chambers under constant light, at 24°C, for seven days. At the end of this period, 6 mL sterile distilled water was added to each Petri dish, which was placed in the refrigerator for 30 min. Then, the colonies were removed from the refrigerator and kept in the laboratory environment, at room temperature, for 30 min, for zoospore release. The suspensions obtained from each plate were transferred to a beaker, and a sample was separated to determine the concentration by using a hemocytometer.

To evaluate SBR severity in the young peach palm plants and compare external and internal symptoms in their stem, 20 young plants were injected at the stem base with a 0.1-mL aliquot of a suspension of 2.2 x $10^6$ zoospores of SA-16 isolate/mL (8). Twenty seedlings were inoculated at the stem base with a 0.1-mL aliquot of sterile distilled water, while other 20 young plants did not receive inoculation or water. The external symptoms of the disease were evaluated based on the descriptive scale (8) (Figure 1), while the internal symptoms were evaluated by transversally cutting the stem according to the methodology developed in the current study, which estimates the area colonized by the pathogen as percentages (zero, 25, 50, 75 and 100%). Internal and external symptoms were evaluated at 5, 10, 15 and 20 days after inoculation. The treatment consisted of inoculated and non-inoculated young plants, in five evaluation periods, arranged in randomized blocks with 10 replicates of each young plant.

To determine the best method for inoculating *P. palmivora* in young peach palm plants, the following methods were compared: Method I - Replacing a hard bark disc by a culture medium disc containing *P. palmivora* mycelium: Mycelium discs of *P. palmivora* oomycetes, in active growth, were cultivated in CA for seven days and inoculated in young peach palm plants. A metal puncher (3 mm diameter) was used to remove bark discs from the stem base, which were subsequently replaced by mycelium pathogen discs. The inoculated area was immediately wrapped with adhesive tape. Young plants that received only culture medium served as control (15). Method II - Immersing root ends in *P. palmivora* zoospore suspension: Six-month-old seedlings showing 10 cm height and 2-3 pairs of leaves were placed in 49-cell polyethylene trays (cell dimensions: 5 cm high, 3.5 x 3.5 cm) and cultivated in pine bark substrate. The seedlings were removed from the trays for root end snipping and returned to the trays, which were immediately placed in plastic boxes where root ends (about 1 cm) were immersed in a suspension of 2.5 x $10^6$ zoospores of SA-15 isolate/mL for one hour. The root ends of other plants were immersed in sterile distilled water, serving as control (8). Method III - Injecting *P. palmivora* zoospore suspension at the stem base: This method consisted in injecting a 0.1-ml aliquot of a suspension of 2.9 x $10^6$ *P. palmivora* zoospores/mL in incisions made at the stem base of peach palm seedlings. A disposable syringe with a hypodermic needle (1.2 x 40.0 mm) was used. Control consisted in injecting a 0.1-mL aliquot of sterile distilled water (8).

The seedlings were kept in a greenhouse at average temperature of 26°C (amplitude of 21 to 31°C) and the disease severity was assessed by using the scale described by Fuzitani et al. (9). Treatments consisting in these three inoculation methods were assessed four times, at 7, 14, 21 and 28 days after inoculation, arranged in a completely randomized design, following a 3 x 4 factorial scheme (methods x evaluation periods) with 10 replicates for each seedling.

To test the aggressiveness of *P. palmivora* isolates, a 0.1-mL aliquot of a suspension of 5.5 x $10^6$ zoospores/mL was injected at the stem base of the peach palm seedling (8). Inocula were obtained by using the previously described methodology and the following isolates: SP, SA-5, SA-9, SA-10, SA-11, SA-12, SA-14, SA-15, SA-16, SA17, SA-30 and SA-31. Treatments consisted of 12 isolates at four evaluation periods, 7, 14, 21 and 28 days after inoculation, arranged in a completely randomized design, following the 12 x 4 factorial scheme (isolates x evaluation times) with 10 replicates for each seedling.

In this experiment, eighteen peach palm progenies were evaluated for their resistance to *P. palmivora*. The progenies were derived from Putumayo race (large fruit), which were collected from Benjamin Constant in Amazonas State and underwent two generations of selection for vigor and absence of thorns (16). Twelve-month-old seedlings were inoculated with a 0.1-mL aliquot of a suspension of 5.5 x $10^7$ zoospores of SA-16 isolate/mL. The seedlings were kept in a greenhouse at average temperature of 26°C, and severity assessments were done by using the described scale (8). The treatments consisted of 18 peach palm progenies and four evaluation periods, at 7, 14, 21 and 28 days after inoculation, arranged in a completely randomized design, following the 18 x 4 factorial scheme (progenies x evaluation time) with 10 replicates of one seedling each.

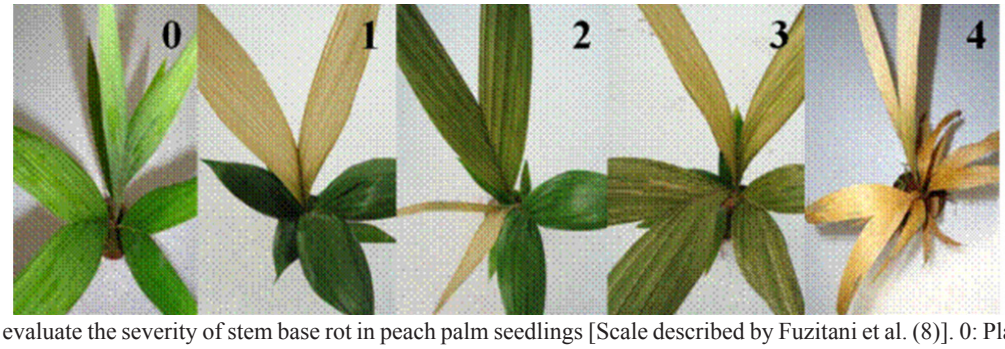


**Figure 1**. Scale to evaluate the severity of stem base rot in peach palm seedlings [Scale described by Fuzitani et al. (8)]. 0: Plant without symptom; 1: Plant with flag leaf and/or first open leaf wilted or yellowed; 2: Plant with flag leaf, first and second leaves wilted or yellowed; 3: Plant with wilted or yellowed leaves; 4: Dead Plant.

Data were subjected to analysis of variance, according to F test, and means of the inoculation methods were compared. The isolates and progenies were compared according to Scott Knott test at 5% probability, using the SISVAR program (7). The program from CanaSis Belan project was used to calculate the area under the disease progress curve (AUDPC).

## RESULTS AND DISCUSSION

Plants inoculated with *P. palmivora* zoospore suspension showed evolution of symptoms, which can be seen in Figure 2, highlighting the comparison between external and internal SBR symptoms at zero, 5, 10, 15 and 20 days after inoculation. External symptoms were characterized by wilting and yellowing of the flag leaf and the first open leaf. During this same period, internal symptoms reached 50% of the damaged tissue area from the stem base. At 10 and 15 days, evolution of external symptoms and internal symptoms reached 75% of the area at the stem base. Finally, the death of seedlings was noted, while the internal tissue showed 100% of the area colonized by *P. palmivora* oomycete.

Seedlings that were injected only with water showed darkening of the stem internal tissue due to tissue oxidation in the area that was injured by the needle during injection, without showing external symptoms of the disease.

Evaluating the inoculation methods, method I, which consisted in substituting a hard bark disc for culture medium containing mycelium, showed that some plants had symptoms characterized by wilting and yellowing of the flag leaf or the first open leaf from the first evaluation to the seventh day. At 21 and 28 days, severity reached an average level of 2.5 and 3.5, respectively, causing the death of the seedling (Figure 3). In this method, death was verified for 15% of the treated control plants.

Although *Phytophthora* seedling inoculation techniques have been reported for different plant species like the papaya tree (15), pineapple

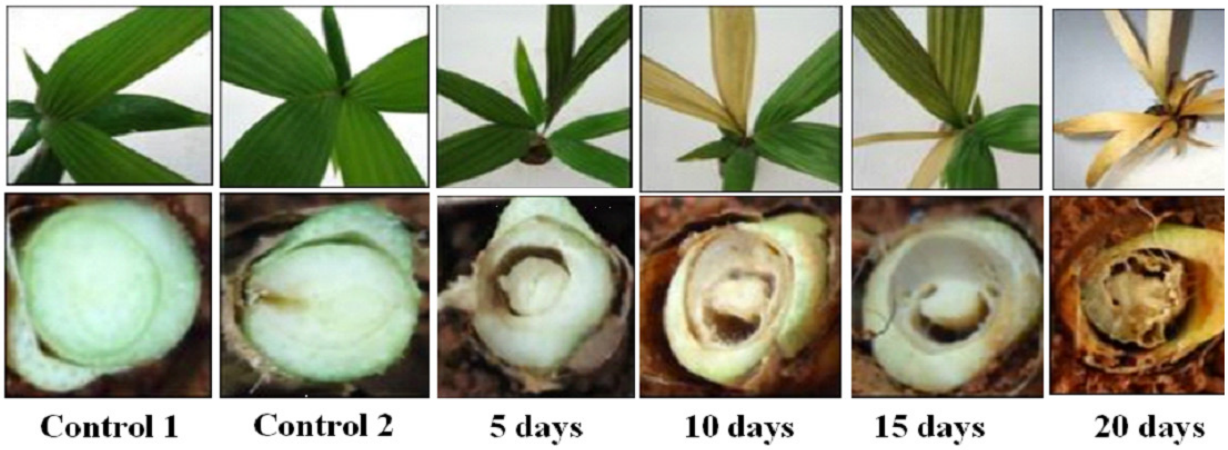


**Figure 2**. External (upper) and internal (lower) symptoms of SBR in young peach palm plants inoculated with *Phytophthora palmivora* zoospore suspension at 5, 10, 15 and 20 days after inoculation.

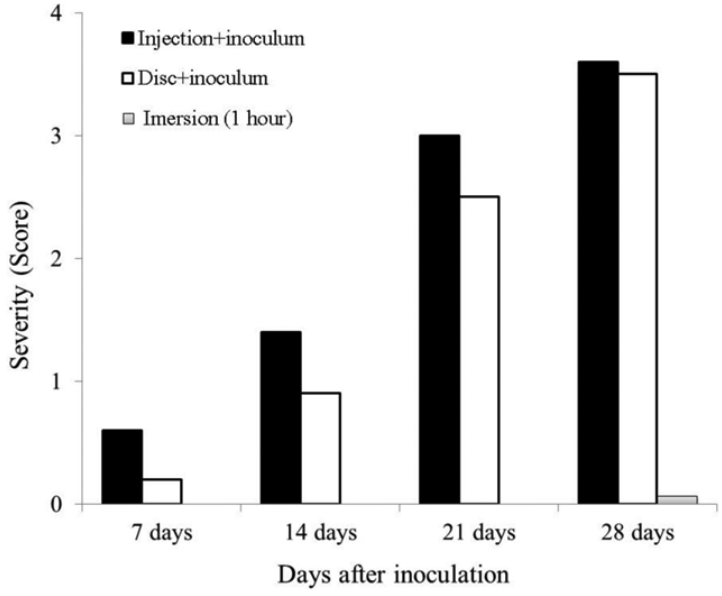


**Figure 3**. Severity of stem base rot in young peach palm plants inoculated with *P. palmivora* by means of zoospore injection, mycelium disc and root end immersion in a zoospore suspension at 7, 14, 21 and 28 days after inoculation. Stem base rot was scored based on a scale from 0 (plant without symptom) to 4 (dead plant) developed by Fuzitani et al. (8).

plant (18) and citrus (4, 17), only Alves et al. (3) and Pizzinatto et al. (13) worked with peach palm using the method of substituting a hard bark disc for culture medium disc containing mycelium. For young citrus plants, the bark substitution method was satisfactory during tests conducted in a greenhouse by Siviero et al. (17). In the present study, however, this method was considered drastic because the injury caused by the puncture at the seedling's stem base caused the death of internal tissues, including the stem of the control treatment.

In method II, in which the root ends were immersed in a suspension of *P. palmivora* zoospores, no symptoms were noted in the plant until the 21st day after inoculation. However, at day 28, the first severity symptoms became visible in the inoculated plants, reaching a level lower than 1 on the descriptive scale. *Phytophthora* species, as well as the other oomycetes, need water for sporulation and movement of zoospores (12). In the present study, only the root apex area were inoculated, requiring more time for the stem base colonization and consequently for the appearance of symptoms of the disease. This method is closest to conditions of natural infection (12, 15).

In method III, in which a *P. palmivora* zoospore suspension was injected at the stem base, symptoms characteristic of the disease were noted, from wilting and yellowing of the first open leaf to the seedling death during all evaluation phases. The internal region of the stem base, where the inoculation occurred, corresponds to the preferential tissue for *P. palmivora* colonization, best simulating the conditions of natural infection, while control plants showed no external symptoms, except for slight darkening around the area injured by the injection needle.

According to Costa et al. (6), the method of injecting *Colletotrichum graminicola* suspension in corn stock proved to be difficult for standardizing the quantity of inoculum to be injected. However, for peach palm, this method was proven faster to yield results and less aggressive, causing less damage to the plant tissue; it was also operationally easier compared to the other tested methods. This is why it was chosen for testing the aggressiveness of isolates, the concentration of zoospores and the resistance of peach palm progenies to *P. palmivora*.

At 28 days after inoculation, the isolates SA-12, SA-17 and SA-SP were significantly less aggressive, compared to the other isolates (Table 1). Note that the areas under the disease progression curve (AUDPC) relating to these isolates, which are 23.90, 21.75, and 29.95, were smaller than those for the other isolates. Regarding SBR incidence, these isolates presented 80, 90 and 90% seedlings showing symptoms of the disease, respectively. Therefore, in relation to the other treatments that reached 100% incidence, the isolates considered most aggressive were: SA-5, SA-9, SA-10, SA-11, SA-14, SA-15, SA-16, SA-30 and SA-31.

The pathogenicity of *P. palmivora* in peach palm has already been shown by Pizzinatto et al. (13) and Santos et al. (14), highlighting differences in the aggressiveness of *P. palmivora* isolates. This behavior explains the SBR severity variations in plants and peach palm clumps, in which the disease evolution is transferred from the mother plant to the offshoots (13).

At 21 days after inoculation, progenies T2 and T7 significantly differed from the others, showing less disease severity (Table 2). At 28 days, progenies T4, T9 and T15 also showed less disease severity. Regarding the area under the disease progress curve, the lowest values were found for progenies T2 (31.1) and T7 (33.0). As no study has been done on the resistance of peach palm to *P. palmivora,* there is a possibility for progenies to acquire resistance to *P. palmivora.*

**Table 1**. Incidence, severity and area under the disease progress curve (AUDPC) for stem base rot in peach palm seedlings inoculated with 12 *Phytophthora palmivora* isolates and evaluated at 7, 14, 21 and 28 days after inoculation.

| Isolates | Severity (Score) | | | | | | | | Mean | | AUDPC | Incidence |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 7 days | | 14 days | | 21 days | | 28 days | | | | | (%) |
| SA-30 | 0.7 | a A | 1.8 | a B | 3.0 | b C | 3.8 | b D | 1.9 | B | 31.5 | 100 |
| SA-31 | 0.8 | a A | 1.7 | a B | 3.0 | b C | 3.5 | b C | 2.2 | C | 36.5 | 100 |
| SA-10 | 0.5 | a A | 1.5 | a B | 2.5 | a C | 3.4 | b D | 2.0 | B | 31.0 | 100 |
| SA-11 | 0.4 | a A | 1.5 | a B | 2.5 | a C | 3.8 | b D | 2.0 | B | 31.4 | 100 |
| SA-12 | 0.2 | a A | 1.1 | a B | 2.0 | a C | 2.9 | a D | 1.5 | A | 23.9 | 80 |
| SA-14 | 0.6 | a A | 1.7 | a B | 3.2 | b C | 3.8 | b C | 2.3 | C | 36.6 | 100 |
| SA-16 | 0.8 | a A | 1.8 | a B | 3.2 | b C | 3.5 | b C | 2.3 | C | 37.0 | 100 |
| SA-17 | 0.4 | a A | 1.0 | a A | 2.2 | a B | 3.0 | a B | 1.6 | A | 21.7 | 90 |
| SA-05 | 0.5 | a A | 1.7 | a B | 3.1 | b C | 3.9 | b D | 2.3 | C | 36.1 | 100 |
| SA-09 | 0.6 | a A | 1.4 | a B | 2.7 | a C | 3.6 | b D | 2.1 | B | 32.0 | 100 |
| SA-15 | 0.7 | a A | 1.5 | a A | 3.4 | b B | 3.6 | b B | 2.3 | C | 36.3 | 100 |
| SA-SP | 0.8 | a A | 1.6 | a B | 2.2 | a C | 3.1 | a C | 1.9 | B | 29.9 | 90 |
| Mean | 0.6 | A | 1.5 | B | 2.7 | C | 3.5 | D | | | | |
| **CV (%)** | **33.7** | | | | | | | | | | | |

Means followed by the same uppercase letter on a row or lowercase letter in a column do not differ statistically according to Scott-Knot test (P=0.05). Stem base rot was scored based on a scale from 0 (plant without symptom) to 4 (dead plant) developed by Fuzitani et al. (10).

**Table 2**. Incidence, severity and area under the disease progress curve (AUDPC) for stem base rot in peach palm progenies inoculated with zoospores of SA-16 isolate of *P. palmivora* and evaluated at 7, 14, 21 and 28 days after inoculation.

| Progenies | Severity (Score) | | | | | | | | Mean | | AUDPC | Incidence |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 7 days | | 14 days | | 21 days | | 28 days | | | | | (%) |
| T1 | 1.0 | a A | 2.5 | b B | 2.6 | b B | 3.8 | b C | 2.5 | c | 39.2 | 100 |
| T2 | 1.0 | a A | 1.7 | a B | 2.1 | a B | 3.3 | a C | 2.0 | a | 31.1 | 90 |
| T3 | 0.9 | a A | 2.5 | b B | 2.9 | c B | 3.8 | b B | 2.5 | c | 40.4 | 100 |
| T4 | 0.8 | a A | 2.1 | a B | 3.0 | c C | 3.5 | a D | 2.4 | b | 37.7 | 100 |
| T5 | 1.0 | a A | 2.6 | b B | 2.8 | c B | 4.0 | b C | 2.6 | c | 41.3 | 100 |
| T6 | 0.6 | a A | 2.1 | a B | 3.0 | c C | 4.0 | b D | 2.4 | b | 38.3 | 100 |
| T7 | 1.0 | a A | 2.0 | a B | 2.2 | a B | 3.2 | a C | 2.1 | a | 33.0 | 100 |
| T8 | 1.5 | b A | 3.0 | c B | 3.0 | c b | 4.0 | b C | 2.9 | d | 46.0 | 100 |
| T9 | 1.8 | c A | 2.2 | a B | 2.5 | b B | 3.5 | a C | 2.5 | c | 38.7 | 100 |
| T10 | 1.2 | a A | 2.7 | b B | 2.8 | c B | 3.8 | b C | 2.6 | c | 41.9 | 100 |
| T11 | 2.0 | c A | 2.5 | b B | 2.8 | c B | 4.0 | b C | 2.8 | d | 43.7 | 100 |
| T12 | 1.4 | b A | 2.8 | c B | 3.0 | c B | 4.0 | b C | 2.8 | d | 44.6 | 100 |
| T13 | 1.9 | c A | 2.6 | b B | 2.9 | c B | 3.8 | b C | 2.8 | d | 44.0 | 100 |
| T14 | 1.3 | b A | 2.5 | b B | 3.0 | c C | 4.0 | b D | 2.7 | c | 42.6 | 100 |
| T15 | 1.4 | b A | 2.6 | b B | 2.8 | c B | 3.6 | a C | 2.6 | c | 41.5 | 100 |
| T16 | 1.0 | a A | 3.0 | c B | 3.0 | c B | 3.7 | b C | 2.8 | c | 43.7 | 100 |
| T17 | 1.5 | b A | 3.0 | c B | 2.9 | c B | 4.0 | b B | 2.8 | d | 45.5 | 100 |
| T18 | 1.3 | b A | 2.4 | c B | 2.9 | c C | 4.0 | b D | 2.6 | c | 41.6 | 100 |
| Mean | 1.3 | A | 2.5 | B | 2.8 | C | 3.8 | D | | | | |
| CV (%) | 17.7 | | | | | | | | | | | |

Means followed by the same uppercase letter on a row or lowercase letter in a column do not differ statistically according to the Scott-Knot test (P=0.05). Stem base rot was scored based on a scale from 0 (plant without symptom) to 4 (dead plant) developed by Fuzitani et al. (10).

The method of injecting a zoospore suspension at the stem base of peach palm seedling was the most efficient and operational method for studying the epidemiology of stem base rot.

*Phytophthora palmivora* isolates SA-5, SA-9, SA-10, SA-11, SA-14, SA-15, SA-16, SA-30 and SA-31 were most aggressive for peach palm seedlings.

Peach palm progenies T2, T4, T7, T9 and T15 show resistance to *Phytophthora palmivora*.

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