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(RESEARCH ARTICLE)



## Alternaria diseases of agricultural crops in Uzbekistan

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### Abstract

In the article the tasks have been set on the inventory of the species composition of fungi of the genus *Alternaria* Nees ex Walroth - pathogen of various *Alternaria* diseases of vegetable crops in Uzbekistan. More than 200 isolates were collected and about 150 monosporous pure cultures were obtained from them. According to preliminary data, they belong to 15 species of the genus *Alternaria* and have been isolated from the 12 most important crop species. It was found that a more accurate identification of species requires the study of a wide range of morphological and biochemical traits. The article discusses the issue of infection of vegetable seeds in Uzbekistan. The composition of micromycetes living on seeds of vegetable and green crops was revealed. In total, 21 species were recorded, of which 9 species includes to *Alternaria* genus.

**Keywords:** Alternaria; Micromycetes; Leaf spot; Disease; Seed infection; Conidiophores; Conidia.

### 1. Introduction

Fungi of the genus *Alternaria* Nees ex Walroth are widespread in nature. Due to their wide ecological amplitude, they have adapted to parasitism on various important crops. Diseases of various cultivated, wild-growing and weed plants, called *Alternaria*, are known to all phytopathologists and the staff of the plant protection system. Species of this genus are found all over the world. Some of them are harmless saprotrophs, while others, parasitic species, cause harmful diseases of crops.

The identification of many micromycetes and, in particular, *Alternaria* species is associated with a number of difficulties, such as the similarity of morphological characteristics of different species and, at the same time, intraspecific variability of traits.

Many *Alternaria* species cause leaf spots and thereby damage the photosynthetic apparatus of the plant, which, if severely infested, leads to significant yield losses, especially in weakened plants of susceptible varieties. It is estimated that the loss of tomato fruit yield in India reached 78% [1]. Studies carried out in Germany have shown that the loss of yield of winter rapeseeds from *Alternaria* can be up to 50% [2]. The dying off of carrot tops caused by *Alternaria* greatly reduces the efficiency of mechanized harvesting of root crops [3, 4].

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*Alternaria* species have been identified on a very wide range of plant substrates. Some species are saprotrophs, but most are parasites with a necrotrophic feeding method and various specializing and degree of parasitism (from facultative parasites to facultative saprotrophs).

The disease is widely known in Uzbekistan as spotting of potatoes and tomatoes. *Alternaria* on potatoes and tomatoes in open ground caused by large-spore *Alternaria* species (*A. solani* in a wide sense). *Alternaria* of cruciferous crops (cabbage, rapeseed, radish, etc.) caused by *A. brassicae*, *A. brassicicola*, and *A. japonica* (syn. *A. raphani*) is widespread everywhere. *Alternaria* on leaves and root crops of carrots (*A. dauci*, *A. radicina*) has been identified in several regions of Uzbekistan. The spread of *Alternaria* at the end of the growing season before harvesting reaches 90-100%, and development up to 15%. *Alternaria* on onions (*A. porri*), pumpkin (*A. cucumerina*) and a number of ornamental plants (calendula, zinnia) are of local importance.

The genus *Alternaria*, previously attributed to imperfect fungi (*Hyphomycetes* species, *Dematiaceae* family), in accordance with the currently accepted system, is considered an anamorph of marsupial fungi of the *Pleosporaceae* family of the order Pleosporales of the Pleosporomycetidae subclass of the Dothideomycetes class [5]. In some *Alternaria* species, a teleomorph (sexual stage) from the genus *Lewia* is known, but the vast majority of species have lost it.

It is known that the disease *Alternaria* can be transmitted by seeds, in this regard, we conducted a study to identify the possibility of transmission of infection by seeds [6]. Sometimes such an infection is not accompanied by the appearance of any symptoms and does not lead to a decrease in the number of seeds and their quality (weight of 1000 seeds, germination). In other cases, the damage from infection is very significant and manifests itself in hollow seed, low viability. During the period of seed germination, the fungus, under favorable conditions for it, can release phytotoxins that reduce germination. Also, seed infection sometimes leads to infection of seedlings and their death. The detrimental effect of seed infection with *Alternaria* species on seedlings of cruciferous crops [7], flax [8], carrots [9], and other agricultural plants has been described.

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## 2. Material and methods

The collection of material was carried out during a route survey on sowing of agricultural crops in farms and in vegetable stores of Tashkent region. Also studied herbarium samples of affected plants stored in the mycological herbarium of the Institute of Botany of the Academy of Sciences of Uzbekistan.

The identification was carried out according to the monographs by M.B. Ellis [10, 11], L.M. Levkina [12], F.B. Gannibal [13]. Reconciliation was done according to the work of M.Sh. Sagdullaeva et al. [14].

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## 3. Results

The aim of this work is to study the representation of various *Alternaria* species associated with agricultural crops for further analysis of morphological and other identification traits. Within the Republic of Uzbekistan, the species of the genus *Alternaria* have been little studied, and their identification was carried out mainly on the basis of specialization to certain substrates and distribution [14]. The analysis of the species composition of the pathogens of *Alternaria* in vegetable crops has not been carried out yet taking into account the modern taxonomic concepts. Many *Alternaria* species cause serious disease in important crops. Carrots (black rot and brown spot), crucifers (black spot), potatoes (early spot), grain crops (black embryo or asymptomatic lesion of kernels) suffer especially strongly from *Alternaria*.

The work has been carried out since 2011. In the course of the research, the crops of grain, vegetables and cotton were examined. In addition to more than 200 strains we collected, the work involved materials stored in the mycology laboratory of the Institute of Botany of the Academy of Sciences of the Republic of Uzbekistan and the experimental station of the Tashkent Agrarian University.

A preliminary analysis of about 150 collected strains by obtaining monosporous pure cultures, showed that they belong to the following species.

- *Lycopersicon esculentum* (tomato) – *A. alternata* (Fr.) Keissler .f. sp. *lycopersici* (black mold on fruits); *A. longipes* (Ell. Et Ev.), Wasson (alternaria), *A. solani* (Ell. Et Mart.) Sor.;
- *Solanum tuberosum* (potato) – *A. solani* (Ell. Et Mart.) Sor. (alternaria), *A. alternata* (Fr.) Keissel (alternaria), *A. tenuissima* (Kunze ex Pers.) Wiltshire;

- *Daucus carota* (carrot) – *A. radicina* Meier, Drecshler & Eddy; (black rot), *A. dauci* (Kuhn) Groves & Skolko, *A. alternata* (Fr.) Keissel;
- *Allium cepa* (onion) – *A. porri* (Ell.) Cif., *A. alternata* (Fr.) Keissel;
- *Brassica oleraceae* (cabbage) – *A. brassicae* Sacc., *A. brassicola* (Schw.) Wiltshire, *A. japonica* Yoshii (alternaria);
- 6. *Capsicum annuum* (pepper) – *A. solani* (Ell. Et Mart.) Sor. (Alternaria of pepper fruit), *A. alternata* (Fr.) Keisse;
- *Solanum melongena* (eggplant) – *A. solani* (Ell. Et Mart.) Sor.;
- *Cucurbita pepo* (pumpkin) – *A. cucumerina* (Ellis & Everh.) Elliot (leaf spot and wilting), *A. longissima* Deighton & MacGarvie, *A. alternata* (Fr.) Keisse;
- *Apium graveolens*, *Anethum graveolens* (celery, dill) – *A. radicina* Meier, Drecshler & Eddy;
- *Triticum aestivum* (wheat) – *A. alternata* (Fr.) Keissel (black embryo), *A. state* of *Pleospora infectoria* Fuckel;
- *Cotton plant* – *A. macrospora* Zimm.
- *Helianthus annuus* (sunflower) – *Alternariaster helianthi* (Hansf.) E.G. Simmons (syn. *A. helianthi*).

21 species of phytopathogenic fungi were identified from vegetable seeds, where, along with the genus *Alternaria* (9 species), the species frequently found on many plants were distinguished: p.p. *Aspergillus*, *Botrytis*, *Chaetomium*, *Cladosporium*, *Fusarium*, *Mucor*, *Helminthosporium*, *Penicillium*, *Stemphillium*, *Trichotecium*, *Verticillium*.

The identified types of the genus *Alternaria* can be classified as plant-specific pathogens. Thus, the species *Alternaria alternata*, *A. alternata* f. sp *lycopersici*, *A. tenuissima*, *A. solani* were found in tomato seeds, *A. alternata* was also found on eggplant seeds, and *A. solani* also lives on potato seeds. On cabbage seeds, *A. brassicae* and *A. brassicola* were noted, which were also isolated from rapeseeds, and *A. brassicola* was also isolated from radish. *A. radicina* was isolated from the seeds of carrots, radishes and dill. The species *A. dauci* was also recorded on carrots. The species *A. porri* was isolated from onion seeds (Table 1).

**Table 1** Micromycetes, identified in seeds of vegetable crops.

No.	Micromycetes species	Vegetable and green crops														
		Tomato	Potato	Cabbage	Carrot	Sweet pepper	Beetroot	Early radish	Rapeseed	Radish	Parsley	Eggplant	Onion	Dill	Celery	Turnip
1.	<i>Alternaria alternata</i>	+										+				
2.	<i>A. alternata</i> f. sp <i>lycopersici</i>	+														
3.	<i>A. tenuissima</i>	+														
4.	<i>A. solani</i>	+	+													
5.	<i>A. brassicae</i>			+					+							
6.	<i>A. brassicola</i>			+					+	+						
7.	<i>A. radicina</i>				+				+					+		
8.	<i>A. porri</i>											+				
9.	<i>A. dauci</i>				+											
10.	<i>Aspergillus niger</i>	+	+	+	+		+			+	+	+		+	+	
11.	<i>Botrytis cinerea</i>		+		+		+									
12.	<i>Chaetomium</i> sp.								+							
13.	<i>Cladosporium herbarum</i>		+	+	+		+							+	+	+
14.	<i>Fusarium solani</i>		+	+					+							
15.	<i>F. lateritium</i>	+														
16.	<i>Mucor mycedo</i>	+	+	+	+		+		+			+		+	+	
17.	<i>Helminthosporium</i> sp	+	+			+				+	+					
18.	<i>Penicillium glaucum</i>	+	+	+	+	+	+				+	+		+	+	
19.	<i>Stemphillium</i> sp	+	+		+											
20.	<i>Trichotecium roseum</i>	+	+	+	+	+	+	+		+	+	+		+	+	+
21.	<i>Verticillium lateritium</i>	+	+	+	+	+	+	+			+	+		+	+	+

#### 4. Discussion

*Alternaria* species have been identified on a very wide range of plant substrates. Some species are saprotrophs, but most are parasites with a necrotrophic feeding method and various specializing and degree of parasitism (from facultative parasites to facultative saprotrophs). As a result of the study, 15 species of micromycetes related to the genus *Alternaria* noted on 12 crops. *Alternaria alternata* was identified on potato, tomato, sweet pepper crops, *A. alternata* f. sp. *lycopersici* only on tomatoes, *A. brassicae* on cabbage, *A. brassicola* on cabbage, turnip, early radish, radishes, *A. capsici-annui* on sweet pepper, *A. dauci* and *A. radicina* on carrot, *A. raphani* on cabbage and radish, *A. porri* on onion, *A. solani* on potatoes, tomatoes, eggplant, beetroot, and *A. tenuissima* were found on tomato and potatoes.

The frequently found species with high degree development of disease are: *Alternaria alternata*, *A. solani*, *A. tenuissima*, the species with medium degree development are *A. brassicae*, *A. brassicola*, *A. dauci*, *A. radicina*, *A. raphani*, *A. porri* and less found species are *A. alternata* f. sp. *lycopersici*, *A. capsici-annui*.

The taxonomy of the genus *Alternaria* Nees and other genera closely related to it has undergone numerous major revisions over almost 200 years. At the moment, the basis of the taxonomy of the genus is the works of E. Simmons, who published the identification key [15]. The differentiation of species according to E. Simmons is based solely on morphological traits. In our opinion, the works of M.B. Ellis [10, 11] made a great contribution to the possibility of identifying fungi with mural spores. However, due to the accumulation of the level of knowledge in molecular biology and general trends in the technology of identification of living organisms, at present, much attention is paid to modern methods of studying DNA. These methods include PCR with specific primers (including quantitative real-time PCR), DNA sequencing, and enzyme-linked immunosorbent assay (ELISA) [13]. According to V.F., Hannibal (2011), it is more expedient to discuss not one genus *Alternaria*, but in general the entire group of fungi close in terms of kinship - *Alternaria* hyphomycetes. *Alternaria* hyphomycetes includes 10 genera with a total volume of about 350 species [13]: *Alternaria* (about 280 species), *Alternariaster* (1 species), *Brachycladium* (2 species), *Chalastospora* (1 species), *Embellisia* (23 species), *Nimbya* (17 species), *Prathoda* (1 species), *Teretispora* (1 species), *Ulocladium* (24 species), *Undifilum* (2 species).

Previously, we carried out work to differentiate morphologically similar genera of micromycetes with mural spores. The identification of representatives of the genus *Alternaria* Nees ex Fr., *Stemphylium* Wallrot, and *Ulocladium* Preuss presents certain difficulties due to their morphological similarity. The appearance of data on several types of conidiogenesis [16] and the recognition of their taxonomic value [17] gave impetus to the development of a new technique for identifying deuteromycetes, forcing researchers to pay attention to the existence of a conidiogenic cell, the nature of conidia formation and proliferation (germination) of conidiophoids. Despite the existing differences in the morphology of conidia of the indicated genera [18, 19, 20], in particular: the presence of chains of spores and apical outgrowth (beak) in *Alternaria* conidia and the absence of those in *Stemphylium* [21]; the presence of the main, medium transverse septum in *Stemphylium* and its absence in *Ulocladium* (synonym - *Pseudostemphylium*) [22], ignoring the features of the sporulation apparatus, can lead to an incorrect result. E.G. Simmons [23] notes the distinctive features for these genera, expressed in the outlines of conidia and proliferation of conidiophores.

In the genus *Alternaria*, the conidiophoid may carry one conidia or spore chain. The base of the conidia is widened, the distal end is narrowed. The ovate form is usually clearly visible at an early stage of development. The shape of the conidia of *Ulocladium* is different, the proximal part is narrower than the distal one. A distinctive feature is the shape of the base of the conidia and the shape of the most spores - inverted ovoid in *Ulocladium* as opposed to ovoid in *Alternaria*. The genus *Stemphylium* differs in the way of proliferation of the conidiophore, the shape of its apical part. The conidiogenic cell is always inflated. A single conidium forms through the terminal pore. The previously formed conidium becomes lateral or disappears. The next conidium is formed through the apex of the secondary outgrowth, and a series of 3 or more proliferations is usually observed.

It is now generally accepted to divide the species of the genus into a group of small-spore, medium- and large-spore fungi. One of the problematic group from the point of view of taxonomy, is the group of so-called small-spore *Alternaria* species. Previously, all representatives of this group of small-spore species were often united under one name - *Alternaria alternata* (syn. *A. tenuis*). A number of attempts to describe the forms or to divide this species into smaller species, slightly differing morphologically, but specialized in different hosts (without proper experimental evidence) have led to the fact that now there are more than 100 names, estimated as small-spore taxa with unclear species characteristics that do not allow distinguish them from each other [15]. Many small-spore species (e.g. *Alternaria tenuissima*, *A. infectoria* and *A. alternata*) have very extensive lists of plant-hosts. At the same time, several related species are very narrowly specialized, which is associated with the ability to synthesize host-specific toxins. Among such pathogens are the causative agents of diseases of the apple tree, Japanese pear, several types of citrus fruits,

strawberries and tomatoes. The rest (not small-spore) species are mostly confined to one or several plant species of one genus, less often several genera, but one family [24, 25].

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## 5. Conclusion

As a result of the study conducted, 15 species of micromycetes were identified that are related to the genus *Alternaria* marked on 12 crops. The composition of micromycetes living on seeds of vegetable and green crops was revealed. In total, 21 species were recorded, of which 9 species include to the genus *Alternaria*. Many of the listed species differ in certain morphological and cultural features from the traits indicated in the determiners, which requires their study for a wider range of morphological and biochemical traits, using possible molecular markers.

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## Compliance with ethical standards

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### *Disclosure of conflict of interest*

No conflict of interest declared.

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