Int.J.Curr.Microbiol.App.Sci (2018) *7*(12): 2080-2087

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 7 Number 12 (2018)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2018.712.238

Characterisation of Isolates of *Tilletia indica* Inciting Karnal Bunt of Wheat *(Triticum aestivum L.)*

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Keywords

Tilletia indica, Karnal Bunt, *Triticum aestivum*

Article Info

Accepted:
15 November 2018
Available Online:
10 December 2018

A B S T R A C T

Wheat is the most important food crop in India. During 2017-18, India's harvesting recorded wheat grain production 99.70 mt from 29.58 mha cultivated area. India's participation in the international wheat trade however is not up to the mark as being a major wheat producer. One of the major constraints in exporting wheat is prevalence of Karnal bunt (KB), internationally quarantined disease in the Indo-gangetic plain, major wheat bowl of the country. Karnal bunt caused by *Tilletia indica* (Mitra) is heterothallic fungus which requires fusion of different mating types for causing infection which results variants in their different potential to cause disease. Keeping this in view present study was undertaken to characterize different isolates of *T. indica* on the basis of mycelial growth, mycelial weight and sporulation. It was observed that there was non-significant variation in mycelial growth and mycelial weight, but significant variation in sporulation was observed among various isolates of *T. indica*. KB1 and KB2 isolates were most sporulating as compared to other isolates of *T. indica*. Characterization of *Tilletia indica* isolates will help in understanding the mechanism(s) of pathogenesis for devising novel strategies for management of Karnal bunt of wheat.

Introduction

Wheat (*Triticum aestivum*) is the most prominently cereal crop grown worldwide (Haung and Roder, 2004), and a basic staple food for human population. After the green revolution, wheat production has increased abundantly. Currently, India has surplus stock of wheat and a potential to generate more surplus. India has produced 99.70 mt wheat from 29.58 mha cultivated area in 2017-18.The prevalence of the Karnal bunt (KB) disease in Northern-western plain zone of India, is barrier on wheat grain export due to the Sanitary and Phytosanitary agreement, such as restricted movement of consignment to other countries (FAO, 1996). Karnal bunt or partial bunt of wheat caused by the heterotallic fungus *Tilletia indica* (syn. *Neovossiaindica*) was first reported from Karnal (Haryana) by Mitra (Mitra, 1931). *T. indica* is a heterothallic fungus belonging to the order Ustilaginales and family Ustilaginaceae (Nagarajan *et al.*, 1997).

Seventy-seven countries had imposed restrictions on import of wheat from the areas where the KB disease occurs (Bonde *et al.,* 2004).

The wheat importing countries have imposed strict quarantine measures and insist on zero tolerance limit (Singh and Gogoi, 2011). In recent years, re-emergence of KB disease in North-western plains of India had seriously affected quality of wheat grain (Gurjar *et al.,* 2016). Karnal Bunt is seed and soil-borne and also has an air-borne sporidial stage. The fungus enters the grain through the germinal end and partially converts the kernels into sori filled with teliospores (Aggarwal *et al.,* 1999). *T. indica* survives in the form of diploid teliospores in or on the seed and in agricultural soil.

Teliospores of *T. indica*germinate to produce primary sporidia or the macro (filiform) conidia are splash dispersed and in turn produce a large quantity of secondary or micro or allantoid spores (Bansal *et al.,* 1983). These spores are the only form that infects the wheat earhead (Dhaliwal and Singh, 1988).

T. indica being heterothallic fungus demands fusion between secondary sporidia of opposite mating types that results in much variation (Duran and Cromaty, 1977). Keeping this in view the studies were conducted undertaken to characterize different isolates of *T. indica*on the basis of mycelial growth, mycelial weight and sporulation.

Materials and Methods

Host and pathogen

Twenty isolates of *T. indica* were cultured and maintained at 16±2 ^{0}C in incubator in Fungal and Molecular Biology Laboratory, Division of Plant Pathology, ICAR-IARI, New Delhi for further use (Table 1).

Comparison of radial mycelial growth of *T. indica*

Potato dextrose agar media was sterilized and poured in 90 mm petriplates. All isolates were inoculated in three petri plates with using a disc of culture bearing germinating spores and incubated at16±2°C in BOD incubator under light and dark conditions. After few days, creamy white growth of fungus appeared. The diameter of fungus colony was measured using scale after 20 days of inoculation and was analysed statistically (Fig. 1).

Comparison of sporulation

A culture disc facing downward was placed at the apical part of the PDA slant and the tubes were incubated at 16±2°C with alternate light and darkness. After 7 to 8 days creamy white growth of fungus appeared showering sporidia downward from the disc which covered the entire slant within a few days. Spore suspension for all 20 isolates using 20 days old slant cultures was used for allentoid spore count through haemocytometer. Allantiod spore of banana shape were infective spores in *T. indica* (Fig. 2). The three replicated data observed on sporulation of all the isolates of *T. indica* was statistically analysed (Fig. 3).

Comparison of mycelial weight

Sterilized potato dextrose broth was suspended in 250 ml sterilized conical flasks. The media was inoculated with all the KB isolates using mycelial bits and incubated at 16±2°C in BOD incubator under light and dark conditions.

Fungal mat was thus harvested on 20th day and filtered through muslin cloth. Fungal mycelium fresh from each flask was weighed using electrical weighing balance. Three replicates were kept for each isolate and was statistically analysed.

Results and Discussion

Comparison of radial mycelial growth of *T. indica* isolates

Data recorded on radial mycelial growth of *T. indica* isolates is presented in Table 2. Among 20 isolates, KB1, KB2, KB8, KB16, KB17 and KB18 had significantly higher radial mycelial growth, with no significant difference between them. Minimum mycelial radial growth was observed in isolate KB19 (1.20 cm).

Isolates of *T. indica* and sporulation

The data on sporulation (allantoid spores) of different isolates of *T. indica* was recorded, statistically analyzed, and is presented in Table 2. Among 20 isolates, KB1 (32×10^4 spores/ml) and KB2 (30.33×10^4 spores/ml) were significantly highly sporulative, with no significant difference between them. Isolate KB13 and KB14 were least sporulative (0.22×10^4 spores/ml).

Mycelial weight of *T. indica* isolates

Data recorded on mycelial weight of the isolates of *T. indica* is presented in Table 2. Mycelial weight was also significantly higher in KB1 (3.28gm) and KB2 (2.90gm), with no significant difference between them. Minimum mycelial weight was observed in isolates KB15 (0.14 gm).

Taking into consideration all above three parameters, KB1 isolate showing highest sporulation count, radial mycelial growth and mycelial weight, was selected for further expression studies.

Table.1. Isolates of *T.indica* collected from North-Western plain zone of India

S. No.	Isolates	Location	Year of collection
1.	KB-1	Aligarh, UP	2015
2.	KB-2	New Delhi	2015
3.	KB-3	Taroari, Karnal, Haryana	2014
4.	KB-4	Pipli, Kurukshetra, Haryana	2014
5.	KB-5	Nilokheri, Karnal, Haryana	2014
6.	KB-6	Sonipat, Haryana	2014
7.	KB-7	Jind, Haryana	2014
8.	KB-8	Panipat, Haryana	2014
9.	KB-9	Bareilly, UP	2015
10.	KB-10	Bhiwani, Haryana	2015
11.	KB-11	Pundri, Kaithal, Haryana	2015
12.	KB-12	Ambala, Haryana	2015
13.	KB-13	Pantnagar, Uttarakhand	2015
14.	KB-14	Sultanpur, UP	2015
15.	KB-15	Allahabad, UP	2015
16.	KB-16	Bulandshahr, UP	2016
17.	KB-17	Saharanpur, UP	2016
18.	KB-18	Mujaffar Nagar, UP	2016
19.	KB-19	Pataudi, Gurgaon, Haryana	2016
20.	KB-20	Shamli, Haryana	2016

Figure.1 Radial mycelial growth of 20 KB (Karnal Bunt) isolates

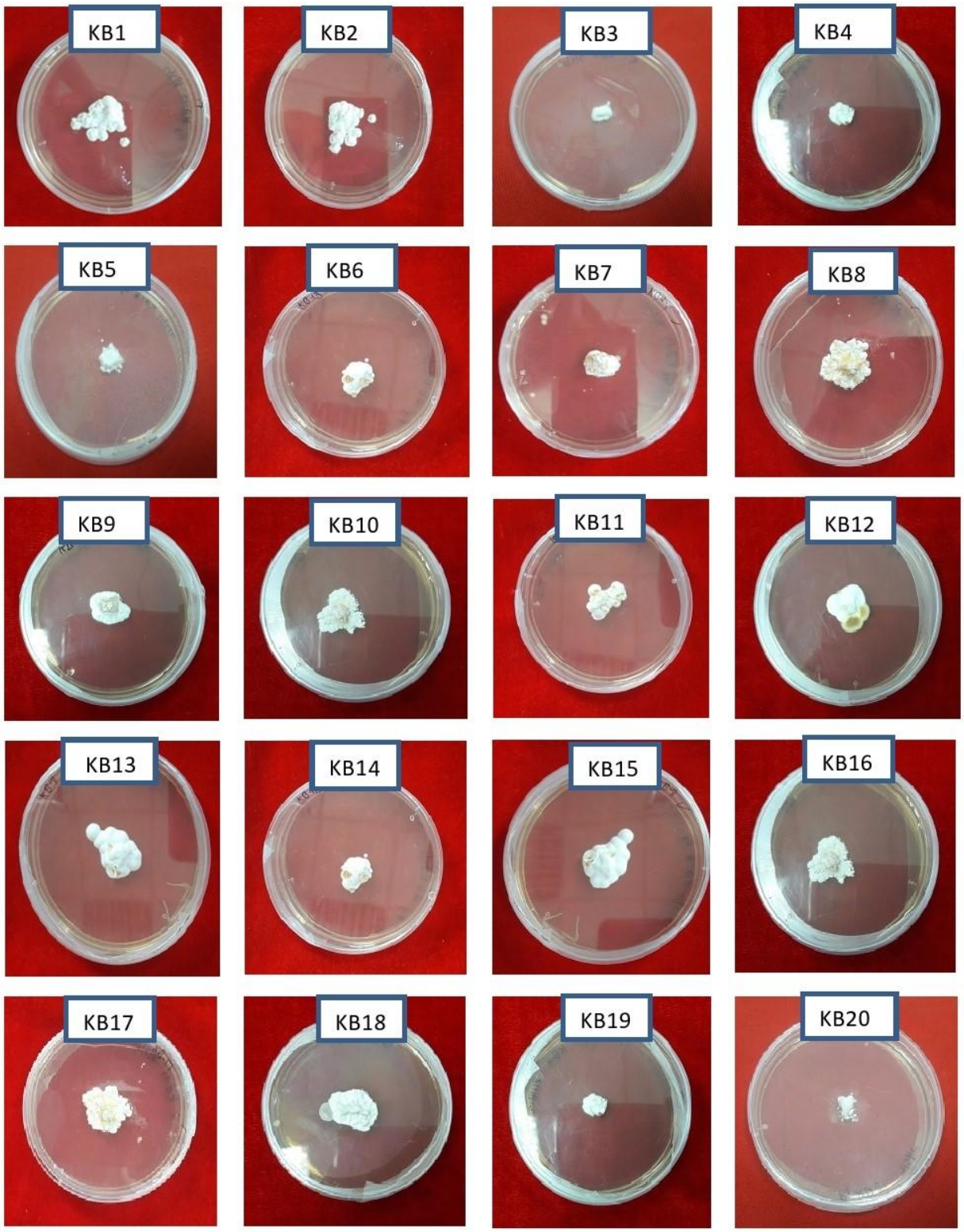


Table.2 Radial mycelial growth, sporulation and mycelial weight of KB isolates

T. indica isolates	Average (cm)	Average (10^4 Spores $ml^{-1)}$	Average (gm)
KB1	2.79	32.00	3.28
KB2	2.78	30.33	2.90
KB3	1.50	3.67	1.43
KB4	1.45	2.78	1.40
KB5	1.56	7.56	1.32
KB6	1.62	6.56	1.88
KB7	1.68	1.66	0.86
KB8	2.80	6.89	1.98
KB9	1.60	14.00	2.10
KB10	1.80	18.78	1.48
KB11	1.76	21.67	0.78
KB12	1.87	16.22	2.52
KB13	1.84	0.22	0.41
KB14	1.78	0.22	0.38
KB15	1.75	2.11	0.14
KB16	2.69	16.78	1.72
KB17	2.78	17.11	2.22
KB18	2.76	2.33	2.21
KB19	1.20	2.89	1.11
KB20	1.30	2.04	0.29
C.D.	0.16	0.12	4.24
SE(m)	0.06	0.04	1.48
SE(d)	0.08	0.06	2.09
C.V.	4.81	4.56	24.96

Figure.2 Microscopic view of allantiod spores of *Tilletiaindica*

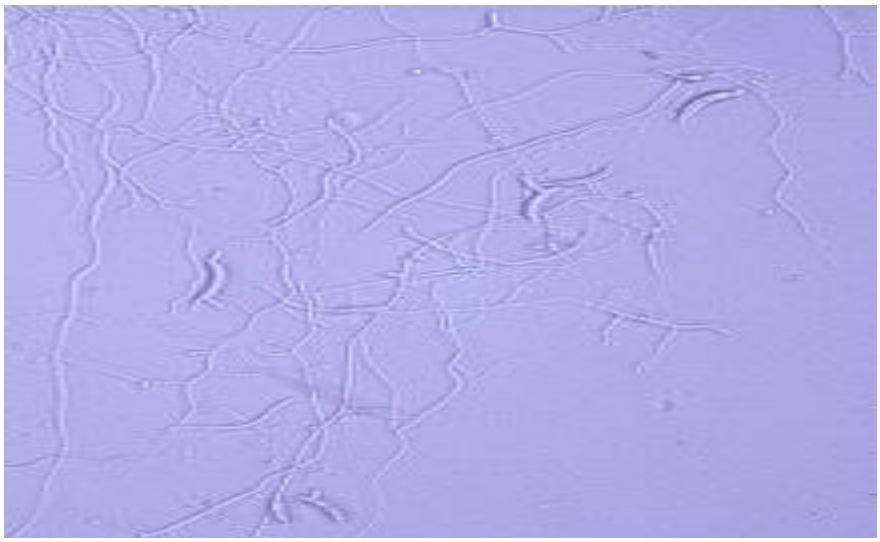
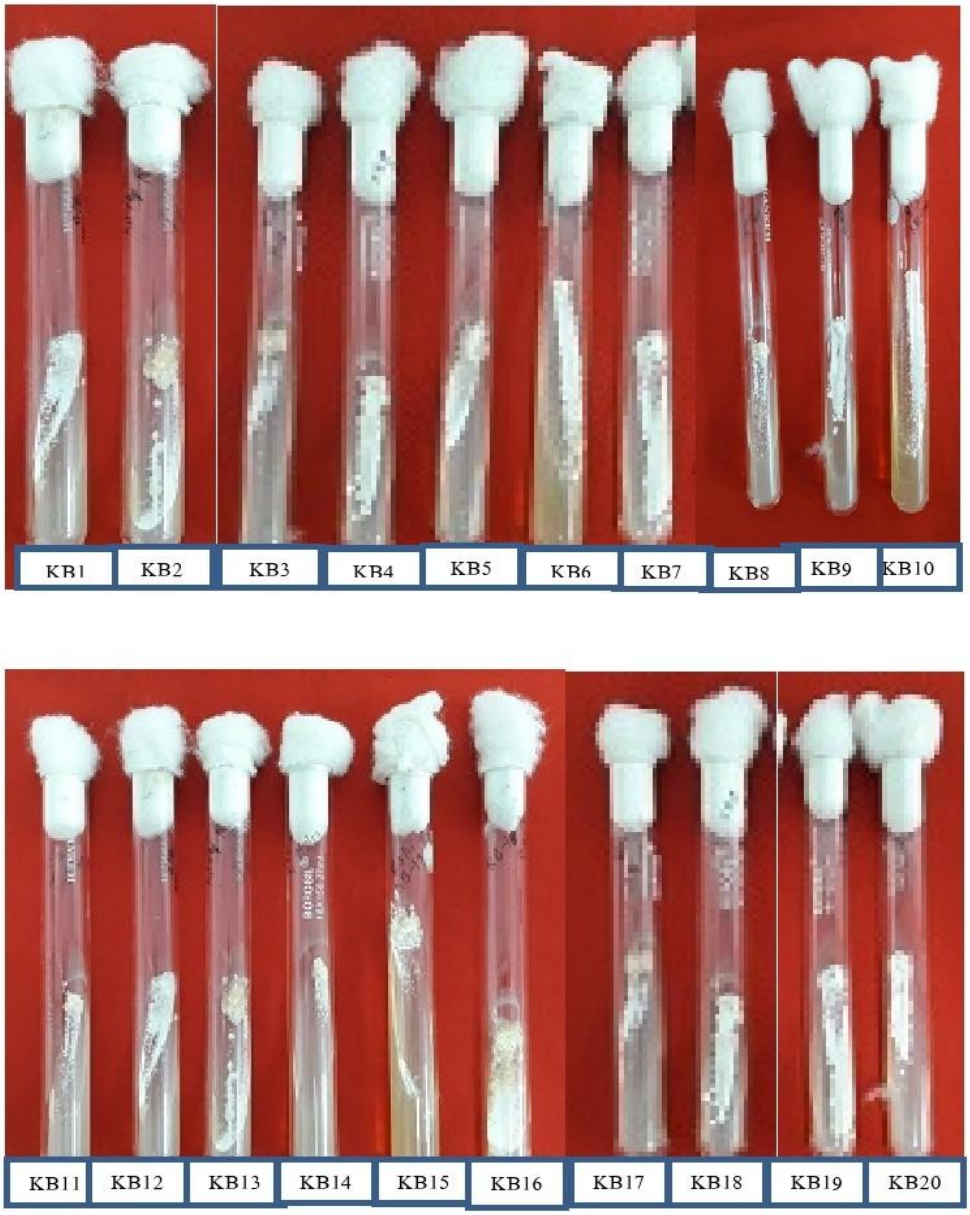


Figure.3 Sporulation of 20 KB (Karnal bunt) isolates



Karnal bunt is an important quarantine disease of wheat not only in India but also in several countries across the world. India being the origin of this disease is targeted more in international wheat trade. The comparative analysis of pathogenic field isolate of *T. indica* from different locations will help in understanding the fungal virulence spectrum.

Selection of effective and sporulative isolate of *T. indica* among 20 isolates was done on the basis of radial mycelial growth, sporulation and mycelial weight. Radial mycelial growth was higher in 6 isolates, with no significant difference between them (CD=0.16). Allantiod spores which are known for causing infection were counted by

haemocytometer. KB1 and KB2 isolates were highly sporulating among 20 isolates. Similarly, KB1 and KB2 have higher mycelial weight, when cultured on potato dextrose broth. KB1 and KB2 succeeded in all three parameters with statistically no significant difference between them, when compared with respective critical difference.

When teliospores of *T.indica* germinate, the single diploid nucleus undergoes meiosis. In general, the colonies of the fungus are brittle, crustaceous, umbonate with wavy margins. Subsequently, from a cushion like structure, two types of secondary sporidia i.e. falcate (allantoid) and filiform sporidia are produced. Most secondary sporidia are mononucleate. Mycelial cells that originate from either type of secondary sporidia are banana shaped, 11.9-13 µm long and 2-2.03 µm wide, and are forcibly discharged. These spores are the only infective entities. The filiform sporidia serve as the reproductive bodies to raise allantoid sporidia in successive generations (Dhaliwal and Singh, 1989).

T. indica is a heterothallic fungus with bipolar incompatibility, controlled by multiple alleles at one locus (Krishna and Singh, 1983; Aggarwal *et al*., 2010). Bonde *et al.*, (1977) suggested that dikaryotization occurs prior to penetration on glume surface. However, Sharma *et al.*, (2008) have observed that the dikaryotization takes place inside the host tissue. Physiologic forms of *T. indica*are known to occur in India. Aujla *et al.*, (1987) differentiated four pathotypes K1, K2, K3 and K4 from different regions of Punjab and Himachal Pradesh on the basis of host pathogen interactions on 17 differentials. KB isolates which showed variation in mycelial growth, mycelial weight and sporulation in present study may also differ in their aggressiveness in causing disease on different wheat varieties. So these isolates should be characterized further to know pathogenic variation and thus most virulent isolate obtained should be used for evaluating the breeding material for identifying resistant sources.

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How to cite this article:

Jagmohan Singh, Rashmi Aggarwal, M.S. Gurjar, Sapana Sharma and Saharan, M.S. 2018. Characterisation of Isolates of *Tilletia indica* Inciting Karnal Bunt of Wheat *(Triticum aestivum L.)*. *Int.J.Curr.Microbiol.App.Sci.* 7(12): 2080-2087.
doi: https://doi.org/10.20546/ijcmas.2018.712.238