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Full Length Research Paper

Phoma negriana, a new invasive pathogen for Moghan's vineyards in Iran

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During disease diagnosing studies in Moghan area, northern west of Iran, symptoms of black rot and necrosis were observed for the first time on *Vitis venifera*. Incidence was first noticed on leaves and stems. Symptoms occurred as small brown spots on leaves and stems, and developed as blackish-brown necrosis/canker so that the black pycnidia extended at the central parts of them. On the fruit, symptoms appeared as mummification of berries that were visible in the infested vineyards until winter time. The loss due to the disease along with downy mildew was remarkable during the studied years and can lead to substantial yield losses in Moghan climate condition. In order to identify the causal agent, the pathogen was isolated from infected leaves and stems. Also pathogenesity test was carried out by inoculating the causal agent to grapevine cuttings. According to culture, pycnidia and conidia characteristics described by Boerema et al. (2004), the results pointed toward *Phoma negriana* Thum as the causative organism.

Key words: fungi, *Phoma negriana*, *Vitis venifera*, Moghan area, Iran.

INTRODUCTION

The symptoms of black rot/canker of grapevine *Vitis venifera* L. on leaves, fruits and stems is common and harmful, and can be attributed to various biotic factors, including bacteria and fungi. Usually grapevine leaves and stems are infected with commonly occurring *Guignardia bidwellii* (haffman et al., 2002), *Plasmopara viticola*, *Sclerotinia sclerotium* (Hall et al., 2002) and *Phoma* spp. Some species of *Phoma* are common opportunistic pathogen of vine, such as *Phoma vitis* (Barbetti and Wood, 1978), *Phoma herbarum* (Krol, 2006) and *Phoma negriana* (Boerema and Doerehbosch, 1979). In Moghan area, significant damage of grapevine leaves and stems is also attributed to *P. viticola* inhibition of growth.

During disease diagnosing studies in Moghan area, northern west of Iran, symptoms of black rot and necrosis have been observed at first time on grapevine in Moghan Junior College of Agriculture vineyards (approximately 20 km south of Parsabade Moghan, Ardabil Province, Iran) as well as other places of Moghan area, during vegetation period in 2004. Incidence was first noticed on leaves and stems, and berries were susceptible to infection after flowering.

The aim of this study was to identify the disease causal agent. For this, the pathogen was isolated from infected leaves, stems and mummifying fruits, and purified. Also pathogenesity test was carried out by inoculating the causal agent to grapevine cuttings.

MATERIALS AND METHODS

In order to isolation of plant pathogenic agents, affected shoots and leaves were collected and transferred to plant pathogenic laboratory. The gathered samples were superficially disinfected for 1 min in 0.5% sodium hypochlorite and washed 3 times in sterile distilled water. They were then next cut into 3 or 4 mm pieces of tissue, and dried with sterilized filter paper. Pieces of infested tissue were planted on potato dextrose agar (Difco) amended with streptomycin (PDA+S) and incubated at 25 ± 1°C for 7 days (Hall et al., 2002).

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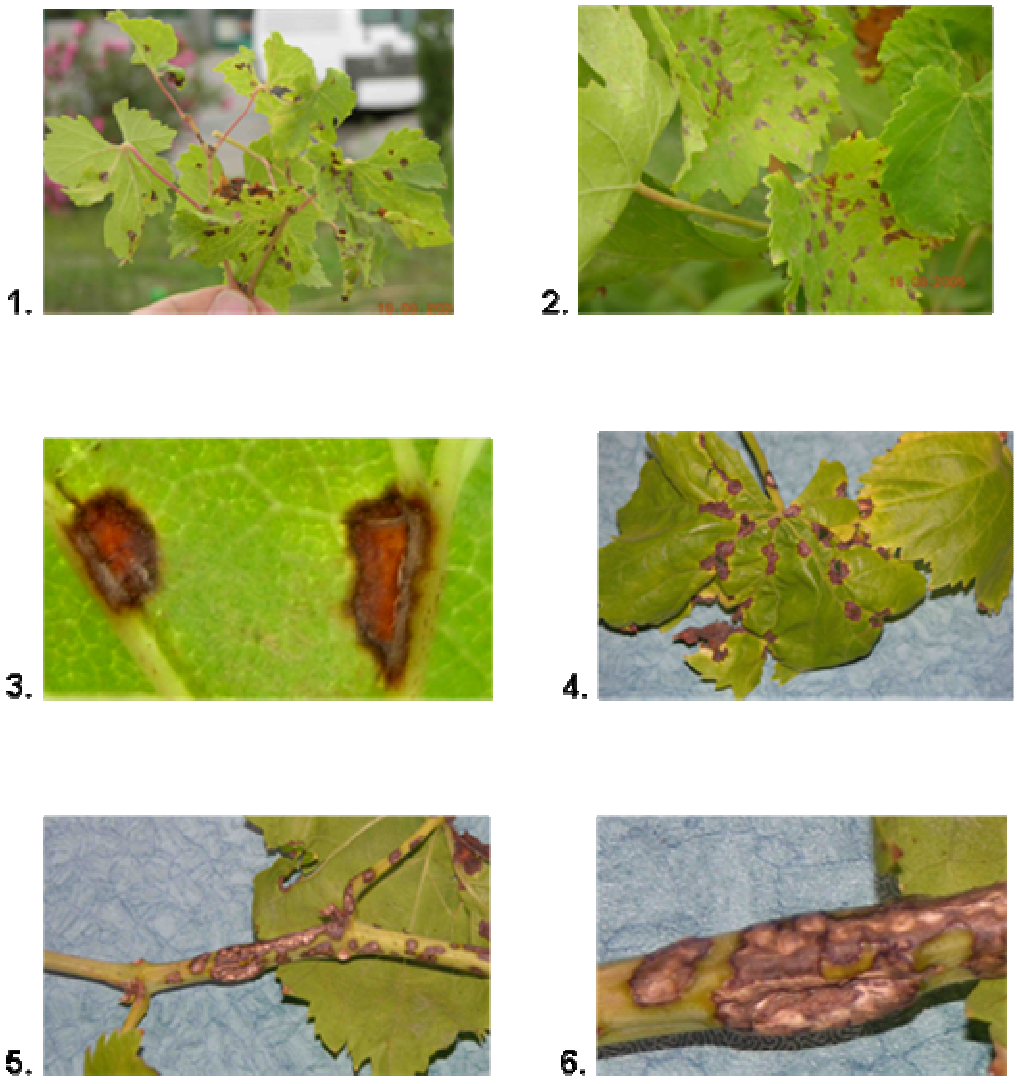

Figure 1. Symptoms of affected leaves and shoots with *Phoma negriana* in Moghan Junior College Agriculture vineyards, Parsabade Moghan, Ardabil, Iran. 1 – 4: Leaf showing bleckish-brown necrotic spots. 5 – 6: Brown to black elongated lesion on shoots.

The developing colonies were isolated and subcultured onto potato dextrose agar. The detected isolates were identified on the basis of microscopic examination of fine morphological diagnostic features and cultured characteristics on potato dextrose agar (PDA), Oat Meal Agar (OA) and Malt Extract Agar (MEA). Analysis of observed symptoms were as described in literature (Boerema et al., 2004; Machowicz-Stefaniak and Krol, 2006).

Pathogenesity study were carried out on some separating grapevines located in Moghan Junior College of Agriculture vineyards that were surface sterilized with 0.5% sodium hypochlorite by spraying on aerial parts of them. A spore suspension containing 10^6 conidia/ml were inoculated using a hand held pre-Val sprayer and enclosed in polyethylene bags overnight (approximately 12 h) to maintain the surface wetness necessary for infection (Hoffman et al., 2002). To prepare inoculums, the identified isolate was grown on plates of PDA for 14 days at 25 ± 1°C under fluorescent light. For harvesting conidia, plates were flooded with 10 ml of sterile distilled water and incubated on the bench top for 30 min. The spore suspension was decanted and stirred for 15 min on a magnetic stir plate to thoroughly mix the spores. The suspension was adjusted to a final concentration of 10^6 conidia/ml with the aid of a hemacytometer.

RESULTS AND DISCUSSION

During vegetation period in 2004, the symptoms occurred as small brown spots on leaves and stems, and developed as blackish-brown necrosis/cancker so that the black pycnidia were extended at the central parts of them (Figure 1). On the fruit, symptoms were arisen as mummification of berries that were visible in the infested vineyards until winter time. The disease was found in the same vineyard in spring 2005 but with higher incidence than the previous year and more vines was affected. The loss due to the disease along with downy mildew was remarkable during the studied years and can lead to sub-

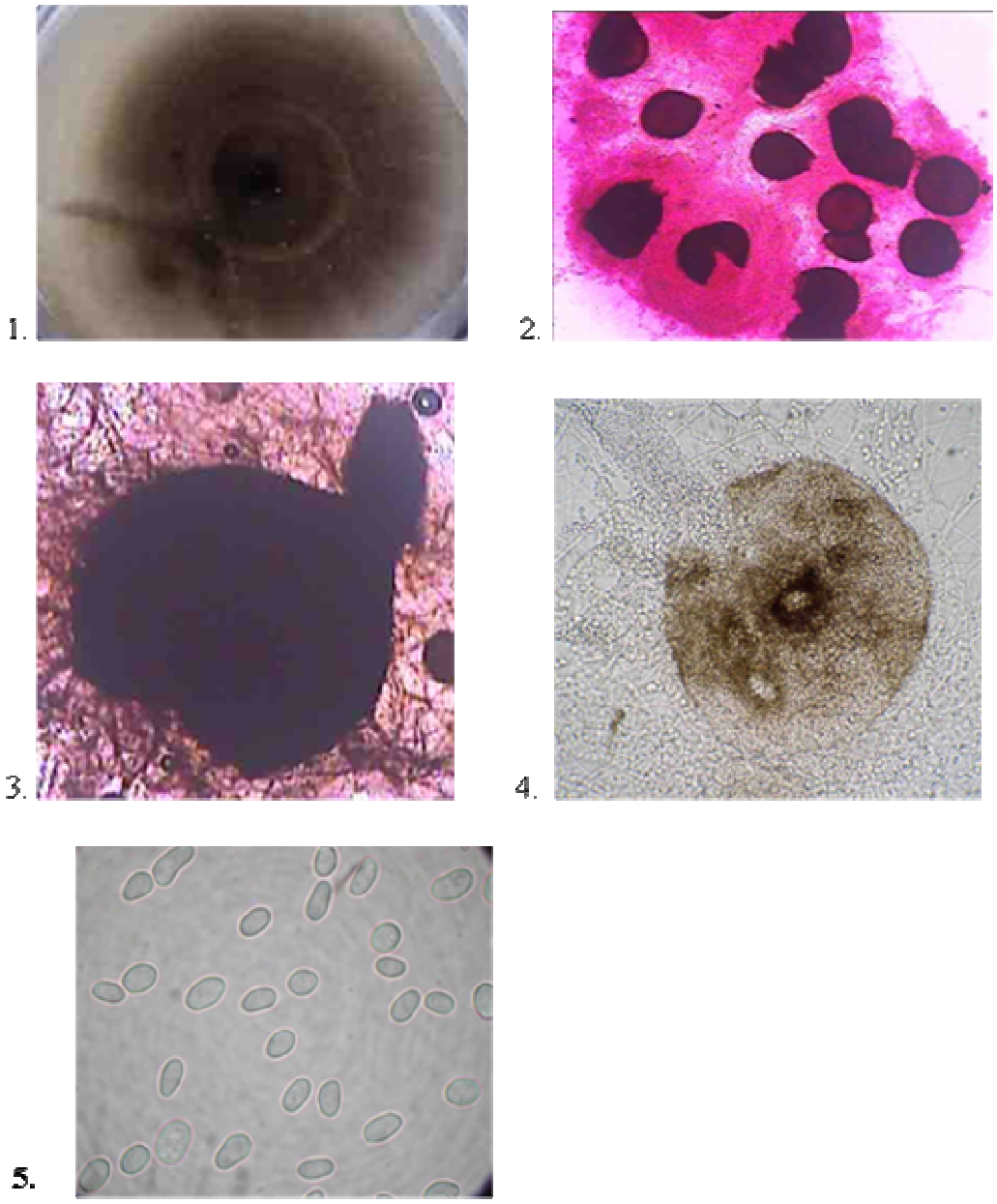

Figure 2. *Phoma negriana* cultured on PDA. 1. Colonies on PDA after growing at 25 ± 1ºC for 14 days in darkness. 2 – 4. Pycnidia from PDA cultures (2, 10X; 3, 400X; and 4, 1000X). 5. Conidia from PDA cultures (1000X).

stantial yield losses under the Moghan climate condition.

The fungus isolated on the affected tissues in all samples were characterized by forming slow growth on OA and PDA (colony diameter on PDA and OA after 7 days were recorded 1.42 and 3.9, cm respectively), gray colonies on OA and MEA and produce abundant black pycnidia, which pose globosely or irregular with 1 - 2 papillae ostioles, 70 - 220 µm in diameter and broadly ellipsoidal conidia with several small and large polar guttules, measuring 4.5 - 8.5 × 2 - 4 µm (Figure 2). Such properties according to Boerema et al. (2004) and Machowicz-stefaniak and Krol (2006) were indicative of *P. negriana* Thuemen (Syn: *Phyllosticta vitis* Saccardo). Specimen of the isolated fungus is housed in the Mohaghegh Ardabili University herbarium.

Spore production, dispersal, infection and continued disease development are favored by warm humid condition in growing regions, like Moghan climate. Crop losses are a result of direct destruction of foliage by the fungus, however fruit losses are significant. Infected vines may be weakened by the disease and is unlikely to result in vine death. It seems that the fungus can be

spread as spores by wind and rain. Asexual spores are formed in flask-shaped bodies which are responsibility for recurring infections within and between vines during the growing season. The conidia are able to disseminate to all new growth of leaves, petioles, shoots, tendrils and berries causing new infection until the end of the season. The critical period for berries infection is from end of flowering to beginning of variation. Sexual bodies were not formed on mummified fruit as well as other infected tissue in late summer and it seems that the pathogen over-winters due to mycelia and pycnidia in grapevines debris in vineyards in Moghan climate condition. There is an urgent need to develop management strategies for controlling this disease. Removing infected debris from the vineyards can help to control of the disease. Although it has been reported in association with leaves, stems and fruits in southern Europe (Boerema and Doerenbosch, 1979) and isolated from decaying grape-vine cutting in the polish nurseries (Stojanovic, 1986), *P. negriana* is recorded here as a new invasive pathogen for Moghan's vineyards in Iran.

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