Mycologia Iranica 11(2): 2024 Original Article DOI:10.22043/MI.2024.367808.1294

Morphological and molecular characterization of *Neopestalotiopsis clavispora*, causing rose stem canker in Iran

M. Nourbakhsh
B. Sharifnabi ✉
Department of Plant Protection, College of Agriculture, Isfahan University of Technology, Isfahan

Abstract: Rose (*Rosa* spp.) is a widely cultivated perennial flowering plant grown in open fields and under controlled greenhouse conditions. This study focused on the isolation and characterization of a pathogen affecting *Rosa hybrida* plants in the Najafabad and Lenjan counties of Isfahan Province, Iran. Infected stems and crowns were collected from the greenhouses between July 2022 and September 2023. The cultivars affected were Samurai and Tanja. Fungal isolation was achieved, followed by morphological characterization and molecular identification by DNA sequencing of the internal transcribed spacer (ITS) regions, translation elongation factor 1-alpha (*TEF1-α*), and β-tubulin (*TUB*). Pathogenicity tests confirmed the ability of the isolate to induce stem canker and leaf spot symptoms in healthy Samurai and Tanja cultivars of rose (*R. hybrida*) plants, thereby fulfilling Koch's postulates. The isolates were identified as *Neopestalotiopsis clavispora* by the morphological and molecular characteristics. To our knowledge, this study reports the first identification of *N. clavispora* as a pathogen causing canker disease and dieback on roses in Iran, emphasizing the need for effective management strategies to protect rose health and mitigate economic losses in the ornamental horticultural sector.

Keywords: Plant disease, Phylogenetic analysis, Morphological identification, Rose stem canker, Pathogenicity.

INTRODUCTION

Roses (*Rosa* spp.) hold substantial economic value as ornamental plants, not just in cut flower production but also in garden cultivation and medicinal applications (Widrlechner, 1981). However, diseases such as stem canker and dieback pose significant threats to rose health. These conditions lead to lesions that can girdle the stem, resulting in wilting and browning of the upper foliage, ultimately affecting the terminal regions of the plant (Sweets, 1982). Currently, the fungal pathogens responsible for rose dieback and stem canker primarily include *Botryosphaeria dothidea* (Jia et al. 2019), *Diaporthe rosiphthora* (Caio et al. 2021), *Trichothecium roseum* (Wright et al. 2007), *Acremonium sclerotigenum* (Mirtalebi et al. 2016), and *Coniothyrium fuckelii* (Zaher et al. 2012). Additionally, emerging species from the genus *Neopestalotiopsis* (*Pestalotiopsidaceae*, *Sordariomycetes*), previously named as *Pestalotiopsis,* have been recognized for their role in causing canker and dieback in roses (Jiang et al. 2018). Steyaert (1949) proposed reclassification of the genus *Pestalotia*, breaking it into three genera: *Pestalotiopsis*, *Pestalotia*, and *Truncatella*. Furthermore, he subdivided *Pestalotiopsis* into four sections of *Monosetulatae*, *Bisetulatae*, *Trisetulatae*, and *Multisetulatae*, based on apical appendage characteristics. Guba (1961) further refined this classification by categorizing *Pestalotia* according to its conidial characteristics. Recent phylogenetic studies focusing on the 28S Large Subunit (LSU) nrRNA gene revealed three main monophyletic groups within *Pestalotiopsis*. This analysis led to the identification of two new genera: *Neopestalotiopsis* and *Pseudopestalotiopsis* (Maharachchikumbura et al. 2014). Morphologically, *Neopestalotiopsis* can be distinguished from the other genera by its unique median cell color and conidiophore structure (Maharachchikumbura et al. 2014).

One notable species, *N. clavispora*, has been associated with rose leaf blotch disease (Feng et al. 2014). It has also been reported to cause canker and twig dieback in southern highbush blueberries in Spain (Borrero et al. 2018).

In Iran, the authors noted the rising incidence of *Pestalotiopsis*-like fungi in different hosts, which they attributed to various species, including *P. trachycarpicola* on croton (Atashi Khalilabad & Fotouhifar 2022), *P. theae* on bananas (Ketabchi, 2014), *N. asiatica* on almonds (Ayoubi & Soleimani 2016a), *N. mesopotamica* and *N. iranensis* on strawberry (Ayoubi & Soleimani 2016b), *P. disseminata* on feijoa (Naeimi et al. 2015), and *P. biciliata* on Eucalyptus (Amirmijani et al. 2024). Mirabolfathi and Ershad (2004) conducted the first and only report of *Pestalotiopsis* sp. as a causal agent

Submitted 25 Nov. 2024, accepted for publication 15 Dec. 2024
✉ Corresponding Author: E-mail: sharifnabib@gmail.com

https://mij.areeo.ac.ir

of stem canker in Iranian roses. The fungal pathogens responsible for stem canker and dieback present significant challenges in horticulture. Understanding their characteristics, alongside developing effective management strategies, is crucial for maintaining rose health. Thus, this study aims to deepen our understanding of *N. clavispora*, particularly its association with stem canker in *Rosa* spp. in Iran through morphological comparisons and phylogenetic analysis.

Table 1. Strains and GenBank accession numbers were used for phylogenetic analysis in this study.

Species	isolates	GenBank Accession Numbers		
		ITS	*TUB2*	*TEF1α*
Neopestalotiopsis acrostichi	MFLUCC 17-1755*	MK764273	MK764339	MK764317
Neopestalotiopsis brachiata	MFLUCC 17-1555*	MK764274	MK764318	MK764340
Neopestalotiopsis chrysea	LSCKS81	OQ392362	OQ410711	OQ410712
Neopestalotiopsis clavispora	CBS_447.73*	KM199374	KM199443	KM199539
Neopestalotiopsis clavispora	RS-PC-67	MZ097377	MZ097380	MZ090098
Neopestalotiopsis clavispora	MZ097377	MZ090100	MZ097382	MZ097379
Neopestalotiopsis clavispora	**Na3**	**PQ586978**	**PQ606055**	**PQ606056**
Neopestalotiopsis cubana	CBS 600.96*	KM199347	KM199438	KM199521
Neopestalotiopsis ellipsospora	GZCC15-0085*	KU500017	KU500010	KU500013
Neopestalotiopsis formicarum	CBS 362.72*	KM199358	KM199455	KM199517
Neopestalotiopsis macadamiae	BRIP 63737c*	KX186604	KX186654	KX186627
Neopestalotiopsis musae	MFLUCC 15-0776*	KX789683	KX789686	KX789685
Neopestalotiopsis saprophytica	CBS_115452*	KM199345	KM199433	KM199538
Neopestalotiopsis sonneratae	MFLUCC 17-1744*	MK764279	MK764345	MK764323
Neopestalotiopsis zimbabwana	CBS 111495*	MW422813	KM199456	KM199545
Pestalotiopsis trachicarpicola	MFLUCC12-0266*	JX399002	JX399033	JX399066
Pseudopestalotiopsis cocos	CBS 27229*	MH855069	KM199467	KM199553

CBS: culture collection of the Centraalbureau voor Schimmelcultures, Fungal Biodiversity Center, Utrecht, The Netherlands; MFLUCC: Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; GZCC: Guizhou Provincial Culture Collection Center, Guizhou, China; BRIP: Queensland Plant Pathology Herbarium, Australia.

Ex-type strains are labeled with the superscript *. Isolate in boldface was sequenced in this study

MATERIALS AND METHODS

Sampling and fungal isolation

From July 2022 to September 2023, 40 samples of infected rose stems exhibiting symptoms of canker and dieback were collected from various greenhouses in the Najafabad and Lenjan counties of Isfahan Province, Iran. Approximately 0.5 cm segments of the stems, encompassing both healthy and diseased tissue, were excised using sterilized scalpels. The samples underwent sterilization by dipping them in 70% ethanol for 1 minute, followed by treatment with sodium hypochlorite (NaOCl) solution at a concentration of 5% for 1 minute, and were then rinsed with sterile distilled water. After surface sterilization, the tissue pieces were placed on potato dextrose agar (PDA) plates and incubated at 25 °C under a 12-hour light/dark cycle for five to seven days to promote fungal growth. To obtain pure cultures of the isolates, the hyphal tip method (Brown, 1924) was employed. A small portion of the actively growing mycelium was carefully collected from the edge of the colony and transferred to a new water agar (WA) plate. Once growth was established, single hyphal tips were subcultured onto fresh PDA plates to eliminate any remaining contaminants and ensure a pure isolate.

Morphological characterization

Morphological characteristics of the fungal colonies were examined following incubation. The colony morphology including color, diameter, texture, and growth pattern was meticulously recorded (Maharachchikumbura et al. 2014). Slide mounts were prepared with the fungal samples stained with lactic acid, and images were captured using a high-resolution BH2 Olympus light microscope equipped with a TrueChrome 4K Pro camera. This facilitated the assessment of morphological characteristics such as conidial size and the presence of apical and basal appendages. The captured images were subsequently imported into Mosaic v. 2 software (https://sios.net.au/software/mosaic-2 which enables precise measurements of various fungal structures. To ensure accuracy, measurements of conidia were obtained from at least 20 conidia per isolate.

DNA extraction, and PCR amplification

For molecular identification, genomic DNA was extracted from the fungal mycelia using the CTAB method, as described by Murray and Thompson (1980). The internal transcribed spacer (ITS) region of rDNA was amplified using ITS1 and ITS4 primers (White et al. 1990). EF1-728F and EF-2 primers were employed to amplify the partial sequence of the translation

elongation factor 1-alpha (*TEF*) gene (O'Donnell et al. 1998; Carbone & Kohn, 1999). The partial β-tubulin (*TUB*) gene was also amplified using the specific T1 and Bt2b primers (Glass & Donaldson, 1995; O'Donnell & Cigelnik, 1997). PCR reactions were performed in a total volume of 10 µl, containing 5 µl of 2× *Taq* master mix Red, 0.5 µl each of forward and reverse primers (10 pmol/µL), 3 µl of nuclease-free water, and 1 µL of genomic DNA (15 ng/µl). The thermal cycling conditions included an initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 52°C (ITS), 57°C (*TEF*), or 55°C (*TUB*) for 30 s, and extension at 72 °C for 1 min, with a final extension at 72 °C for 5 min (Weir et al. 2012; Maharachchikumbura et al. 2014). The PCR products were electrophoresed on 1% agarose gel and visualized under UV light using a Vilber Lourmat SSM-930 gel documentation system.

Fig. 1. The observed symptoms caused by the fungus *Neopestalotiopsis clavispora* in the rose greenhouses of Najafabad and Lenjan counties of Isfahan Province a, b. on the Samurai cultivar; c. on Tanja cultivar.

Sequencing, and phylogenetic analysis

The amplicons were directly sequenced by Pishgam Biotech Company using Sanger sequencing. The obtained sequences were edited using chromas 2.6.6 software (https://www.technelysium.com.au/chromas.html) and then compared with those available in the National Center for Biotechnology Information (NCBI) GenBank database using the Basic Local Alignment Search Tool (BLAST) to confirm the identity of the sequences.

The phylogenetic tree was constructed by incorporating the newly obtained sequences alongside those from ex-type specimens cited in earlier studies (Maharachchikumbura et al. 2014; Daengsuwan et al. 2021). For phylogenetic analysis, the sequences were aligned using Clustal W (Thompson et al. 1994). *Pseudopestalotiopsis cocos* (CBS 272.29) and *Pestalotiopsis trachicarpicola* (MFLUCC 12-0266) were used as outgroups (Table 1). A maximum likelihood approach was then employed to construct the phylogenetic tree, applying the General Time Reversible (GTR) model to account for nucleotide substitution rates in MEGA v. 6 (Tamura et al. 2013). Statistical support for the inferred relationships was assessed through bootstrap analysis with 1,000 replicates.

Pathogenicity test

To fulfill Koch's postulates, pathogenicity tests were conducted by inoculating healthy Samurai and Tanja cultivars of rose (*R. hybrida*) plants with isolated fungi. Conidial suspensions were prepared by collecting conidia from cultures grown on potato dextrose agar (PDA) for seven days and diluting them in sterile distilled water to achieve a concentration of 10^6 conidia/mL. Small wounds were created on the tissues of six, eight-week-old healthy rose plants, and 20 µL of conidial suspension was applied to the wounds on both stems and leaves. Two control plants were treated with sterile distilled water to account for external factors that affect plant health (Bhunjun et al. 2021). The inoculated plants were covered with plastic bags and maintained in a controlled environment with 16-hour light cycles and temperatures ranging from 20 to 25 °C. Over four weeks, symptoms were monitored, noting the emergence of stem canker, wilting, and leaf necrosis. Small pieces of necrotic tissue from the edge of each lesion were cut and placed on PDA. The pathogen was isolated from inoculated plants and compared with the primary recovered isolate to confirm Koch's postulates.

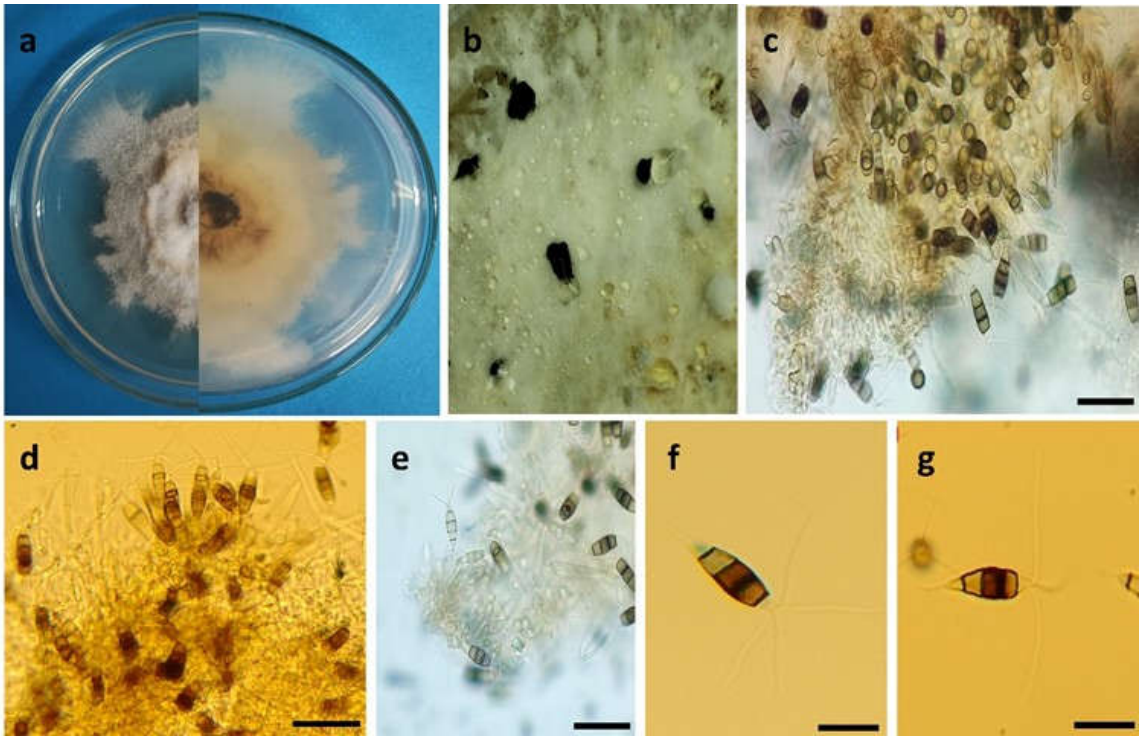

Fig. 2. Morphological characteristics of *Neopestalotiopsis clavispora*. a. Colony characteristics of fungi on PDA media; b. Conidiomata sporulating on PDA; c-e. Conidiogenous cells; f, g. Conidia. Scale bars: c-e = 100 µm; f, g = 10 µm.

RESULTS

Morphological characteristics and DNA sequence analysis

In this study, two isolates of *Neopestalotiopsis* (Na3 and Ln1) were identified as the causal agents of canker affecting the stems of rose varieties: Samurai (Ln1) and Tanja (Na3) (Fig. 1). The morphological characteristics (Fig. 2) led to the initial identification of the pathogen as belonging to the genus *Neopestalotiopsis* (Maharachchikumbura et al. 2014). Blast search and sequence analysis revealed a striking similarity, with over 99% sequence homology between isolate Na3 and multiple sequences of *Neopestalotiopsis clavispora*. The resulting phylogenetic tree displayed robust clustering of isolate Na3 with the type strain of *N. clavispora* (CBS_447.73), supported by a high bootstrap value of 100% (Fig. 3). The new sequences have been deposited in GenBank under the following accession numbers: PQ586978 (ITS), PQ606055 (*TUB*), and PQ606056 (*TEF1-α*).

Based on morphological and molecular characteristics the causal agent of rose canker obtained from single conidia was identified as *Neopestalotiopsis clavispora* and characterized as follows:

Neopestalotiopsis clavispora (G.F. Atk.) Maharachch., K.D. Hyde & Crous, stud. Mycol. 79: 138 (2014).

Cultures were incubated on potato dextrose agar (PDA) at 25 °C under a 12-hour day/night regime. Following incubation, the fungal colonies on PDA appeared white with irregular edges, exhibiting a wavy surface and masses of black conidiomata (Fig. 2a). Small black spots, identified as acervuli, were observed on the surface of the aerial mycelial layer (Fig. 2b). The whitish mycelia produced black, smooth, globular acervuli containing slimy spore masses. The colony diameter reached 70 ± 5 mm after a seven-day incubation period. Conidia were fusiform or clavate, either straight or slightly curved, consisting of five cells and four septa, measuring 18.4–27 × 6.3–8.2 µm. The three median cells were multicolored, featuring progressively darker walls; the second cell from the base was pale brown, the third cell was darker brown, and the fourth cell was the darkest. The basal cells were conical and hyaline,

Submitted 25 Nov. 2024, accepted for publication 15 Dec. 2024
✉ Corresponding Author: E-mail: sharifnabib@gmail.com

https://mij.areeo.ac.ir

each featuring a single hyaline appendage at the base, measuring 5.8–6.8 µm. The apical cells were hyaline and subcylindrical, bearing two or three hyaline appendages, and measuring 19.2–29.5 µm (Fig. 2f, g).

Pathogenicity test

Seven days post-inoculation, isolate Na3 exhibited symptoms including stem cankers and leaf spots. Over the following 14 days, these cankers and leaf spots progressed to a necrotic state, characterized by the formation of black globular acervuli filled with spore masses. In contrast, no visible symptoms were observed in the control stems and leaves (Fig. 4). Koch's postulates were fulfilled through the successful re-isolation and microscopic identification of *N. clavispora*.

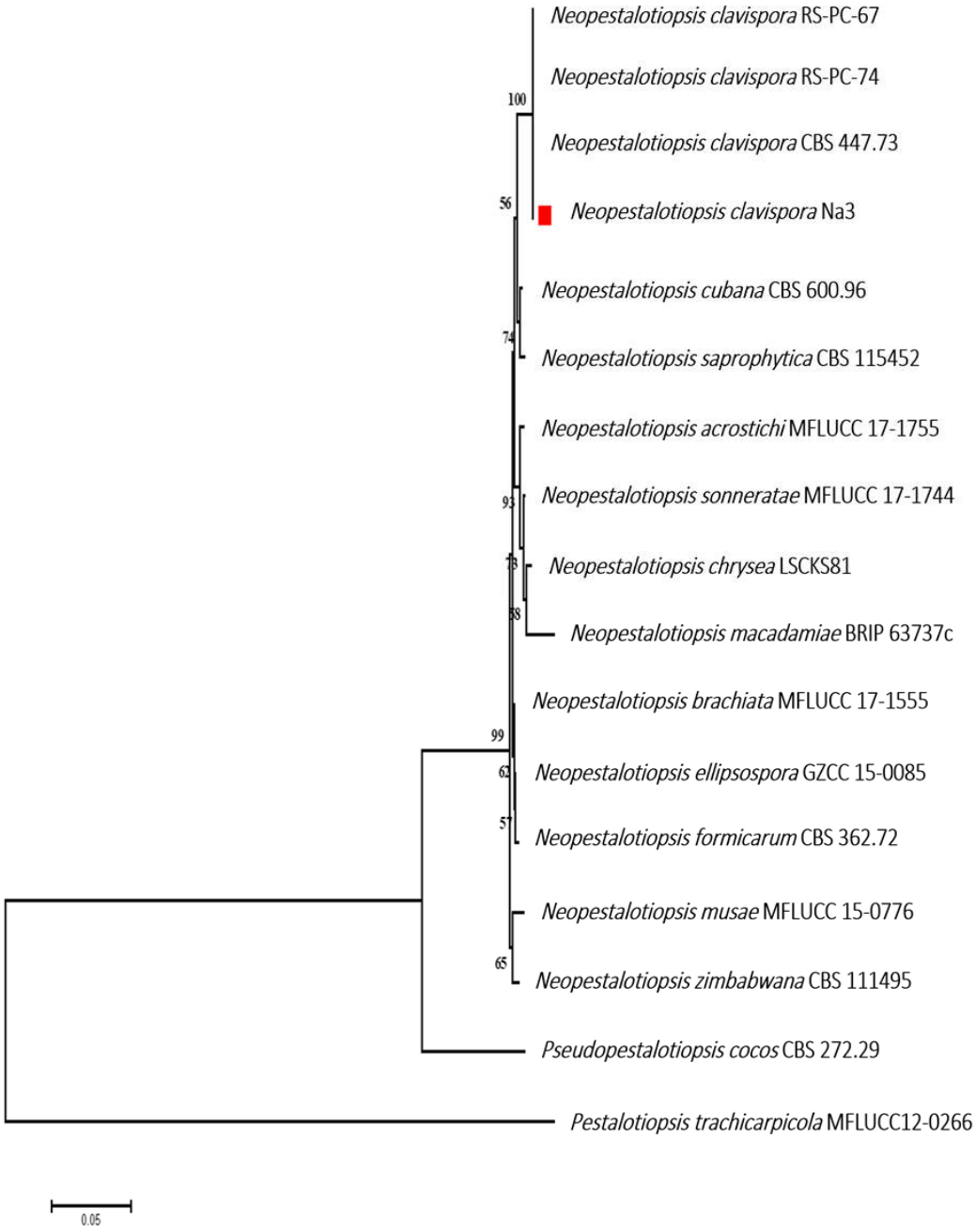

Fig. 3. Phylogram generated from maximum likelihood analysis based on combined ITS, *TEF1-α*, and *TUB* sequence alignments of 17 isolates of *Neopestalotiopsis*, *Pestalotiopsis*, and *Pseudopestalotiopsis*. Bootstrap values > 50% (1000 replicates) of the ML analysis were exhibited above/below the branches. The Iranian rose isolate is marked with a red square.

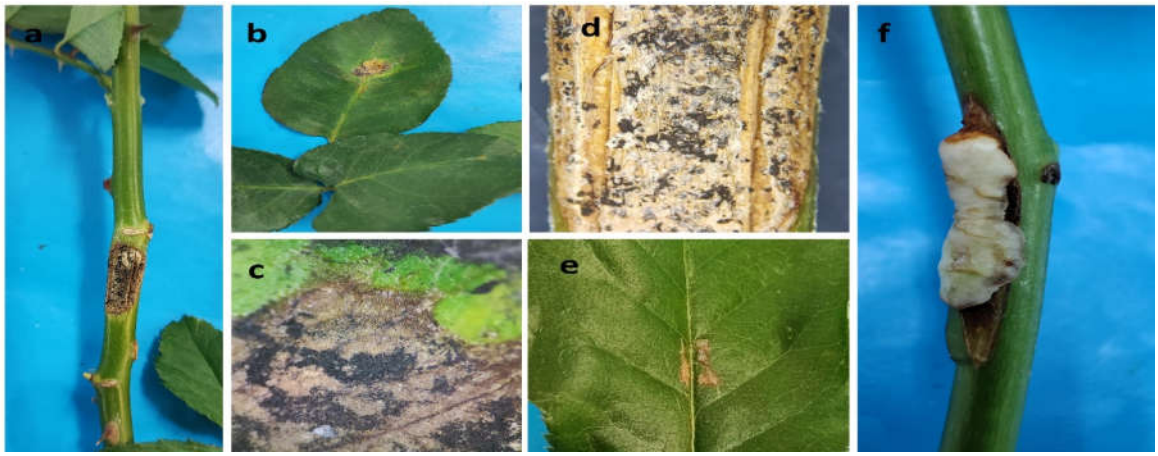

Fig. 4. Lesions of infection by *Neopestalotiopsis clavispora* (Na3) on the stems and leaves of Tanja varieties with small black acervuli at 10 days post-inoculation under the greenhouse condition (20 to 25 °C). (a, d) The stem surface symptoms were inoculated with a conidial suspension. (b, c) Leaf surface symptoms inoculated with a conidial suspension. (e, f) Control stems and leaves.

DISCUSSION

Through comprehensive morphological and molecular analyses, coupled with pathogenicity assessments, this study demonstrates that *N. clavispora* is associated with rose canker and dieback disease. To our knowledge, this is the first report of *N. clavispora* as a causative agent of rose canker in Iran. Another species of the genus *Neopestalotiopsis,* including *N. asiatica,* has been identified as the causal agent of leaf spot on sweet almond (*Prunus dulcis*) in Isfahan province, Iran (Ayoubi & Soleimani 2016a). In another study, a new species of *Neopestalotiopsis*, named *N. iranensis*, was isolated from rotted strawberry (*Fragaria ananassa*) fruits and lesions in Kurdistan province, Iran, characterized by distinct morphological features and confirmed through phylogenetic analysis, alongside the first report of *N. mesopotamica* as a pathogen of strawberries (Ayoubi & Soleimani 2016b). Morphologically, *Neopestalotiopsis* can be distinguished from *Pseudopestalotiopsis* and *Pestalotiopsis* by its distinctive versicolorous median cells. Additionally, *Neopestalotiopsis* feature is the indistinct conidiophores that are often reduced to conidiogenous cells. While these three genera share overlapping morphological traits, distinguishing them based solely on morphology can be challenging (Maharachchikumbura et al. 2014; Liu et al. 2017). *Neopestalotiopsis* spp. exhibit widespread distribution and an extensive host range (Reddy et al. 2016), typically initiating pathogenesis via insect vectors, wounds, or natural openings (Keith et al. 2006; Daengsuwan et al. 2020; Pornsuriya et al. 2020). Notably, *N. clavispora* has demonstrated a remarkable capacity to infect over 50 plant species across 27 families, inducing severe foliar diseases (Qiu et al. 2020). Its pathogenic effects extend to various plant hosts, manifesting as canker and twig dieback in blueberry (Borrero et al. 2018), flower blight in *Anthurium andraeanum* (Daengsuwan et al. 2020), root and crown rot in strawberry (Obregón et al. 2018), leaf spot in macadamia (Santos et al. 2019), and apple leaf spot (Shi et al. 2024). Furthermore, seven *Neopestalotiopsis* species: *N. clavispora*, *N. palmarum*, *N. rosicola*, *N. concentrica*, *N. subepidermalis*, *N. rosae*, and *N. versicolor,* have been implicated in leaf blotch and stem canker of roses (Feng et al. 2014; Maharachchikumbura et al. 2014; Jiang et al. 2018; Peng et al. 2022). *Neopestalotiopsis clavispora* is distinguishable from other *Neopestalotiopsis* species causing rose diseases through molecular analyses and morphological characteristics. Specifically, *N. clavispora* possesses longer and fewer tubular apical appendages compared to *N. rosicola* (19-32 µm vs 12-23 µm), differs from *N. subepidermalis* in having a smaller basal appendage (3-5.5 µm vs 7-7.5 µm), and exhibits larger conidia than *N. concentrica* (20-24×6.5-8.5 vs 14-18.5 ×4.5-5 µm) (Maharachchikumbura et al. 2014, Peng et al. 2022).

ACKNOWLEDGMENTS

The authors acknowledge the financial support of Isfahan University of Technology.

REFERENCES

Amirmijani, A., Fotouhifar, K., Atashi Khalilabd, A., and Seyedi, N. 2024. *Pestalotiopsis biciliata*, a new record for funga of Iran. Mycologia Iranica 11(1): 67-74.

Atashi Khalilabad, A., and Fotouhifar, K. B. 2022. Morphological and molecular characterization of a novel *Pestalotiopsis trachycarpicola*, causing Garden Croton leaf spot in Iran. Mycologia Iranica 9(1): 59-66.

Ayoubi, N. and Soleimani, M. J. 2016a. Morphological and molecular identification of *Neopestalotiopsis asiatica* causing leaf spot on sweet almond. Journal of Plant Pathology 98(2): 321–325.

Ayoubi, N. and Soleimani, M. J. 2016b. Strawberry fruit rot caused by *Neopestalotiopsis iranensis sp. nov*., and *N. mesopotamica*. Current Microbiology 72: 329-336.

Bhunjun, C. S., Phillips, A. J., Jayawardena, R. S., Promputtha, I. and Hyde, K. D. 2021. Importance of molecular data to identify fungal plant pathogens and guidelines for pathogenicity testing based on Koch's Postulates. Pathogens 10(9):1096.

Borrero, C., Castaño, R. and Avilés, M. 2018. First report of *Pestalotiopsis clavispora (Neopestalotiopsis clavispora)* causing canker and twig dieback on blueberry bushes in Spain. Plant Disease 102(6): 1178-1178.

Brown, W. 1924. A method of isolating single strains of fungi by cutting out a hyphal tip. Annals of Botany 38(150): 402-404.

Caio, P., Bruno, F., Carlos, A. P. and Robert, B. 2021. *Diaporthe rosiphthora* sp. nov.: Yet another rose dieback fungus. Crop Protection 139: 105365.

Carbone, I. and Kohn, L. M. 1999. A method for designing primer sets for speciation studies in filamentous ascomycetes. Mycologia 91(3): 553-556.

Daengsuwan, W., Wonglom, P. and Sunpapao, A. 2020. First report of *Lasiodiplodia theobromae* causing spadix rot in *Anthurium andraeanum*. Journal of Phytopathology 168(2): 129-133.

Daengsuwan, W., Wonglom, P., Arikit, S. and Sunpapao, A. 2021. Morphological and molecular identification of *Neopestalotiopsis clavispora* causing flower blight on *Anthurium andraeanum* in Thailand. Horticultural Plant Journal 7(6): 573-578.

Feng, Y. R., Liu, B. S. and Sun, B. B. 2014. First report of leaf blotch caused by *Pestalotiopsis clavispora* on *Rosa chinensis* in China. Plant Disease 98(7): 1009-1009.

Glass, N. L. and Donaldson, G. C. 1995. Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. Applied and Environmental Microbiology 61(4): 1323-1330.

Guba, E.F. 1961. Monograph of *Pestalotia* and *Monochaetia*. Harvard University Press, Cambridge. MA. USA.

Jia, J. Y., Li, X. H., Zhang, W., Zhou, Y. Y. and Yan, J. Y. 2019. First report of *Botryosphaeria dothidea* associated with stem canker on *Rosa chinensis* in China. Plant Disease 103(12): 3280.

Jiang, N., Bonthond, G., Fan, X. L. and Tian, C. M. 2018. *Neopestalotiopsis rosicola* sp. nov. causing stem canker of *Rosa chinensis* in China. Mycotaxon 133(2): 271-283.

Keith, L. M., Velasquez, M. E. and Zee, F. T. 2006. Identification and characterization of *Pestalotiopsis* spp. causing scab disease of guava, *Psidium guajava* L. in Hawaii. Plant Disease 90: 16-23.

Ketabchi, M. 2014. First report of banana fruit rot by *Pestalotiopsis theae*. Iranian Journal of Plant Pathology 50(1):103–104.

Liu, F., Hou, L., Raza, M. and Cai, L. 2017. *Pestalotiopsis* and allied genera from *Camellia*, with description of 11 new species from China. Scientific Reports 7(1): 866.

Maharachchikumbura, S. S., Hyde, K. D., Groenewald, J. Z., Xu, J. and Crous, P. W. 2014. *Pestalotiopsis* revisited. Studies in Mycology 79(1): 121-186.

Mirabolfathi, M. and Ershad, D. 2004. Twig and cane canker of rose in the greenhouses of central area of Iran. Iranian Journal of Plant Pathology 40: 84.

Mirtalebi, M., Banihashemi, Z., Sabahi, F. and Mafakheri, H. 2016. Dieback of rose caused by *Acremonium sclerotigenum* as a new causal agent of rose dieback in Iran. Spanish Journal of Agricultural Research 14(4): 24.

Murray, M. and Thompson, W. 1980. Rapid isolation of high molecular weight plant DNA. Nucleic Acids Research 8: 4321-4326.

Naeimi, S., Javadi, L. and Javadi, A. R. 2015. First report of *Pestalotia disseminata*, the causal agent of feijoa fruit rot in Iran. Mycologia Iranica 2(1):75-76.

Obregón, V. G., Meneguzzi, N. G., Ibañez, J. M., Lattar, T. E. and Kirschbaum, D. S. 2018. First report of *Neopestalotiopsis clavispora* causing root and crown rot on strawberry plants in Argentina. Plant Disease 102(9): 1856.

O'Donnell, K., Kistler, H. C., Cigelnik, E. and Ploetz, R. C. 1998. Multiple evolutionary origins of the fungus causing Panama disease of banana: concordant evidence from nuclear and mitochondrial gene genealogies. Proceedings of the National Academy of Sciences 95(5): 2044-2049.

O'Donnell, K. and Cigelnik, E. 1997. Two divergent intragenomic rDNA ITS2 types within a monophyletic lineage of the fungus *Fusarium* are non-orthologous. Molecular Phylogenetics and Evolution 7(1): 103-116.

Peng, C., Crous, P. W., Jiang, N., Fan, X. L., Liang, Y. M. and Tian, C. M. 2022. Diversity of *Sporocadaceae* (Pestalotioid fungi) from *Rosa* in China. Persoonia-Molecular Phylogeny and Evolution of Fungi 49(1): 201-260.

Pornsuriya, C., Chairin, T., Thaochan, N. and Sunpapao, A. 2020. Identification and characterization of

Neopestalotiopsis fungi associated with a novel leaf fall disease of rubber trees (*Hevea brasiliensis*) in Thailand. Journal of Phytopathology 168(7-8): 416-427.

Qiu, F., Xu, G., Zheng, F. Q., Zhou, J., Zheng, L., Miao, W. G. and Xie, C. P. 2020. First report of *Neopestalotiopsis clavispora* causing leaf spot on macadamia (*Macadamia integrifolia*) in China. Plant Disease 104(1): 288.

Reddy, M. S., Murali, T. S., Suryanarayanan, T. S., Rajulu, M. G. and Thirunavukkarasu, N. 2016. *Pestalotiopsis* species occur as generalist endophytes in trees of Western Ghats forests of southern India. Fungal Ecology 24: 70-75.

Santos, C. C., Domingues, J. L., Santos, R. F. D., Spósito, M. B., Santos, A. and Novaes, Q. S. 2019. First report of *Neopestalotiopsis clavispora* causing leaf spot on Macadamia in Brazil. Plant Disease 103(7): 1790-1790.

Shi, J., Li, B., Wang, S., Zhang, W., Shang, M., Wang, Y. and Liu, B. 2024. Occurrence of *Neopestalotiopsis clavispora* causing Apple leaf spot in China. Agronomy 14(8): 1658.

Steyaert, R. L. 1949. Contribution à l'étude monographique de *Pestalotia* de Not. et *Monochaetia* Sacc. (*Truncatella* gen. nov. et *Pestalotiopsis* gen. nov.). Bulletin Jardin Botanique Etat Bruxelles 19: 285–354.

Sweets, L. E., Pfleger, F. L., Morgan, F. C. and Mizicko, J. R. 1982. Control of fungi associated with cankers of greenhouse roses. Plant Disease 66: 491-494.

Tamura, K., Stecher, G., Peterson, D., Filipski, A. and Kumar, S. 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. Molecular Biology and Evolution 30(12): 2725-2729.

Thompson, J. D., Higgins, D. G. and Gibson, T. J. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Research 22(22): 4673-4680.

Weir, B. S., Johnston, P. R. and Damm, U. 2012. The *Colletotrichum gloeosporioides* species complex. Studies in mycology 73: 115-180.

White, T. J., Bruns, T., Lee, S. J. W. T. and Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR protocols: a guide to methods and applications 18: 315-322.

Widrlechner, M. P. 1981. History and utilization of *Rosa damascena*. Economic Botany 35: 42-58.

Wright, E. R., Pizzingrilli, P., Caligaris, M. V. and Cabral, D. 2007. Rose dieback caused by *Trichothecium roseum* in Argentina. Plant Disease 91(5): 631-631.

Zaher, E., Attia, M. and Reyad, N. E. H. 2012. Rose stem canker caused by *Coniothyrium fuckelii* Sacc. in Egypt. Egyptian Journal of Phytopathology 40(2): 39-55.

تعیین خصوصیات مورفولوژیکی و مولکولی *Neopestalotiopsis clavispora* عامل بیماری شانکر ساقه گل رز در ایران

مهدی نوربخش، بهرام شریف نبی✉

گروه گیاه‌پزشکی، دانشکده کشاورزی، دانشگاه صنعتی اصفهان، ایران

چکیده: رز (*Rosa* spp.) یک گیاه گل‌دار چندساله است که به‌طور گسترده در فضای باز و شرایط گلخانه‌ای کشت می‌شود. اکثر بیماری‌های رایج این گیاه ناشی از بیمارگرهای قارچی هستند که خسارات اقتصادی قابل‌توجهی را به همراه دارند. در این مطالعه، طی بازه زمانی تیر ۱۴۰۱ تا شهریور ۱۴۰۲، نمونه‌برداری از ساقه‌های دارای علائم شانکر و سرخشکیدگی ارقام رز هلندی (*Rosa hybrida*) تانجا و ساموراییی در شهرستان‌های نجف آباد و لنجان استان اصفهان انجام شد. جداسازی قارچها با استفاده از روش‌های خالص‌سازی و کشت نمونه صورت گرفت و سپس ویژگی‌های ریخت‌شناسی و تجزیه و تحلیل نواحی ITS، *TEF1-α* و *TUB* مورد بررسی قرار گرفت. آزمون‌های بیماری‌زایی توانایی جدایه‌ها را در ایجاد علائم شانکر و لکه‌برگی تأیید کردند. همچنین، با جداسازی مجدد گونه‌های قارچی از علائم نکروتیک، اصول کخ اثبات شد. بر اساس ویژگی‌های مورفولوژیکی و مولکولی، جدایه‌ها به‌عنوان *Neopestalotiopsis clavispora* شناسایی شدند. بر اساس یافته‌های این پژوهش، این نخستین گزارش از *N. clavispora* به عنوان عامل شانکر و سرخشکیدگی گل رز در ایران است.

کلمات کلیدی: بیماری گیاهی، آنالیز فیلوژنتیکی، شناسایی مورفولوژیکی، شانکر ساقه رز، بیماریزایی.

مکاتبه کننده: بهرام شریف نبی Email: sharifnabib@gmail.com

تاریخ دریافت: ۱۴۰۳/۰۹/۰۵ تاریخ پذیرش: ۱۴۰۳/۰۹/۲۵