The occurrence of *Alternaria* species on cabbage in Iran

Tahere RAHIMLOO, Youbert GHOSTA

University of Urmia
Urmia, Iran
E-mail: ghoosta@gmail.com

Abstract

In a study on *Alternaria* species, associated with cabbage black spot disease in Urmia, West Azerbaijan, Iran, cabbage fields were inspected during the growing seasons of 2012–2013 and leaves with suspected symptoms of *Alternaria* infection were collected. A total of 135 isolates with *Alternaria* characteristics were obtained from 10 main growing areas in this region and studied taxonomically. On the basis of morphological characteristics of the studied isolates, 8 species viz. *Alternaria arbusti*, *A. brassicicola*, *A. destruens*, *A. infectoria*, *A. perangusta*, *A. tenuissima*, *A. turkisafria* and *A. vaccinii* were identified. *Alternaria tenuissima* with 53 isolates, *A. brassicicola* with 38 isolates and *A. destruens* with 29 isolates had respectively the highest frequency among the identified species and distributed all over the studied area. *A. arbusti* and *A. perangusta* each with only one isolate had the lowest frequency. Results obtained from the pathogenicity tests showed that all the tested isolates from identified species were pathogenic on cabbage leaves, although there were statistically significant differences in virulence of the tested isolates. *A. brassicicola* isolates had the highest virulence and *A. arbusti* and *A. vaccinii* isolates had the lowest virulence. Isolates belonging to other species had moderate virulence. Except *A. brassicicola* which had been previously reported from cabbage in Iran, all other seven species are reported for the first time from cabbage as the causal agents of black spot disease. Also, *A. arbusti*, *A. perangusta* and *A. vaccinii* are reported as new species for Iran mycoflora.

Key words: *Alternaria* blight, Black leaf spot, *Brassica oleracea*, pathogen, taxonomy, virulence.

Introduction

White headed cabbage (*Brassica oleracea* L. var. *capitata* L. f. *alba* DC) is a biennial herb in the *Brassicaceae* family which is commonly grown in temperate regions. The cultivation history of cabbage goes back to 600 B.C. Several insect pests and plant pathogens attack this plant worldwide, and where pest management programs were not implemented appropriately, severe attack this plant worldwide, and where pest management programs were not implemented appropriately, severe losses could occur both in the field and in the store. One of the most common and important plant pathogens which causes qualitative and quantitative losses and reduces plant shelf-life is cabbage black spot disease which is caused by *Alternaria* species. It is reported that the yield loss caused by *Alternaria* blight disease on oilseed Brassicas is up to 60% (Kolte, 2002). A complex of three *Alternaria* species viz. *A. brassicicola*, *A. brassicaceae* and *A. japonica* has been reported as the causal agents of black spot disease of crucifers (Peruch et al., 2006; Reis, Boiteux, 2010). During the study aimed to identify causal agents of dark leaf spot disease of the four brassica crops grown in Thailand, *A. brassicicola* was the only identified species based on morphological and molecular characteristics (Pattanamahakul, Strange, 1999). In a survey of species causing *Alternaria* leaf spots on Brassica species in Pernambuco, *A. brassicaceae* was found in all Chinese cabbage fields, while *A. brassicicola* was found in all fields of cabbage, cauliflower and broccoli, an indication of host preferences (Michereff et al., 2012). In a recent study to determine pathogen species diversity on oilseed Brassicas, *Alternaria brassicaceae*, *A. brassicicola* and *A. alternata* were isolated from infected samples and *A. brassicaceae* was the dominant species (Aneja et al., 2014). The genus *Alternaria* is a cosmopolitan fungus with worldwide distribution that includes saprobic, endophytic and animal/plant pathogenic species (Grisoni et al., 2011; Polizzotto et al., 2012). Many species of *Alternaria* are known as important plant pathogens causing plant diseases and postharvest rots on a wide range of crops (Woudenberg et al., 2013). Some taxa are gaining prominence as emerging human pathogens particularly in immunocompromised patients or as airborne allergens (Hoog de et al., 2000; Thomma, 2003; Simmons, 2007). Host range is varied and the genus ranks 10th among fungi causing plant diseases and postharvest rots on a wide range of crops (Woudenberg et al., 2013). Some taxa are gaining prominence as emerging human pathogens particularly in immunocompromised patients or as airborne allergens (Hoog de et al., 2000; Thomma, 2003; Simmons, 2007).
A. brassicicola was reported from Ardabil, Qom, Karaj, Lahijan, Urmia, Tabriz, Sarab and Tooskeran (Ghosta, 2004; Ershad, 2009), and A. japonica was reported from Urmia (Ghosta et al., 2003). In spite of widespread occurrence of cabbage black leaf spot disease and its economic losses, there is not any specific study on the disease, species composition of the causal agents and the pathogenicity of different species in Urmia or other main cabbage growing areas of Iran.

The objective of the present study is to isolate, identify and characterize the pathogenicity of different Alternaria species from cabbage in Urmia, West Azerbaijan, one of the main cabbage growing areas in northwest part of Iran.

Material and methods

Isolation and purification of fungal isolates. During the growing seasons (summer and autumn) of 2012–2013, cabbage fields in Urmia, West Azerbaijan, Iran were inspected and plants with suspected symptoms of Alternaria infection were collected. Infected leaves were placed separately in paper bags, transported to the laboratory and incubated at 4°C. Isolation of the fungi was done immediately. Leaves were washed thoroughly under tap water, and then surface sterilized with 0.5 percent sodium hypochlorite solution for 5 min, rinsed again with sterile distilled water and blotted dry on sterile paper towels. Leaves were cut into small pieces and plated on potato dextrose agar (PDA). Some of the leaf samples were placed in Petri plates containing a moistened sterile filter paper. Petri plates were incubated in the dark at 23–25°C and the fungi growing up from the margins or surfaces of the plant samples were transferred into new PDA plates. In the cases that fungal conidiophores and conidia were formed on the collected plant samples, the isolated fungi was done by transferring single spores by fine sterile needle and plated on PDA. Purification of the isolated fungi was done by transferring single spores or hyphal tips onto fresh PDA plates. Purified fungi were grown in potato carrot agar (PCA) slants containing a 0.5 percent sodium hypochlorite solution. In the inoculation sites, a small lesion was made with sterile needle and an agar plugs (diameter 5 mm²) were placed on the lesion mycelial side down. In controls, agar plugs without fungal mycelia were used. Inoculated leaves were placed in plastic containers containing wet, three layers sterilized filter paper. The containers were incubated at 20–22°C for 14 days. Each experiment included three replicates per treatment. All experiments were repeated twice. Isolations were made from all inoculated leaves and the isolates were identified as described initially to fulfil Koch’s postulates. Lesion diameters around the inoculated sites were measured and used in comparisons. Results were analyzed using SPSS software program and means were compared using Tukey’s test.

Results and discussion

Fungal isolates. Cabbage leaves with dark spots were found in all visited fields in the studied area. Isolates with Alternaria characteristics were dominant among the isolated fungi, although some isolates with Stemphylium, Cladosporium and Penicillium characteristics were also found among the isolated fungi. Overall, 135 isolates with Alternaria characteristics were obtained from different locations (Table 1).

Species identification. Species identifications were done based on morphological characteristics. Eight species viz. Alternaria arbusti, A. brassicicola, A. destruens, A. infectoria, A. perangusta, A. turkisafria, A. tenuissima and A. vaccinii were identified. Among the identified species, A. tenuissima, A. brassicicola and A. destruens with 53, 38 and 29 isolates had respectively the highest frequency and distribution, and A. arbusti and A. perangusta each with one isolate, had the lowest frequency. Except A. brassicicola which had been previously reported from cabbage in Iran, all the other seven species are reported for the first time from cabbage and cabbage is as matrix nova for these species. Also, A. arbusti, A. perangusta and A. vaccinii are reported as new species to Iran mycoflora. Identified species are described briefly alphabetically.

1. Alternaria arbusti (Simmons, 1993). Mean colony diameter of the studied isolate on PCA medium at 23–25°C after 7 days is 40 mm. Colony colour is grey. Aerial hyphae are low. Primary conidiophores are erect and simple, mostly with one conidiogenous locus, rarely 2–4. Conidia ovoid to long ellipsoid, produced in short chains and have relatively long secondary conidiophores. Transverse septa are thick and darker brown than the outer conidial wall. Conidia have 3–6(–8) transverse and 1–2 longitudinal septum in the wider part, 25–45 × 8–16 µm (Fig. 1). This species was reported previously from leaf spots of Pyrus pyrifolia and cherry fruit cv. ‘Rainier’ (Simmons, 1993). One isolate was obtained from cv. ‘Emanzadeh’ in this study. This is the first report of the presence of A. arbusti in Iran and cabbage is as new host for this species.
2. Alternaria brassicicola (Schw.) (Wiltshire, 1947). Mean colony diameter of different isolates on PCA medium at 23–25°C after 7 days is 55 mm. Colony colour is olivaceous to dark brown. Primary conidiophores arise directly from agar surface, mostly simple, with a single conidiogenous site, up to 125 µm length. Conidia are borne on long branched chains; initial conidia large, ellipsoid to ovoid, 30–45 × 10–12 µm; younger conidia in a chain are smaller, ovoid, 12–25 × 6–8 µm. Conidia have 5–7 transverse septa, usually without longitudinal septum, rarely with 1–2 longitudinal/oblique septa at the wider part. Transverse septa are thick and darker brown than the outer conidial wall (Fig. 2). This species had been previously reported in Iran and other parts of the world from cabbage and other taxa of Cruciferae, Digitalis purpurea, Phaseolus coccineus, Thymus vulgaris, Artemisia sp. and plant species in the genera of Linum, Papaver, Scrozonera and Phaseolus (Simmons, 2007; Ershad, 2009). 38 isolates were obtained in this study from all visited areas.

Table 1. Identified Alternaria species, isolates and their frequencies

<table>
<thead>
<tr>
<th>Species</th>
<th>Isolate</th>
<th>Species frequencies</th>
</tr>
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<tbody>
<tr>
<td>A. arbusti</td>
<td>EM6</td>
<td>0.74%</td>
</tr>
<tr>
<td>A. brassicicola</td>
<td>VZ3, EM1, EM2, EM4, LR2, LR10, KA8, HA11, HA12, HA13, HA14, HA15, QA13, QA14, QA15, QA16, QA17, QA18, QA19, QA20, QA21, QA24, QA25, QA27, QA30, QA31, UF3, UF5, TP1, TP2, TP3, TP4, TP5, TP6, TP11, TP15, TP16, TP17</td>
<td>28.14%</td>
</tr>
<tr>
<td>A. destruens</td>
<td>EM9, EM11, EM12, EM23, LR3, LR9, LR13, LR14, LR15, LR16, LR17, KA4, KA5, KA10, HA10, QA3, QA4, QA6, QA7, QA8, QA23, TP7, TP14, NA2, G02, G05, GT1, GT3, GT4</td>
<td>21.48%</td>
</tr>
<tr>
<td>A. infectoria</td>
<td>EM10, UF1, UF2, LR19, HA6, QA9, QA11</td>
<td>5.18%</td>
</tr>
<tr>
<td>A. perangusta</td>
<td>LR8</td>
<td>0.74%</td>
</tr>
<tr>
<td>A. tenuissina</td>
<td>VZ2, EM8, LR4, LR5, LR6, LR7, LR11, LR18, KA1, KA3, KA6, KA7, KA11, HA3, HA5, HA7, HA17, HA18, HA19, HA20, HA23, QA1, QA2, QA3, QA5, QA10, QA12, QA22, QA32, QA33, QA34, QA35, QA36, UF7, UF8, TP8, TP9, TP10, TP11, TP12, TP13, TP18, G03, G04, G06, G07, PC, PC1, PC2, PC3, NA2, NA3, NA4</td>
<td>39.25%</td>
</tr>
<tr>
<td>A. turkisafria</td>
<td>VZ1, HA2, HA13</td>
<td>2.22%</td>
</tr>
<tr>
<td>A. vaccinii</td>
<td>EM7, HA4, UF4</td>
<td>2.22%</td>
</tr>
</tbody>
</table>


Figure 1. Alternaria arbusti: colony on potato carrot agar (PCA) after 7 days (A), sporulation pattern (B) and conidial chains and conidiophores (C) (scale bar = 50 μm). (For colour figures, please visit http://www.zemdirbyste-agriculture.lt/1023_str44/)

Figure 2. Alternaria brassicicola: colony on potato carrot agar (PCA) after 7 days (A), sporulation pattern (B) and conidia (C) (scale bar = 50 μm)
3. *Alternaria destruens* (Simmons, 1998). Mean colony diameter of different isolates on PCA medium at 23–25°C after 7 days is 57 mm. Colony colour is brown. Conidia are produced on short unbranched chains (4–8 conidia), rarely a short conidial branch with 1–2 conidia arises from a body cell of catenate conidium. Most conidia in chains appear beakless, but a few have a relatively long secondary conidiophore up to 60 µm. These prominent secondary conidiophores inserted in short, simple chains of conidia are diagnostic character of this species among the small-spored *Alternaria*. Conidia ovoid to ellipsoid, dark brown, 20–40 × 8–12 µm, with 3–5 transverse septa and rarely one longitudinal septum in the widest part (Fig. 3). This species was reported for the first time from *Cuscuta gronovii* Wild (Simmons, 1998) and from sunflower, eggplants, barley and mango in Iran (Ershad, 2009). 29 isolates were obtained from cabbage in this study in all visited areas and cabbage is a new host for this species.

![Figure 3. *Alternaria destruens*: colony on potato carrot agar (PCA) after 7 days (A), sporulation pattern (B) and conidial chains, primary and secondary conidiophores, long conidiophores between conidia (arrowheads, C) (scale bar = 50 µm)](image1)

4. *Alternaria infectoria* (Simmons, 1986). Mean colony diameter of different isolates on PCA medium at 23–25°C after 7 days is 66 mm. Primary conidiophores which arise from aerial hyphae or arise directly from agar surface are simple and have several geniculations. Conidia are produced on short branched chains (4–10 conidia). Primary conidia usually are the largest conidia. Conidia are ellipsoid to long ovoid, 30–45 × 8–15 µm, with 3–6 transverse septa and 0–2(–3) longitudinal septa. Apical cell of primary conidia usually generate secondary conidiophores with variable length and several geniculations with branching chains of secondary conidia (Fig. 4). This species had been previously reported from sunflower, barley, apple, soybean, canola, kidney bean and wheat in Iran (Ershad, 2009). In this study, 7 isolates were obtained from cabbage in cvs. ‘Emamzadeh’, ‘Qasemieh’, ‘Lake Road’, ‘Hosseinabad’, ‘Vazirabad’ and ‘Nazloo’. Cabbage is reported as a new host for this species.

![Figure 4. *Alternaria infectoria*: colony on potato carrot agar (PCA) after 7 days (A), sporulation pattern (B) and conidial chains and secondary conidiophores (arrowhead, C) (scale bar = 50 µm)](image2)

5. *Alternaria perangusta* (Simmons, 1999). Mean colony diameter of the studied isolate on PCA medium at 23–25°C after 7 days is 64 mm. Colony colour is brown. Primary conidiophores are short; conidia are produced in simple, long chains (10–18 conidia); conidia are long-narrow-ellipsoid, dilute yellow-tan; outer walls punctuate, with 3–5 transverse septa and without longitudinal septa, rarely with 1 longitudinal septum, 17–40 × 6–8 µm. Younger conidia in chains are small, ovoid and with only one transverse septum (Fig. 5). One isolate from cv. ‘Lake Road’ was obtained in this study. This species was reported originally from *Citrus paradisi* Macfad. × *C. reticulata* Blanco cv. ‘Minneola’ (Rutaceae) (Simmons, 2007) and is reported for the first time from cabbage. Also this species is new to Iran mycoflora.

6. *Alternaria tenuissima* (Kunze) (Wiltshire, 1933). Mean colony diameter of different isolates on PCA medium at 23–25°C after 7 days is 62 mm. Colony colour is brown. Primary conidiophores arise directly from agar surface, up to 70 µm length and have 1–3 geniculations. Conidia are produced in simple, short chains (4–8 conidia), are ovoid to obclavate, 20–45 ×
Conidia have 3–5 transverse septa and 1–2 longitudinal septa in the wider part (Fig. 6). This species was reported from different hosts and environments (Simmons, 2007; Ershad, 2009), but for the first time is reported from cabbage in Iran. 53 isolates were obtained in this study from all the visited locations and it had the highest frequency among the identified species.

**Figure 5.** *Alternaria perangusta*: colony on potato carrot agar (PCA) after 7 days (A), sporulation pattern (B) and long conidial chains and narrow conidia (C) (scale bar = 50 μm)

**Figure 6.** *Alternaria tenuissima*: colony on potato carrot agar (PCA) after 7 days (A), sporulation pattern (B) and conidial chains and conidia (C) (scale bar = 50 μm)

7. *Alternaria turkisafria* (Simmons, 1999). Mean colony diameter of different isolates on PCA medium at 23–25°C after 7 days is 55 mm. Colony colour is brown. Primary conidiophores are short and arise directly from agar surface, up to 65 μm length. Conidia are borne in long simple chains (10–20 conidia). First formed conidia are long-ellipsoid and younger conidia are long-ovoid. Conidia are medium yellow brown, with 3–5(–8) transverse septa and one longitudinal septum at the widest part, 15–50(–63) × 8–10 μm (Fig. 7). Three isolates were obtained in this study from cvs. ‘Hosseinabad’ and ‘Vazirabad’. This species was reported from citrus plants in Turkey, South Africa and Iran (Simmons, 1999; Ghosta, 2004) and is reported for the first time from cabbage.

**Figure 7.** *Alternaria turkisafria*: colony on potato carrot agar (PCA) after 7 days (A), sporulation pattern (B) and conidia (C) (scale bar = 50 μm)

8. *Alternaria vaccinii* (Simmons, 2007). Mean colony diameter of different isolates on PCA medium at 23–25°C after 7 days is 55 mm. Colony colour is light gray. Primary conidiophores arise directly from agar surface, up to 125 μm length. Conidia are produced in moderately long, simple or branched chains (8–10 conidia); secondary conidiophores short or more commonly long (up to 150 μm). Conidia are ovoid or subellipsoidal, dilute brown, 13–35 × 8–12 μm, with 2–6 transverse septa and usually no longitudinal septa or rarely with one longitudinal septum at the widest part (Fig. 8). This species was originally reported from
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Vaccinium sp. (Simmons, 2007). Three isolates in this study were obtained from cvs. ‘Emamzadeh’, ‘Kavsi’ and ‘Vazirabad’. This is the first report of the occurrence of *A. vaccinii* from cabbage and is reported for the first time for Iran mycoflora.

**Pathogenicity tests.** Disease symptoms were seen as chlorosis and necrosis of leaf tissues around the inoculated sites after 14 days. No symptoms were seen in controls (Fig. 9).

On the basis of lesion dimensions, infected area was calculated based on ellipsoid formula. Comparison of data means showed that, although all the isolates were pathogenic on cabbage leaves, there were statistically significant differences among the virulence of the tested isolates from different species (Table 2).

Isolates belonging to *A. brassicicola* had the highest and *A. arbusti* and *A. vaccinii* isolates had the lowest virulence. Isolates belonging to the other species had moderate virulence (Fig. 10).

Until now, pathogenicity of *A. brassicicola, A. brassicae* and *A. japonica* are reported on cabbage plants from different locations and in this study; the pathogenicity of 7 other *Alternaria* species was confirmed. Species composition associated with black spot disease in different studies is somewhat different. In most studies on the identification of *Alternaria* species from brassicaceous hosts, *A. brassicicola, A. brassicicola* and *A. japonica* were reported (Rop et al., 2009; Kumar et al., 2014), although in more recent study, *A. alternata* was also reported as the causal agent of the disease (Aneja et al., 2014). In a study on *Alternaria* species infecting brassicaceae in the Brazilian neotropics, *A. brassicicola* was prevalent on *Brassica oleracea* complex, whereas *A. brassicaceae* was prevalent on *Brassica rapa* complex and weed species (Reis, Boiteux, 2010). In a study to determine *Alternaria* species causing black spot disease on cabbage and kale in Kenya, *A. brassicicola* and *A. japonica* were identified as the causal agents. *A. brassicicola* was predominant in cabbage and *A. japonica* was predominant in kale fields (Rop et al., 2009). In a study on black spot disease of canola in Iran, 5 different species of *Alternaria* were isolated and identified from diseased plants and *A. brassicicola* and *A. japonica* had the highest virulence. Also *A. japonica* was the dominant species (Nourani et al., 2008). In the revised studies on *Alternaria* species infecting Solanaceae, Caryophyllaceae and several other

**Figure 8.** *Alternaria vaccinii*: colony on potato carrot agar (PCA) after 7 days (A), sporulation pattern (B) and conidia and long secondary conidiophores (arrowheads, C) (scale bar = 50 μm)

**Figure 9.** Pathogenicity tests of different species of *Alternaria* on detached cabbage leaves cv. ‘Koikaze’ under laboratory conditions: control (A), *A. brassicicola* (B), *A. destruens* (C), *A. infectoria* (D), *A. turkisafria* (E), *A. tenaxssima* (F), *A. perangusta* (G), *A. vaccinii* (H) and *A. arbusti* (I)

**Table 2.** ANOVA analysis of pathogenicity of various species of *Alternaria* on cabbage cv. ‘Koikaze’ leaves

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Sum of squares</th>
<th>Mean squares</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>7</td>
<td>2313.841</td>
<td>330.549</td>
<td>1910.687</td>
<td>0.000</td>
</tr>
<tr>
<td>Error</td>
<td>16</td>
<td>2.769</td>
<td>0.173</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>23</td>
<td>2316.610</td>
<td></td>
<td>1910.687</td>
<td>0.000</td>
</tr>
</tbody>
</table>

DF – degrees of freedom, F – the test statistic, P – the probability of a value of F greater than or equal to the observed value
host families, a lot of new species of *Alternaria* were identified and described (Simmons, 2000; 2007). Based on the revised species concept in the genus *Alternaria* and controversies in the past for identification of species in this large and complex genus, it is necessary to do more work on the identification of species composition in complex diseases. The results of this study showed that there are several species of *Alternaria* pathogenic on cabbage in the studied area, so more studies need to be done to determine *Alternaria* species diversity associated with cabbage black leaf spot disease in other cabbage growing regions. Due to the differences in pathogenicity of different species of *Alternaria* isolated from cabbage, it may be noted in studies related to breeding resistant varieties and other control strategies against black spot disease.

**Conclusions**

1. Eight species of *Alternaria* were identified from cabbage black spot disease in the studied area and their pathogenicity was confirmed based on Koch's postulates.

2. *A. tenuissima, A. brassicicola* and *A. dastruens* are dominant species of *Alternaria* on cabbage plants in Urmia, West Azerbaijan.

3. *A. brassicicola* isolates are identified as highly virulent and *A. arbusit* and *A. vaccinii* isolates as weakly virulent on cabbage plants.

4. Cabbage is reported as matrix nova for 7 *Alternaria* species, viz. *A. arbusit*, *A. dastruens*, *A. infectoria*, *A. perangusta*, *A. tenuissima*, *A. turksafria* and *A. vaccinii*.

5. Three species of *Alternaria*: *A. arbusit*, *A. perangusta* and *A. vaccinii* are reported for the first time for Iran mycoflora.

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**Figure 10.** Diseased leaf area of cabbage cv. ‘Koikaze’ leaves, 14 days after inoculation with different species of *Alternaria*


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T. Rahimloo, Y. Ghosta
Urmijos universitetas, Iranas

Santrauka
