The Pharma Innovation Journal 2023; 12(8): 2186-2192

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(8): 2186-2192

www.thepharmajournal.com
Received: 16-05-2023
Accepted: 15-06-2023

Abhishek Kumar
Teaching Cum Research Associate, Department of Biotechnology and Crop Improvement, Rani Lakshmi Bai Central Agricultural University, Gwalior Road, Near Pahuj Dam, Jhansi, Uttar Pradesh, India

Gohar Taj
Department of Molecular Biology and Genetic Engineering College of Basic Science and Humanities, GB. Pant Univ. of Agric. and Tech. Pantnagar, Udham Singh Nagar, Uttarakhand, India.

Anil Kumar
Department of Biotechnology and Crop Improvement, Rani Lakshmi Bai Central Agricultural University, Gwalior Road, Near Pahuj Dam, Jhansi, Uttar Pradesh, India

Corresponding Author:
Abhishek Kumar
Teaching cum Research Associate, Department of Biotechnology and Crop Improvement, Rani Lakshmi Bai Central Agricultural University, Gwalior Road, Near Pahuj Dam, Jhansi, Uttar Pradesh, India

Tilletia indica in wheat showed the high of amount protein and more biomass production induced by host factors

Abhishek Kumar, Gohar Taj and Anil Kumar

Abstract
The Karnal bunt disease is a very important disease of wheat which is stable food for human beings caused by *Tilletia indica* (*T. indica*). This disease profoundly affects wheat yield attributes since it causes the bed smell due to the presence of trimethyl amines. Therefore it is directly related to the wheat trade and leads to key interest among the researchers to understand more about the pathogen *T. indica*. However, this pathogen showed high variation in morphological, biochemical, and developmental forms. Observed results suggested that the variability among the monopodial culture was mostly because of genetic factors. Moreover, variability is also because of internal factors apart from external environmental factors. Therefore here it is very obvious to understand more about some internal factors of the host so-called host factors which are much pronounced in monosporidial strains.
Host factors are heat labile and have a profound effect on the pathogen because of nutritional and hormonal effects. How the host factors responsible for pathogenesis is not elucidated till now. In the present study, we could observe that the host factors play an important role in the total amount of protein in a time-kinetic manner among, the different monopodial strains of *Tilletia indica*. We also observed that host factors treated strain showed more biomass production apart from an increase in the radial diameter of monopodial culture. This new observation will open up new horizons to know more about the pathogenesis that leads to the quarantine of Karnal bunt disease. Moreover, it will also provide morphological markers specific to the strain of *Tilletia indica* this could be useful for investigating the levels of pathogenesis apart from protein biochemistry and finally management of Karnal bunt disease.

Keywords: Biomass, radial diameter, Host factors, protein amount and *Tilletia indica*

1. Introduction
Among the cereal crops wheat is one of the most momentous cereals not only in India but also in the world (Singh *et al* 2018) [52]. The total production of wheat in India was about 112.74 million tons in 2022-23 (https://www.cnbctv18.com/economy/indias-wheat-output-set-to-touch-a-new-record-at-11274-million-tonnes-in-2022-23-agriculture-ministry-16766831.htm).
To fill the required demand of the tremendous growing population, it needs to produce more than 112.74 million tons of wheat in future. Therefore it required good agronomical management practices to achieve the pecunious task of wheat production. Here one important practice is the management of the disease which is directly related to yield loss mostly caused by the fungus *Tillitia indica* and the name of the disease is Karnal bunt (Kumar *et al* 2019) [30].
Pathogen infection showed that tissue specificity and the boot emergence stages (S2) were more susceptible than the other two stages boot stage (S1) and seed forming stage (S3) (Bains, 1994) [5]. The infected grain later produces the teliospore which emits a bad odor due to trimethylamine (Sekhon *et al*., 1980; Singh and Bedi, 1985) [48, 57]. The disease was first time observed by Mitra in 1931 [33] on wheat caused by the fungus *Tillitia indica* (Mitra 1931[33] and 1935) in Haryana apart from, Punjab, Rajasthan, H.P, J. and K, U.P, Uttrakhand practices, and West Bengal as well as the Mexican state of Sonora Pakistan, Iraq, Afghanistan, and Nepal in the worlds.
Severely infected kernels showed a reduction in seed germination, the palatability of food, and toxicological aspects because of mycotoxins (Bhat *et al*., 1980) [10]. Yield loss studies suggest a low impact of the disease on production not only in India but also abroad like Mexico. It is found that different variability has been observed in the seed grains infect ability (Mitra, 1931; Mundukur 1943, Gill *et al*., 1931) [33, 35]. Not all the ears in a stool carry the disease and even in the same ear; only a few grains are smutted (Bedi *et al*., 1949; Pal, 1966; Joshi *et al*., 1980) [9, 26, 46].

Due to the irregular distribution of infected grains in the ear, it has been presumed that the result of air-born local infection takes place. As the grains reach maturity, the outer glumes spread out slightly, and the inner glumes of the spikes expand. The spore mass remains covered by the pericarp for some time but later rupture exposing the black powder to the atmosphere. The presence of foul smell due to trim ethylamine secreted by teliospores is a very prominent and peculiar feature in this disease and therefore the disease had been mentioned by some early workers as stinking smut. Fungus spores are both soil or seed-borne and can survive for years (Bedi *et al*., 1949; Aujla *et al*., 1977; Khetrapal, 1980; Joshi *et al*., 1980) [9, 1, 27, 26]. The fungus can be survived with germinating teliospores, primary sporidia, or secondary sporidia under laboratory conditions. In old culture (in potato dextrose agar), filiform / thread-like structures similar to primary sporidia have been observed, (Krishna and Singh, 1983) [29]. It has been shown that variability in sporidia can be because of influenced by the types of culture conditions. Depending upon the cultural conditions, an allantoid type of sporidia can produce a filiform type or vice versa also true (Dhaliwal and Sigh, 1988) [16]. Singh and Singh (1986) [54] found that czapeck's agar amended with wheat germ/wheat embryo/wheat seedling, supported growth of the fungus. While wheat grain dextrose agar supported maximum sporulation and least vegetative growth. However, wheat grain dextrose agar + 0.2% yeast extract supported both moderate growth and sporulation. (Singh and Singh, 1986) [54]. Singh and Singh (1992) [53] reported that glucose yeast extracts agar supported maximum growth while autoclaved wheat grains supported maximum sporulation. The growth and sporulation occur at 10-400 C with optimum at 200 C and at pH level 6.0. Maximum sporadic production was recorded in continuous fluorescent light (1000 lux). Proper aeration and a light amount are required for sporidia production (Singh and Singh, 1989; Singh *et al*., 1990) [59, 55].

The promycelium is occasionally branched and sporadical whorl normally develops from the tip of only one branch but rarely arises from more than one point. Primary sporidia arise as small finger-like projections from the tip of promycelium which increase in size and finally acquire filiform shape. Mitotic division of the daughter nuclei has also been reported in the promycelium. Each primary sporidium receives one nucleus which undergoes a further mitotic division (Fuentus, 1989; Holton, 1951) [55]. The primary sporidia or macro (filiform) conidia are splashed, dispersed, and in turn, produce a large quantity of secondary or micro allantoid spores. The secondary sporidia are released forcibly and are produced in numerous quantities when the leaf wetness tends to dry. These spores are the only forms that infect the wheat ear head (Dhaliwal and Sigh, 1988; 1989) [16, 55]. In *T. indica* heterothallism demands fusion between secondary sporidia that are compatible. The rapid vegetative multiplication of the allantoid spores and the heavy sporulation increases the probability of successful fusion of possible opposite mating types (Nagarajan *et al*., 1977) [40].

The Mycelial of the fungus is an important vegetative part of its survivability because it plays a significant role in the perpetuation of the life cycle. During its life cycle mycelia has to manifest different appearances in the form of homokaryon and dikaryon and represented in haploid and diploid conditions respectively. It has been shown that teliospore is a diploid status however promycelium is a haploid phase that is produced from teliospore. Monokaryon try to fuse with different compatible monokaryon depending upon other factors and the genes are responsible for this coupling process known to be multiallelic in nature. Therefore its molecular regulation bit complex the understanding the whole process of fusion which finally leads to the whole phenotype of the fungus.

Here we have shown that the factors on monosporidial culture of *Tilletia indica* lead to more biomass production and increase the radials and diameter apart a high amount of protein accumulation in a time-dependent manner. Our data showed that host factors induce the pathogenesis in wheat in response to more amount of biomass production of *Tilletia indica*.

2. Materials and methods

The important materials required here with the methodologies as mention below:

2.1 Collection of wheat varieties

Here the Karnal bunt susceptible Wheat variety seeds WH-542 were collected from Punjab Agricultural University, Ludhiana (Punjab), and the same were grown in G. B. Pant University, Pantnagar, and spikes of S-2 stage samples were collected.

2.2 Collection of fungal strains

For our study, we used KBPN6, KB3, and JK (JK msb) strain of Karnal bunt which was collected from G.B. Pant Uni. of Agriculture and Technology, Pantnagar and from IARI New Delhi respectively.

2.3 Preparation of media

Fungus was cultured by using potato dextrose media in appropriate environmental conditions. Here both modified PDA (Potato Dextrose Agar) solid and modified PDB (Potato Dextrose Broth) liquid were used for the culture of the fungus. In the case of liquid media, the above mention media were dissolved with the appropriate amount of distilled water. After proper dissolving the media same was transferred to a different conical flask containing about 50 ml of media. For solid media, PDA was used and preparation was done as above mention with the incorporation of solidifying agent (2% agar). After the adjusting appropriate volume both the solid and liquid broth same were autoclaved. The same solid media was used to prepare culture plates as well as slants. The different content of the media such as PDB Broth (24 g), Glycerine (10 g), KH2PO4 (0.1 g), Mg SO4 (0.5 g), NaCl (50 mg), and Yeast Extract (5.0 g) was used to get the optimal result and observation. Prepared modified PDA media sterilized by autoclaving. Cool the media at <45˚c, added streptomycin 50 ug/ml to prevent bacterial growth. (Streptomycin Preparation: Dissolve 0.1g of streptomycin in 1 ml of sterilized distilled water and filter sterilized it and store at 4˚c).

2.4 Development of monosporidial culture of *Tillitia indica*

The monosporidial strain was developed at Pantnagar University by the continued culture from the base isolates of all three stains of tilitia indica such as (KB3, JK, and KBPN). Sporidia were collected by the decanding method from isolates (KB3, JK, and KBPN) which have been cultured on modified PDA medium separately at 7 days and 14 days of

intervals after the full growth of the strain on the same media. After the proper observation of solitary-grown sporidia with the help of a microscope, again all three strains were cultured individually for further studies in the form of monoculture.

2.5 Harvesting of the Mycelium

The potato dextrose broth was prepared and aliquots about the 10.0 ml broth in each conical and same were autoclaved with proper care. After that autoclaved flasks were inoculated from the mother culture with mycelia disk and the same were grown inside the Biological Oxygen Demand (BOD) incubator at 22±2 ^{0}C desired temperature under the diurnal light and dark phases. Then these flasks containing the broth were used for various purposes such as protein isolation at respective time intervals. Similarly in the same environmental conditions of BOD but in humidified state petri plates grown containing modified PDA were inoculated with fungal discs taken from fungal colonized plates. After the proper growth and development of fungus mycelial in lab conditions, it was properly harvested in 7 days and 14 days time intervals both in the presence of host factors and in the absence of host factors. To get a good amount of the mycelia mat fungus was filtered with muslin cloth and collected in a separate clean flask. Thereupon collected mycelia were washed several times first in 0.05 molar PBS maintaining a pH of 7.2 and then similarly same were washed with autoclaved deionized water several times. Then it was collected again in a separate clean flask as a wet-weight mycelium and the same was lyophilized for 5 hours to reduce the moisture for proper long-time storage. Finally, it was stored in a different chamber of freeze depending upon the use of mycelia for the lab analysis.

2.6 Development of the Acetone collects from susceptible host

First of all susceptible host of wheat variety was collected from the field at the booting stage of spike and after that same was washed with tap water about the 50 g of the plant sample grind in fine powder with the help of liquid nitrogen and pestle and mortar. After that same sample dissolved in chilled acetone in the range of one-tenth. From the day forward chill was filtered after the five hours of treatment with shaking and after proper packing, the collection was stored in frize for further use. The collected solution was consequently dried with the help of an evaporator. After that same material was dissolved in 1/10th volume of autoclaved distilled water aseptically inside the laminar airflow and it was filtered with the help of filter paper to maintain the conta-free acetone collection. Consequently the same was used in preparation of the culture mediums.

2.7 Treatment of homogenized strain using host factors

Here the different monosporidial culture of all three stains was separately added in the culture flask containing the wheat acetone collect as it is mentioned just above in para 2.3 including the media broth of modified PDA in the ratio of 1: 10 as acetone collect and media broth respectively. Consequently, the same was collected after proper growth of the mycelial after the completion of 14 days.

2.8 Preparation of fungal extracts

After the treatment of monospridial culture with acetone collect fungal mycelial was harvested separately in a flask of all three strains such as KB3, JK, and KBPN after the 7 days, 14 days, 21 days and 30 days of treatment.

2.9 Preparation of the protein extraction buffer

The protein extraction buffer has been prepared with the help of the following used chemicals for a total volume of 100 ml and the pH was adjusted to about the 7.5 with help of hydrochloric acid Tris base (0.06 g), Ethylene diamine Tetra Acetic acid in sodium (1.861 g), ethylene glycol-bis (β-aminoethyl ether)-N, N, N′, N′-tetraacetic acid (0.38 g), TritonX-100; 0.5%, β Mercaptoetanol (0.3 g), phenylmethyl sulfonyl fluoride (34.8 g), Ascorbic acid (0.39 g), Polyvinylpyrrolidone (0.4 g), and distilled water were used as a makeup volume 100 ml and same was kept inside the freezer.

2.10 Isolation of fungal protein

After the harvesting of the fungal mycelial about 2 g of the same fungal mycelial was crushed in fine powder with the help of liquid nitrogen and petite and mortar after that about 2 ml of protein extraction buffer was added to each sample. Lysate was centrifuged at 12000 rpm at 4 °C for 30 minutes. Supernatant were collected and consequently, aliquots were made and stored at -20 °**C** for further use.

3. Result and discussion

3.1 Media components play important roles in the morphological variation of *Tilletia indica*

For our study, we have selected three base strains such as KB3, JK msb, and KBPN6 to understand more about the disease of Karnal bunt. We observed the monosporidial cultures of all three strains showed considerable variation among each other. It is known that in general, phenotypic characters are well modulated by environmental factors. However, the phenotypic features detected in terms of time kinetic studies are in good accordance with these molecular characterization features. Here we tried to understand more about how the morphological variation could be influenced by monosporidial strains of *T. indica*. The fungus could grow in a variety of synthetic and semi-synthetic culture media. However, Glucose Yeast Extract Agar (GYEA) supported the maximum colony growth. The fungus produced abundant secondary sporidia on autoclaved wheat and rice grains. GYEA supported the profuse sporulation of most of the isolates; while some of them sporulate best on Potato Dextrose Agar (Singh and Singh, 1989) [55]. This result clearly suggested that these strains are nutrients specific apart from some common requirements. Wheat grains dextrose agar was one of the important factors for the sporulation of the fungus (Singh and Singh, 1986) [54]. Liquid cultures have been also employed for the same work. The advantage of liquid culture over solid is that they produce sporidia more rapidly for inoculation (Singh and Singh, 1986) [54]. It was found that Potato Dextrose Broth (PDB) and PDB supplemented with sucrose or sucrose plus dextrose, consistently produced abundant sporidia (Warham and Cashion 1984) [61]. Sporidia produced in liquid cultures are considered as secondary sporidia since they resulted from primary sporidia produced on the promycelium of the germinating teliospores. These secondary sporidia are similar to the primary sporidia morphologically (i.e. they are filiform); they are referred to as filiform secondary sporidia to distinguish them from allantoid secondary sporidia produced on solid media (Singh and

Singh, 1986) [54].
With kind regards

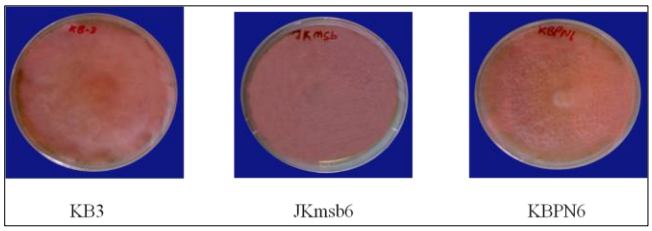

Fig 1: Obtained culture homogeneity of different strains of *Tilletia indica*

Here in the figure: 3.1 showed that the monosporidial culture of base isolate of KB3, JK msb and KBPN6 different morphological variation. Among the three strsin KB3 strain showed fast radial growth with respect to other two monosporidial culture of *T. indica*. The colour of KB3; mycelia were creamy white and showed floppy type of texture of mycelia. Monosporidial strain of JKmsb possesses moderate radial growth as compare to base isolate of KB3 due to its slow growth rate as well as also compared to monosporidial culture of KBPN6.The texture of mycelia were smooth and cremy white in colour. Similarly in KBPN6 strain showed dense growth and creamy white colony colour of monosporidial culture. However the radial growth was very fast as compared to base isolate of KB3 and monosporidial culture of JK msb. Therefore from this observation it is clearly suggested that morphological variations could be because of inherent characteristic of individual base isolates.

3.2 Host factors induce the larger radial diameter and biomass production on monosporidial culture of *Tilletia indica.*

To understand more about the how the of host factors play crucial role in radial growth development different strains of the fungus were culture on solid media including the host factor and without the use of host factors. Ii has been observed that the radial diameter were more in host factors treated strain of monosporadia culture (table 3.1) as compared to without host factors treatment irrespective of the three strain such as KB3, JK msb and KBPN6) of *T. indica*. Similarly all three monosporidal cultures of fungal strains were cultured on liquid culture media with and without the use of host factors (WH 542, S_2S). Myceliums of fungus from the liquid cultures were harvested after the 14 days of the treatment and mycial biomass were measured. In below table 3.1 and the figure 3.2 clealy showed the total biomass production were more in host factors treated funal strain and highly fluctuated in all three strains of monosporiadal culture of *Tillitia indica*. Here total biomass production, as well as radial diameter, was more in host factors treated base isolates as compared to non-treated host factors independent of different base isolates.

Table 1: Effect of host factors on growth of fungal cultures.

Strains/ Isolates	Mycelial Biomass (gram % age on wet basis at 14 days)		Radial Diameter (mm) (at 14 days)	
	With the treatment of host factors	Without treatment of host factors	Without treatment of host factors	With the treatment of host factors
KB 3	6.59	7.79	22.0	34.0
JKmsb	6.62	8.99	22.0	33.0
KBPN 6	7.72	8.62	25.0	38.0

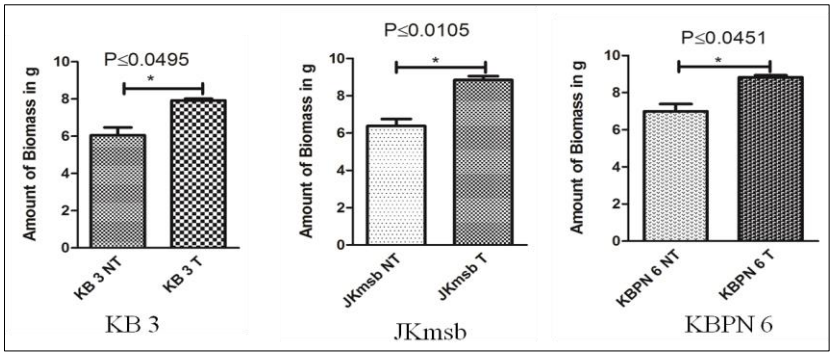

Fig 2: The amount of biomass production were more in hosts factors traded homogenize culture of different strain of *Tilletia indica*

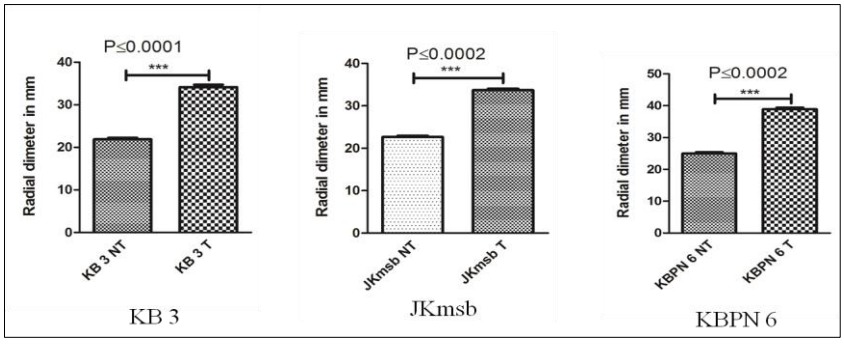

Fig 3: The radial diameter was more with the presence of hosts factors in a different strain of *Tilletia indica*

This observation has been made as a general conclusion to *T. indica* because of the involvement of host factors which finally leads to growth promotion in mycelium. The same result has been correlated with the acetone extracts prepared from the S2 stage of inflorescence and found to be growth promotory and induced mycelination *T. indica* (Rana *et al.* (2001) [45]. The growth promotory activity may be attributed to the fact that the wheat plant at anthesis possesses a rich source of nutrients or hormones that might help the synchronization of its growth and parasitic development of fungus. Here, our result is correlated to other fungi like the *Ustilago violacea* which has been grown in controlled environmental conditions and *was* reported by Day *et al.* 1981 interestingly the parasitic behavior was specific to the host plants only. Surprisingly here the Host factors also stimulated the increase the mycelial formation besides the improvement and expansion of conjugation peg as compared to without the host factor in the same host U. *violacea* in the mating phase.

3.3 Time kinetics studies on the alteration of total amount mycelial protein of *Tilletia. indica* treated with host factors

To determine the impact of host factors on protein amount the fungal strains were cultured on liquid media with and without the host factors isolated from the susceptible host WH542, S_2S). Fungal mycelia from liquid cultures were harvested and total proteins were isolated according to the specified protocol as mentioned in the materials and methods. After the analysis of our data as for concerns to mycelia protein, we could observe more protein with the treatment of the host factors as compared to without the host factors irrespective of the strain of fungus as mentioned in table 4.2. The low protein contents on the 7th day were observed in all three strains of *T. indica* (0.70mg/g, 1.2 mg/g, and 2.4 mg/g wet mycelia) without the host factors as compared to treatment of the host factors (1.3 mg/g, 2.7 mg/g and 3.2 mg/g). However KBPN6 strains here exhibited quite high protein content (3.2 mg/g) with the host factors in comparison without the host factors at 7 days (2.4 mg/g) respectively and showed an increase of about 85%. However, in Jkmsb the protein content increased 125% in the presence of host factors, but KBPN6 showed only a 33.3% increments. It is also correlating fact that host factors induce higher temporal protein expression in *Tilletia indica* (Kumar *et al* 2019) [30] and also factors induce higher immunomodulatory protein in *Tilletia indica* (Kumar *et al* 2019) [30].

Table 2: Protein content of fugal mycelia of all three strains grown with and without host factors at the wet basis in a time kinetics manner

Fungal strain and Treatments	Total soluble protein content in mycelia (mg/g of mycelia wet basis)									
	7 Days					14 Days				
	Replicates					Replicates				
	R1	R2	R3	Mean ± SE	% increase	R1	R2	R3	Mean± SE	% increase
KB 3 (N.T.)	0.6	0.7	0.8	0.7±0.4	86	1.6	1.7	1.6	1.6±0.2	
KB 3 (T.)	1.2	1.3	1.4	1.3±0.4		2.0	2.1	2.0	2.0±0.2	25.0
JKmsb (N.T.)	1.2	1.3	1.2	1.2±0.2	125	1.8	2.1	2.1	2.0±0.8	45.0
JKmsb (T.)	2.6	2.9	2.7	2.7±0.9		2.7	3.1	2.8	2.9±0.2	
KBPN6 (N.T.)	2.2	2.5	2.5	2.4±0.4	33.3	2.7	2.6	2.9	2.8±0.4	17.8
KBPN6 (T.)	3.2	3.1	3.2	3.2±0.9		2.7	2.8	2.6	2.7±0.4	
Fungal strain and treatments	**21 Days**					**30 Days**				
	Replicates					**Replicates**				
	R1	**R2**	**R3**	**Mean ± SE**	**% increase**	**R1**	**R2**	**R3**	**Mean ± SE**	**%increase**
KB 3 (N.T.)	2.1	2.0	2.2	2.1±0.8	0.0	2.3	2.1	2.0	2.1±0.9	19.9
KB 3 (T.)	2.1	2.2	2.1	2.1±0.4		2.4	2.2	2.3	2.3±0.4	
JKmsb (N.T.)	2.1	2.5	2.4	2.3±0.8	15	2.1	2.5	2.4	2.3±0.4	30.4
JKmsb (T.)	3.2	3.4	3.0	2.3±1.2		3.1	3.2	2.9	3.0±0.4	
KBPN 6(N.T.)	2.9	2.2	2.0	2.0±0.4	34.0	2.4	2.5	2.6	2.3±0.6	31.6
KBPN 6 (T.)	2.4	2.1	2.2	3.1±0.2		2.7	2.6	3.4	2.9±0.4	

The fast-growing strains indicated low protein content at 30th days compared to the 21st days but slow-growing strains at 30th days indicated constitutively higher protein contents compared to the 21st days. The prominent protein variations are indicated in all three strains.

4. Conclusion with future prospective

Here we conclude that several of the pathogenicity of the fungus *Tillia indica* in the susceptible variety of wheat could be possible because of so many reasons, among them Host factor(s) could be one of the crucial factors for the incidence of the diseases. Although a clear mechanism of the disease severity is not clear how the presence of Host factors influences the growth of the mycelium fungus pathogen under the lab conditions. Moreover, the real molecular mechanism of the incidence of the disease because of the fungus is not clear, although many mitogens activated protein is known for the activation of diseases in the plant system. Furthermore, the nature of the plausible factors also needs to be understood more with respect to disease incidence in the sucetable host. However, from our present observations, we could understand that more mycelia protein is there in the presence of Host factors in the fungus pathogen of T. *indica.* Hence it is very obvious here at least one can know more about the high hostility of the fungus pathogen on the susceptible host of wheat variety in the presence of the host factors which finally leads to the progress of the disease in the infected wheat grains. These observations further confirm the hypothesis that the host factor(s) exerts growth factors like effect on plant pathogenic fungi, *T. indica*. However, the integrative approach is still more valid to understand more about host factors for pathogenesis and the nature of host factors at the molecular level apart from biochemical and physiological aspects.

5. Acknowledgment

We are extremely thankful to our Govind Ballabh Pant University of Agriculture Sciences &Technology Pantnagar

for providing space to growing Karnal bunt susceptible wheat varieties and for the lab work as for as concern to the molecular biology of the fungal pathogen *Tillitia indica.*

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

1. Aujila SS, Sharma YR, Chand K, Swawney SS. Influence of weather factors on the incidence and epidemiology of Karnal bunt disease of wheat in the Punjab. Indian J. Ecol. 1977;4:71-74.
2. Aujila SS, Grewal AS, Gill KS, Sharma I. A screening technique for Karnal bunt disease of wheat. Crop Imp. Bansal R, Singh DV, Joshi LM. Germination of teliospores of Karnal bunt of wheat. Seed Res. 1983;11:258-261.
3. Aujla SS, Sharma I Compatibility systems in N. indica. Indian Phytopathol. 1990;43:222-223.
4. Aujla SS, Sharma I, Gill KS, Grewal HS. Establishment of *Neovossia indica* in wheat kernel. Plant Disease Res. 1988;3:62-63.
5. Bains SS. Influence of wheat spikes maturity on susceptibility to infection and growth of sporidia of N, indica. Curr. Sci. 1994;58:576-578.
6. Bedi KS, Dhiman JS. Spread of *N. indica* in a wheat ear. Indian Phytopathol. 1984;37:335.
7. Bedi PS, Meeta M. Effect of Karnal bunt on wheat and germination of wheat grains and subsequent metabolism of seedlings. Indian Phytopathol. 1981;37:335-337.
8. Bedi PS, Meeta M, Dhiman JS. Effect of Karnal bunt on weight and germination of wheat grains and subsequent metabolism of seedlings. Indian Phytopathol. 1981;34:330-305.
9. Bedi SKS, Sikka MR, Mundukur BB. Transmission of wheat bunt due to *Neovossia indica* (Mitra) Mundukur. Indian Phytopath. 1949;2:20-26.
10. Bhat RV, Rao D, Roy DN, Vijayaragavan M, Tulpule PG. Toxicological evaluation of Karnal bunt of wheat. J Food Safety. 1980;5:105-111.
11. Bhat RV, Rao D, Roy DN, Vijayaragavan M, Tulpule PG. Toxicological evaluation of Karnal bunt affected wheat. Indian J food safety. 1983;5:105-111.
12. Channon AG. Tests of Fungicides against *Alternaria brassicola* and studies on some factors enhancing attack by the fungus. Ann. Appl. Biol. 1970;65:481-484.
13. Chou MC, Preece TF. The effect of pollen grains on infections caused by Botrytis cinerea Fr. Ann. Appl. Biol. 1968;62:11-22.
14. Dhaliwal HS, Gill KS, Randhawa AS, Sharma SK. Karnal bunt *N. indica* (Mitra) Mundkar disease of wheat. Wheat Int. Ser. 1983;46:24.
15. Dhaliwal HS, Singh DV. Production and Interrelationship of two types of secondary sporidia of N. indica. Current Sci. 1989;58:614-618.
16. Dhaliwal HS, Singh DV. Interrelationship of two types of secondary sporidia of *N. indica.* Phytopathol; c1988. p. 41276.
17. Duran R, Cromarty R. *Tilletia indica*: A heterothallic wheat bunt fungus with multiple alleles controlling incompatibility. Phytopathology. 1977;67:812-815.
18. Economic Survey; c2020.
19. Endo RM, Oertti JJ, Stimulation of fungal infection of bent grass. Nature. 1964;201:313.
20. Fokkema NJ. The influence of pollen on the development of Cladosporium herbarum in the phyllosphere of rye. Neth. J. Plant Physiol. 1968;74:159-165.
21. Fuentus DG, Duran R. *Tilletia indica*: Cytology and teliospore formation `*in vitro* and in immature kernels. Canadian j. of Botany. 1986;4:1712-1719.
22. Gill KS, Sharma I, Aujla SS. Karnal bunt and wheat production. Punjab Agricultural University, Ludhiana; c1993. p.153.
23. Goates BJ. Histology of infection of wheat by *T. indica.* The Karnal bunt pathogen. Phypathol. 1988;77:371-375.
24. Holton CS. Methods and results of studies on heterothallism and hybridization in *Tilletia caries* and T. foetida. Phytopathol. 1951;41:511-521.
25. Holton CS. Observations on *Neovossia indica.* Indian Phytopath. 1949;2:1-5.
26. Joshi LM, Singh DV, Srivastava KD. Present status of Karnal bunt in India. Indian Phytopathol. 1980;33:147-147.
27. Khetrapal RK, Agarwal VK, Chauhan KPS. Studies on the influence of weather conditions on the incidence of black-point and Karnal bunt of triticale. Seed Res. 1980;8:108-110.
28. Krishna A, Singh RA. Effect of physical factors and chemicals on the teliospore germination of *Neovossia indica*. Indian Phytopath. 1982;35:448-453.
29. Krishna A, Singh RA. Cytology of teliospore germination and development in *Neovossia indica*, the incitant of Karnal bunt of wheat. Indian Phytopathol. 1983;36:115-123.
30. Kumar Abhishek, Gohar Taj and Anil Kumar. Host factors induces immunomodulator effect on pathogenicity of *Tilletia indica* in wheat Journal of Pharmacognosy and Phytochemistry. 2019;SP5:21-25.
31. Kumar Abhishek, Gohar Taj and Anil Kumar. Host factors of wheat altered the temporal protein expression of *Tilletia indica* in wheat Journal of Pharmacognosy and Phytochemistr. 2019;SP5:360-364.
32. Mehdi V, Joshi LM, Abvral VP, Tidies on Chapetti quantity. VI Effect of wheat grains with bunts on the quality of Chapattis. Bull. Grain Tech. 1973;11:195-197.
33. Mitra M. New bunt on wheat in India. Ann. Appl. Biol. 1931;18:178-179.
34. Mitra M. Striking smut (Bunt) of wheat with special reference to *Tilletia indica* Mitra. Indian J Agric. Sci. 1935;5:51-74.
35. Mundukur BB. A second contribution towards knowledge of Indian Ustilaginales. Trans. Brit. Mycol. Soc. 1943a;24:312-336.
36. Mundukur BB. Karnal bunt air borne disease. Curr. Sci. 1943b;12:230-249.
37. Munjal RL. Studies on Karnal bunt of wheat. Ph. D. Thesis. Punjab University, Chandigarh, India; c1970.
38. Munjal RL. Status of Karnal bunt (*Neovossia indica*) of wheat in Northern India during 1968-69 and 1969-70. Indian J. Mycol. Plant Path. 1975;5:185-187.
39. Nagarajan S. Epidomology on Karnal bunt of wheat incited by *Neovossia indica* and an attempt to develop a disease prediction system. Technical Report, Wheat Programme CIMMYT, Mexico; c1991. p. 1-62.

40. Nagarajan S, Aujla SS, Nanda GS, Sharma I, Goel LB, Kumar J, *et al*. Karnal bunt (*Tilletia indica)* of wheat–a review. Review of Plant Pathology. 1977;76:12.
41. Partridge JE. Karnal bunt of wheat. Dept. of Plant Pathology, University of Nebraska – Lincoln; c1997.
42. Prescott JM. Sporidia trapping studies of Karnal bunt. Pro. of the 5th Biennial Smut Workers' Workshop held at Cuidad Obregon, Sonora, Mexico; c1986, p. 22.
43. Rai RC. Studies on loss estimation and chemical toxicological evaluation of wheat. Ph. D. thesis, G.B. Pant Uni. of Agric. and Technology. Pantnagar, India; c1983. p 136.
44. Rai RC. Singh A. A note on the viability of wheat seeds infected with Karnal bunt. Seeds Res. 1978;6:188-190.
45. Rana Manish, Charu Arora, Basant Ram and Anil Kumar. Floral specificity of Karnal Bunt Infection Due to Presence of Fungal Growth-Promotory Activity in Wheat Spikes. J. Plant Biol. 2001;28(3):283-290.
46. Raper JR. Genetics of sexuality in higher fungi. Ronald Press, New York; c1966.
47. Royer MH, Rytter J. Artificial inoculation of wheat with *Tilletia indica* from Mexico and India. Plant Dis. 1985;69:317-319.
48. Sekhon KS, Gupta SK, Bakshi AK, Gill KS. Effect of Karnal bunt disease on Chapatti making qualities of wheat grains. Crop Improv. 1980b;7:174-149.
49. Sekhon KS, Saxena AK, Randhawa SK, Gill KS. Effect of Karnal bunt disease on quality characteristics of wheat. Bull. Grain Tech. 1980a;18:208-212.
50. Singh A. Karnal bunt – a major threat to wheat cultivation. Indian Farmers' digest. 1981;14:3-4.
51. Singh D. A note on the effect of Karnal bunt infection on vigor of wheat seed. Seed Research. 1980;8: 81-82.
52. Singh J, Singh, Aggarwal R, Gurjar MS, Sapana S, Saharan MS. Characterisation of Isolates of *Tilletia indica* Inciting Karnal Bunt of Wheat (*Triticum aestivum* L.); c2018. p.7-12.
53. Singh M, Singh A. Growth and sporulation of *Neovossia indica* in wheat callus culture. Indian Phytopathol. 1992;45:113-115.
54. Singh M, Singh A. Effect of culture media and antimicrobial agents on growth and sporulation of *Neovossia indica.* Indian J Mycol. Plant Pathol. 1986;16: 331-334.
55. Singh SM. Factors affecting growth and sporidiation of *Neovossia indica*. The incitant of Karnal bunt of wheat. Indian phytopathol. 1989;42:317.
56. Singh M, Ram B, Singh S. Studies on Karnal bunt in relation to teliospores germination and losses caused by it. Proc. Third Intern. Symp. Plant Path.Indian Research Institute. New Delhi, December; c1981. p.14 – 18.
57. Singh PP, Bedi PS. Effect of Karnal bunt infection on gluten characteristics and protein fractionation of wheat grains. Annals of Applied Biology. 1985;1:223-225.
58. Singh RA, Krishna A. Susceptible stage for inoculation and effect of Karnal bunt on viability of wheat seed. Indian Phytopath. 1982;35:54-56.
59. Singh S, Singh PP, Bedi PS. Effect of light and aeration on sporidial potential of *T. indica* in axenic culture. Pl. Dis. Res. 1990;5:166-169.
60. Strange RN, Majer JR, Smith. Isolation and identification of choline and betaine as the two major components in anthers and wheat germ that stimulate *Fusarium graminearum in vitro*. Physiol. Plant Pathol. 1974;4:277-290.
61. Warham EJ, Cashion NL. Evaluation of inoculation methods in the green house and field. Proceedings of conference on Karnal bunt of wheat, April 16- 18, 1984. Guided Bregon, Sonora, Mexico, CIMMYT; p.1984. p. 11-13.
62. Warren RC. Attempts to define and mimic the effects of pollen on the development of lesions caused by Phomabetae inoculated onto sugar beet leaves. Ann. Appl. Biol. 1972;71:193-200. https://www.cnbctv18.com/economy/indias-wheat-output-set-to-touch-a-new-record-at-11274-million-tonnes-in-2022-23-agriculture-ministry-16766831.htm.