Biology and epidemiology of *Tilletia indica* inducing Karnal bunt (partial bunt) of wheat (*Triticum aestivum*) in arid regions

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**ABSTRACT:** Wheat (*Triticum aestivum* L.) fields were surveyed during April-May 2007, 2008, 2012 and 2013, to see the incidence of Karnal bunt of wheat (Kb) caused by *Tilletia indica* in south of Iran. The assessments of disease indicated that there were no teliospores in soils. The experiments were conducted to investigate a substitute for teliospore which survive and produce secondary sporidia. In *vitro* teliospores on water agar, a type of tiny translucent spores, were seen to reproduce by budding on lids of Petri-dishes several weeks after germination. Floccose colonies producing allantoids sporidia were initiated after mounting the lids over fresh potato dextrose agar medium. On dehydrated colonies of fungus initiated from teliospores on water agar, kept for a period of 2 years at dry condition (10-14% RH; 10-45°C), small radiated colonies were seen to be formed producing secondary sporidia under humidity provided from a piece of moistened cotton wool. Similar tiny spores were captured on slides used as trap mounted over the samples of teliospores free soils under humid conditions. While a number of tiny spores germinated into colonies producing secondary sporidia, numerous secondary sporidia were also seen on the traps among tiny spores which seemed to be released and deposited on slides from soil. Thus, results indicated that wheat fields have been infested through decades by a moiety of *T. indica*; so called minisporidia, providing secondary sporidia to initiate disease at favorite conditions in south of Iran.

**Key words:** Karnal bunt, monokaryotic life cycle, soil-borne plant pathogen, *Tilletia indica*, wheat diseases

Karnal bunt (Kb) (partial bunt) of wheat caused by *Tilletia indica* Mitra (= *Neovossia indica* (Mitra) Mundkur) is a minor disease of wheat (Weise, 1987). However, losses could be high in certain weather conditions. In 1996, Iran lost more than 100,000 ha of wheat crop due to this disease. The epidemic happened when an outbreak occurred in - arid southeast of the country (Torabi et al., 1996). Disease incidence was 2-28%. In most of the fields, at the time of harvesting, black fogs produced from the release of teliospores which deposited into the soil. Since then, Kb is found in seed samples at very low incidence, and in very few kernels. Low infection results in low if any supply of teliospores to soil, as small sori are difficult to rupture by mechanical pressure of harvesters. This results in soil depletion from teliospore in time, as in a survey conducted since 1997, for a period of five years, the number of teliospores in 325 wheat fields of three provinces (Fars, Hormozghan and Kerman) was reduced from 350-4150 in 1997 to 0-36 in 2000 per 25 g of dry soil (Malhipour, unpublished data).

Mundkur (1943) was the first who postulated that Kb is initiated after local infection of florets via air-borne secondary sporidia that originate from germinated teliospores at or near to the soil surface. Since then, teliospore has regarded as the only survival unit for *T. indica* (Krishna and Singh, 1983; Singh, 1994). Studies indicated in different soil types that teliospores survive 2-5 years (Krishna and Singh, 1983; Bonde *et al.*, 2004). Considering the longevity of teliospores of *T. indica*, the questions are: is there any other source (s) than teliospores to survive, providing secondary sporidia necessary for infection in dry areas? What was the source of inoculum in 1996 outbreak in south of Iran? Are there any risks of future outbreaks in such areas?

The objectives of this study were to find out the state of Kb incidence related to biology and epidemiology of *T. indica* during the last 7 years period (2007-2008 and 2012-2013) in arid regions in south of Iran.

**MATERIALS AND METHODS**

**Sampling**

The study was carried out in regulated areas, namely Dorz-e-Saiban of Lar (55°. 25'. 16" N; 27°. 51'. 53" E) and Fork-Abshor of Darab (55°. 13'. 22" N; 28°. 17'. 04" E) districts, as an arid regions with absolute maximum temperature reaching 47-50°C in southeast of Fars province, Iran. During harvesting period (April-May) in 2007, 2008, 2012 and 2013, 50 wheat fields were surveyed in each region and 2 kg seed samples were collected from different parts of the piles before transporting to the silos. Samples were inspected visually for the presence of partially bunted kernels. Microscope slides were prepared from teliospores obtained from the sorus on the infected seeds to verify *T. indica*. The soil samples were also taken subsequently from fields with positive partial bunt kernels. Each sample (approximately 2000 g) consisting of 10 sub-samples were taken from 0-5 cm soil level on M or W pattern. Soil samples were air-dried and stored inside glass jars. Size-selective
producing allantoids secondary sporidia. During this period there were no spores seen deposited on the inner surface of lids, and no colonies developed on PDA after mounting the lids on media. During second time period (10-15 days), allantoids secondary sporidia were seen deposited on inner surface of lids. A number germinated into single hyphae producing more of its kind and filiform secondary sporidia on the sides. At this time period, floccose colonies were initiated after mounting lids on PDA. In the third time period (15-20 days) on the lids, a number of secondary sporidia were seen to start autolysis which continued in the fourth time period (20-30 days) also. Meantime, tiny translucent spores (0.8-1.6µm) were seen to be produced from fragmentation of filiform secondary sporidia and reproduce by budding. Floccose colonies appeared 2-3 days after replacing the lids on fresh PDA.

On the inner surface of the lids, more secondary sporidia produced and germinated into colonies producing allantoids and filiform secondary sporidia in sealed than unsealed plates in second time period (10-15 days). Meantime, sealed condition favoured more autolysis of secondary sporidia, but more production of tiny spores from fragmentation of filiform secondary sporidia (Fig. 1a). However, multiplication of tiny spores was a phenomenon which continued on inner side of lids in humid (sealed plates) and dry (unsealed plates) conditions. Tiny spores that reproduced by budding in sealed and unsealed plates were only propagules observed on inner surface of Petri lids at the end of 60 days. Mounting the lids on fresh PDA, white floccose colonies started to appear on the media after 2-3 days producing secondary sporidia. In dry conditions on inner side of lids of dried cultures were seen to increase in a unique pattern of colonies with a certain boundary (Fig. 1b).

**Longevity in vitro**

Linear and star-like colonies appeared in 3 days after 2 years on dehydrated WA media and on the inner sides of Petri-dishes wall under humid conditions producing secondary sporidia (Fig. 1c).

**Trap**

Numerous tiny spores similar to those produced in culture, beside secondary sporidia, were seen deposited onto glass slides used as trap over the soil samples (Fig. 1d,e). A number of tiny spores germinated into linear colonies produced secondary sporidia on glass slides. The result of *in vitro* studies indicated that after germination of teliospores of *T. indica*, translucent tiny spores (minisporidia), were produced from fragmentation of filiform secondary sporidia, which were multiplied by budding. Assessments of soil samples from arid regions indicated that instead of teliospores, minisporidia survive to produce secondary sporidia. This moiety of fungus persist dryness and withstand high temperatures. The results implies that when soil is infested somehow by teliospores, monokaryotic cycle characteristic of
Basidiomycota (Anonymous, 2014), starts to ensure indefinite contamination of soil through the production of tiny spores. These spores are able to germinate in a short time producing secondary sporidia at 20-22°C and >70% RH, conducive to disease development. After infection dikaryotic cycle is started which ends up with the formation of teliospores. Tiny spores are only moiety of the fungus by which T. indica survives in soil of dry regions indefinitely, but Kb is induced in plant to an extent determined by epidemiological studies (Nagarajan, 1991; Mavi et al., 1992). Therefore, once Kb is seen in arid region, it is sign of soil contamination by T. indica minisporidia. In these regions, Kb usually occurs as a minor disease in microclimates.

Tiny spores are similar to secondary sporidia. They not only reproduce by budding, but they forcibly discharge and germinate into colonies producing secondary sporidia. On the contrary, tiny spore persists and reproduces under high temperatures and dry conditions, but secondary sporidia do not. The results confirm those of Smilanick et al. (1989).

Minisporidia are light enough to be carried by wind and settle on soil to contaminate the lands or be trapped by rough surfaces and in cracks etc., where they increase by budding. However, Kb occurs in places where susceptible hosts are present at right developmental stage and climate conditions favourable for infection and subsequent development of the fungus inside plant tissue. This might be the reason Kb is present in scattered areas around the world.

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