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Puccinia pimpinellae, a New Pathogen on Anise Seed in Egypt

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Abstract: Routine seed health inspection of anise seeds showed *Puccinia pimpinellae* to be a commonly observed fungus on seed samples collected from different locations and the commercial markets of Egypt. Symptoms were shown as black discolorations on seeds. Masses of uredio- and teliospores of the fungus were visually seen. In some samples, a seed washing technique was essential to inspect for the presence of the fungal spores. This is the first report of *Puccinia pimpinellae* as a seed-borne pathogen of anise in Egypt.

Key words: *Puccinia pimpinellae*, anise, seed-borne fungi, microscopic examination, washing test

INTRODUCTION

Anise (*Pimpinella anisum* L.) is an annual plant that belongs to the family *Apiaceae*. It's native to the Eastern Mediterranean, Western Asia and North Africa regions. The plant is fragrant and widely used in medicine and as a food flavoring (Chevallier, 1996). In Egypt, its cultivation has become more widespread to supply the increasing needs of medicinal industries and export markets.

In 2009, it was reported that rust caused by *Puccinia pimpinellae* Mart. has become one of the most prevalent and destructive diseases of anise plants in Egypt (Saber *et al*., 2009). It was assumed to be specific to anise among the members of the *Apiaceae* family.

Puccinia pimpinellae is an autoecious, microcyclic rust (uredial-telial in the life cycle) characterized by rust-colored uredial pustules on the upper and lower leaf surfaces, being more predominant on the underside of the leaf. The infection extended to stem, flowering buds, inflorescence and seeds. Severe infection may cause leaves to curl upwards, dessicate , turn brown and drop prematurely. Flowering set, fruit pill and fruit size can be reduced if early infection is severe. It adversely affects the germination, quality and weight of seeds.

Puccinia pimpinellae on anise plants was reported in the USDA (1960). Recently, Reichling and Bomme (2004), in the UK and Saber *et al*. (2009) in Egypt, reported *P. pimpinellae* as the causative pathogen of anise rust. Additionally, the fungus was reported on other wild *Apiaceae* plants *viz*., *Laserpitium prutenicum* in Germany (Scholler, 1996) and on wild chervill (*Anthrisscus sylvestris* L.) in Canada (Darbyshire *et al*., 1999).

Reports on the seedborne mycoflora of anise are scanty (Mathur and Manandhar, 2003). We are not aware of any available information on the incidence of a seed-borne rust fungus in anise.

This study is based on five years of survey to record the incidence of rust infection on anise seeds grown in Egypt and to discuss the testing methods involved in detecting this pathogen. Descriptions and certified taxonomic studies of the fungus were also considered.

MATERIALS AND METHODS

Samples: Fifty seed samples of anise (*Pimpinella anisum* L.) collected from commercial markets in different regions of Egypt including Alexandria, Assiut, Cairo, Damietta Dakhlia and Gharbia from 2000 to 2005 were used in this study.

Detection of seed-borne rust fungus on anise: Two methods, microscopic examination and a washing test, were used to detect rust spores on anise seeds following the procedures used by Agrawal and Mathur (1992) to detect teliospores of Karnal bunt disease of wheat.

Microscopic examination: Three 1,000 seed subsamples (approx. 2 g each) were taken from each of fifty seed samples of anise and carefully examined under a

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stereoscopic microscope (12-50X) to detect rust spores on the seeds. The heavily infected seeds, with the pericarp covered by pustules of spores or ruptured and filled with masses of spores, were counted and picked out. Percentage of infection was determined for each sample. The healthy-looking seeds were used for the washing test to detect non-pustulated spores on the seed.

Washing test: When it was difficult to detect non-pustulated rust spores by direct microscopic examination of the seed surface, the washing test was utilized with the same subsamples previously examined. Each seed replicate (2 g) was transferred to a 250 mL conical flask containing 25 mL of water. One drop of detergent was added to facilitate removing spores from the seed surface. The seeds were then shaken for 20 min using a mechanical shaker at 200 rpm. The resultant suspension was centrifuged for 20 min at 4000 rpm. The supernatant was discarded and the sediment was dispersed in 5 mL of 2% glycerol. The number of spores was counted microscopically by using a haemocytometer slide. The spore number per gram of seeds was calculated using the following equation:

$$\text{No.of spores per g} = \frac{\text{No. of spores per mL} \times 5(\text{stock volume, mL})}{2(\text{sample size, g})}$$

Identification of rust fungus: To examine the morphology and structure of uredinia and telia, heavily infected seeds were selected and carefully examined under a stereoscopic binocular microscope. Urediniospores and teliospores were scraped from the erupted pustules and mounted in a drop of lactophenol solution on a microscopic slide. A total of 50 spores were randomly chosen and observed, under an Olympus BH 100 microscope, for their morphological features. Measurements were made with a Leica Q-Win Image Analyzer. To observe germ pores in urediniospores, the spores were placed in a drop of lactic acid on a microscope slide, heated to boiling for a few seconds and mounted with an additional drop of lactophenol solution with aniline blue. The spores on the slide were smashed by applying gentle pressure on a cover slip over the preparation. Identification of the rust fungus was carried out according to Saber *et al*. (2009).

RESULTS AND DISCUSSION

Appearance of *Puccinia pimpinellae* on anise seeds: The infection of anise seeds by the rust fungus was observed microscopically. Infected seeds showed symptoms of brown and black discolorations, which were masses of uredio- and teliospores of the fungus, *Puccinia pimpinellae*. The degree of discoloration varied considerably from one seed to another (Fig. 1). In some cases, infected seed coats were intact and the spores of the fungus could be observed only when the discolored areas were pierced with a needle. In the case of severely affected seeds, most of the pericarp was destroyed and the mixed uredio- and teliospore mass was clearly seen (Fig. 2).

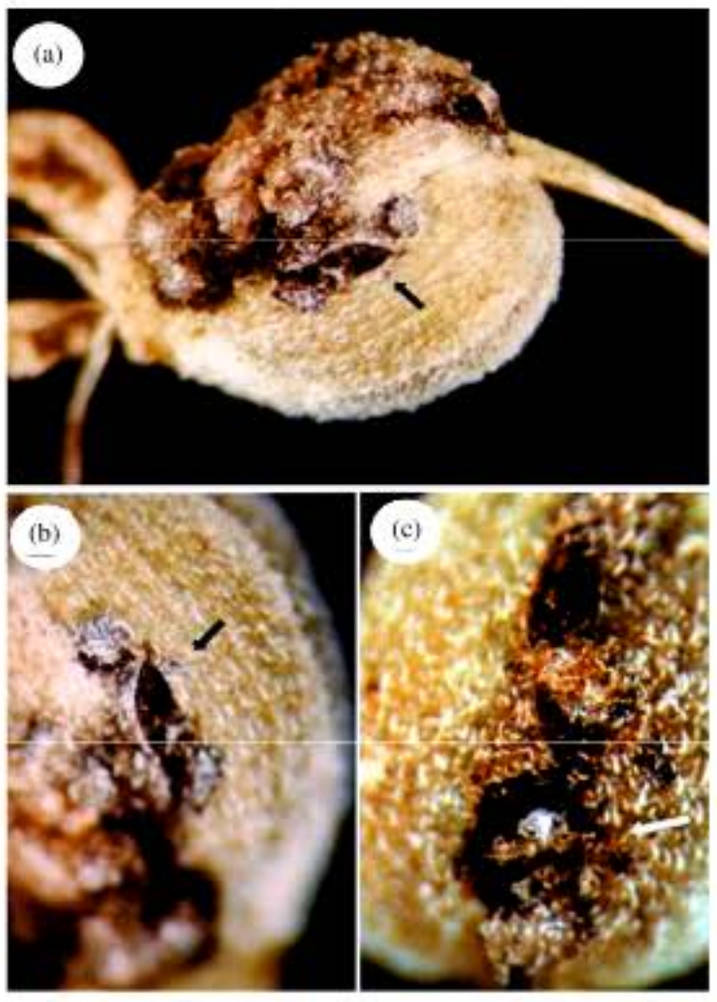

Fig. 1: Stereoscopic micrograph of a rust fungus *Puccinia pimpinellae* on an anise fruit showing the rust spores appearing from the ruptured pericarp (arrows; a, X25: b and c X40)

Fig. 2: Stereoscopic micrograph of a rust fungus appearing in a pustule from ruptured pericarp on an anise pedical (arrow; a, X100). (a) Typical uredinio and (b) teliospores mounted in water (X400)

Microscopic examination: Results in Fig. 3 show that among 50 anise seed samples, 13 samples (representing 26% of the seed lots) were found infected with *P. pimpinellae*. The highest incidence of infection (10.4%) was shown in seed sample No. 20., followed by samples No. 5 and 31 (8.5 and 8.3% infection, respectively). These samples were collected from Dakhlia province. However, the overall average of infected seed rust incidence ranged from 1.7-10.4%.

When using visual inspection to detect rusted seeds, only the seeds with prominent symptoms were recognized.

Washing test: Healthy-looking seeds were examined by the washing test. Thirteen samples were found infected by rust spores (Fig. 4). Two types of spores, uredio- and teliospores, were found in the ruptured anise pustules. The types of spores and their presence in each sample varied from one sample to another. Testing showed that urediospores varied from 6.50 to 42.50×10^4 spores per gram of seed. Samples No. 20 and 23 showed the highest count of urediospores (42.50×10^4 and 40×10^4 spore/g, respectively). Teliospore count ranged from 4.0 to 29.0×10^4 spores per gram. Samples No. 40, 23 and 8 showed the highest spore counts (28.0×10^4, 26×10^4 and 22.5×10^4 spores per g, respectively), while no significant variation among the others was seen. Samples No. 23 and 20, collected from Dakhlia province, showed the highest spore count (66 and 64.5×10^4 spores/g, respectively). The incidence of urediospores in the tested samples was much higher than the incidence of teliospores.

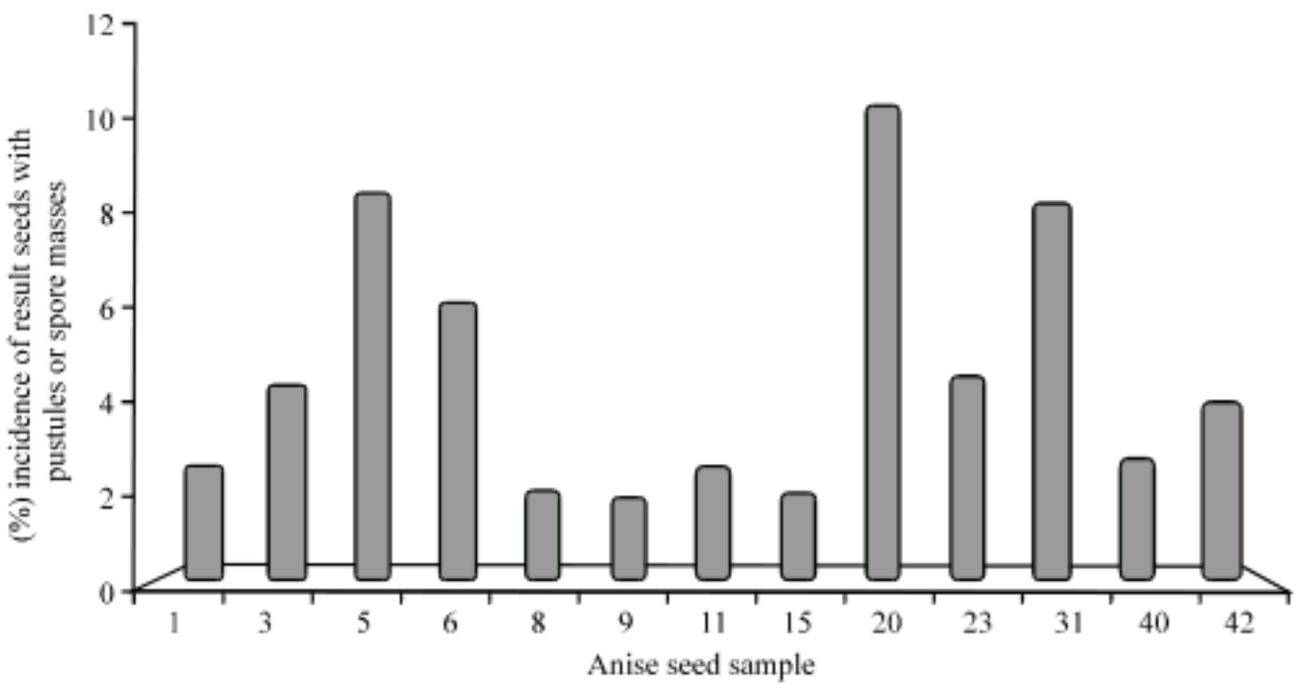

Fig. 3: Percentage of rust incidence on anise seeds infected by *Puccinia pimpinellae*. LSD = 0.870 at p≤0.05

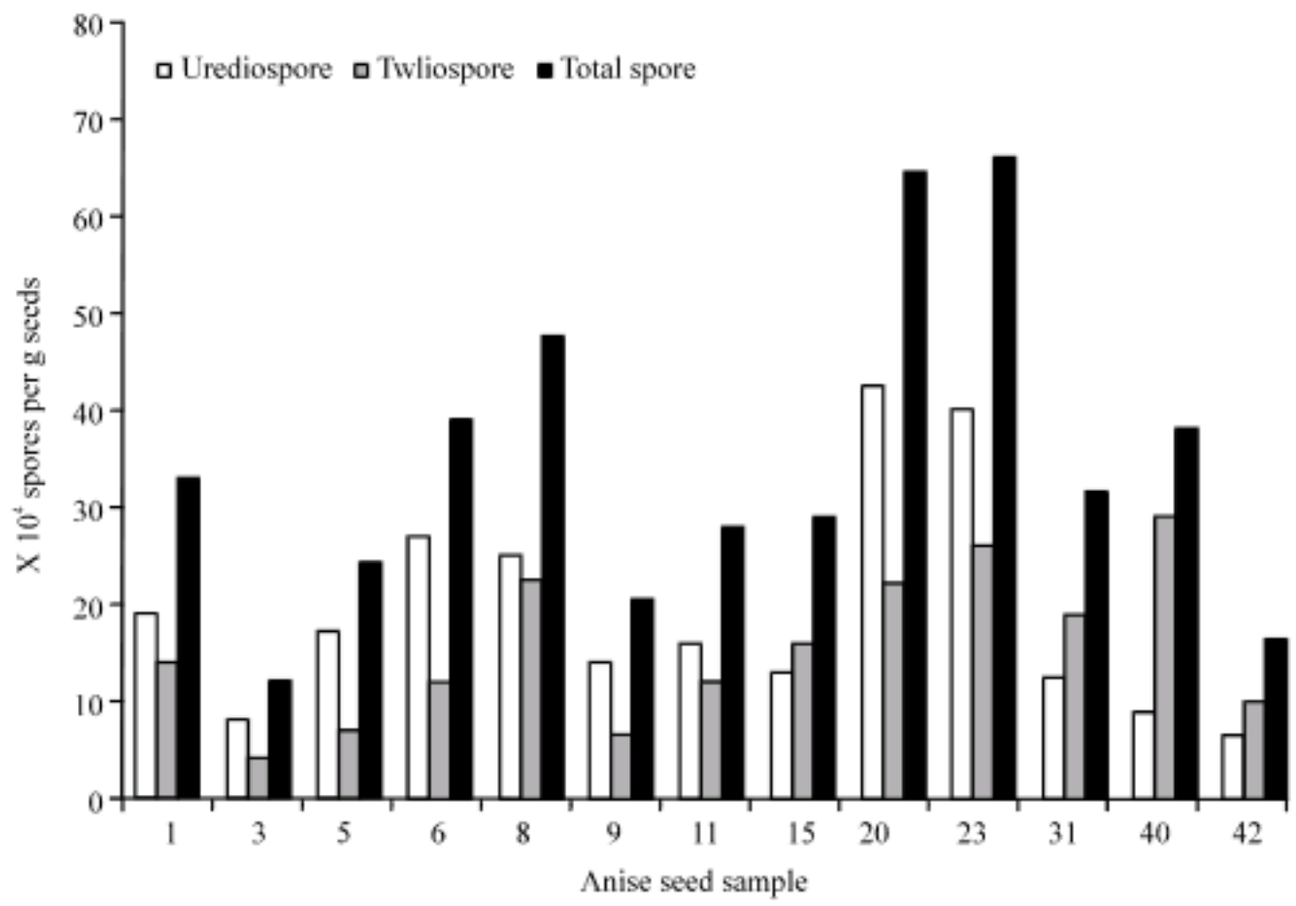

Fig. 4: Number of uredio- and teliospores of *Puccinia pimpinellae* detected on contaminated samples of anise seed. LSD at p≤0.05 = 7.937 for urediospores, 6.27 for teliospores and 13.454 for total spores

The results presented here show that washing was the most appropriate technique for detecting *P. pimpinellae*. It is capable of revealing urediospores and teliospores even when they are present in small numbers and in places where they cannot be seen under the microscope. This is an important consideration, especially when the seed samples are small-sized, as is the case in plant quarantine laboratories where the same sample may have to be tested by more than one method.

It has been reported that urediospores and teliospores could be transmitted through seed (black stem rust, *P. graminis* of wheat). The teliospores may germinate and develop into basidiospores which infect *Berberis*. Also, it has been reported that urediospores may germinate and infect new hosts (Neergaard, 1979). *Puccinia carthami* was reported as a seed-borne rust fungus in safflower. Black teliospores appear at the end of the crop growth cycle and can infest seeds or persist on the soil. These spores produce basidiospores, which then infect seedlings developing from infected seed or seedlings grown in a field that had safflower the previous year. Aeciospores and teliospores initiate the foliar stage of the disease on older plants and are wind-borne. The foliar infection is characterized by chestnut-brown pustules (containing urediospores) on cotyledons, leaves and bracts. These can re-infect the foliage in a number of recurring cycles (Klisiewicz, 1977; Bruckart, 1999).

In general, the use of rust-free seed is recommended, but even uncontaminated seed may become infested from seed cleaning and handling equipment.

Taxonomic identity of *Puccinia pimpinellae*: The fungus was uredial-telial in its life cycle. Both uredia and telia were formed on the abaxial surface of the seeds. The microscopic investigation revealed that urediospores were globose or subglobose-oblong and 23-31×22-27 µm in size. The walls were cinnamon-brown, uniformly echinulate and 2-3.5 µm thick at sides and up to 6 µm at the apex, with three equatorial germ pores (Fig. 2b).

Teliospores are formed within the uredinia or exclusively in the telia. The teliospores are two-celled, mostly broadly ellipsoid, obovoid-ellipsoid or oblong-ellipsoid, rounded at both ends but less prominently round at the pore, slightly constricted at the septum and 30-43×9-27 µm in size. The walls are chestnut-brown, smooth and 2-3.5 µm thick at sides and up to 4 µm thick at the apex. One germ pore was located in each cell: the upper pore apical and the lower variable, often near the pedicel. The pedicel was 6-16 µm (11 µm) long and basal, fragile, hyaline and persistent (Fig. 2c). Comparison of the observed characteristics of the fungus under discussion with the descriptions and morphological characteristics of rust fungi lead to the conclusion that this fungus was taxonomically identical to *Puccinia pimpinellae*.

Puccinia species are obligate parasites and attack many families of angiosperms (Neergaard, 1979). *Puccinia alli* occurs in seeds of *Allium* sp. (Alcock, 1931; Campacci, 1950), while black stem rust occurs profusely as sori in seeds of wheat (Hungerford, 1920). *Puccinia striiformis* (*P. glumarum*) causes yellow rust in wheat (Naumova, 1960). However, seed transmission of *P. graminis* and *P. striiformis* has not been proven, although It is suspected (Neergaard, 1979; Mathur and Manandhar, 2003).

The isolation of *Puccinia pimpinellae* associated with anise seeds is the first report of this rust in Egypt.

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