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Review Article

Chemotherapy of karnal bunt of Wheat: A Review

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Abstract

Bread wheat (*Triticum aestivum* L.) is the main staple food crop and major source of nutrition for the people of Pakistan. Many factors contribute to low yield in Pakistan, diseases being one of them. Karnal bunt (partial bunt) of wheat caused by *Tilletia indica* (Mitra) Mundkur, causes considerable losses in the yield of wheat. The disease not only reduces the weight of grains, but also deteriorates its quality and makes it unacceptable for human and animal consumption. The pathogen infects the ovaries in the emerging wheat heads and converts the grains partially or completely into dark colored powdery masses of teliospores. The diseased fields emit a foul smell like that of rotten fish due to production of trimethylamine. Karnal bunt can reduce wheat yields. Karnal bunt differs from other diseases of wheat in that the pathogen infects plants during anthesis and it sporulates on the same generation of the host which it infects. Neither all spikes of plant nor all grains in spike are affected by the disease and usually a few irregularly distributed kernels are bunted. Its spores remain viable in soil for a long period. Teliospores are very resistant to adverse environmental conditions. Although many control strategies have been suggested for the management of karnal bunt disease and the strategies include seed treatment with hot water and solar energy, seed treatment with fungicides and soil drenching with fungicides, however, the results were not convincing. The cheapest and the most feasible method of Karnal bunt control is the use of host resistance and breeding for varieties resistant to karnal bunt disease. Control of karnal bunt has now become a major concern in Pakistan due to scarcity or non-availability of resistance in commercial wheat varieties under cultivation. The gravity of the situation of the disease calls for evaluation of fungicidal toxicants against the disease for its management. As the pathogen is soil, seed and air-borne, it can penetrate locally into host plant, so application of spray fungicides is very critical. Epidemiological factors have great influence on the epidemic development of karnal bunt disease. Wheat is vulnerable to karnal bunt fungus only during a 2-3 week windows of its physiological development stages if the environmental conditions happen to be conducive during this short period for successful infection and the weather favourable for the disease development does not exist every year. For the better management of this disease following line of work was designed: i) Survey for the incidence of karnal bunt disease of wheat in major wheat growing areas of the Punjab was conducted, ii) Screening of advanced lines/varieties of wheat was done by artificial inoculation, iii) Determination of correlation was done of environmental factors with karnal bunt disease of wheat, iv) A disease predictive model was designed to determine the relationship of environmental factors with disease incidence. However, an integrated approach is practicable and conductive for the better management of karnal bunt.

Keywords: Wheat, karnal bunt, biology, symptoms, management strategies, epidemiological factors

Introduction

Bread wheat (*Triticum aestivum* L.) is the main staple food crop and major source of nutrition for the people of Pakistan. It is grown in winter months of November to April on an area of 27.69 million hectares (Anonymous, 2007a). Average wheat yield of Pakistan is 2.38 mt/ha, which is

extremely low as compared to other wheat producing countries of the world such as Ireland, Denmark, United Kingdom, France, Egypt and Saudi Arabia having 7.86, 7.83, 7.78, 6.23, 6.15 and 4.48 mt/ha, respectively (Anonymous, 2007b). Many factors contribute to low yield in Pakistan, diseases being one of them. Karnal bunt (partial bunt) of wheat caused by *Tilletia indica* (Mitra) Mundkur, causes considerable losses in the yield of wheat. The disease not only reduces the weight of grains, but also deteriorates its quality and makes it unacceptable for human and animal consumption (Gopal and Sekhon, 1988). According to Pamela *et al*. (2004) it fulfills the following criteria of its inclusion in the list of emerging infectious diseases (EIDs) of plants, (i) it has increased incidences, geographical distribution and host range (ii) it has changed its pathogenesis (iii) it has been newly evolved or newly recognized. The emergence of plant EIDs is similar to those of humans, wildlife and domestic animals (Pamela *et al.*, 2004). Modern changes in the agricultural pattern, implementation of modern farming techniques, ecosystem, intensification and globalization stimulate the attacking mechanisms of the pathogen, which pose threats to agriculture or biodiversity conservation.

According to Dobson and Foufopoulos (2001) understanding of factors driving the plant EIDs require knowledge of host-parasite biology, use of multiple-host system models parameterized for a large number of environmental, ecological and biological factors.

Karnal bunt was first detected in 1931 at karnal in Haryana, India and hence it is called Karnal bunt (Mitra, 1931). It bears many names such as Karnal bunt, new bunt, partial bunt, incomplete bunt, Indian bunt and stinking smut. Singh *et al*. (1989) reported that Karnal bunt was a disease of wheat, durum, rye and triticale (a hybrid of wheat and rye). Though the disease is native to South Asia but subsequently it has been reported from Iran, Syria, Afghanistan, Iraq, Mexico (Joshi *et al*., 1983), Nepal (Singh and Dhaliwal, 1989) and United States (Ykema *et al*., 1996). The disease remained less damaging till late 1970 but subsequently severe epidemics started occurring coinciding with the change over to high yielding, irrigated, semi dwarf and high fertilizer input farming.

In early 50s, Karnal bunt disease was considered to be a disease of minor importance and it was confined to hills of Pakistan. In 1971, the disease was reported from Sialkot, Gujranwala and Mardan districts during the crop years of 1966-71 (Hassan, 1971) and the frequency of disease ranged from traces to 2.0 percent. The disease remained endemic for considerable period of time in the Northern area of Pakistan and later it spread to south and was reported as far as Jhang, Khanewal and Muzaffargarh district of the Punjab (Bhatti and Ilyas, 1986; Asma *et al*., 2012). A little later the disease became wide spread throughout the Punjab and was prevalent in 23 districts with a frequency range of 0.32 to 3.50 per cent (Ilyas *et al*., 1989a). At present almost all commercial varieties of wheat under cultivation are susceptible to Karnal bunt and the disease incidence is aggravating with the passage of time. The disease reduces wheat yields and can cause a fishy, unpalatable odor and taste in wheat flour, reducing flour quality (Bonde *et al*., 1997; Sekhon *et al*., 1980; Singh and Bedi, 1985). Since karnal bunt affects the international trade of commercial wheat grain and movement of germplasm, the presence of the disease can cause economic loss to wheat exporting countries (Bonde *et al*., 1997; Shakoor, 2009). The yield and quality losses are generally minor; the most economic loss can be attributed to the quarantine status of the pathogen (Babadoost, 2000; Butler, 1990).

The pathogen infects the ovaries in the emerging wheat heads and converts the grains partially or completely into dark colored powdery masses of teliospores. The diseased fields emit a foul smell like that of rotten fish due to production of trimethylamine. Karnal bunt can reduce wheat yields. There is no estimate of losses, due to this disease, occurring in Pakistan; however, survey in India conducted during the years of heavy disease revealed a total loss of 0.5 percent, but in some fields where 89 percent of the kernels were infected, the yield losses ranged from 20-40 percent in highly susceptible varieties (Anonymous, 2004). Brennan *et al.* (1990) estimated the economic losses from Karnal bunt of wheat in Mexico to be US $ 7.02 million per year. Besides yields losses, Karnal bunt can reduce wheat flour quality due to fishy, unpalatable odour and taste, if a

grain lot contains 1-4 percent infected seed (Bonde *et al*., 1997; Hussain *et al*., 1988; Mehdi *et al*., 1973; Sekhon *et al*., 1980). If in a grain lot 5 percent of the grain is infested, the quality of the flour recovery and chemical changes in composition of flour and gluten contents cause poor dough strength (Sekhon *et al*., 1980; Gopal & Sekhon, 1988). Karnal bunt is also a disease of quarantine interest and it affects the international trade of commercial wheat grain and movement of wheat germplasm through out the world. Thus presence of diseased grain in wheat lots can cause economic loss to wheat exporting countries (Bonde *et al*., 1997; Babadoost, 2000; Butler, 1990).

Karnal bunt differs from other diseases of wheat in that the pathogen infects plants during anthesis and it sporulates on the same generation of the host which it infects. Neither all spikes of plant nor all grains in spike are affected by the disease and usually a few irregularly distributed kernels are bunted (Mitra, 1935; Bedi *et al*., 1949; Dhaliwal *et al*., 1983). Infection of individual kernels varies from small points of infection to completely bunted kernels but completely infected ones are rare (Chona *et al*., 1961). The embryo of the infected kernels usually remains undamaged except when infection is severe (Cashion and Luttrel, 1988) but the endosperms of the kernels get shrunken to varying degrees. At maturity severely infected kernels are filled with teliospores of the pathogen which serve as primary source of inoculum. The spikes of infected plants are generally reduced in length with less number of spikelets (Mitra, 1937). During harvest, infection sori are broken resulting in contamination of healthy seeds, soils, equipment, machinery and the vehicles with the liberated spores. The spores may be blown by wind for long distances.

Karnal bunt differs from other diseases of wheat in that the pathogen infects plants during anthesis and it sporulates on the same generation of the host which it infects. Neither all spikes of plant nor all grains in spike are affected by the disease and usually a few irregularly distributed kernels are bunted (Krishna and Singh, 1983b; Babadoost *et al*., 2004; Bonde *et al*., 2004). Although many control strategies have been suggested for the management of Karnal bunt disease and the strategies include seed treatment with hot water and solar energy, seed treatment with fungicides and soil drenching with fungicides (Anonymous, 2005), however, the results were not convincing. The cheapest and the most feasible method of karnal bunt control is the use of host resistance and breeding for varieties resistant to karnal bunt disease.

Control of Karnal bunt has now become a major concern in Pakistan due to scarcity or non-availability of resistance in commercial wheat varieties under cultivation. The gravity of the situation of the disease calls for evaluation of fungicidal toxicants against the disease for its management. As the pathogen is soil, seed and air-borne, it can penetrate locally into host plant, so application of spray fungicides is very critical. Epidemiological factors have great influence on the epidemic development of karnal bunt disease. Wheat is vulnerable to karnal bunt fungus only during a 2-3 week windows of its' physiological development stages if the environmental conditions happen to be conducive during this short period for successful infection and the weather favourable for the disease development does not exist every year (Workneh *et al*., 2008; Asma *et al*., 2012). Therefore spraying for the disease every year would be unnecessary waste of time and resources. Characterization of environmental conditions conducive to partial bunt disease is required to decide the timing of fungicide application. Development of a disease predictive model for the chemotherapeutic control of this disease was the main objective of the studies which was accomplished with following line of work:

1. Survey for the incidence of Karnal bunt disease of wheat in major wheat growing areas of the Punjab.
2. Screening of advanced lines/varieties by artificial inoculation.
3. Determination of correlation of environmental factors with Karnal bunt disease of wheat.
4. Development of a disease predictive model to determine the relationship of environmental factors with disease incidence.

Taxonomic status

Tilletia indica (Mitra) Mundkur (synonym *Neovossia indica* Mundkur) belongs to class *Ustilaginomycetes*, phylum *Basidiomycota*, order *Ustilaginales*, family *Tilletiaceae* and genus *Tilletia* specie *indica*. Black, dusty-appearing teliospors of the fungus gave it the name "smut" (Bonde *et al*., 1997). The class name is

derived from "ustulatus", meaning burned, in suggestion to the blackened appearance of the infected plants (Carris, *et al*., 2006). Cereal-infecting species of *Tilletia* that produce teliospores within the ovaries of their host plants are generally called bunt fungi, also considered to be derivation of word burned. Mundkur (1938, 1940) stated that *T. indica* "probably belongs to the genus *Neovossia*" based on the large number of non-conjugating basidiospores produced by the fungus. Based on a detailed taxonomic study Krishna and Singh (1982) justified its placement in *Neovossia indica*. However, western scientific literature prefers to designate the name of causal agent of Karnal bunt as *Tilletia indica* (Duran, 1972).

Symptoms of the disease

The Partial bunt is too much difficult to detect under field conditions, and generally careful examination revealed evidence of disease (Morris *et al.,* 1997, and Bonde *et al.*, 1997). Only a few kernels of some wheat heads become partially infected, except, in severe cases in which whole of the kernels in an ear are replaced with fungal sorus. There may be a slight swelling or darkening of infected florets. Therefore, karnal bunt is easiest to detect by observing the harvested grains carefully. The pathogen converts the infected ovary into a sorus where a mass of dark brown coloured teliospores are produced. Small sori are generally formed in longitudinal furrow, leaving the rest of seed unaffected. However, in severe cases the major part of the endosperm along the longitudinal furrow may get spoiled. Partially infected grains occur in clusters of spikelets and many such aggregate clusters may be present in a spike (Nagarajan *et al.*, 1997).

In a standing wheat crop, infected spike can be detected by the shiny silvery black spikelets with glumes spread apart and swollen ovaries. The infected grains emit a fishy odor due to trimethylamine and wheat products from severely infected grains are unpalatable. Cashion and Luttrell, (1988) reported that the pathogen (*Tilletia indica*) did not invade the embryo and the mycelium growth was limited to the pericarp. Transmission electron microscope (TEM) study revealed that the mycelium proliferated in the pericarp by disintegrating the middle layers of parenchymatous cells and prevent fusion of the outer and inner layers of pericarps, with the seed coat. The mycelial mat formes a compact hymenium-like structure which gives rise to short, septate stalks with single teliospores (Roberson and Luttrell, 1987).

Host range

Tilletia indica infects bread wheat (*Triticum aestivum* L.), durum wheat (*Triticum turgidum* Desf.), and Triticale (x *Trititicosecale* Wittm.) under natural conditions (Fuentes-Davila *et al.*, 1996). The wild wheat species *Aegilops geniculata* Roch (as *T. ovatum*), *Aegilops sbaronensis* Eig., *A. pereggrina* (Hack.) Maire and Weiller var. peregrine (as *T. variabilis*), and *"Triticum scerrit"* are reported as hosts for *Tilletia indica*, (Aujla *et al.*, 1985). With artificial inoculation, *T. indica* infects triticale, 3 species of *Triticum* L., 11 species of *Aegilops* L., 2 species of *Bromus* L., 3 species of *Lolium* L., and *Oryzopsis miliacea* (L.) (Royer and Rytter, 1988). Host range lists for *Tilletia* species contained naturally occurring hosts and artificially inoculated hosts (Warham *et al.*, 1986) and hosts resulting from synonymy of similar species (Duran and Fischer, 1961).

Biology of the *Tilletia indica*

Dispersal of teliospores:

Dispersal of karnal bunt pathogen is documented by means of its' teliospores. The long distance dissemination of teliospores contaminating wheat seeds or grains is believed to be in a manner similar to other bunt disease of wheat (Wilcoxson & Saari, 1996). These bunted seeds are known as reservoir of teliospores. Besides being seed-borne the spores can be carried to new areas through machineries and hand tools. Teliospores cling to plant parts, clothes, farm equipments, vehicles, threshers, and combine harvesters. They may also be dispersed by rain water and animals, including insects and birds, both as surface contaminants and through feces. Teliospores may be transferred to new areas in the form of wind-blown inoculums (Bonde *et al.*, 1987). Teliospores also germinate successfully after ingestion by livestock and insects like grasshoppers, providing another mean of dissemination (Smilanick *et al.*, 1985b).

Teliospore dormancy

Most freshly collected teliospores are dormant and unable to germinate (Mitra, 1931; Banasal *et al.*, 1983;

Smilanick *et al.*, 1985a). The highest rate of germination occurres with one year old teliospores (Mathur and Ram, 1963; Kiryukhina and Shcherbakova, 1976). Three types of teliospore dormancy have been noted in *T. indica*. Teliospores taken from freshly harvested grains germinate poorly compared with several months to a year or more old bunted grains. (Bansal *et al*., 1983; Mitra, 1935; and Rattan and Aujla, 1990). This type of dormancy is known as postharvest dormancy that occurs in *T. horrida* also (Chahal *et al*., 1993). The second type of dormancy is long-term dormancy, in which germination rarely exceeded 50% under optimal laboratory conditions and it remained between 15%–30% in most reports, although teliospores were stored for more than one year. This type of dormancy, contributes to teliospore survival under field conditions. The third type of dormancy can be induced by cold temperature. Dry teliospores of *T. indica* kept at −18◦C progressively lost the capability to germinate over a period of 12 weeks of treatment (Zhang e*t al.*, 1984). The teliospores in these experiments were plated immediately after treatment and non-germ inability was thought to indicate lethality. However, the inhibition of germination was minimized if teliospores were plated after a 20-day "thawing" period at 22◦C rather than immediately after the cold treatment (Chahal and Mathur, 1992). Cold-induced dormancy had also been observed in experiments performed over 4 to 8 days of freezing at 0◦C (Sindhartha *et al.*, 1995). Bedi *et al*. (1990) noted that with the passage of time germination of teliospores is affected, 4-14 month old teliospores had maximum germination which declined with further aging. Similar dormancy also occurs in *Tilletia horrida* (Chahal *et al*., 1993). Cold-induced dormancy of the dwarf bunt pathogen *T. controversa* Kuhn also occurs annually in natural field environments of temperate climates (Hoffmann, 1982; Hoffmann and Goates, 1981).

Nature of teliospores and their survival in the soil

Due to the hardy nature of teliospores of *T. indica* it remains viable in the soil for a long period of time. Teliospores of *T. indica*, *T. horrida*, and other bunt species that have been examined have relatively thick cell walls with four distinct layers, an endosporium, a thin middle layer, an exosporium from which the ornamentation develops, and an outer sheath (Hess and Gardner, 1983; Nawaz and Hess, 1987; Roberson and Luttrell, 1987). The teliospore wall resists toxic gases and liquids including methyl bromide and chloropicrin (Smilanick *et al*., 1988), hydrogen peroxide (Smilanick *et al.*, 1994), propionic acid, ozone (Rush *et al.*, 2005), and degradation in the digestive tract of animals (Smilanick *et al.*, 1986). This enables teliospores of *T. indica* to survive for several years in hot desert to cold temperate field environments (Babadoost *et al.*, 2004; Bonde *et al.*, 2004; and Chib *et al*., 1990). Under typical laboratory conditions, *T. indica* teliospores can survive for at least 16 years (Bonde *et al.*, 1997).

Teliospores are globose to subglobose in shape, 22-49µm in size, reticulate with spines and are surrounded by a thick cover sheath of 2-4 microns (Nagarajan *et al*., 1997). The peridium or sheath is fragile, fractured structure and is easily identifiable under the electron microscope. The second sheath episporium is reticulate and has numerous curved spines while the third layer is endosporium. Mature teliospores are dark brown and immature spores are light brown (Bonde *et al.*, 1996.) Some hyphal fragments on mature teliospores may be present. Pale orange to mainly dark, reddish brown to opaque-black and densely ornamented with sharply pointed to truncate spines, occasionally with curved tips make the distinguishable morphological characteristics of *T. indica* compared with other two similar species *T. horrida* and *T. walkri* (Anon., 2004; Asma *et al*., 2012).

The teliospors of *Tilletia indica* are very resistant to adverse environmental conditions and have been reported to survive in the contaminated soil for 2 to 5 years as reported by various research workers (Chib *et al.*, 1990; Krishna and Singh, 1983; Singh *et al.*, 1990). Teliospore, being the only source of survival in the soil provides the basis of pathogen spread and play a vital role in the perpetuation of disease. Bonde *et al.* (2004) conducted an experiment to study the survival of teliospores of *Tilletia india* in different types of soils. A teliospore longevity study was initiated in Kansas, Maryland, Georgia, and Arizona. Soil from each location was with *T. indica* teliospores and placed in polyester mesh bags. The bags were placed within soil from same location with in polyvinyl chloride pipes. Pipes were buried in the respective plots such that the bags were at 5, 10 and 25-cm depths. Each pipe was open at the both ends to allow interaction with the outside environment; however, it

was fitted with screens preventing possibility of teliospore escape. In the Karnal bunt–quarantine area of Arizona, bags of infested soil also were placed outside the pipes. Teliospore-infested soil from each location was will furnished with dry conditions in a laboratory. During the first 2 years, viability of the teliospores of karnal bunt declined more rapidly in pipes than outside pipes, and more rapidly in fields in Kansas and Maryland than in Georgia or Arizona. After 2 years, viability declined equally. In the laboratory over 3 years, viability decreased significantly more rapidly in dry soil from Kansas or Maryland than in dry soil from Georgia or Arizona, while pure teliospores remained unchanged. Consequently, Bonde *et al*. (2004) concluded that soils, irrespective of weather, affect teliospore longevity.

Teliospores of *T. indica* are introduced into the soil at harvest and may persist there for 45 months (Krishna and Singh 1983; Bonde *et al.*, 1997; Singh, 1994 and Warham, 1986). Teliospores on the soil surface germinate and produce primary and secondary sporidia which, under favourable environmental conditions, infect plants at flowering (Bonde *et al.*, 1997; Singh 1994 and Warham, 1986). Chib *et al.* (1990), and Gill *et al.* (1993a) reported that in India, *T. indica* teliospores survived for 3 years on soil surface in the field, but spores buried 20 cm deep decreased to low viability after 2 years. In another experiment Krishna and Singh (1983) investigated that in India, survival of teliospores on the soil surface and at depths of 7.5 and 15 cm was 45, 39, and 27 months, respectively. It was reported that viability of the teliospores of *Tilletia indica* decreased with increasing burial depth from 5 to 20 cm (Rattan and Aujla, 1990; Sidharta *et al.*, 1995). Teliospores survived better in loamy sand soil than in clay and sandy loam soil (Rattan and Aujla, 1990). Singh (1994) and Smilanick *et al.* (1989) demonstrated that the survival of pathogen is longer in dry soil than in wet soil in the form of teliospores. In laboratory conditions storage teliospores retained their viability for 5 to 7 years (Kiryukhina and Scherbakova, 1976; Mathur and Ram, 1963 and Zhang *et al*., 1984.).

Study was conducted by Babadoost *et al.* (2004) for the assessment of survival of *T. indica* teliospores in a location in the northern United States. Soils differing in texture and other characteristics were collected from four locations, equilibrated to –0.3 mega pascal (MPa), and infested with teliospores of *T. indica* to give a density of 10^3 teliospores per gram of dry soil. Samples (22 g) of the infested soil were placed in 20-µm mesh polyester bags, which were sealed and placed at 2, 10, and 25cm depths in polyvinyl chloride tubes containing the same field soil as the infested bags. Tubes were buried vertically in the ground at Bozeman, in October, 1997. Soil samples were assayed for recovery and germination of *T. indica* teliospores 1 day and 8, 20, and 32 months after merging of teliospores into soil. Teliospores recovered from soil samples were 90.2, 18.7, 16.1, and 13.3% after 1 day and 8, 20, and 32 months after assimilation of teliospores into soil, respectively, and was significantly ($P < 0.01$) affected by soil source. The percentage of teliospore recovery from soil was the greatest in loam soil and lowest from a silt loam soil. The rate of teliospores recovered from soil was not significantly affected by depth of burial and the soil source–depth interaction during the 32-month period. The percentage of germination of teliospores was significantly ($P < 0.01$) affected by soil source and depth of burial over the 32-month period. The mean percentage of teliospore germination at 1 day, and 8, 20, and 32 months after incorporation into soils was 51.3, 15.1, 16.4, and 16.5%, respectively. In another experiment, samples of silty clay loam soil with 5×10^3 teliospores of *T. indica* per gram of soil were stored at different temperatures in the laboratory. After 37 months of incubation at 22, 4, –5, and –18°C, the rates of teliospore recovered from soil were 1.6, 2.0, 5.7, and 11.3%, respectively. The percentage of spore germination from soil samples was highest at –5°C. Microscopy studies revealed that disintegration of teliospores began after breakdown of the sheath-covering teliospore. The results of this study showed that teliospores of *T. indica* could survive in Montana for more than 32 months and remained viable.

Teliospore germination

Most locally infecting bunt fungi have echinulate, verrucose, or tuberculate teliospores that germinate at approximately 20^0C–25^0C (Castlebury *et al*., 2005). Though each teliospore generally produces one promycelium (Mitra, 1931), several promycelia may arise from a single teliospore (Krishna and Singh, 1981; Mitra, 1931; Warham 1988a; Rivera-Sanchez and Fuentes-Davila, 1997). Promycelia vary in length 500 µm and bear at the apex a whorl of 32 to 128 or more primary sporidia (Mitra, 1931). Variation in the

length of promycelial tips may occur (Peterson *et al.*, 1984).

The effects of physical factors that influence teliospore germination in *T. indica* and *T. horrida* has been studied extensively (Chahal *et al.,* 1993; Dupler *et al.*, 1987; Krishna and Singh, 1983). Dupler *et al*. (1987) and Smilanick *et al.* (1985a) reported that germinating teliospores were remarkably durable, strong and resilient during the process even with wide swings in pH, temperature, and soil moisture including freezing and desiccation (Dupler *et al.,* 1987, Biswas, 2003). The basidium (also called a "promycelium") emerges through the ruptured wall of the teliospore and either produces basidiospores instantly, or elongates to over 500 µm in length, depending on the species and prevailing environmental conditions. Elongating basidia form retraction septa, confining the cytoplasm to the apical region. A terminal whorl of 10 to 150 primary basidiospores, also called primary sporidia, is formed. Castelebury *et al.* (2005) reported that the number of basidiospores formed per basidium varied considerably among different taxa and not all locally infecting species produce a large number of basidiospores, and the number of basidiospores also varied considerably within a species (Castlebury and Carris, 1999; Mitra, 1931; and Teng, 1931).

Basidiospores may elongate slightly after detachment, and become curved or undulate before germinating to form either hyphae or a sterigma-like structure on which a haploid, uninucleate, allantoid sporidium also called "ballistospore" (Ingold, 1996) was asymmetrically formed. The allantoid sporidia formed by *T. indica*, *T. horrida*, and other locally infecting *Tilletia* species studied had the same morphology and forcible discharge (Ingold, 1996, 1997). Allantoid sporidia may germinate directly via germ tubes or repetitively to produce additional sporidia. In culture, passively dispersed, filiform sporidia were formed from short, lateral sporogenous cells on the hyphae. Allantoid sporidia were the primary infective agents of *T. indica* but had not been studied in other locally infecting bunts. The role of the passively dispersed, filiform sporidia in the infection process is unclear.

Dispersal of sporidia from soil level to ear head

Prescott (1986) reported that the microconidia or crescent shaped allontoid spores are the product of macroconidia produced by the chlamydospors present in the soil. The microconidia get deposited on the lowermost leaves by current of air and splash. The maximum trapping of the microconidia occurres in air samples during early morning hours when 100 % relatively humidity (RH) prevails. Nagarajan, (1991) explained that micro-conidia in the presence of leaf wetness produce a secondary crop of spores and as the leaf surface dries, these spores get dispersed to higher/upper leaves. Having climbed up the leaves through monkey jumps, they reach the flag leaf, some also get wind deposited and if at the boot emergence stage coincides with a mild drizzle or rain, and the micro-conidia get washed down into the sheath. Bedi *et al.* (1949) revealed that with presence of free water, once again crops of the microsporidia are produced. Before complete ear emergence, if more number of rains and favourable weather occurs causing run down, then more severe karnal bunt develops. Occasionally, microconidia get lodged on the floret parts at the time of anthesis and if conditions are favourable they infect to produce karnal bunt sorus on individual grains.

According to Bains and Dhaliwal, (1988) spikelets of wheat crop inoculated with sporidial suspension of karnal bunt pathogen (*N. indica*) at the booting stage, produced secondary sporidia after incubation (intact/detached) under favourable moist conditions in the laboratory. Sporidia were also released from inoculated spikes in the field where sporidial release exhibited diurnal periodicity. With the help of electron microscope it was confirmed that viable and infective sporidia trapped in different experiments were invariably of the allantoid type. Maximum sporidia developed on the outer glumes of florets. More sporidia were captured between 5 to 6 o'clock than later parts of the day but no sporidia were trapped between 14 to18 o'clock. However, they could be trapped at any time of the day from the detached spikes incubated in the laboratory under favourable and moist conditions. Sporidia developed at 15 and 20°C but not at 30°C. These findings indicated that repeated cycles of sporidial production in spikes provided more inoculum than expected from soil-borne teliospores of *N. indica.*

Infection process

The karnal bunt disease cycle is initiated with the germination of the teliospores at or near the soil surface, producing basidiospores that form hyphae,

allantoid sporidia, and successive generations of sporidia. According to Prescott, (1986) the diurnal release of *Tilletia indica* sporidia occurs in the presence of high relative humidity mainly between 1800 and 0800 h but the most favorable at approximately 0200 to 0300 h . Some sort of "defense mechanism" has been suggested that insures the existence of sufficient basidiospores at the time of heading, such as a factor that triggers teliospores to germinate when the plant is heading (Warham 1988; Shakoor, 2009). Otherwise, teliospores would undergo a hypothesized "suicidal germination", if they germinate when the host is not in a susceptible condition (Rush *et al*., 2005, and Stein *et al.*, 2005).

Preliminary infection by *T. indica* occurs when sporidia accumulate on spikes and germinate to produce hyphae that enter stomata (Goates, 1988). Hyphae then penetrate inter-cellular to the base of the floret and enter into the periderm of nascent kernels passing through the funiculus. Scanning electron microscopy studies revealed that the stomata of the rachis could also be essential path way for primary infection (Dhaliwal, 1989). However, solid information collected during the movement of hyphae within spikes indicates the rachis was not a regular site for initial infection (Rattan and Aujla, 1991). Nagarajan *et al*. (1997) stated that basidiospores or hyphae of *T. indica* frequently fuse to form a dikaryotic mycelium prior to attack on the host plants, and basidiospore fusion during culture has been reported (Krishna and Singh, 1983).

During the study of infection process of *T. indica* visible hyphal anastomosis was only vary infrequently observed prior to penetration into the wheat plant, was not considered a normal means of dikaryon formation (Goates, 1988). Most probably, haploid hyphae are capable of infecting the host plant but the dikaryotic state must be attained for the development of teliosporogenesis (Duran and Cromarty, 1977). The stage at which the fusion of two nucleus started in the *T. indica* was not identified. The method of penetration and timing of dikaryon formation in *T. horrida* are unknown, although it is suspected that sporidia lodge on the feathery stigma and enter into the style to reach at the chalazal end of ovary.

Frequently the embryo is not colonized except in sever infection when the embryo is killed, and infected seed grains are often able to germinate (Fuentes-Davila, *et al.,* 1996). Teliospores of *T. indica* arise from sporogenous cells that generate a thin hymenial stratum on the surfaces of the cavities formed by the partition of the inner and outer layers of the pericarp and by the separation of the inner pericarp from the seed coat (Roberson and Luttrell, 1987). Teliospores develop at the terminal portion of sporogenous hyphae, where the dikaryotic cytoplasm becomes surrounded by a septum and karyogamy occurs during enlargement of teliospore of *T. indica* primary cells (Fuentes-Davila and Duran, 1986). This was basically the same procedure observed in *Tilletia* species infecting systemically (Trione *et al*., 1989). The process of teliosporogenesis in karnal bunt pathogen was influenced by temperature. Even after flourishing introduction of infection, utmost diurnal temperatures of 35^{o}–40^{o}C after the grain-filling stage decrease teliosporogenesis significantly.

Goates and Hoffmann (1987) reported that during initial stages of teliospore germination, the diploid nucleus undergoes meiosis followed by several rounds of mitosis, producing abundant haploid nuclei prior to the development of the basidium. In *T. indica*, about 64 to 128 nuclei were observed migrating into the basidium. Nuclei migrate into developing basidiospores, split synchronously, and one daughter nucleus returns to the basidium. These nuclei in the basidium then either entered empty basidiospores or degenerated. The sorting of 64 or more nuclei that were tightly grouped at the tip of the basidium followed by orderly migration of 1 nucleus into each of approximately 100 basidiospores is a remarkable feat of cellular mechanics. Each haploid cell of the basidiospore can produce hyphae, or form allantoid or filiform sporidia. According to Fuentes-Davila (1984) and Duran and Cromarty (1977), T. *indica* is heterothallic and bipolar with four alleles controlling mating and pathogenicity. Variable numbers of chromosomes can be found among monospore isolates from single teliospores of *T. indica*, indicating that chromosomal alteration or differential segregation occurs during meiosis.

Epidemiology and disease prediction models

Karnal bunt disease spreads from season to season and various stages of disease development begin when teliospores of the disease become dislodged from infected kernels during harvest, become airborne, and settle in the field or spread to adjacent and remote

areas with wind currents or through other resources. The classic work of Mundkur (1943) and Bedi *et al*. (1949), revealed that natural infection by *T. indica* occurs through airborne sporadic (inoculum) during heading, but there is considerable disagreement on plant stages that are susceptible, and on the most susceptible stage. Results from a lot of studies provide the justification of susceptibility only during specific periods within boot swelling to anthesis stage (Aujla *et al.,* 1986; Bains, 1994; Nagarajan 2001; Rush *et al.*, 2005). However, Goates, (1988), reported that under natural conditions, the spikes were within the boot and airborne sporidia could not reach the glumes, where hyphal penetration was initiated. According to the recent studies of Goates, (2006) with karnal bunt disease of wheat revealed that infection from airborne inoculum can occur when florets begin to emerge from the boot up to the soft dough stage of wheat kernel, and infection peak up after spikes had completely emerged, but before the on set of anthesis. These results are contradictory to studies claiming susceptibility prior to spike emergence (Kumar and Nagarajan, 1998; Nagarajan, 2001; Sharma *et al.*, 1998 and Bains, 1994) and it was the first report of susceptibility much beyond anthesis.

According to Indu-Sharma and Nanda, (2003) teliospores of karnal bunt when suspended over water, started germinating from 15 October onwards to March under field conditions. Environmental conditions were conducive frequently for the disease at vulnerable stage (ear emergence). Prolonged tenure of mist, fog or rain for 20 days in December postponed the teliospore germination and the sporidia lost the potential to cause the disease after a lapse of 45-50 days. Nutritional component of the medium in which sporidia were cultured, affected the disease inducing potential.

Under specific climatic conditions of heavy dew or light rain, it appears that flag leaf and the boot sheath may be essential for normal infection (Aujla *et al*., 1986 and Kumar and Nagarajan, 1998). Dhaliwal *et al.* (1983) reported that after the primary infection, spread to adjacent florets had been occurred as late as the dough stage. The results of various studies on teliospore germination representing the wide range of environmental conditions conducive for teliospore germination indicate that it is not a limiting factor for disease development. The production of allantoid sporidia, which are the infective agents of the disease, seemed to be the primary factor in epidemiology. Bedi *et al.* (1990) reported that the optimum temperature and pH for germination and production of sporidia were 20^{o}C and 8.0 respectively. He further described that pre-incubation storage of teliospores at 4^{o}C for one week significantly enhanced their capacity to produce sporidia where as storage at 40^{o}C or more were not conducive. White fluorescent light enhanced germination and no sporidia were formed under complete darkness.

Disease-prediction models have been developed in the karnal bunt disease affected countries that integrate the climatic factors that influence significantly production of sporidia including temperature, humidity, solar radiation, and rainfall (Jhorar *et al*., 1993; Jhorar *et al.*, 1992 and Mavi *et al*., 1992). Germinating teliospores produces sporidia possessing thin cell walls that are considered to be short-lived and transitory structures sensitive to drought (Aujla *et al*., 1985; Nagarajan *et al*., 1997 and Smilanick *et al*., 1989). According to Smilanick *et al*. (1989), sporadia of *T. indica* at 95% relative humidity, survived no longer than 14 h. However, according to the experiments conducted by Goates (2006), *T. indica, T. horrida*, *T. walkeri*, and *T. caries* sporidia were remarkably durable. Sporadia collected from natural discharge in air dried conditions were viable for 30 days at 10–20% RH at 20°–22°C and after 60 days at 40%–50% RH at 18◦C, and newly formed sporidia were commonly observed germinating within 18 h of rehydration. It was observed that sporadia could survive in common field even, after several diurnal periods containing temperature above 38 ^{o}C associated with relative humidity below 10% and then rapidly produced hyphae under humid rainy conditions to infect host plants.

Similar results were obtained in field experiments over 46 days at temperatures often exceeding 40^{o}C and relative humidity as low as 10% (Lori *et al*., 2006). It was concluded by these results that teliospore germination prior to a vulnerable stage of host plant. The inoculum remains viable in dry field conditions, which can regenerate rapidly during humid conditions normally associated with disease.

According to Sansford, (1998), weather extremes (extremely hot, extremely dry, or very humid and cold conditions) are not favorable for development of karnal bunt disease and are considered to be limited to moderately cool climates (Jhorar *et al*., 1992;

Sansford, 1998; and Diekmann, 1998). However, *T. indica* had never been reported outside of its' allocation in the southwestern US and Mexico in spite of the historic movement of wheat grains throughout the North America. This relatively limited distribution of karnal bunt could be attributed to specific weather conditions affecting on the life cycle of the fungus (Diekmann, 1998).

Krishna and Singh, (1982) reported the optimum environmental conditions for germination of teliospores of *T. indica* and concluded that 15-20^{0}C as adequate temperature under alternate light and dark regime. Singh, (1994) suggested that successful infection was dependent upon the suitable weather conditions during flowering stage (inflorescence) of wheat plants, which is considered the most vulnerable stage to infection, and optimum temperature range for teliospores germination is 15 to 25^{0}C. Smilanick *et al*. (1985a) reported that the optimum temperature after 3 week incubation in continuous light was 15 to 20^{o}C, over a pH range of 6.0 to 9.5. Moisture was a critical factor in determining weather in disease development (Singh, 1994; Smilanick *et al.,* 1985a and Gill *et al.,* 1993a). Singh (1994) reported that 82 % relative humidity and preferably free water was required for the teliospores germination. He further added that with high humidity and rainy weather during 2-3 week window at flowering, infection of wheat kernels and individual seed grains increases. In the laboratory tests, survival of teliospores was noted after freezing over several months, but with delayed or reduced germination, (Chahal and Mathur, 1992; Zhang *et al*., 1984). According to Diekmann, (1993) moisture at the time of flowering may be the most critical element for the establishment of karnal bunt disease in U.S.A. Prediction made from environmental data and prerequisite of infection by pathogen suggest that, under current climatic conditions, karnal bunt would never cause major crop losses in the U.S.A.

Generally, teliospores at or very near the soil surface germinate in optimum conditions, and consequently release secondary sporidia that become air-borne and infect the host plant at flowering. However, the exact details of many steps of the disease development are still poorly understood and are considered a matter of opinion or conjecture. Krishna and Singh, (1983) reported that teliospores of *T. indica* survived longer on the soil surface than in soil, and teliospore survival decreased with increased soil depth. No teliospores survived after 27 months at a depth of 15 cm in Pantnagar, India. But in the experimental studies of Babadoost, *et al*. (2004) it was indicated that teliospores survived for more than 32 months at all three depths (2, 10, and 25 cm) in Montana. There was non significant influence of soil depth on teliospore recovery from soil. Even after a period of twenty months, germination of teliospores recovered from various soil samples buried at 25 cm was significantly higher than those of samples buried in the soil at depth of 2 and 10 cm. The differences between the report by Krishna and Singh, (1983) and the results from studies of Babadoost *et al*. (2004) may be consequently attributed to the effects of soil moisture and temperature on survival, viability and germination of teliospores, as reported by Rattan and Aujla, (1992). When Babadoost *et al*. (2004) stored infested soil samples with the teliospores of karnal bunt fungus in the laboratory in Bozeman, teliospore revival decreased as long as the soil samples were wet. It was also pointed out that temperatures at depths of 2 and 10 cm were higher than those at 25 cm during summer.

The Pest Risk Analysis Panel of the North American Plant Protection Organization (NAPPO) estimated the risk of Karnal bunt disease, establishment in commercial wheat growing regions of North America during October 2001. Critical factor of environments conducive to induction of disease development were acknowledged in coincidence with time of occurrence, in phonological terms, with potential disease severity to categorize areas at risk of disease establishment. Data were stratified by the phonological window matching to the critical stage of wheat anthesis which was considered the vulnerable period for each country across North America. Data were analyzed, characterized, and related to disease triangle including to production features, like yield and cropping constituency of wheat, climatology, known disease distribution (survey results), relevant wheat production technology, cultivation practices, chronological disease dynamics and environmental conditions leading to epidemics of disease. The risk model confirmed that majority of the wheat growing areas in North America were not highly susceptible to the establishment of the disease most of the time. Limited area had been acknowledged where risk may be occurred medium or high. By the analysis of existing climatic conditions and planting patterns during winter and spring wheat it was concluded that vulnerable

period did not generally coincide with environmental factors conducive to karnal bunt disease at the greater part of North America. The majority of wheat production territories in Canada and the United States keep up a correspondence to the lowest risk category for the disease. This was true for both winter and spring cultivated regions of both countries (Anon., 2001).

Various models to estimate risk of establishment of karnal bunt in different countries have been developed (Kehlenbeck *et al.,* 1997; Diekmann 1998; Murray and Brennan, 1998; Sansford, 1998; Baker *et al.,* 2000).

The Humid Thermal Index (HTI) model as a tool for determining potential disease distribution

On the basis of prevailing climatic conditions, there are examples for determination, prediction and mapping the potential distribution of diseases. An example of mapping the potential distribution of disease based on relevant environmental indices is provided by studies of Karnal bunt disease of wheat. The disease has been established in regions of southern USA. A number of pest risk areas (PRAs) had been selected to determine whether the disease could be established other areas through grain shipments. The HTI is a disease forecasting model developed by (Jhorar *et al.,* 1992). In this model he found a high correlation between a humid thermal index (HTI) and karnal bunt disease development over a period of 19 years in the central areas of Punjab, in India. The HTI can be defined as the relative humidity (RH) in mid-afternoon divided by the maximum daily temperature, calculated at the time between first awn visible to half inflorescence emergence.

This model was used in the European Union (EU) sponsored to karnal bunt programme as a device to determine potential distribution within Europe (Sansford *et al*., 2006). The HTI model has also been as a source to predict potential induction of karnal bunt in Australia (Murray & Brennan, 1998; Stansbury & McKirdy, 2002) and South Africa (Stansbury & Pretorius, 2001). The model was devised when it was considered that there was a strong positive relationship between disease intensity and both average daily temperature and relative humidity during the month of anthesis of wheat crops (Jhorar *et al*., 1992; Mavi *et al.,* 1992). An HTI between 2.2 and 3.3 was shown to be particularly favourable for the disease.

The HTI has been used to determine prevailing environmental conditions prior to anthesis of European wheat crops for infection and this finding has been extrapolated to predict that disease could establish on susceptible wheat varieties in many areas of Europe (Baker *et al*., 2005; Sansford *et al*., 2006). After the analysis of long-term, average data, the HTI model predicts that a number of places in Pakistan, India, Iran, Arizona and South Africa had unfavorable climatic conditions for karnal bunt despite the disease being present. This contradiction is justified by the reasons that local temperature and relative humidity levels are modified by irrigation (Stansbury & McKirday, 2002). Secondly in some parts of the Punjab wet and humid conditions favor teliospores germination, consequently inoculum is depleted at the stage of crop development when the wheat is not susceptible to infection (Sharma & Nanda, 2003).

Teliospores at or near the soil surface possess a level of hypersensitivity to environmental conditions and soil moisture and temperatures of around 5–20^{o}C can stimulate germination (Sansford, 1998). According to Smilanick *et al*. (1985b) only the teliospores that germinate within the 2 mm of the soil surface will release sporidia that are capable of spreading to canopy of host plants. However if favourable conditions are present for the germination of teliospores other than at the vulnerable period of crops for infection, teliospores undergo 'suicidal germination' and the teliospore population will decrease without inducing disease. In contrast, if suboptimal climatic conditions for teliospores germination remain dominant for most of the growing season of crop, teliospores are more likely to survive longer with the potential to become an inoculum source for next crop infection (Bonde *et al*., 2004).

A long dry season in hot arid and semiarid climate may be an important suboptimal weather element or device to inhibit 'suicidal germination'. The dark-coloured, hardy nature, thick-walled teliospores endure harsh, dry summer conditions during the post-harvest period (Singh *et al*., 2003). Dormancy accompanied by dry period ensured that large numbers of teliospores remained un-germinated until water stimulates germination during the next growing season. Seasonal rains or irrigation water are required for wheat production in dry regions and, because teliospores act as a reservoir of inoculum by releasing sporidia in areas where the disease is prevalent.

However, the situation is different in environments in Europe where rain is more evenly distributed throughout the year than in warmer regions. Consequently, persistent moisture may also stimulate microbial antagonism and degradation that may affect viability and reduce the longevity of teliospores. Therefore, in spite of favourable weather circumstances, prior to anthesis of wheat crops there may be no great reservoir of inoculum capable of sustaining the disease because of a gradual depletion of teliospore reserves. Even if infection did occur under favourable low teliospore population levels would result in reduced disease incidence and increase the possibility of pathogen extinction (Bonde *et al*., 2004; Garrett & Bowden, 2002).

Diekmann, (1993) reported three temperature related parameters as sufficient for discriminating environmental indices where the disease had established and those where it had not. They were (1) the difference between mean daily maximum and minimum temperature in the month of planting, (2) the mean daily maximum temperature in the month of flowering and (3) the mean daily minimum temperature in the coldest month of the year. Due to some drawback this model it was criticized by (Sansford *et al.,* 2006) as it was not based on temperature parameters only, no rainfall or soil moisture was included as an essential parameter.

Jones (2007) concluded that the climate throughout the year is important and not just climatic conditions prior to antheis. There is a range of sometimes conflicting information available on how abiotic factors during rest of the year influence the survival of pathogen in establishment of karnal bunt disease of whereas summarized by Warham, (1986). With respect to the suitability of the climate in the Pest Risk Analysis (PRA) area, Warham, (1986) came to conclusion that low temperature and high humidity were essential at wheat anthesis for the infection to occur, while dry weather, high temperature and bright sunlight were not favorable for infection process. Rainfall is necessary but rain on its' own at flowering was not sufficient to cause infection, suggesting that a specific combination of environmental factors was required. Crop irrigation is an additional factor favouring disease.

Jhorar *et al.* (1992) devised a model for the prediction of karnal bunt disease in the central Punjab, India, by an empirical method. A study of the associations between incidence of karnal bunt disease and meteorological factors, using chronological meteorological data and data pertaining to disease intensity for Ludhiana district, in the central plains of the Punjab, was made for the reproductive stage of wheat crop. The period studied corresponded to flag leaf emergence (starting 12 February in Ludhiana) and subsequent stages of host plant ending on 18 March. This corresponded to the most important period for the pathogen, when teliospors that germinated at the time could lead to the production of infective sporidia that survived to infect the host and for the karnal bunt to develop.

The first meteorological model used to assess the risk of karnal bunt infection was developed by Jhorar *et al.* (1992). They showed that there were non-significant relationships between the karnal bunt disease development and maximum daily temperature and sunshine duration during the vulnerable stages leading up to anthesis for wheat (for India this was the 9^{th}-11^{th} standard meteorological weeks (SMW). "The two most important factors were determined to be mean maximum daily temperature (r =0.88) and 3 pm RH (r =0.93). The sunshine duration (r = -0.73) was negatively related, while number of rainy days (r = 0.71) were positively related. Regression analysis showed that evening relative humidity and maximum temperature can be put in a disease model as independent variables in simple regression equations. The data were then used to develop a HTI forecasting suitability for disease establishment and spread. The HTI was the monthly average 3 pm RH, divided by the average monthly maximum temperature, for the month leading up to anthesis of wheat crop.

The most significant relationship was found between disease intensity and evening relative humidity (A), maximum temperature (B), and the "Humid Thermal Index" (A/B). A best-fit model was developed for forecasting the severity of karnal bunt in the central Punjab thus:

(1) DI = -0.8 + 1.5 HTI
(2) HTI = ERH ÷ TMX

Where DI = Disease index, HTI = Humid Thermal Index, ERH = evening relative humidity recorded at 14.30 hrs (average of the third, fourth, and fifth reproductive weeks), TMX = maximum temperature (average of third, fourth and fifth reproductive weeks).

Jhorar *et al*. (1992) concluded that the HTI during this part of growing period varied between 1 and 5; the lowest values occurring in extremely dry and warm environment, and highest values representing extremely humid and cold weather, neither of which favoured the disease. An HTI of 2.2-3.3 throughout the third and fourth week of the study period favoured the disease. These conditions resulted from frequent cloudiness and irregular showers which could be predicted, thus permitting a disease forecast to be made.

The second meteorological model for assessment of optimum conditions for the karnal bunt disease was developed for the Pacific Northwest, USA by Smiley, (1997) who noted that temperature and relative humidity were essential for the region, but stressed the significance of specific weather factors such as suitable rainfall and related humidity levels that were essential for teliospore germination, secondary sporidial reproduction, its penetration and infection. Smiley, (1997) reported that utility of these events being synchronized with three to four week vulnerable period leading up to wheat anthesis. Suitable rain and humidity events were defined as: "1. Measurable rain (>3 mm) occurring on each of two or more successive days; 2. At least 10 mm were being collected within 2-day interval 3. Average daily RH must also exceed 70% during both rainy days".

In order to predict the likely risk the pathogen establishing Pest Risk Analysis (PRA) area, the 1996 UK PRA applied the HTI used climatic data from individual meteorological stations in UK and showed that conditions during the "heading" period (broadly speaking May and June) were favourable for infection and disease development, i. e. the majority of the calculated HTI values fell within 2.2 and 3.3. Kehlenbeck *et al*. (1997) also calculated the HTI for the wheat growing areas of Germany and found that some of the southern areas had HTI values which fell within optimum range for disease development.

Murray and Brennan, (1998) also used this methodology for Australia. They assessed the locations for potential development of karnal bunt in Australia. Data for average monthly maximum and minimum temperatures and 3 pm relative humidity were obtained from the Bureau of Meteorology (http://www.bom.gov.au) for 122 wheat growing regions in Australia. The data were compiled up to the end of 1996. Times of sowing and stage for anthesis development were collected from agronomists and wheat breeders of concerned region. The humid thermal index (HTI) was computed for each month coinciding anthesis occurrence at each location, as the ratio of the monthly average 3 pm relative humidity to the monthly average maximum temperature. It was noted that out of 122 sites within Australian wheat belt, 67 had HTIs conducive for the karnal bunt disease establishment.

The location was estimated for potential development of disease as follows: "(i) if HTI < 2.2, locality is too hot or too dry (ii) if 2.2 £ HTI £ 3.3, site is appropriate for karnal bunt disease (iii) if HTI > 3.3, site is too cold or too wet of the 122 localities, 46 were of category (i), 67 were of category (ii) and 9 were of category (iii). The 67 "reasonable" sites were through the southern wheat growing regions from Western Australia to central New South Wales. Category (i) sites or localities were in Queensland, northern New South Wales, the Victorian and South Australian Mallee, and the northern inland wheat belt of Western Australia. The cool and wet localities, category (iii), were outside the main wheat producing areas, on the south coast of Western Australia, southern Victoria and the north coast of Tasmania.

As the sowing time and growth development stages of wheat crop vary from country to country, region to region and even locality to locality with in a country therefore, life-cycle of the pathogen differ with time between Asian and European countries. The Australian, United Kingdom, and German studies were conducted using individual data. For the improvement of this work further, Baker *et al*. (2000) undertook provisional meteorological mapping using interpolated environmental data (1961-90) with adjustments in the calculation to allow for the lack of mid-afternoon relative humidity measurements. The result showed that for June, an area covering much of central and southern Englang had HTIs falling between 2.2 and 3.3 which was therefore, considered using this factor alone, favourable for karnal bunt development in the crops phonologically susceptible at that time.

There is an existence of strong relationships between environmental conditions at anthesis and establishment of karnal bunt disease of wheat for sites in India. Mavi *et al*. (1992) developed a model based

on the average maximum temperature during mid to late anthesis (-ve correlation), the "evening relative humidity" (presumably 2:30 pm, +ve corr.) and sunshine duration (-ve) during early to late anthesis, and the number of rainy days in early anthesis (+ve). Although this model has R^2 of 0.89, it is likely to be location specific due to the inclusion of sunshine hours and therefore not directly applicable to Australian conditions.

Diekmann, (1993) launched "geophytopathology" methodology to develop a correlation between karnal bunt likelihood and (i) the distinction between the mean maximum and minimum temperature in the month of crop sowing; (ii) the average daily minimum temperature in the coldest month of the year; and (iii) the average daily maximum temperature at anthesis of wheat crop. Diekmann (1993) compared localities in the world where *T. indica* did and did not occur for the development of the model.

Stansbury & McKirdy, (2002) compiled two previously established meteorological modeling techniques while determining areas in Western Australia (WA) where circumstances were conducive for infection of wheat crop by karnal bunt pathogen. A strong correlation (r = 0.83) was found between the rainfall model, and the Humid Thermal Index (HTI) model, which used average-monthly data. Rain fall model was developed and based on the per cent opportunity of at least three Suitable Rain Events (SRE) during the vulnerable stages (August to October) of wheat crop. It was concluded that northern wheat growing belts are too hot and dry (HTI < 2.2, chance of SRE 15–27%), southern regions are unimportant due to too cold and/or wet (HTI > 3, chance of SRE 68–97%), eastern regions are marginal to too hot and dry (HTI around 2.2, chance of SRE 24–50%), and western wheat growing sites are appropriate (HTI between 2.2 and 3.3, chance of SRE 41–78%). Analysis indicated that between and within year infection due to *T. indica* was more likely to establish if anthesis occurred in northern areas in August, in October in southern regions, in September in eastern belts, and in August, September, and October in western and south-eastern wheat growing localities.

Stansbury and Pretorius (2001) used the meteorological modeling techniques to determine suitable condition for the development of infection, and influence of irrigation schedules in South African wheat growing belt for the favourable environment for karnal bunt pathogen. Only rainfed spring wheat in the western and southern wheat production areas of the Western Cape experienced climatic suitability to *Tilletia indica* development. Humid Thermal Indexes (HTI) experienced an appropriate range of 2.2 to 3.3 (HTI range 2.09-3.20, mean 2.58) and most regions indicated at least one Suitable Rain Event (SRE) (range 0.78-2.44) during the vulnerable period of crop. In distinction to central and eastern wheat production belts in South Africa were dry and warm under natural circumstances (HTI range 0.61-1.67, mean 1.06) possessing less than one SRE (range 0-0.63) for early and mid-sowing wheat crop in these regions. It was concluded that the late sowing of wheat in irrigated areas experienced less conducive climatic conditions for the prevalence of karnal bunt pathogen. This may not be applied to wheat growing regions where crops are irrigated very 24 h, as minimum critical relative humidity (RH) for the security and the chances of survival of *T. indica* spores would potentially be increased.

A linear disease prediction model was devised by Nagarajan, (1991) for the climatic suitability of *Tilletia indica* infection by using average weekly weather had an R^2 value of 0.89 indicating an appropriate degree of fitness. For the condition of North West India, Y= 0.4381+2.97a-2.77b-0.09c=0.13d; where Y= is the predicted karnal bunt severity on bread wheat and the variable a to d, represent rainfall duration between 15-20 February, rainfall during 2-28 February, amount of rain days between 5-20 February, amount of rain between 22-28 February, respectively. When an analogous exercise was carried out for Mexico where karnal bunt disease developed in Sinaloa and Sonora states, the R^2 value was 0.91 and the model when validated was found to indicate a reliable forecasting Y=-0.71+1.6A +0.95B+1.07C+0.20D; where A to D stand for the number of rainy days between 1-7 February, 8-14 February, and 22-28 February; and total magnitude of rainfall between 21-28 February, respectively. Though there are minor differences between both the models for fractional coefficient values, the common denominator is that the number of rainy days during a particular period of February when the ear head emerges.

Sources of genetic resistance against the disease

The most successful, efficient and economic method of disease control is through host plant resistance. Screening is carried out by creating artificial epiphytotic circumstances at boot leaf stage. Studies with *Tilletia indica* aimed at developing trustworthy and practical methods of inoculation for screening germplasm have demonstrated the highest rate of infection occurred by hypodermically injecting a suspension of sporidia into the wheat boot at awns-emerging stage, or slightly before (Aujla *et al*., 1982, Aujla *et al*., 1986; Royer and Rytter, 1988; Bains, 1994). Krishna and Singh (1982a) adopted injection technique at several phonologic stages of wheat growth from panicle initiation to early milky stage using sporidial suspension and concluded that the most susceptible stage for inoculation was when awns were just emerging. During the artificial inoculations in wheat crop in the field, use of more precise propagules of the allontoid secondary sporadia had required, in order to attain reliable high level of infection (Singh, 1988; Fuentes-Davila *et al.*, 1993).

The susceptibility of bread wheat to karnal bunt fungus had been well acknowledged by the work of Fuentes-Davila *et al*. (1992) achieving infection levels greater than 50 % under artificial conditions; therefore it is significant to continue evaluating new advanced lines and cultivars, as a measure to overcome the problems, and guidelines for the release of commercial use. However, bread wheat cultivars that had shown constantly low levels of infection were identified to occur (Fuentes-Davila and Rajaram, 1994). According to Bedi *et al.* (1949) and Fuentes-Davila *et al.* (1992) sources of resistance against the karnal bunt disease were also present in durum wheat and triticale germplasm under natural and artificial inoculated conditions.

Villareal *et al*. (1994) during an evaluation with three crop cycle under artificial conditions concluded that 49 % synthetic hexapolids (SH) were immune to the disease. Villareal *et al*. (1996) identified 4 SHs as immune sources during a work to evaluate elite bread wheat lines, synthetic hexaploid wheat derivatives, commercial varieties and candidates to release for resistance against karnal bunt disease of wheat under artificial inoculation. Mujeeb, *et al.* (2006) evolved immune synthetic hexaploid wheat by crossing (hybridizing) randomly high yielding durum wheat cultivars with several *Aegilops tauschii* accessions. The F1 combinations were advanced by conventional breeding protocols to F8. Crosses between *Triticum aestivam* and *Aegilops tauschii* accessions resulted in F1 hybrids with 2n=3X=21, ABD plants. These hybrid seedlings after colchicine treatment led to hexaploid C-0 (2n=6X=42, AABBDD) seed formation. Stable plants with 42 chromosomes called synthetic hexaploids were screened against biotic stress. An Elite SH group of 95 advanced lines was prepared from the initial 420 SH wheats. These 95 germplasm lines were distributed by CIMMYTs' germplasm bank. Supplementary repositories were at the Kansas, State University Wheat Genetic Resource Faculty, Manhattan, Kansas, USA and at National Agricultural Research Center, Islamabad, Pakistan. High level of resistance in the elite SH wheat were maintained during the karnal bunt stress screening. The durum cultivars involved in these SH combinations were generally susceptible under the severe greenhouse inoculation tests. The corresponding SH wheat showed immunity under similar conditions.

Singh *et al*. (2003) in his studies for mapping a resistance gene effective against karnal bunt of wheat reported that most sources of genetic resistance to KB were traced in China, India and Brazil, Gill *et al*. (1993), Fuentes-Davila *et al*. (1995) and Singh *et al*. (1995b) also had same conclusions.

In India, Indu-Sharma *et al.* (2005) reported the genetics of karnal bunt (KB) resistance in populations derived from crosses of four resistant stocks (HD 29, W 485, ALDAN 'S'/IAS 58, H 567.71/3*PAR) and a highly susceptible cultivar, WH 542. The plant materials screened for KB response consisted of F2, BC1 and RILs from all 'Resistant' x 'Susceptible' crosses and RILs from the six possible 'Resistant' x 'Resistant' crosses as well as the parents and F1s. The screening was performed under optimal conditions for disease development with a mixture of isolates from North Western Plains of India using the widely followed syringe method of inoculation. The KB scores of the F1 from the four 'Resistant' x 'Susceptible' crosses indicated partial dominance of resistance. Genetic analysis revealed that HD 29, W485 and ALDAN 'S'/IAS 58 each carried two resistance genes whereas 3 genes were indicated in H 567.71/3*PAR. The six 'Resistant' x 'Resistant' RIL sets showed that the genes in the four resistant stocks

were different and that there may be as many as nine genes governing KB resistance in the four parents.

Aujla *et al.* (1989) devised rating scale for identifying wheat cultivars resistant to karnal bunt disease of wheat. They categorized wheat varieties on the basis of severity and response to *T. indica* into five classes ranging from highly resistant to highly susceptible and expained the procedure for calculating the coefficient of infection with the help of grades of infection and numerical values of infected grains. Dhaliwal *et al.* (1986) concluded that germplasm of wild wheats, and *Aegiolops agropyron* possess the resistance to various diseases including karnal bunt disease of wheat. *Triticum urartua* was resistant to *T. indica* and some *Aegiolops*.

Warham, (1988b) conducted experiments for screening against karnal bunt to locate the source of resistance in wheat, triticale, rye and barley. None of the bread wheat lines were immune to karnal bunt disease.

Gartan, *et al*. (2004) during detection of multiple resistance sources concluded that among 100 advanced breeding lines of wheat (*T. aestivum*) and triticale (*Secale cereale*), the genotypes HS450, HS455, HPW232, VL861, PW731, PW733, PW738 and PW739 had multiple resistance against a number of wheat diseases including yellow (*Puccinia striiformis* var. *striiformis*), brown rusts (*Puccinia recondita*), powdery mildew (*Erysiphe graminis*) and Karnal bunt (*Tilletia indica*). The evaluation of these genotypes may be used in crossing programme with other agronomically superior varieties to incorporate the genes of desirable characters to obtain high yielding, disease resistant varieties. Souza *et al.* (2005) reported a resistant germplasm line IDO602 (Reg. no. GP-776, PI 620628), resulting from a first backcross of the hard white spring wheat Borloaug M95 onto the hard red spring wheat Westbred 926, in Idaho, USA, and delivered it in February 2003 for use in research and crop improvement programmes. It is semi-dwarf wheat with resistance to karnal bunt (*T. indica*) and stripe rust (*Puccinia striiformis).*

Sharma *et al*. (2004) obtained a karnal bunt resistant wheat stock ('KBRL22') germplasm from a cross of two resistant lines ('HD29' and 'W485'). They used it as a donor for introgression in KB-free trait into 'PBW343' (an 'Attila' sib), the most widely grown wheat variety in India. The numbers of KB-free and KB-affected plants in BC1, BC2, BC3 and BC4 as well as the F2 were obtained after artificial inoculations. The segregation pattern in these generations clearly indicated two independently segregating, dominant genes which jointly confer the KB-free attribute.

Kaur and Nanda (2002) evaluated wheat genotypes WL 711, WL 1562, HD 2329, PBW 343, PBW 396 and WH 542 along with resistant genotype HD 29 for susceptibility to karnal bunt disease (*T. indica)* during 1995-2002 by artificial inoculation. Results showed that WL-711 was the most susceptible to disease. Singh *et al.* (2003) obtained resistant sources of wheat from Advanced Varietal Trials and other sources like CIMMYT and were tested at hot spot multi-locations under artificially inoculated conditions for Karnal bunt (*T. indica*) establishment during 1990/91-1993/94 in Indian Punjab, Himachal Pradesh, Haryana, New Delhi and Uttar Pradesh, India. Of the 66 entries, 18 were resistant lines, namely HD 29, HD 30, HD 2385, RAJ 2296, WL 1786, WL 6975, WL 7247, HW 502, PBW 34, PBW 225, W 285, W 382, W 388, W 485, DWL 5010, ND 589, ND 602 and HP 1531. PBW 34 and PBW 225 were released varieties. Nineteen lines received from CIMMYT were also resistant to karnal bunt. HD 29 and HD 30 had already been registered by the National Bureau of Plant Genetic Resources, New Delhi, as INGR 99012 and 99011, respectively.

Indu-Sharma (2001) demonstrated that out of 43,680 germplasm lines of bread wheat (*Triticum aestivum* L. emend. Fiore & Paol.) tested against karnal bunt disease of wheat, 744 lines expressed stability for resistance. Out of 188 strains possessed multiple resistances to karnal bunt, brown (*Puccinia recondita* Rox.ex. Desm.) and yellow (*P. striiformis*) rusts and were agronomically superior. Six karnal bunt-free ('KBRL 10', 'KBRL 13', 'KBRL 15', 'KBRL 18', 'KBRL 22', 'KBRL 24') and 3 high-yielding Karnal bunt-resistant wheats ('W 7952', 'W 8086', 'W 8618') were developed by pyramiding of Karnal bunt-resistant genes and pedigree method of breeding respectively.

Jafari *et al*. (2000) inoculated spikes of 26 wheat advanced cultivars/lines by injection of a suspension of secondary sporidia of *T. indica* at booting stage. Coefficient of infection and percentage of infected grains for each entry were estimated, they were ranked

into four groups for their responses to the disease. None of the entries was completely resistant the karnal bunt disease and most of them were vulnerable. Cultivars Pastour, N-75-3 and N-75-5 were partially resistant. Darab-2 with 68.1% of infected grains and a coefficient of infection of 44.1 was the most susceptible cultivar, Atrak and Niknejad very susceptible and Falat susceptible. A significant correlation was found between coefficient of infection and percentage of infected grain for each entry and the regression model was calculated.

Beniwal *et al*. (1999) studied the effect of three different sowing dates on karnal bunt development was studied in ten commercially grown wheat varieties in India during 1995-96. The coefficient of infection was greater for crops sown on 16 November than for crops sown on 16 and 31 December. The varieties C.06 (31.96%), WH 147 (31.16%), HD 2009 (26.93%) and HD 2329 (20.93%) had a greater mean coefficient of infection compared to WH 283, HD 2285 and WH 896. Weather parameters including mean temperature (19.5 ^{o}C), relative humidity (62.6%) and rainfall (66.9 mm) spread over 6 rainy days from the date of inoculation to harvesting for crops sown on 16 November made them more susceptible to infection than those sown on 31 December, which had a mean temperature of 23^{o}C, relative humidity of 53.7%, and 31.6 of mm rain (3 rainy days only).

In Pakistani wheat germplasm generally there is scarcity of resistance against karnal bunt disease of wheat. Out of 141 wheat varieties/lines evaluated by Ahmad *et al*. (1999) at the Wheat Research Institute, Ayub Agricultural Research Institute, Faisalabad, Pakistan, during 1993-94, four lines, T-91731, T-911734, T-91736 and T-91740, remained free from karnal bunt infection caused by *N. indica* (*T. indica*). T-92733, T-91729 and D-91690 were moderately resistant while 51 showed a moderately susceptible response. Thirty nine varieties/lines were susceptible and 44 highly susceptible. Iftikhar *et al*. (1988) during screening of wheat germplasm against *T. indica* concluded that out of eighty cultivars evaluated only three entries T.C. L.83740, V-86354 and V-86257 were totally free from every kind of infection and thus they were declared to be immune. However, none of the entries was found to fall in resistant class. Eight germplasm lines, i, e. V-85003, V-85028,V-85255, V-85409, V-86231, V-86326, V-86369 and V-86371 exhibited moderately resistant, 10 moderately susceptible (V-83134, V-83156-3, V-83035, V-84021, V-85195, V-85165, V-85060, V-83152-1,V-85283 and V-86357), 21 susceptible (Dirk,Mexi-Pak-65, SA-75, B. Silver, Lyp.-73,Pb.85, C-591, C-518, C-271, C-217, C-273, Yaccura, Suitleg-86, Sandal, V-85096, V-84658, V-86115, V-86061, V-852992,V-86184,V-87240) and 38 were highly susceptible (Arz, Pavan, LU.26, Lu.26 S, LU.26 S-1, WL-711, Indus-79, Pak-81, Pb.81, BWp-79, K.Noor-83, Morrocco, Potowar, Barani-70,Chenab-79, SA-42, Pb.76, C-228, Fsd-85, Wandanak, Pari-75, Chakwal-86, Barani-83, V-83171, V-84140, V- 85078, V-85205, V-85072-1, V-84133, V-85054, V-86215, V-86299, V-85405, V-86303, V-86124, V-86124, V-86240 and V-87239.

Chemical control of the disease

Karnal bunt is very difficult to control when it is present in wheat area. Seed treatments that are used to control other bunt and smut diseases of wheat are generally ineffective because these only protect the wheat crop at seedling stage. The fungus *T. indica*, rather infecting the host plant or seedling as in the case with other smuts, infects the seed in the head as it emerges. Several attributes of the etiology of karnal bunt and the teliospores of *T. indica* have made control of it a very complex problem. Cultural practices that reduce the karnal bunt incidence, such as delay in sowing date, reduced nitrogen fertilization or reduced planting density, only affect modest reduction in karnal bunt incidences and may themselves reduce yields (Gill *et al*., 1993a; Singh, 1994; Warham and Flores, 1988; and Rivera-Castaneda *et al*., 2001). Due to the hardy nature teliospors, are sturdy, long-lived and very resistant to chemical and physical treatments (Smilaninick *et al*., 1988 and Warham, 1988a). They are seed borne and protected by the sorus and remainder of the partially bunted seeds typical of disease. According to Smilanick *et al.* (1989) teliospores masked in the soil persist longer than those of present on soil surface and are more sheltered from harsh climatic conditions and chemical treatments. Seed and soil treatments applied for the control of karnal bunt have been only partially successful. Seeds treatment with fungicides does not kill the teliospores but inhibit their germination (Hoffmann, 1986 and Warham *et al*., 1989). They have reported that fungicides applied to soil at seedling had not reduced the disease, probably because the infection originated from airborne infectious sporidia from the teliospores that germinated outside the test plots. According to

Agarwal *et al*. (1993) numerous chemical compounds have actively against this pathogen by reducing the germination of teliospores. However, Anonymous (1997) proposed that the chemical seed treatments have proved ineffective in killing teliospores with the exception of mercurial compounds which are banned in most countries. Seed treatment does little to eliminate soil-borne inoculum. So in case of chemical control the only valid and the most effective option is the application of foliar fungicides at anthesis stage.

According to Smilanick *et al.* (1987) seed treatment fungicides do not protect wheat plants from infection when seed are planted in teliospore-infested soil, and do not persist long enough within the plant to inhibit the infection of florets. During the evaluation of fungicides they estimated that out of eight seed-applied and 16 foliar-applied fungicides against the karnal bunt of wheat more than 80 % control was obtained with two applications of either Propiconazole or etaconazole or four application of mancozeb or copper hydroxide when these fungicides were applied to wheat crop when the spikes were still enclosed (feeks' growth stage), with the awns emerged about 1cm. Best control of propiconazole and etaconazole was obtained when they were applied 72 hours after inoculation rather than before inoculation.

Foliar application of the fungicide has given control of karnal bunt disease of wheat to some extent. Two or more application of propiconazol at or after spike emergence reduced the incidence of disease by 95 % (Aujla *et al.*, 1989; Singh, 1994). Sharma *et al.* (2005) evaluated new fungicides in greenhouse experiments to determine efficacy against the karnal bunt disease of wheat and durum wheat, caused by *T. indica*. The treatments comprised: 0.05, 0.10, 0.20, 0.40 and 0.80% Folicur (tebuconazole); 0.05, 0.10 and 0.20% Contaf (hexaconazole); 0.05, 0.10 and 0.20% Tilt (propiconazole); 50, 100 and 200 g a.i. thifluzamide/ha; and the control, applied at 48 h after inoculation of sporidial suspension. Infected and healthy grains were counted in the inoculated ear heads and the percent infection was calculated. Folicur at 0.20%, Contaf at 0.10%, Tilt at 0.10% and 100 g a.i. thifluzamide/ha resulted in more than 90% karnal bunt control, while Folicur at 0.40% and 0.80%, and Contaf at 0.20% resulted in 100% bunt control.

Rivera-Castaneda *et al.* (2001) during *in vitro* studies have reported the effectiveness of few commercial fungicides in inhibiting teliospore germination of *T. indica.* A number of native plants extracts from Sonora, Mexico, were tested to determine their antifungal activity against *T. indica*. Dichloromethane (DCM) and methanol (MeOH) extracts were incubated with the fungus to measure inhibition of mycelial growth. DCM extracts from *Chenopodium ambrosiodes* and *Encelia farinosa* reduced mycelial colony growth of the fungus, but total inhibition was obtained with 500 mg/ml of the DCM extract from *Larrea tridentata*. Teliospores subjected to this treatment showed no viability when transferred to fresh culture media. None of the evaluated extracts stimulated fungal growth. The extract from *Larrea tridentata* illustrated potential as control agent for *T. indica*.

A study was carried out by Singh *et al.* (2000) from 1996 to 1999 wheat cropping season to evaluate fungicides for the control of karnal bunt (*T. indica*) through foliar spray under field conditions to ensure healthy wheat seed production. Using a knapsack sprayer the highly susceptible cultivar HD 2329 was sprayed with following fungicides: propiconazole, hexaconazole, tricyclazole, flusilazole, thiophanate-methyl, cymoxanil and carbendazim at 0.05 and 0.1% dosage level. Among the fungicides, the maximum disease control (99.8%) was achieved by two sprays of propiconazole (0.1%) whereas a single spray controlled (97.68%) disease, followed by hexaconazole (94.40%) in the post-inoculation treatment. In the pre-inoculated spray treatments, maximum disease control (99.78%) was achieved with two sprays of propiconazole, while a single spray provided 96.46% control followed by hexaconazole (92.87%). None of the fungicides assured in a complete control of the disease. Flusilazole controlled the disease by 70.25-83.76%, but was phytotoxic to the wheat crop. Tricyclazole, cyamoxanil, carbendazim and thiophanate-methyl resulted non-significant in disease control. Two sprays of propiconazole (0.1%) at 15 days interval reduced the disease from 19.83 to 0.02 and 18.66 to 0.04% in post-inoculation and pre-inoculation treatments, respectively, which is below the level of international seed certification standard for foundation seed (0.05%).

Goel *et al*. (2000) reported that a foliar spray of propiconazole (Tilt 25 EC) at 250 and 500 ml/ha, applied at the boot leaf stage, decreased Karnal bunt

disease in wheat up to 78 and 87% respectively, in multilocation trials conducted during 1988-93 at New Delhi, Ludhiana and Gurdaspur (Punjab). Consequently it increased grain yield and was therefore, efficient for the management of this disease in the field. According to Salazar *et al*. (1997) out of 11 fungicides applied in varying combinations and concentrations to control *T. indica* infection of wheat in Mexico, propiconazole resulted the best disease control. However, based on costs and quarantine regulations in northwest Mexico, the use of propiconazole would not be economically profitable. Agarwal *et al*. (1993) found that a range of fungicides that gave significant control of sporidia on wheat crop if applied at the early heading stage. These included mancozeb, carbendazim, fentin hydroxide, bitertanol and propiconazole. Findings from current EU (European Union) research project (Anon., 2004b) reported that some active ingredients, particularly azoxystrobin as well as propiconazole were effective against both pre and post-infection by *T. indica* when applied as a single spray treatment at flag leaf ligule just visible (first awn visible) or caryopsis watery ripe stage.

Krishna and Singh (1982) evaluated the fungicides for the control of karnal bunt of wheat. In a glass-house test against *T. indica* Plantavax (oxycarboxin), Vitavax (Carboxin), Bayletan (triadimefon) and Bavistan (carbendazim) gave 82-87.55 % control when sprayed on the ear heads two days before inoculation of a sporidial suspension into the boot leaf. Krishna and Singh (1983) reported the chemical that enhanced the germination of teliospores of *T. indica*. Out of 16 treatments tested for obtaining abundant and early germination of teliospores from the diseased grains of wheat, the best were citrus and tomato juice, ether and hexan.

Mancozeb and Metalaxyyl are considered broad spectrum fungicides, and exhibited a strong antisporolant activity against three major divisions of the fungi, Oomycota, Ascomycota and basidiomycota when applied before inoculation rather than post infection (curative applicative application). Metalaxyl (260 µg/ml) also provide complete control of disease when used in protectant mode (Ammerman *et al*., 1992; Baldwin *et al.*, 1996; Gold *et al*., 1996 and Mitsuhiro *et al*., 1999).

Conclusion

Although many control strategies have been suggested for the management of karnal bunt disease and the strategies include seed treatment with hot water and solar energy, seed treatment with fungicides and soil drenching with fungicides, however, the results were not convincing. The cheapest and the most feasible method of Karnal bunt control is the use of host resistance and breeding for varieties resistant to karnal bunt disease. However, an integrated approach is practicable and conductive for the better management of karnal bunt.

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