

Integrated Disease Management (IDM) Modules against karnal bunt (*Tilletia indica*) of wheat

Comment [S1]: The term is not helpful in this title !

Abstract

Comment [S2]: The abstract text is not explaining about what was made on this experiments !!

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Wheat, (*Triticum aestivum* L.) belonging to family "Gramineae" and genus "*Triticum*", is one of the world's most widely cultivated food grain crop, due to its wider adaptability to different agro-climatic and soil conditions. Karnal bunt (*Tilletia indica*) is an important wheat disease with implications for wheat grain quality and inflicts changes in chemical composition of infected grains. IDM modules evaluated under pot and field condition revealed that all the thirteen modules were significantly effective and observed lower disease incidence of karnal bunt. Module M₁₁ and M₈ recorded nil (0.00%) disease incidence in both condition. The maximum incidence was recorded (0.425%) and (0.81%) in M₁₂ followed by M₅ (0.395%) and (0.68%) whereas minimum incidence was observed in M₉ (0.002%) and (0.05%) under pot and field respectively. However, in the field maximum yield (44.65 q/ha) and test weight (36.04 g) was recorded in module M₁₁, followed by (44.30q/ha) and (35.91g) in M₈, while, minimum yield (40.35q/ha) and test weight (35.30g) was observed in M₁₂.

Comment [S3]: Unnecessary and repeated with the introduction

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Comment [S4]: This wheat disease is of worldwide importance as...

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Comment [S5]: The term modules does not help – combined treatments of Inform general / summarizes IDM combined treatments of each on seed, soil and plants !

Comment [S6]: The conclusions of what were the better IDM treatment is not based on robust statistical analyses, so the abstract is vague and resume only on show the better results ! It does not explain so, it does not convince ! What is Mxmodules ? The authors have to be able to summarize and at same time, to explain better the IDM treatments !

Keywords: Karnal bunt, *Tilletia indica*, Incidence, Module, Treatments

Introduction

Wheat, (*Triticum aestivum* L.) belonging to family "Gramineae" and genus "*Triticum*", is one of the world's most widely cultivated food grain crop, due to its wider adaptability to different agro-climatic and soil conditions. Globally, the nutritive rich cereals are grown altogether in 215.48 million hectare area with the annual production 731.40 million metric tons. In India, during 2020-21, wheat has been cultivated in 30.22 million hectare and production has made another landmark achievement by producing 99.9 million metric tons with an average national productivity of 33.71 q/ha. (Anonymous, 2021). Many factors of biotic and abiotic stresses pose serious threats to sustain production, productivity and quality of wheat in Indian subcontinent and through-out the world (Chatrath et al., 2007). Out of these different biotic stresses, karnal bunt (*Tilletia indica*) is an important wheat disease with

Comment [S7]: Worldwide economic amounts / importance ?

Comment [S8]: Space between words and sentences are lacking in many places. It seems to be a computer keyboard problem and need to be checked.

Comment [S9]: What is the economic value for India ?

implications for wheat grain quality and inflicts changes in chemical composition of infected grains (Aujla *et al.*, 1980). Karnal bunt is also a disease of quarantine interest and it affects the international trade of commercial wheat grain and movement of wheat throughout the world. With the advent of new stringent laws in import/export, there is zero tolerance limits on shipment of wheat from karnal bunt prone regions (Butler, 1990). The loss due to Karnal Bunt is difficult to estimate because the disease reduces seed quality, inflicts changes in the chemical composition of infected grains and renders seed useless for consumption. Nevertheless, in India, loss in yield due to the disease has been calculated as $\frac{1}{3} \times \text{yield} \times \text{per cent infection}$ as the disease covers one third of an area under wheat cultivation in India (Munjal, 1975). The financial losses caused by the disease are substantial, ranging from 5-20%. The losses in grain quality due to Karnal bunt have serious economic repercussions, even though the losses in grain yield are minimal. The economic importance of this disease is generally not measured by the loss in grain quantity but by grain quality which gets deteriorated due to *Tilletia indica* (Sharma *et al.*, 2021).

Comment [S10]: For where ?

Comment [S11]: The introduction is limited on the importance of wheat crop and the smut disease, but it not bring a good review on IDM strategies for bunt karnal disease control !! A pertinent review is missing ?

Methods and Materials

An experimental trial (field and pot) for the evaluation of different Integrated Disease Management (IDM) Modules (treatments) against karnal bunt of wheat was conducted at Crop Research Center Chiraudi (CRC), Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut during the year 2021 and 2022. The trial was conducted in 4.0x3.0 m² plots under irrigated condition with recommended package and practices. The experiment was laid out in a Randomized Block Design (RBD) with 3 replications and 13 treatments (Modules) using karnal bunt susceptible variety HD-2967. When crop reached to boot leaf stage each of the ten heads in each plot was inoculated (4-5 times at one day intervals) with 3 ml of sporidial suspension of *T. indica* (10,000 spores/ml). Sterilized water sprayed in sub-plots served as control. After 48 hours of artificial inoculation, each fungicide was sprayed at their recommended dosage rates at two crop stages first at boot stage and second at ear emergence stage (Table 1). Inoculated plants were tagged and labeled and field moisture was maintained. At maturity, the inoculated heads were harvested, hand threshed and percent seed infections for each module was determined. The data collected included the numbers of infected and uninfected seeds every ten spikes to evaluate the incidence of the disease (% seeds infected). The incidence of the disease for each entry was calculated by using the following formula Aujla *et al.* (1989).

Comment [S12]: The same description for field and pot (or plot?) experiments are confusing, as well the experimental unit in each case are not described. I do not know until here if is pot or plot experiment !!!

Comment [S13]: Parcels of 4x3 meters or of 12 square meters ? 4x3 m² does not make sense! Also what is the meaning of package here ?

Comment [S14]: Replication and experimental unit ?

Comment [S15]: How the moisture was maintained ? On field or on pot/plot?

Comment [S16]: Visualized seed with symptoms and signals ? Teleospores in mass ? Explain better please !

Comment [S17]: calculate

Comment [S18]: entry = experimental unit ? => need operational definition here !

$$\text{Percent disease incidence} = \frac{\text{Number of bunted grains in 10 spike}}{\text{Total number of grain in 10 spike}} \times 100$$

And Percent Disease control was calculated with the help of the following formula:

$$\text{Percent disease control} = \frac{\text{PDI in control} - \text{PDI in treated}}{\text{PDI in control}} \times 100$$

Whereas, PDI = Percent Disease Incidence



Fig 1.1. Culture of *Tilletiaindica* in PDB, 2. Artificial inoculation of spore suspension, 3. Fungicide spray on experimental field and 4. Infected grains of wheat

Comment [S19]: This technique was not explained on M&M text !!

Table 1: Details of the IDM modules:

Module	Seed Treatment	Soil Treatment	Foliar Spray
M ₁	Hot water (52 ⁰ C) for 10 min.	<i>Pseudomonas fluorescens</i> (cfu 2×10 ⁸ /g) @ 5Kg/ha. + Vermicompost @ 10t/ha.	Propiconazole 25% EC @ 0.1% at ear emergence
M ₂	Thiram 75% DS @ 2.5 g/Kg seed	<i>Bacillus subtilis</i> (cfu 2×10 ⁸ /g) @ 5Kg/ha. + Vermicompost @ 10t/ha.	Tebuconazole 25.9% EC @ 0.1% at ear emergence
M ₃	Late sowing	<i>Pseudomonas fluorescens</i> (cfu 2×10 ⁸ /g) @ 5Kg/ha. + Pressmud @ 10t/ha.	Azoxystrobin 23% SC @ 0.1% at ear emergence
M ₄	Hot water (52 ⁰ C) for 10 min.	<i>Bacillus subtilis</i> (cfu 2×10 ⁸ /g) @ 5Kg/ha. + Pressmud @ 10t/ha.	Tebuconazole 25.9% EC @ 0.1% at ear emergence
M ₅	Salt (NaCl) @ 20%	<i>Pseudomonas fluorescens</i> (cfu 2×10 ⁸ /g) @ 5Kg/ha. + Vermicompost @ 10t/ha.	Azadirachtin (Neem Oil) @ 0.3% at ear emergence
M ₆	Thiram 75% DS @ 2.5 g/Kg seed	<i>Bacillus subtilis</i> (cfu 2×10 ⁸ /g) @ 5Kg/ha. + Vermicompost @ 10t/ha.	Azadirachtin (Neem Oil) @ 0.3% at ear emergence
M ₇	Hot water (52 ⁰ C) for	<i>Pseudomonas fluorescens</i> (cfu 2×10 ⁸ /g) @	Azadirachtin (Neem Oil) @ 0.3% at ear

Comment [S20]: Titles are so incomplete - not self-explained. The same criticism are valid for the rest of tables and figures titles !

	10 min.	5Kg/ha. + Pressmud @ 10t/ha.	emergence	
M ₈	Salt (NaCl) @ 20%	<i>Pseudomonas fluorescens</i> (cfu 2×10 ⁸ /g) @ 5Kg/ha. + Vermicompost @ 10t/ha.	Bootstage	Ear emergence
			Propiconazole 25% EC @ 0.1%	Azadirachtin (Neem Oil) @ 0.3%
M ₉	Thiram 75% DS @ 2.5 g/Kg seed	<i>Bacillus subtilis</i> (cfu 2×10 ⁸ /g) @ 5Kg/ha. + Vermicompost @ 10t/ha.	Tebuconazole 25.9% EC @ 0.1%	Azadirachtin (Neem Oil) @ 0.3%
M ₁₀	Hot water (52°C) for 10 min.	<i>Pseudomonas fluorescens</i> (cfu 2×10 ⁸ /g) @ 5Kg/ha. + Vermicompost @ 10t/ha.	Azoxystrobin 23% SC @ 0.1%	Azadirachtin (Neem Oil) @ 0.3%
M ₁₁	Thiram 75% DS @ 2.5 g/Kg seed	<i>Bacillus subtilis</i> (cfu 2×10 ⁸ /g) @ 5Kg/ha. + Vermicompost @ 10t/ha.	Propiconazole 20% EC @ 0.1%	Azoxystrobin 23% SC @ 0.1%
M ₁₂	Late sowing without treatment			
M ₁₃	Control	Control	Control	

Results and Discussion

IDM modules evaluated under pot condition revealed that all the thirteen modules were significantly effective and observed lower disease incidence of karnal bunt (Table 2). Module M₁₁ [seed treatment with Thiram 75% DS @ 2.5 g/kg seed, soil treatment with *Bacillus subtilis* (CFU 2×10⁸/g) @ 5Kg/ha. + Vermicompost @ 10t/ha. and Propiconazole 20% EC @ 0.1% booting stage+ Azoxystrobin 23% SC @ 0.1% at ear emergence stage] and M₈ [seed treatment with salt (NaCl) @ 20%, soil treatment with *Pseudomonas fluorescens* (cfu 2×10⁸/g) @ 5Kg/ha. + Vermicompost @ 10t/ha and Propiconazole 25% EC @ 0.1% at booting stage and Azadirachtin (Neem Oil) @ 0.3% at ear emergence stage] recorded nil (0.00%) disease incidence. After control the maximum incidence was recorded (0.425%) in M₁₂ [late sowing] followed by M₂ (0.395%) [Seed treatment with salt (NaCl) @ 20%, soil treatment with *Pseudomonas fluorescens* (cfu 2×10⁸/g) @ 5Kg/ha + Vermicompost @ 10t/ha. and foliar spray with Azadirachtin (Neem Oil) @ 0.3% at ear emergence] whereas minimum incidence was observed in M₉ (0.001%). [seed treatment with Thiram 75% DS @ 2.5 g/Kg seed, soil treatment with *Bacillus subtilis* (cfu 2×10⁸/g) @ 5Kg/ha + Vermicompost @ 10t/ha and foliar spray with].

Table 2: Evaluation of IDM modules against *T. indica* (Pot)

Comment [S21]: Most of space on test was wasted describing again, and again the combination of IDM treatments. There is many repetition of treatments description with are desgusting (stressful) for the reader.

Modules	Disease Incidence (2021)	Disease Incidence (2022)	Plant Disease over Control (2021)	Plant Disease over Control (2022)	Average DI (2021-22)
M ₁	0.026	0.028	94.90	94.71	0.027
M ₂	0.019	0.022	96.27	95.84	0.020
M ₃	0.210	0.200	58.82	62.26	0.205
M ₄	0.090	0.110	80.39	79.24	0.100
M ₅	0.390	0.400	23.52	24.52	0.395
M ₆	0.250	0.270	50.98	49.05	0.260
M ₇	0.310	0.300	39.22	43.39	0.305
M ₈	0.000	0.000	100	100	0.000
M ₉	0.000	0.005	100	99.05	0.002
M ₁₀	0.150	0.140	70.58	73.58	0.145
M ₁₁	0.000	0.000	100	100	0.000
M ₁₂	0.430	0.420	15.68	20.75	0.425
M ₁₃	0.510	0.530	0	0	0.520
CD at 5%	0.03	0.03			

Comment [S22]: Statistics are missing !what is CD ? Legends are missing also !

Studies on management of karnal bunt were conducted to evaluate thirteen IDM modules, under field condition revealed that all the modules were significantly effective and recorded lower disease incidence of karnal bunt (Table 3). However, under natural condition module M₁₁[seed treatment with Thiram 75% DS @ 2.5 g/Kg seed, soil treatment with *Bacillus subtilis* (cfu 2×10⁸/g) @ 5Kg/ha. + Vermicompost @ 10t/ha. and Propiconazole 20% EC @ 0.1% booting stage+ Azoxystrobin 23% SC @ 0.1% at ear emergence stage] and M₈[seed treatment with salt (NaCl) @ 20%, soil treatment with *Pseudomonas fluorescens* (cfu 2×10⁸/g) @ 5Kg/ha. + Vermicompost @ 10t/ha. and Propiconazole 25% EC @ 0.1% at booting stage and Azadirachtin (Neem Oil) @ 0.3% at ear emergence stage] observed nil (0.00%) incidence followed by M₉ [seed treatment with Thiram 75% DS @ 2.5 g/Kg seed, soil treatment with *Bacillus subtilis* (cfu 2×10⁸/g) @ 5Kg/ha + Vermicompost @ 10t/ha. and foliar spray with Tebuconazole 25.9% EC @ 0.1% at booting stage and Azadirachtin (Neem Oil) @ 0.3% at ear emergence stage] (0.005%) was recorded. The other modules were recorded higher disease incidence viz., M₁₂(0.81%) [Late sowing].

M₅(0.68%) [Seed treatment with salt (NaCl) @ 20%, soil treatment with *Pseudomonas fluorescens* (cfu 2×10⁸/g) @ 5Kg/ha + Vermicompost @ 10t/ha. and foliar spray with Azadirachtin (Neem Oil) @ 0.3% at ear emergence]and
M₇(0.60%) [seed treatment with Hot water (52⁰C) for 10 min., soil treatment with *Pseudomonas fluorescens* (cfu 2×10⁸/g) @ 5Kg/ha + Pressmud @ 10t/ha and foliar spray with Azadirachtin (Neem Oil) @ 0.3% at ear emergence].

Table 3: Evaluation of IDM Modules against karnal bunt of wheat (Experimental Field)

<u>ModuleCom</u> <u>bined IDM</u> <u>Treatments</u>	Disease Incidence	<u>Plant Disease over</u>	Disease	<u>Plant Disease over</u>	Average
	2021	control (2021)	Incidence (%) ? 2022	control (2022)	Disease Incidence(%) ?
M ₁	0.15	83.14	0.17	80.46	0.16
M ₂	0.09	89.88	0.11	87.35	0.10
M ₃	0.47	47.19	0.45	48.27	0.46
M ₄	0.21	76.40	0.20	77.01	0.20
M ₅	0.67	24.71	0.60	20.68	0.68
M ₆	0.53	40.45	0.56	35.63	0.54
M ₇	0.59	33.71	0.61	29.88	0.60
M ₈	0.00	100	0.00	100	0.00
M ₉	0.04	95.50	0.06	93.10	0.05
M ₁₀	0.33	62.92	0.35	59.77	0.34
M ₁₁	0.00	100	0.00	100	0.00
M ₁₂	0.83	6.74	0.79	9.19	0.81
M ₁₃	0.89	0	0.87	0	0.88
CD at 5%	0.06	0.06			

Comment [S23]: Plant disease over control ?
Explain ...what is the unit ?Percentage ?

Data from table 4 also revealed that all the modules were significantly effective and recorded higher yield and test weight compared to unprotected control. However, the maximum yield and test weight was recorded (44.65 q/ha) and (40.59g) in module M₁₁ [seed treatment with Thiram 75% DS @ 2.5 g/Kg seed, soil treatment with *Bacillus subtilis* (cfu 2×10⁸/g) @ 5Kg/ha. + Vermicompost @ 10t/ha. and Propiconazole 20% EC @ 0.1% booting stage+

Azoxystrobin 23% SC @ 0.1% at ear emergence stage] followed by (44.30 q/ha) and (40.38 g) M₈ [seed treatment with salt (NaCl) @ 20%, soil treatment with *Pseudomonas fluorescens* (cfu 2×10⁸/g) @ 5Kg/ha. + Vermicompost @ 10t/ha and Propiconazole 25% EC @ 0.1% at booting stage and Azadirachtin (Neem Oil) @ 0.3% at ear emergence stage], after control the minimum yield and test weight was observed (40.35q/ha) and (37.50 g) in M₁₂ (Late sowing) respectively.

Table 4: Effect of IDM modules on yield (q/ha) and 1000 grain weight (test weight) of wheat

Module	Yield (q/ha)		Increase yield over control		Average yield (2021-22)	1000 grain weight (gram)		
	2021	2022	2021	2022		2021	2022	Ave.
M ₁	43.60	43.20	9.00	7.73	43.40	39.92	39.68	39.80
M ₂	43.90	43.70	9.75	8.98	43.80	40.09	39.87	39.98
M ₃	42.30	42.10	5.75	4.98	42.20	39.19	38.76	38.97
M ₄	43.10	42.80	7.75	6.73	42.95	39.86	39.39	39.62
M ₅	40.60	40.50	1.50	0.99	40.55	37.96	37.71	37.83
M ₆	41.90	41.70	4.75	3.99	41.80	38.77	38.43	38.68
M ₇	41.50	41.30	3.75	2.99	41.40	38.23	38.09	38.16
M ₈	44.40	44.20	11.00	10.22	44.30	40.44	40.33	40.38
M ₉	44.10	43.90	10.25	9.47	44.00	40.26	40.11	40.18
M ₁₀	42.70	42.50	6.75	5.98	42.60	39.63	39.17	39.40
M ₁₁	44.80	44.50	12.00	10.97	44.65	40.67	40.52	40.59
M ₁₂	40.30	40.40	0.75	0.75	40.35	37.56	37.47	37.50
M ₁₃	40.00	40.10	0	0	40.05	37.10	37.21	37.15
CD at 5%	5.20	5.18				1.78	1.72	

DI= Disease Incidence, Ave. = Average

Karnal bunt pathogen is predominantly soil-borne rather than seed-borne like the dwarf bunt; seed treatment with fungicide is ineffective for the total eradication of infection (Hoffmann, 1982; Brooks and Buckley, 1977). However, it might reduce the likelihood of infection (El-Naimiet *al.*, 2000). The biggest issue with utilize existing

Comment [S25]: Inform unit

Comment [S24]: Explain better and inform the unit !

fungicides is that when the chemical washes off the spore, karnal bunt spores may develop (Sharma & Basandrai, 2000).

Foliar sprays of Propiconazole, Tebuconazole, Hexaconazole, Thifluzamide, Diniconazole etc. were shown to be effective against natural infection in India (Singh *et al.*, 1999).

All the fungicides singly or in combinations significantly increased the disease control and yield increased as compared to control. Efficacy of such combination products in managing many fungal diseases has been reported by various workers across the world (Wanyera *et al.*, 2009; Muniraj *et al.*, 2017). Different fungicides vary in their efficacy to control karnal bunt. Nagy and Moldovan (2006) reported that fungicides containing Difenoconazole (Dividend 030 FS 1.0 l/t), Tebuconazole (Raxil 060 FS 0.5 l/t), Fludioxonil + Epoxiconazole (Maxim Star DS 1.5 kg/t), Tebuconazole + Thiram (Raxil T 515 FS 2.0 l/t) had a very good efficiency in controlling the common bunt even under artificial infections. Singh *et al.* (2000) compared the efficacy of different fungicides found that the maximum disease control (99.8%) was achieved by Propiconazole (0.1) by a single spray controlled (96.46%) disease, followed by Hexaconazole (92.87%) in the post-inoculation treatment. Singh *et al.* (2018) evaluated the efficacy of seven fungicides viz; Tilt 250, Folicur, Bavistin, Thiram, Vitavax, Benlate and Dithane M-75 of different groups against *Tilletia indica* and reported that out of seven, two fungicides viz; Tilt 250 EC and Folicur to be the most effective as they have inhibited the fungal growth completely.

CONCLUSION

Among all the 13 modules (treatments) evaluated at field conditions, the best module was found to be effective, against karnal bunt of wheat was module M₁₁ [seed treatment with Thiram 75% DS @ 2.5 g/Kg seed, soil treatment with *Bacillus subtilis* (cfu 2×10⁸/g) @ 5Kg/ha. + Vermicompost @ 10t/ha. and Propiconazole 20% EC @ 0.1% booting stage + Azoxystrobin 23% SC @ 0.1% at ear emergence stage] and M₈ [seed treatment with salt (NaCl) @ 20%, soil treatment with *Pseudomonas fluorescens* (cfu 2×10⁸/g) @ 5Kg/ha. + Vermicompost @ 10t/ha. and Propiconazole 25% EC @ 0.1% at booting stage and Azadirachtin (Neem Oil) @ 0.3% at ear emergence stage] observed nil incidence (0.00%), whereas, minimum disease incidence was observed in M₉ (0.005%) [Seed treatment with Thiram 75% DS @ 2.5 g/Kg seed, soil treatment with *Bacillus subtilis* (cfu 2×10⁸/g) @ 5Kg/ha. + Vermicompost @ 10t/ha and foliar spray with Tebuconazole 25.9% EC @ 0.1% at booting stage and

Comment [S26]: The negative or of minor grade results were not discussed or summarized on text ! Even, what could be the major practices or sub-IDM treatments resulting on better bunt Karnal disease control ? The seed treatment ? What seed treatment – the fungicide effect ? Or the soil treatment ? It is possible to discuss the relative dimension of each IDM treatment on M11 or M8 ? What was the more efficacious treatments: on seed, soil or plants n?

Azadirachtin (Neem Oil) @ 0.3% at ear emergence stage]. Similar results were also recorded with test weight (40.59g) and yield (44.65q/ha) in module M₁₁ and M₈ (40.38g & 44.30q/ha).

FUTURE SCOPE

The fungus *Tilletia indica* causing Karnal bunt disease of wheat is a serious concern for import of wheat to Karnal bunt free countries. The grain quality deterioration caused by Karnal bunt fungus is known to have serious implications in the world wheat trade due to strict quarantine regulations. Understanding pathogen population is an important aspect for exploiting resistance, especially when dealing with a heterothallic fungus. Several diagnostic techniques have been devised for the accurate identification of the fungus. This seed, soil and air borne pathogen have less chemical control measures so an integrated disease management strategy is the best approach to combating the disease.

Comment [S27]: As the results, many space on conclusion was wasted on the repeated details of the treatment description. The author have to discover a way to summarize the IDM combined treatments or using another compost acronyms... p.ex. ST (seed treatment), STT seed treatment with thiram, STT+SOBs+FSTA (seed treatment with thiram plus soil treatment with *B.subtilis* plus fungicidal spraying with tebuconazole and azoxystrobin... or something similar !

Comment [S28]: These are not future scope for this specific research. These are future scope for the wheat crop in general. Unnecessary scope here. The authors need to bring proposals for the next step of the trials of the current reseachs to improve the results or to optimize de IDM control practices of wheat bunt karnaldisease !

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