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Characterization of New *Bipolaris* Spp.: The Causal Agent of Rice Brown Spot Disease in the North of Iran

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ABSTRACT

Brown spot is one of the most important seed-borne diseases of rice. It causes qualitative and quantitative damages on rice. Although this disease prevails throughout the rice growing areas of Iran, there is no precise information about its dispersal, species and the rate of damage. Therefore, this study was carried out in order to identify the genus and species of rice brown spot agent in Guilan. To do so, at first some samples were collected from paddy fields in Guilan. In order to isolate the fungus from disease tissues the obtained samples were cultured on potato dextrose agar (PDA) medium and by this, 342 isolates were isolated. Isolates were cultured due to sporulation on culture medium of tap water agar (TWA) + wheat straw. Conidium and conidiophore morphology and the process of conidium formation and pattern of its germination were studied in order to identify the taxonomy. According to the results, isolates were belonged *Bipolaris oryzae, B. victoriae, B. indica and B. bicolor*. The total isolates include of 10% *B. oryzae*, 85% *B. victoriae*, 2% *B. indica* and 3% *B. bicolor*. Pathogenicity test of isolates in these four species was done in desiccator, which revealed the pathogenicity of the species and their ability to cause brown spot on rice.

Key Words: Rice; Brown spot; *Bipolaris*; New species; Characterization

INTRODUCTION

Brown spot is one of the most important diseases of rice in Guilan province in the north of Iran (Safari Motlagh *et al*., 2006). It causes seedling blight and damages the foliage and panicles of rice, particularly when rice is grown in nutritionally deficient or otherwise un-favorable soils (Marchetti & Peterson, 1984). At first the causal agent of brown spot disease was named by Breda de Haan *Helminthosporium oryzae* (Gangopadhyay & Padmanabhan, 1987). Nowadays the graminicolous *Helminthosporium* species are divided into three genera based on colony, conidiophore and conidial morphology, type of conidial germination and the type of hilum structure: *Bipolaris, Drechslera and Exserohilum*. Their telemorphs were from ascomycetes and consist of: *Cochliobolus, Pyrenophora and Setosphaeria*, respectively (Sivanesan, 1987). Brown spot was reported for the first time in Iran at 1957 (Behdad, 1982). The causal agents of brown spot disease in Mazandaran province reported include *Bipolaris oryzae, B. sorghicola and B.* sp. (Khosravi, 1998), in while in Fars and Kohgiloyeh and Boyerahmad provinces causal agents include *B. oryzae, B. tetramera and Exserohilum rostratum* (Razavi, 1992). The goal of this research was to identify the genus and species of fungus of rice brown spot agent in Guilan province.

MATERIALS AND METHODS

Collection and culture of fungal isolates. Leaves and panicles with symptoms of brown spot were sampled from five locations in each field. Each sampled location was approximately 5 × 8 m and locations were approximately 35 m apart. Leaves and panicles were transferred to the laboratory and then gradually isolated the causal agents from disease samples following the method of Xia *et al.* (1993). Samples were trimmed, washed by sterile distilled water and placed on potato dextrose agar in petri dishes at 25–30°C for 2-3 days. TWA + wheat straw medium was used for sporulation. Petri dishes were then incubated at 20-26°C in the dark or artificial light supplied by fluorescent light on a 12 h light/dark photoperiod for 10-20 days (Sivanesan, 1987). For avoid of bacterial contamination sulfate streptomycin antibiotic was used (Safari Motlagh *et al*., 2006). Conidia were single–sporulated and then placed onto sterilized filter.

Papers were incubated in sterilized vials at freezer with -20°C (Safari Motlagh *et al*., 2006). A total of 342 isolates were collected.

Study and identification of fungi. Morphological studies were carried out on TWA + wheat straw medium. Each of filter papers were placed onto PDA medium for 2-3 days. Then, the section of colonies was transferred to TWA +

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Table I. Disease rating caused by 4 species of *Bipolaris*

Species	Disease rating
B. oryzae	2.822
B. victoriae	2.171
B. indica	2.025
B. bicolor	2.002

Fig. 1. Colony of *Bipolaris oryzae*

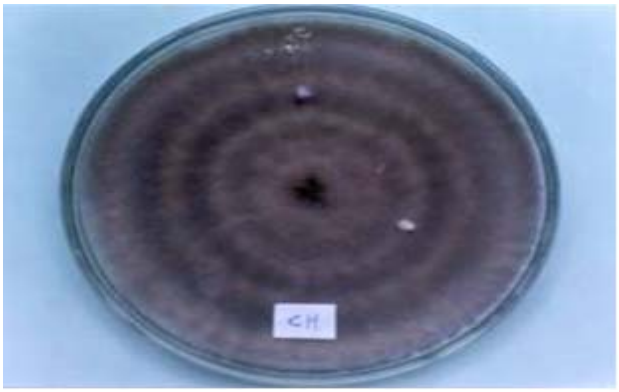


Fig. 2. Conidiophore of *B. oryzae* (×460)

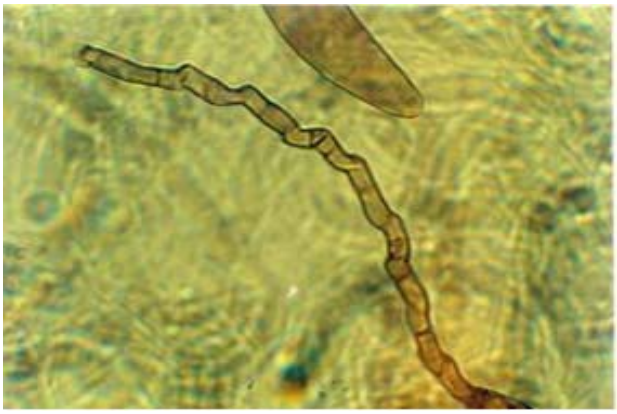
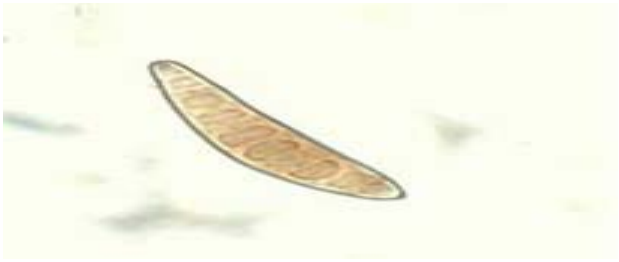


Fig. 3. Conidium of *B. oryzae* (×460)



wheat straw medium for about 10-20 days in incubator at 26°C and 12 h photoperiod. Afterwards, morphological observations were taken based on colony, conidium and conidiophore morphology, type of conidial germination and structure of hilum (Ellis, 1971; Sivanesan, 1987).

Pathogenicity tests. Pathogenicity tests were carried out in a desiccator. In each of two desiccators (one desiccator as control) 2 petri dishes were placed each carrying 10 seeds of rice cultivar khazar. Seeds were then sterilized at Banmary at 52-57°C and cultivated in sterilized sand and were incubated at 25°C. Distilled water was added to perti dishes. Following 16-18 days, seedlings containing two foliages waere inoculated by suspension of spores. The concentration of conidia was adjusted to approximately 40 000 mL^{-1}. To increase the surface adsorbtion, 1% Tween–20 was applied. Evaluation of symptoms was performed 7 days after inoculation. Therefore, standard evaluation system for rice and Horsfall–Barratt was applied (IRRI, 1996).

$$\text{Diseaserating} = \frac{(N_1 \times 1) + (N_2 \times 2) + ... + (Nt \times t)}{(N_1 + N_2 + ... + N_t)}$$

N= Number of leaves in each of rate, t= Number of treatments.

RESULTS AND DISCUSSION

A total of 342 collected isolates belonged to Bipolaris Shoemaker. These isolates were divided into 4 groups based on morphological characters, as follows:

Characteristics of the first group. Grey to dark grey conidial colonies grew and spread rapidly (Fig. 1). Aerial mycelium was fluffy, cottony, grey olivaceous with brownish tinge. Conidiophores were single or in small groups, straight to flexuous, sometimes geniculate, pale to mid brown or olivaceous brown, pale towards the apex, septate 430–580 × 4–7 μm (average 500×5 μm) (Fig. 2). Conidia were usually curved, navicular, fusoid or obclavate, occasionally almost cylindrical, pale to mid golden brown, smooth, 5–12 distoseptate, 46.5–125×10–26 μm and hillum was minute dark or light, often protruding, slightly papillate (Fig. 3 & 4). The first septum was sub-median, the second delimited the basal cell and the third formed toward the apex of the conidium. Conidia germinated from polar cells and germ tube from the basal cell usually emerged immediately adjacent to the hilum and grows in the direction of the long axis. The general characteristics of this group are similar to *Bipolaris* Shoemaker (Shoemaker, 1959; Alcorn, 1983 & 1988), but special characteristics, such as shape and color of colony, morphology of conidium and conidiophore are similar to *B. oryzae* (Ito & Kurib) Drechsler ex Dastur (Ellis, 1971; Sivanesan, 1987). This species contains 10% of isolates.

Characteristics of the second group. Conidial colonies grew, spread, grey to dark grey (Fig. 5). Aerial mycelium was fluffy, cottony and pale to mid yellowish. Conidiophores were single or in small groups, straight to flexuous, sometimes geniculate above, pale to mid brown, smooth, septate, 50–40×5–9 μm (Fig. 6). Conidia were slightly curved, broadly fusiform or obclavate fusoid, pale or mid golden brown, smooth, 4–13 (mostly 8–10) distoseptate, 32–143×9–24 μm and hilum was minute but not protrudiated (Fig. 6). Type of conidium formation and germination of this group are similar to the first group (Fig. 7). The general characteristics of this group are similar to

Fig. 4. Hilum of *B. oryzae* (×1200)

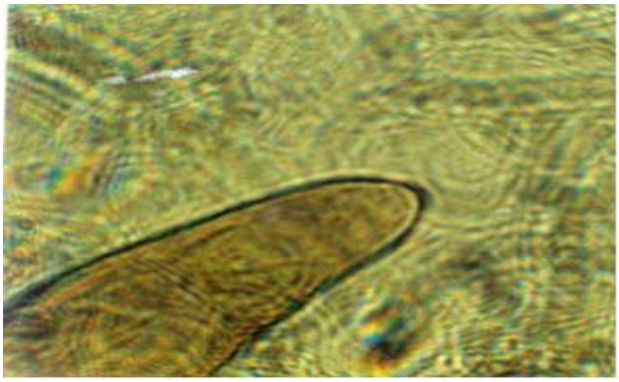


Fig. 5. Colony of *B. victoriae* (×230)

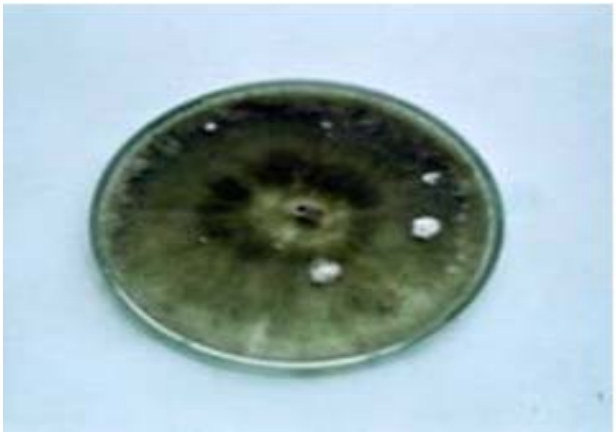


Fig. 6. Conidiophore and conidium of *B. victoriae* (×230)

Bipolaris Shoemaker, but special characteristics such as morphology of conidium and conidiophore also the structure of hilum are similar to *B. victoriae* Nelson (Nelson, 1961; Ellis, 1971; Sivanesan, 1987). This species contains 85% of isolates.

Characteristics of the third group. Colonies were effuse, dark blackish brown and velvety (Fig. 8). Aerial mycelium was fluffy, pale brown to dark brown. Conidiophores were

Fig. 7. Germination of conidium of *B. victoriae* (×460)

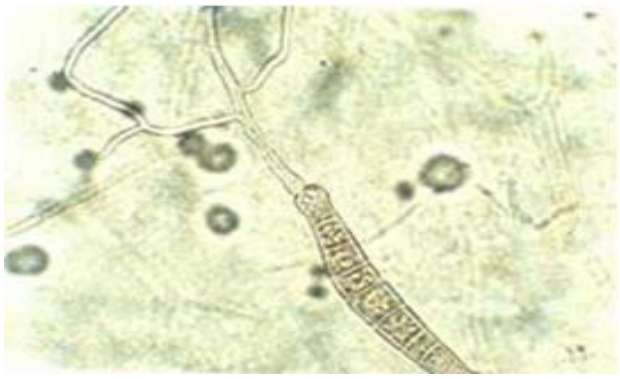


Fig. 8. Colony of *B. indica*

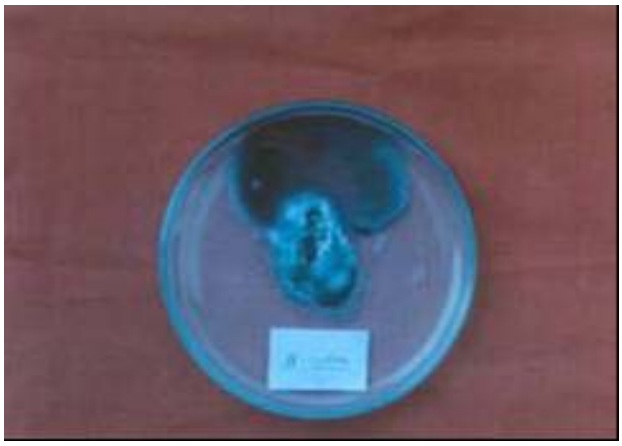
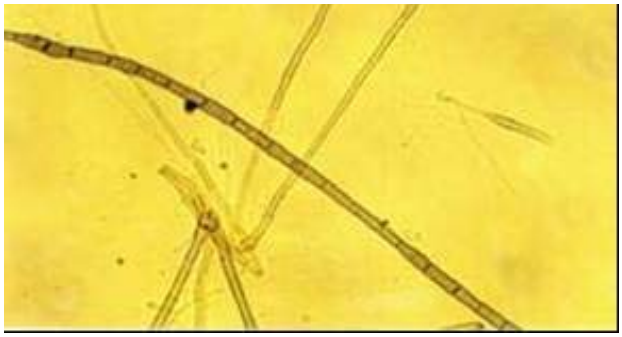


Fig. 9. Conidiophore of *B. indica* (×460)



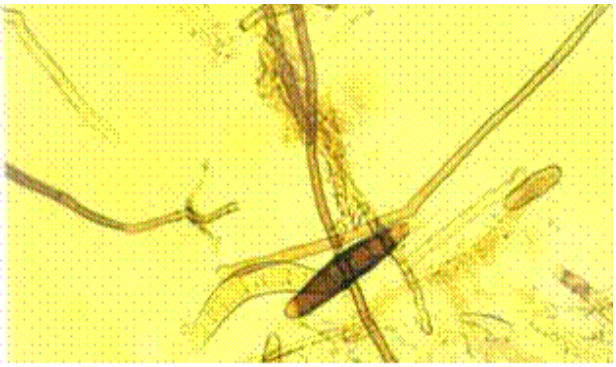
single, straight or flexuous, mid to dark olivaceous brown, smooth, septate, cylindrical, 260–350×7–10 μm (Fig. 9). Conidia were straight, shortly clavate or more rarely broadly ellipsoidal, with a markedly protuberant hilum or the hilum without protuberant in some conidia, mid to dark olivaceous brown, smooth, 35–69×12–25, 4–8 distoseptate (mostly 6) (Fig. 10). The general characteristics of this group corresponded with *Bipolaris* Shoemaker, but special characteristics corresponded with *B. indica* Rai, Wadhwani and Tewari (Sivanesan, 1987). This species consists 2% of isolates.

Characteristics of the fourth group. Colonies were effuse,

Fig. 10. Conidium of *B. indica* (×460)

Fig. 11. Colony of *B. bicolor* (×230)

Fig. 12. Conidiophore and conidium of *B. bicolor* (×230)



grey to blackish brown and velvety (Fig. 10). Aerial mycelium was fluffy, yellow, pale brown to dark brown. Conidiophores were single or in small groups, straight to flexuous, septate, smooth, occasionally upper part geniculate, golden brown, 300–400×5–10 µm (Fig. 11). Conidia were straight or rarely curved, cylindrical or rather broader in the middle, tapered towards the ends rarely obclavate, 3–8 (mostly 6) distoseptate, 31–95 × 9–17 µm central cells of mature conidia were often dark brown but end cells hyaline or very pale and frequently cut off by a very dark septum and hilum was 3–5 µm (Fig. 12). The general characteristics of this group were corresponded with *Bipolaris* Shoemaker, but special characteristics corresponded with *B. bicolor* Poul and Parbery (Ellis, 1971; Sivanesan, 1987). This species consists 3% of isolates.

Pathogenicity tests. The symptoms created by these species were necrotic spots to leaf death. The first symptoms of *B. oryzae, B. victoriae and B. indica* appeared 4 days after inoculation and the first symptoms of *B. bicolor*, 2 days after inoculation.

The results indicated that not only the symptoms but also the virulence in these species was different (Table I). Ocfemia (1924) reported the first symptoms of *B. oryzae* 24 h after inoculation (Ou, 1985). Sato (1965) observed that the color of infected cells changed 17–20 h after inoculation (Sato, 1965). Gangopadhyay and Padmanabhan (1987) showed that no pathogenic changes were correlated with variety of strain and conidium morphology (Gangopadhyay & Padmanabhan, 1987). Whereas, Razavi (1992) reported that aggressiveness of *B. oryzae* is more than that of *B. tetramera* and *Exserohilum rostratum* (Razavi, 1992). Although there was differences between severity symptoms caused by different species of *Bipolaris*, but these differences were not significant. Screening of species is difficult, because the shape and size of conidium and conidiophore and the number of septum in this fungus is affected by environmental factors such as pH levels, light, temperature, sucrose and concentration of azote (Harding, 1975; Teviotdales & Hall, 1976; Trainor & Marthinson, 1978; Elliot, 1989). Therefore, the evaluation of taxonomy of this fungus by morphological method needs further precise and detailed studies.

CONCLUSION

The results showed that not only the symptoms but also the virulence in these species were different. The virulence in *B. oryzae* was more than *B. victoriae* and the latter was more than *B. bicolor*. Also, variability between various species and particularly within species are so great that they get the taxonomists mixed up. Thus, the morphological integrated studies along with molecular methods based on DNA are amended.

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