

**FIRST REPORT OF *PHYTOPHTHORA NICOTIANAE*
ON *SALVIA FRUTICOSA* AND REACTIONS OF SOME
VARIETIES**

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Abstract

Wilting and dieback symptoms were observed in *Salvia fruticosa* Mill (Anatolian sage) in commercial production fields in Antalya province in Türkiye in 2021. When the infected areas were examined, symptoms of root and crown root rot, yellowing, wilting, curling of the leaves, besides complete wilting and dieback of plants in severe infections were observed. Isolations were performed from root and crown root of infected plants using selective mediums, and were identified based on cultural and morphological features and rDNA sequences of ITS region. As a result of isolations and identification studies, it was determined that the causative agent of the disease was *Phytophthora nicotianae*. Pathogenicity of *P. nicotianae* was tested by soil inoculation on *Salvia fruticosa* seedlings. Two months after inoculation, yellowing of leaves, general wilting, shortening of hairy roots, serious reductions in root density and death of some plants were detected in plants in this study. As a result of variety reaction studies, significant root density and weight loss were observed in Turgut and Karik varieties. Root density and weight loss were less in Uysal than in the other varieties. This study is the first record that *P. nicotianae* causes disease in *Salvia fruticosa*.

Key words: dieback, *Phytophthora nicotianae*, variety reaction, *Salvia fruticosa*

Introduction. *Salvia* is the largest genus of plants in the sage family Lamiaceae, with nearly 1000 species in the world, mostly in America, Asia and the

Mediterranean [1]. There are 97 *Salvia* species, four subspecies and eight varieties in Türkiye, which is one of the countries where sage is collected the most in the world. Fifty-one of these species are endemic [2]. Among these species, *Salvia fruticosa* Mill (Anatolian sage) is a shrub-like perennial species belonging to the genus *Salvia*, from the dicotyledon class (Magnoliopsida), the Lamiaceae family. It spreads naturally in Italy, Sicily, the Balkans, Cyrenaica and western Syria, and in the Western Aegean, Northwestern and Western Mediterranean regions in Türkiye [3]. *Salvia fruticosa* is a plant that can grow between 0–1350 m a.s.l., spreads in maquis or phrygana ecosystems and islands, can reach a height of 160 cm, and blooms in March-May. The leaves are whitish gray to silvery in colour and hairy in the young development stage. Next to the main leaves, there are lateral leaflets that are more or less developed on one or both sides [4]. A light yellow or colourless oil called ‘apple oil’ is obtained from the leaves of the plant. The reason why it is called apple oil is the presence of galls (gal) at the ends of some branches of the plant, which look like apples and are about 2–3 mm long, 2 mm wide and thick [4, 5]. This species, which has been used by collecting from flora for many years, has an important place both in the domestic market and in the foreign market [5]. *Salvia fruticosa* is a medically and economically important species of the genus *Salvia*, which is well known in Anatolia (the Asian part of Türkiye).

Anatolian sage and the other *Salvia* species have been prepared as tea, ointment, tincture or extract in traditional folk medicine since ancient times, as a pain reliever, expectorant, carminative, sedative, antiperspirant, externally wound healing. These medicinal plants are used in the treatment of colds, bronchitis, tuberculosis, menstrual disorders and stomach ailments. In recent years, because of its beneficial properties such as healing headache, rheumatism, epilepsy, hepatitis, insomnia, diarrhea, cough, gastritis, respiratory diseases, lung diseases, spleen diseases, nervous diseases, some skin diseases, cardiovascular diseases, cholesterol-lowering, diuretic, antispasmodic properties, it is also a plant that is the centre of attention [6]. *Salvia fruticosa*, apart from its medicinal use, is used in aromatherapy, perfumery and cosmetics, as a natural insect repellent, as a spice in fish, meat, soups and stews; as a flavouring in pickles, cheese, beverages, and as an antioxidant in processed foods [5]. Anatolian sage (*Salvia fruticosa*) and large-flowered sage (*Salvia tomentosa*) species constitute a large part of sage export in Türkiye [7].

Phytophthora genus, as a member of Oomycetes, is an important fungi group causing severe disease in agricultural and forest plants. Some species of this genus have been reported to cause disease in fruit, vegetables and forest trees in Türkiye [8–10]. There is only one study that *Phytophthora cryptoea* causes disease in *Salvia officinalis* in Türkiye [11]. *Phytophthora cryptoea* causes root and crown rot in *Salvia officinalis* and *Salvia leucantha* [12]. There is no record of *Phytophthora nicotianae* Breda de Haan causing disease in *Salvia* species. This study is the first to reveal that *P. nicotianae* causes disease in *Salvia fruticosa*.

Being a soil-borne pathogen, wild agent and having wide host range, *Phytophthora* species cannot be suppressed easily. Negative side effects of chemical control have been increasing on human health and the environment, and chemical control methods are more expensive and laborious [13]. In addition, chemical control is not recommended for medicinal and aromatic plants consumed as oil, extract and tea in many areas. Therefore, finding of resistant varieties against *Phytophthora* disease will make a significant contribution to sage growers.

In this study the definition of the agent *P. nicotianae* isolated for the first time from a sage (*Salvia*) plant in Türkiye and the reactions of the most produced varieties in Türkiye against the disease caused by *P. nicotianae* were investigated.

Materials and methods. Sampling and isolation of fungi. Leaf chlorosis, root rot, wilt and dieback in commercial *Salvia fruticosa* fields were observed in 2021 in Antalya province in Türkiye. Diseased plant samples were taken from 9 different fields. Necrotic roots were washed in running tap water and air-dried, plant parts 2–4 mm long were taken from infected tissues in diseased roots, and crown roots and plated onto semi selective medium (PARP) [14] without superficial disinfestation for *Phytophthora* isolation. Plates were incubated at 22 °C in the dark and developing hyphae were observed after 3–4 days under a microscope. Small pieces were taken from the hyphae tips of the developing colonies and transferred to carrot juice agar (CA) and V8 fruit juice agar and pure cultures were obtained.

Identification. All isolates were cultured on CA (Carrot juice agar), CMA (Corn meal agar), MEA (malt extract agar), PDA (potato dextrose agar) and V8 juice agar, and their structures such as hyphae, chlamydospore and sporangium were examined and they were diagnosed classically, taking into account the diagnostic keys [15]. Two isolates were also identified through molecular analysis. When half of the surface of the V8 and CA juice agar was covered with mycelia, agar disks with a diameter of 4–5 mm were taken from the growing tips and placed in Petri dishes with a diameter of 6 cm to which 7 mL of rainwater was added to ensure the formation of sporangia. After 5 days of incubation, the ability of the isolates to proliferate at 35 °C was determined in CA and PDA.

In molecular identification. Internal transcribed spacer (ITS) regions of rDNA of two *Phytophthora* isolates were amplified using the universal primer pairs ITS 4 and ITS 6 [16]. DNA isolations were made by using Blood and Tissue Kit (QIAGEN), according to the instructions for use. Each PCR reaction contained 3 µL of template DNA, 1 µL of each primer and 12 µL Taq DNA Polymerase 2× Master Mix (Ampliqon). The PCR reaction mix was adjusted to a final volume of 25 µL with ultrapure water. PCR amplifications were carried out on the ABI Veriti (Applied Biosystem) thermal cycler. The following cycling protocols were used: an initial denaturing of 94° for 3 min; 35 cycles of 94° for 30 s, annealing at 58.5° for 1 min, an extension at 72° for 45 s; and final extension of 72° for 7 min [17]. PCR products were analyzed by electrophoresis on a 2% agarose gel

prepared with Pronosafe (Conda, Spain) DNA dye and visualized under UV transilluminator. Sequence analyses were performed by BM Lab (Ankara, Türkiye). Then Blast analyses of two isolates were made using these sequences on NCBI (<http://www.ncbi.nlm.nih.gov>) to find the closest matches. The sequences of the two isolates were registered to GenBank.

Pathogenicity test. *Salvia fruticosa* Mill. (Anatolian sage) was propagated clonally by tip cuttings. Prepared cuttings of approximately 10 cm in length were treated with powdered NAA and rooted in a 1:1 ratio of peat + perlite. Cuttings rooted in about one month were transplanted into 0.2 L pots. For inoculum preparation wheat seeds were soaked in water for two days and filtered. It was then filled into flasks and autoclaved for two consecutive days. It was then inoculated with agar discs from 4–5 day old cultures (HA) and incubated in the dark at 20–22°. After three weeks, the trial was set up after *P. nicotianae* had completely covered the wheat seeds. While five plants were inoculated with *P. nicotianae*, five plants were used as controls. Five pots of 0.5 L were used. Four percent inoculum was added to the peat + perlite (3:1) mixture and the plants were planted in this mix. After a month the pots were kept in water for two days. In the evaluation of pathogenicity test, the root weights of infected plants were compared with the root weights of control plants [17]. Symptomatic tissues were taken from the margin of the lesions and placed on PARP to re-isolate the pathogen. Root weights were subjected to analysis of variance (ANOVA), based on a completely randomized design, and means separated using the LSD Test.

Reaction of varieties study. In the variety reaction study, the reactions of Karık, Turgut and Uysal Anatolian sage varieties against *P. nicotianae* were tested. Three-month-old seedlings were used in the study. The varieties were obtained from the Western Mediterranean Agricultural Research Institute in Antalya, Türkiye. Ten seedlings, five with inoculum and five without inoculum, were used for each variety in the experiments. The inoculum was developed on wheat seeds as in pathogenicity. It was added to the 4% peat + perlite mixture (3:1, v/v) at a rate of 4% and filled in 2-liter pots. Plants were incubated for 4 months. Then the plants were removed and dry and fresh weights were measured. For dry weight measurement, their fringe roots were cut, dried in an oven at 65 °C for one day and their weights were measured. As a result of the study, the root weights of infected plants for each variety in both fresh and dry roots were compared with the root weights of uninfected plants, and the percentages of reduction in roots were calculated according to the formula [18] as in pathogenicity. Re-isolation was made as in pathogenicity.

Percent reduction in root weight (wt) = $\frac{[(\text{Uninfected root wt}) - (\text{infected root wt})]}{[\text{Uninfected root wt}]} \times 100$.

Statistical analysis. After angle transformation of the data, results were subjected to analysis of variance (ANOVA), based on a completely randomized design, and means separated using the Tukey Test. Statistical analysis was carried out using JUMP 10.0 (SAS Institute Cary, NC, USA).

Results. Isolation and identification. Eleven *Phytophthora* isolates were obtained from necrotic root tissues of symptomatic plants taken from nine commercial Anatolian sage areas in Antalya province. Firstly, isolates were identified on the basis of colony colour, morphology, mycelial characteristics, and dimensions of sporangia. The isolated fungal colonies showed white and cottony growth (Fig. 1). As a result of cultural and morphological characteristics of the isolates, they were identified as *Phytophthora nicotianae* (Table 1). Maximum temperature growth of isolates was $> 35^{\circ}\text{C}$. Identifications of the isolates were also confirmed with molecular analysis using DNA ITS sequences.

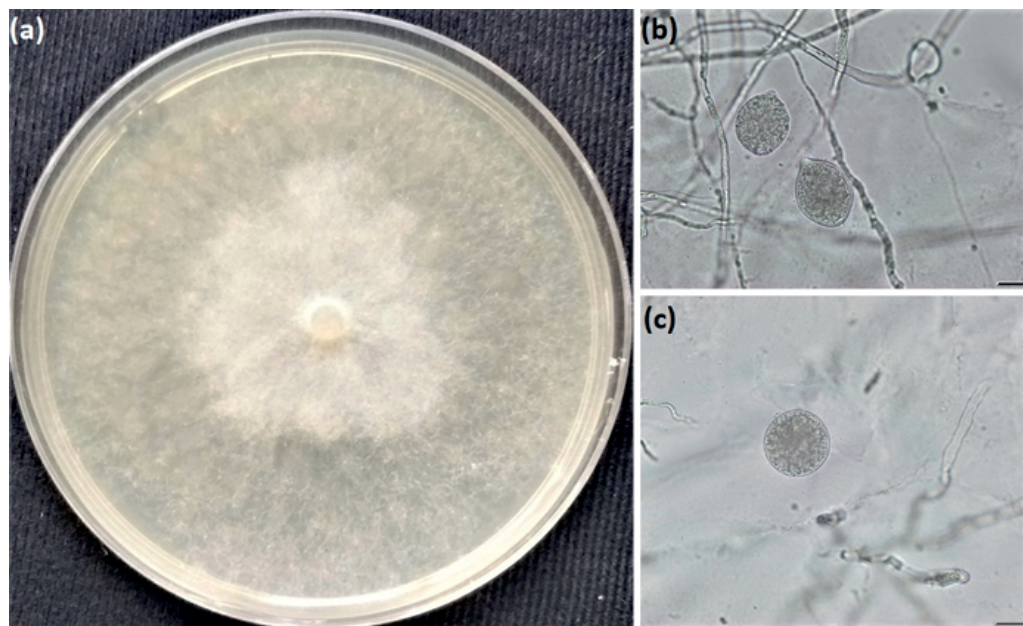


Fig. 1. Undistinctive colony pattern on V8A (a); caducous sporangia with short pedicel (b); and chlamydospore (c) of *Phytophthora nicotianae*. Bar = 20 μm

Sequences of two isolates in this study were registered to GenBank with their OR512082 and OR512083 Accession numbers. As a result of the blast analysis performed in NCBI, the isolate OR512082 was 100% similar to the isolate MG570058.1, and the isolate OR512083 was 100% similar to the isolate MT472132.1 in the GenBank.

Pathogenicity test. As a result of the pathogenicity study, *P. nicotianae* isolates caused severe root and crown root lesions as well as serious reductions in root density and weight in sage plants. The symptoms of wilting and dieback were observed on the leaves and the whole plant in the above-soil parts of the plants (Fig. 2). Root weights of control plants were significantly different from those inoculated by *P. nicotianae*. Compared to control plants, the percentage of root weight reduction in diseased roots averaged 77.78%.

T a b l e 1

Cultural and morphological characteristics of the isolates of
Phytophthora nicotianae obtained from sage roots in Antalya, Türkiye

	<i>P. nicotianae</i>
Sexuality	Heterothallic
Sporangia	
Shape	Usually ovoid and obpyriform, sometimes almost spherical
Papillae	Papillate, sometimes with double papillae
Length/breadth ratio	1.4
Length/breadth mean (µm)	56.6 × 40.2
Total range (µm)	49.0–66.2 × 33.3–46.6
Internal proliferation and nesting	No
Caducity	Sometimes caducous with short pedicel
Hyphal swellings	No
Chlamydospore	Yes
Sporangium base	Rounded
Lateral attachment	Yes
Colony pattern	Not distinctive

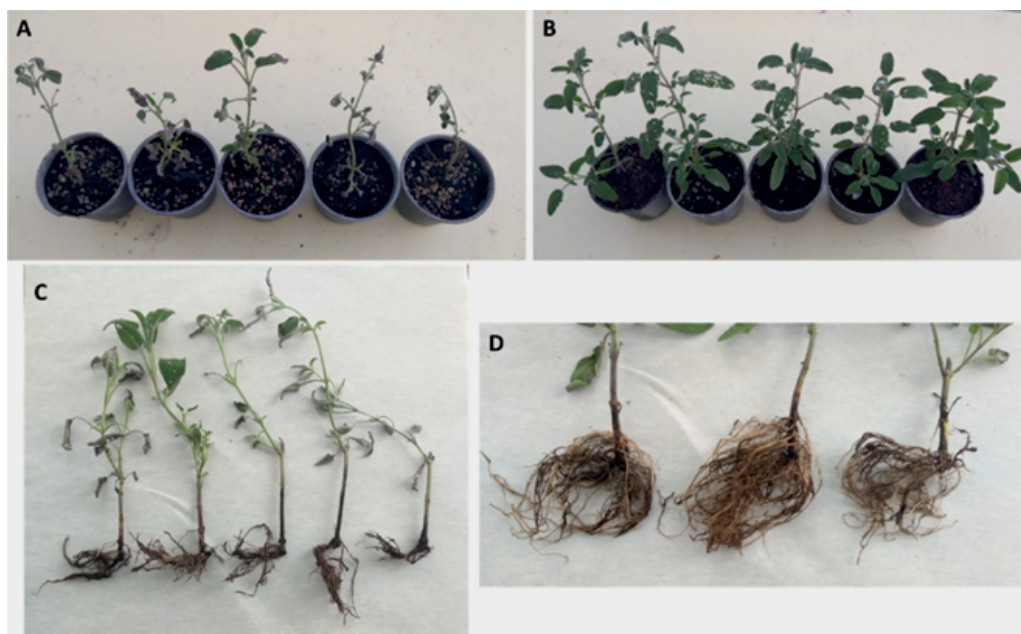


Fig. 2. Images from pathogenicity study with *Salvia fruticosa*: A – infected plants in pots; B – uninfected plants in pots; C – infected roots and crown roots; D – uninfected roots and crown roots

Reaction of varieties study. In the study, comparing infected with uninfected plants, reductions were observed in root weights of all three cultivars, but the highest loss was in Turgut variety and it was determined as the most sensitive cultivar (Fig. 3).



Fig. 3. Images from the reaction studies of three different varieties of *Salvia fruticosa* against *P. nicotianae*: A – Karık variety – infected; B – Karık variety – uninfected (control); C – Turgut variety –infected; D – Turgut variety – uninfected (control); E – Uysal variety – infected; F – Uysal variety – uninfected (control)

Considering the fresh weights, root weight loss in Turgut variety took the first place with an average of 67.09% and was determined as the most sensitive variety against *P. nicotianae*. This was followed by Karık and Uysal varieties with root weight losses of 60.31% and 57.37%, respectively. Root weight losses in dry weights were also similar to fresh weights. The highest loss was determined in Turgut variety with 50.45%, the lowest loss was determined in Uysal variety with 42.14% (Table 2) .

Discussion. This study presents the first record of *P. nicotianae* causing root and crown root rot, wilt and dieback on *Salvia fruticosa* (Anatolia sage) in Antalya, Türkiye. There are only two studies on the diseases that cause problems in *Salvia* species in Türkiye. In a study conducted in Türkiye, sudden damping-off symptoms were observed in the plants of *Salvia officinalis* L. in Antalya and Izmir

T a b l e 2

Fresh and dry root weight averages of varieties inoculated
with *Phytophthora nicotianae*

Variety	Average percentage decrease in fresh root weight (%)		Average percentage decrease in dry root weight (%)	
Turgut	67.09	A*	50.45	A*
Karık	60.31	AB	47.88	AB
Uysal	57.37	B	42.14	B

*Means followed by the same letter are not statistically different
according to Tukey test ($P < 0.05$)

provinces and it was determined that the agent causing the disease was *P. crypto-gea* [11]. In the other study conducted in Türkiye, it was reported that *Fusarium oxysporum*, *F. solani*, *F. moniliforme* and *Rhizoctonia solani* caused collapse of *Salvia officinalis* (medicinal sage) [19]. It is seen that *P. nicotianae* causes more damage to cultivated plants in tropical and subtropical regions. Antalya is a province in the south of Türkiye, which has a coast to the Mediterranean Sea and has a tropical climate. It was determined that *P. nicotianae* caused serious damage to agricultural areas in this province. In a study conducted on pomegranate in Türkiye, *P. nicotianae* was isolated from necrosed plant tissues in the roots and from the soil around the roots of diseased trees. After the first symptoms of the pathogen such as yellowing, wilting and growth retardation in pomegranate plants, some plants started to dry in the following days [17]. Similarly, it was reported that 84 *Phytophthora* spp. obtained from tomato production areas with root and crown rot symptoms in Antalya, 80 of them belong to *P. nicotianae* and four to *P. capsisi* species [10]. The agent also causes damage to agricultural areas in countries with tropical climates such as India and Greece. It has also been reported to cause fruit rot in pomegranates [20]. When studies on *Salvia* species other than *S. fruticosa* were examined, it was reported that *Phytophthora crypto-gea* causes root and crown rot in *Salvia officinalis* and *Salvia leucantha* species [12]. There is no record of *Phytophthora nicotianae* causing disease in *Salvia* species.

As a result of pathogenicity studies, it was observed that the pathogen caused severe destruction and decay in the capillary roots of plants. As a result of these decays, serious reductions in root density and weight, yellowing and wilting of leaves and even death in some plants were observed in above-ground organs. Similarly, ÇAKIR et al. [11], stated that *P. cryptogena* caused necrosis on roots and sudden damping-off in *Salvia officinalis* plants. Because the fungus is a difficult agent to control, and the disadvantages of chemical control in medicinal and aromatic plants, it is important to develop and implement alternative control methods. In this study, the resistance of Karık, Turgut and Uysal varieties, which are widely commercially produced in Türkiye, against the fungus was tested and

it was determined that the Uysal variety was more resistant and promising against the disease than the other two varieties.

Conclusions. When studies conducted around the world on *P. nicotianae* are examined, it is determined that the agent generally causes problems in many cultivated plants in regions with tropical and subtropical climates. There are few studies on the presence and damage of *Phytophthora* species in *Salvia* species and other medicinal and aromatic plants; the data that exist are that they are detected in regions with hot and humid climates. Since the extracts and oils of medicinal and aromatic plants are used in many areas such as medicine, cosmetics, food and tea production, it is important to develop human health and environmentally friendly methods of combating diseases that cause product losses in this plant. Using resistant varieties is just one of them. It is necessary to increase studies on the presence of both *P. nicotianae* and other *Phytophthora* species in medicinal and aromatic plants, their harms and non-chemical control methods.

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