Indian Journal of Agricultural Sciences **95** (4): 376–382, April 2025/Article
https://doi.org/10.56093/ijas.v95i4.157555

Assessment of fungal bioagents against *Tilletia indica* causing Karnal bunt of wheat (*Triticum aestivum*)

SANGALE SMITA[1], MALKHAN SINGH GURJAR[1]*, MUTHYALA MAHENDRA[1] NATASHA KASHYAP[1], JITENDRA KUMAR[1], SURESH M NEBAPURE[1], TUSHAR KANTI BAG[1], VIRENDRA SINGH RANA[1] and MAHENDER SINGH SAHARAN[1]

ICAR-Indian Agricultural Research Institute, New Delhi 110 012, India

Received: 06 October 2024; Accepted: 10 January 2025

ABSTRACT

Wheat (*Triticum aestivum* L.) is an important cereal crop grown worldwide. Among biotic, *Tilletia indica* is a quarantine pathogen, causing Karnal bunt of wheat and it has a zero-tolerance limit. The present experiment was conducted during 2023 and 2024 at ICAR-Indian Agricultural Research Institute, New Delhi to assess the efficacy of few fungal biocontrol agents against *T. indica*. Up on *in vitro* and *in planta* conditions, 18 *Trichoderma asperellum* and 15 *Trichoderma harzianum* isolates were assessed against *T. indica*. Using the well diffusion assay with crude extract of *Trichoderma* spp., the highest percent inhibition was shown by *T. asperellum* 6413 (T_{17}) with 42.07% and 63.97% inhibition of Ti12 and Ti18 isolates of *T. indica,* respectively followed by *T. asperellum* 8619 (T_2) with 37.93% inhibition of Ti12 and 61.07% inhibition of Ti18 isolates of *T. indica* under *in vitro* analysis. *T. harzianum* isolates were not found effective. The GC-MS analysis revealed the presence of several volatile organic compounds (VOCs) were in *T. asperellum* 6413 (T_{17}) and *T. asperellum* 8619 (T_2). However, volatile organic compounds, viz. heptanes, 2, 3-Butanediol, and 1-Hexanol, having antifungal properties were common in both the *T. asperellum* 6413 and *T. asperellum* 8619 and 2 H-Pyran-2-one, acetic acid and toluene were exclusively present in *T. asperellum* 8619. In addition, *in planta* studies with two fungal biocontrol agents revealed that pre-and post-inoculation application of *T. asperellum* 6413 (29.49% and 30.75%) showed the significant inhibition of Karnal bunt pathogen.

Keywords: Biocontrol, Karnal bunt, *Tilletia indica*, *Trichoderma* spp., Volatile organic compounds, Wheat

Globally, wheat (*Triticum aestivum* L.) is the most important staple food crop. India is the second largest wheat-producing country after China. The major wheat-growing states in India are Uttar Pradesh, Punjab, Haryana, Madhya Pradesh, Rajasthan, Bihar and Gujarat. It has an acreage of 30.40 million ha with average production and productivity of 110.5 million tonnes and 3.55 million tonnes/ha, respectively (Anonymous 2022–23). Many biotic stresses like yellow rust, leaf rust, black rust, spot blotch, loose smut and Karnal bunt (KB) diseases affect the wheat crop. Among which, KB is an important quarantine disease, caused by *T. indica*. It was first reported from Haryana in 1931 (Mitra 1931). The KB is mostly prevalent in north-western plain zone of India. Biosecurity risk is applied to wheat-exporting nations due to zero tolerance limits (Singh *et al.* 2020, Gurjar *et al.* 2021). The disease has also been documented in few other nations (Gurjar *et al.* 2021). The pathogen is heterothallic in nature. The sexual recombination happens when compatible allantoid sporidia derive in contact which leads to wide diversity in the pathogen (Gurjar *et al.* 2019, Singh *et al.* 2020).

The field-level detection of KB is very much difficult due to absence of noticeable symptoms and further management practices are delayed and difficult. The dormancy of teliospores plays important role in disease development (Manakkatt *et al.* 2024). Due to the disease's complex infection, the cultural practices and fungicide applications are limited (Pandey *et al.* 2018, Gurjar *et al.* 2018). The foliar spray of fungicide like propiconazole 0.1% at the spike emergence stage was found effective. Until now, very few resistant varieties like, “PBW 343” and “WH542” were developed through backcrossing and are popular in the NWPZ of India (Sharma *et al.* 2015). However, these varieties may become susceptible over time due to evolution of pathogen. Limited studies on biocontrol have been carried out against *T. indica*. Keeping in that view, the host resistance, biological control approaches can be explored for the management of Karnal bunt. Therefore, the present study was aimed to identify the effective biocontrol agents against Karnal bunt of wheat under *in vitro* and *in planta* assays.

[1]ICAR-Indian Agricultural Research Institute, New Delhi.
*Correspondence author email: malkhan_iari@yahoo.com

MATERIALS AND METHODS

Biocontrol agents used for well diffusion assays: The present experiment was conducted during 2023 and 2024 at ICAR-Indian Agricultural Research Institute, New Delhi. All the fungal antagonists were used in the study included 15 *Trichoderma harzianum* isolates (ITCC No-8617, 8016, 8103, 8361, 8681, 8621, 7349, 7357, 7338, 6914, 7838, 7230, 7077, 6276, 8366) and 18 *Trichoderma asperellum* isolates (ITCC No-8549, 8619, 8369, 8541, 8547, 8518, 7903, 8516, 8607, 8687, 8686, 8614, 7041, 6585, 7828, 8272, 6413, 7885). Extremely pathogenic isolates of *T. indica* (Ti12 and Ti18) from Rajasthan, India were used in the study. The well diffusion assay of inhibition of *T. indica* with different strains of *Trichoderma* was used for the study (Magaldi *et al.* 2004). All the strains of *Trichoderma* were grown in conical flasks containing broth media and incubated @220 rpm at 25°C in the shaker incubator up to 15 days. Thereafter, the crude extract was filtered firstly with Whatman filter followed by Millipore filter (Millex® -LG), pore size of 0.3 µm. Then was kept at 4°C in refrigerator. The Ti12 and Ti18 Ti isolates were cultured in PDA media up to 10 days (18 ± 1°C). The inoculum was prepared with sterile water into the culture tubes. Further, the inoculum was filtered by double-layer muslin cloth to remove the media and then the optimum concentration spore (1×10^4 sporidia/ml) was measured by the haemocytometer. Fresh spore suspension of *T. indica* was poured over the PDA plates using the sterile spreader and a 6 mm diameter cavity was made at the centre by a sterilized cork borer. 20 µl crude extract of *Trichoderma* spp. was poured into the cavity, and petri plates were incubated (18 ± 1°C). As negative control, PDA containing plates were also kept without adding *Trichoderma* crude extract into the cavity. A positive control plate was prepared by pouring 20 µl propiconazole 0.1% into cavity. These plates were then sealed with parafilm. After the 10th day of incubation, pathogen growth was measured in different treatments. The experiments were conducted in three replications. The percent inhibition of pathogen was observed (Vincent 1947):

$$\text{Percent inhibition of } T.\ indica = [C–T/C] \times 100$$

Where C, Radial growth of *T. indica* (mm) in control; T, Radial growth of *T. indica* (cm) in the presence of crude extract of *Trichoderma* spp.

Extraction of secondary metabolites from Trichoderma spp. *and gas chromatography-mass spectroscopy (GC-MS) investigation*: *T. asperellum* 6413 *and T. asperellum* 8619 were chosen based on their effectiveness *in-vitro* assay. They were grown in the test tube containing PDA media at 25 ± 1°C up to 7 days. The small bit of mycelia was taken and inoculated into broth media (500 ml Erlenmeyer flasks). These flasks were kept at 25 ± 1°C in the shaker incubator (220 rpm) up to 15 days. After that, the mycelial growth was removed using the Whatman filter paper. Ethyl acetate was applied as a solvent for the extraction of secondary metabolites. For that similar quantity of culture filtrate and ethyl acetate (99% pure) was added and then transferred to a separating funnel (1000 ml) and swirled gently for 15–20 sec. After swirling, the solution was kept undisturbed for 30 min. The organic phase and aqueous phase was transferred to separate flasks and the process was repeated three times. The aqueous phase was concentrated by the rotary evaporator (Heidolph) at 175 rpm and 45°C. The concentrated aqueous phase was transferred to 20 ml sterile glass vials and ethyl acetate was completely dried at room conditions. For later usage, the dried concentrates were weighed and kept at 4°C. To prepare 1000 ppm solution, the concentrates were added in ethyl acetate, then volatile organic compounds (VOCs) were identified.

The VOCs of *T. asperellum* 6413 and *T. asperellum* 8619 were determined with a GC-MS-QP2010 Ultra (Shimadzu Corporation, Japan). It uses a single quadrupole gas chromatograph and an advanced scanning speed protocol (ASSP™) mass spectrometer for processing samples. The components were separated using the column (30 m, 0.25 mm, film thickness 0.25 m) and identified the compounds. On the basis of mass and retention index, the NIST (National Institute of Standard and Technologies) library was used to analyse the VOCs in each spectrum.

In planta *assay using selected biocontrol agents*: *In planta* assay was conducted to find out promising bio agents against *T. indica*. Three different assays namely pre-inoculation treatment with the biocontrol agents, post-inoculation treatment with biocontrol agents and combination of pre- and post- inoculation treatments were conducted under net house conditions. Highly susceptible wheat genotype, viz. Sonalika was taken for this experiment. Culturing of the potential *Trichoderma* strains was done by growing them in the test tubes containing PDA. The conidial suspension was prepared by adding 10 ml sterile water in the test tube, scrapped with a needle, and then filtered through a muslin cloth. The conidial concentration was measured with a haemocytometer and adjusted to 10^8 conidia/ml. Similarly, *T. indica* inoculum was made with water to *T. indica* culture tube, scrapped with the help of a needle and then filtered through a muslin cloth. The spore concentration was measured with a haemocytometer and adjusted to 10^4 sporidia/ml. Fungicide propiconazole 0.1% as kept as positive control for foliar treatment.

In pre-inoculation treatment, at the boot leaf (Z-49) phase of wheat (Zadoks *et al.* 1974) biocontrol conidial suspension (10^8 conidia/ml) was injected into five ear heads with a hypodermic syringe. Propiconazole @0.1% sprayed on five ear-heads served as positive control. After 24 h, the same ear heads were also inoculated with the pathogen (10^4 sporidia/ml) into the ear heads using a hypodermic syringe in the evening period (Aujla *et al.* 1987). For post-inoculation treatment, at the boot leaf (Z-49) phase of wheat, (Zadoks *et al.* 1974) plants were inoculated with *T. indica* in the evening period (Aujla *et al.* 1987). After 24 h, five ear-heads were inoculated with the pathogen also injected with *Trichoderma* conidia suspension (10^8 conidia/ml) by a hypodermic syringe. Propiconazole 0.1% was sprayed on five ear-heads as positive control. For pre- and post-inoculation treatment,

at the boot leaf (Z-49) phase (Zadoks *et al.* 1974) of wheat, conidial suspension (10^8 conidia/ml) was injected into five ear heads of plants by a hypodermic syringe. After 24 h, the same plant was also inoculated with the pathogen by injecting 1 ml spore suspension (10^4 sporidia/ml) into the ear heads using a hypodermic syringe in the evening period (Aujla *et al.* 1987). After 24 h of pathogen inoculation, post-inoculation biocontrol was applied. Maximum RH was maintained via mist for the two-weeks after treatments. At maturity, harvesting was performed separately and KB grains were classified into five categories (0–IV) based on the area of endosperm converted into teliospores of *T. indica*. The per cent of infection and the coefficient of infection (COI) was observed (Aujla *et al.* 1989), and per cent disease control was also calculated. The data were statistically analysed (Panse and Sukhatme 1967) with the standard approach. The OP-STAT web server was used to carry out the statistical analysis and Duncan's multiple-range test (DMRT) was used to find the significance level.

RESULTS AND DISCUSSION

In vitro *studies of biocontrol agents*: Among isolates of *Trichoderma* spp., the highest per cent inhibition was shown by *T. asperellum* 6413 (T_{17}) with 42.07% inhibition of Ti12 and 63.97% inhibition of Ti18 isolates of *T. indica* followed by *T. asperellum* 8619 (T_2) with 37.93% inhibition of Ti12 and 61.07% inhibition of Ti18 isolates of *T. indica* (Table 1 and Fig. 1). Both the isolates of *Trichoderma* were found significant. The next best result was shown by *T. asperellum* 8518 (T_{18}) with 30.6% inhibition of Ti12 and 61.03% inhibition of Ti18 isolates of *T. indica*. The *T. harzianum* isolates were not found effective i.e. zero percent inhibition (Fig. 1). Propiconazole 0.1% was showed 100% inhibition of *T. indica*. Previously, limited studies have been carried out to inhibit *T. indica* using fungal antagonists. Earlier, the germination of teliospores and secondary sporidia of *T. indica* was inhibited by culture filtrates of *Trichoderma pseudokoningii*, *T. lignorum*, *T. koningii*, *Gliocladium roseum*, *G. deliquescens*, *G. virens* (Amer *et al.* 1998).

Analysis of volatile organic compounds (VOCs) of effective Trichoderma *spp.*: The present study of GC-MS analysis revealed the existence of many VOCs in *T. asperellum* 6413 (T_{17}) and *T. asperellum* 8619 (T_2) (Table 2). These organic volatile compounds were further categorized into functional groups i.e. ketones, hydrocarbon, esters, ether, alcohol, acids, aldehyde, siloxane and peroxides. After analysis of VOCs of *T. asperellum* 6413, the maximum retention time was for the cyclooctasiloxane compound followed by cycloheptasiloxane, cyclopentane and hydroperoxide which was 35.72 min, 30.60 min, 11.40 min

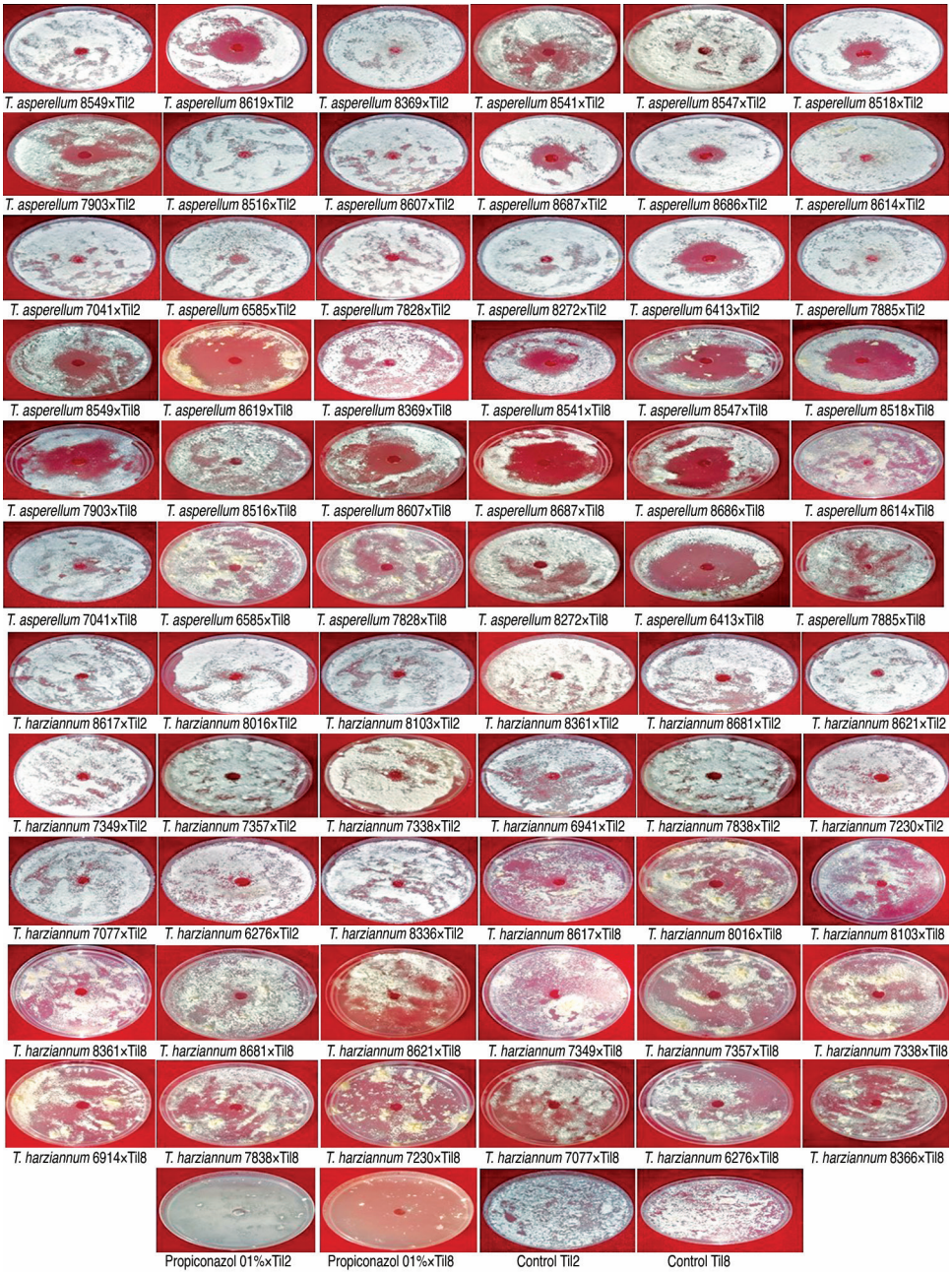

Fig.1 Well diffusion assay of *T. asperellum* and *T. harzianum* against *T. indica.*

Table 1 Bio efficacy of *T. asperellum* and *T. harzianum* isolates against *T. indica in vitro*

Treatment	Ti12		Ti18	
	Radial growth (cm)	% inhibition	Radial growth (cm)	% inhibition
T_1, *T. asperellum* 8549	8.5[a]	0[i]	8.5[a]	34.37[h]
T_2, *T. asperellum* 8619	5.4[f]	37.93[c]	4.67[d]	61.07[c]
T_3, *T. asperellum* 8369	8.5[a]	0[i]	8.5[a]	0[k]
T_4, *T. asperellum* 8541\	6.62[c]	19.13[g]	6.493[b]	24.53[j]
T_5, *T. asperellum* 8547	8.5[a]	0[i]	8.5[a]	60.33[d]
T_6, *T. asperellum* 8518	5.9[e]	30.6[d]	3.267[f]	61.03[c]
T_7, *T. asperellum* 7903	6.2[d]	24.7[f]	3.23[f]	41.53[g]
T_8, *T. asperellum* 8516	8.5[a]	0[i]	8.5[a]	0[k]
T_9, *T. asperellum* 8607	8.5[a]	0[i]	5.8[c]	31.23[i]
T_{10}, *T. asperellum* 8687	6.11[d]	29.07[e]	3.53[e]	56.17[e]
T_{11}, *T. asperellum* 8686	6.86[b]	18.47[h]	4.64[d]	42.4[f]
T_{12}, *T. asperellum* 8614	8.5[a]	0[i]	8.5[a]	0[k]
T_{13}, *T. asperellum* 7041	8.5[a]	0[i]	8.5[a]	0[k]
T_{14}, *T. asperellum* 6585	8.5[a]	0[i]	8.5[a]	0[k]
T_{15}, *T. asperellum* 7828	8.5[a]	0[i]	8.5[a]	0[k]
T_{16}, *T. asperellum* 8272	8.5[a]	0[i]	8.5[a]	0[k]
T_{17}, *T. asperellum* 6413	4.73[g]	42.07[b]	3.37[f]	63.97[b]
T_{18}, *T. asperellum* 7885	8.5[a]	0[i]	8.5[a]	0[k]
T_{19}, *T. harzianum* 8617	8.5[a]	0[i]	8.5[a]	0[k]
T_{20}, *T. harzianum* 8016	8.5[a]	0[i]	8.5[a]	0[k]
T_{21}, *T. harzianum* 8103	8.5[a]	0[i]	8.5[a]	0[k]
T_{22}, *T. harzianum* 8361	8.5[a]	0[i]	8.5[a]	0[k]
T_{23}, *T. harzianum* 8681	8.5[a]	0[i]	8.5[a]	0[k]
T_{24}, *T. harzianum* 8621	8.5[a]	0[i]	8.5[a]	0[k]
T_{25}, *T. harzianum* 7349	8.5[a]	0[i]	8.5[a]	0[k]
T_{26}, *T. harzianum* 7357	8.5[a]	0[i]	8.5[a]	0[k]
T_{27}, *T. harzianum* 7338	8.5[a]	0[i]	8.5[a]	0[k]
T_{28}, *T. harzianum* 6914	8.5[a]	0[i]	8.5[a]	0[k]
T_{29}, *T. harzianum* 7838	8.5[a]	0[i]	8.5[a]	0[k]
T_{30}, *T. harzianum* 7230	8.5[a]	0[i]	8.5[a]	0[k]
T_{31}, *T. harzianum* 7077	8.5[a]	0[i]	8.5[a]	0[k]
T_{32}, *T. harzianum* 6276	8.5[a]	0[i]	8.5[a]	0[k]
T_{33}, *T. harzianum* 8366	8.5[a]	0[i]	8.5[a]	0[k]
T_{34}, Propiconazole 0.1%	0[h]	100[a]	0[g]	100[a]
T_{35}, Control	8.5[a]	0[i]	8.5[a]	0[k]
CD at 5%	0.105	0.528	0.12	0.676
SEM	0.037	0.187	0.042	0.239

Table 2 Volatile organic compounds identified from *T. asperellum* 6413 and *T. asperellum* 8619 using GC-MS approach

Compound	Functional group	Molecular formula	Retention time (min)	% area
T. asperellum 6413				
Heptane	Hydrocarbon	C_7H_{16}	3.405	6.82
Furan, tetrahydro-2,5-dimethyl-	Ether	$C_6H_{12}O$	3.553	7.13
Cyclohexane, methyl-	Hydrocarbon	C_7H_{14}	3.816	0.83
Oxetane, 2,2,4-trimethyl-	Ether	$C_6H_{12}O$	4.02	1.69
Toluene	Hydrocarbon	C_7H_8	4.738	0.53
Furan, 2-butyltetrahydro-	Ether	$C_8H_{14}O$	4.876	1.44
3-Hexanone	Ketone	$C_6H_{12}O$	5.27	2.62
2-Hexanone	Ketone	$C_6H_{12}O$	5.27	2.625
2,3-Butanediol	Alcohol	$C_4H_{10}O_2$	5.43	0.65
3-Hexanol	Alcohol	$C_6H_{14}O$	5.55	2.34
1-Hexanol	Alcohol	$C_6H_{14}O$	5.55	2.34
Ethanone, 1-(3-ethyloxiranyl)-	Ketone	$C_7H_{12}O_2$	8.47	2.78
2,2,5,5-Tetramethyl-3-hexanone	Ketone	$C_{10}H_{20}O$	8.731	2.19
Tetrahydrofuran-2-one	Ester	$C_4H_6O_2$	9.162	0.57
3-Hydroxy-3-methyl valeric acid	Acid	$C_6H_{12}O_3$	9.771	0.76
Hydroperoxide, 1-methylpentyl	Peroxide	$C_6H_{14}O_2$	10.79	24.37
Cyclopentane, 1-acetyl-1,2-epoxy-	Ketone	$C_7H_{10}O_2$	11.406	2.11
Cycloheptasiloxane, tetradecamethyl-	Siloxane	$C_{14}H_{42}O_7Si_7$	30.603	1.83
Cyclooctasiloxane, hexadecamethyl-	Siloxane	$C_{16}H_{48}O_8Si_8$	35.724	1.43
T. asperellum 8619				
1-Hexanol	Alcohol	$C_6H_{14}O$	5.55	1.51
1-Methoxy-2-propyl acetate	Ether	$C_6H_{12}O_3$	10.15	8.94
2(3H)-Benzofuranone,	Ester	$C_8H_6O_2$	30.17	2.5
2,2,5,5-Tetramethyl-3-hexanone	Ketone	$C_{10}H_{20}O$	8.73	1.31
2,3-Butanediol	Alcohol	$C_4H_{10}O_2$	5.43	0.65
2-Hexanone	Ketone	$C_6H_{12}O$	5.28	1.71
2H-Pyran-2-one,	Ester	$C_5H_4O_2$	26.36	7.16
3-Hexanol	Alcohol	$C_6H_{14}O$	5.55	1.51
3-Hexanone	Ketone	$C_6H_{12}O$	5.28	1.71
4-Methyl-5-decanol	Alcohol	$C_{11}H_{24}O$	11.40	1.48
9-Undecenal, 2,10-dimethyl-	Aldehyde	$C_{13}H_{24}O$	38.06	3.66
Acetamide, N-acetyl-N,N'-	Amide	C_2H_5NO	9.77	0.64
Acetic acid, cyano-	Acid	$C_3H_3NO_2$	5.56	0.06
Cubenol	Alcohol	$C_{15}H_{26}O$	36.43	1.76
Cycloheptasiloxane, tetradecamethyl-	Siloxane	$C_{14}H_{42}O_7Si_7$	30.60	2.66
Cyclohexane, methyl-	Hydrocarbon	C_7H_{14}	3.82	0.34
Cyclooctasiloxane, hexadecamethyl-	Siloxane	$C_{16}H_{48}O_8Si_8$	35.72	3.59
Ethanone, 1-(3-ethyloxiranyl)-	Ketone	$C_7H_{12}O_2$	8.47	1.83
Furan, 2-butyltetrahydro-	Ether	$C_8H_{14}O$	4.87	1.13
Furan, tetrahydro-2,5-dimethyl-	Ether	$C_6H_{12}O$	3.55	5.62
Heptane	Hydrocarbon	C_7H_{16}	3.40	4.41
Hydroperoxide, 1-ethylbutyl	Hydroperoxide	$C_6H_{14}O_2$	10.79	16.8
Oxetane, 2,2,4-trimethyl-	Ether	$C_6H_{12}O$	4.02	1.15
Pentan-2-ol, 4-allyloxy-2-methyl-	Alcohol	$C_{10}H_{20}O_2$	9.16	0.38
Toluene	Hydrocarbon	C_7H_8	4.74	0.36

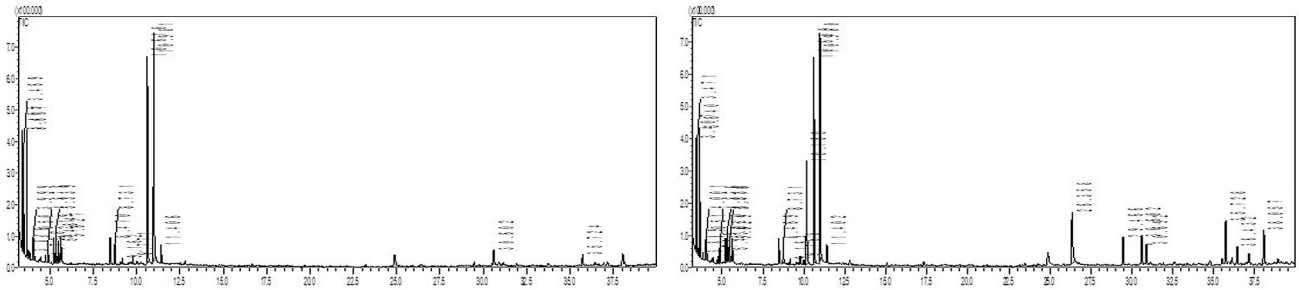

Fig. 2 GC-MS spectrums for the volatile organic compounds of *T. asperellum* 6413 and *T. asperellum* 8619.

and 10.79 min, respectively (Table 2). The area coverage was found maximum (24.37%) for the compound hydroperoxide (Fig. 2). Investigation of VOCs of *T. asperellum* 8619, the maximum retention time was for 9-Undecenal then cubenol, cyclooctasiloxane, 2(3H)-benzofuranone which was 30.17 min, 30.60 min, 30.17 min and 26.35 min, respectively (Table 2). The area coverage was found maximum for the compound hydroperoxide which was 16.89% (Fig. 2). Most of the VOCs were common in *T. asperellum* 6413 and *T. asperellum* 8619. However, the compounds that were found as antifungal activity from the previous studies (Weisskopf 2013, Srinivasa *et al.* 2017, Calvo 2020, Tilocca *et al.* 2022, Shanmugaraj *et al.* 2023) were analyzed for their presence or absence in both the *T. asperellum* 6413 and *T. asperellum* 8619 isolates. We found that volatile organic compounds like heptanes, 2, 3-Butanediol and 1-Hexanol having antifungal properties were present in both the isolates of *Trichoderma* whereas, 2H-Pyran-2-one, acetic acid and toluene were found only in *T. asperellum* 8619. It indicated that these compounds may have role in inhibition of *T. indica*. In earlier reports, *P. fluorescens* showed the significantly highest inhibition (74–82.5%) against *T. indica* under *in vitro* (Vajpayee *et al.* 2015) but they have not conducted *in planta* studies. *Trichoderma* spp. produces the different kind of VOCs involved in antagonist activities (Ruangwong *et al.* 2021).

In planta *assays of biocontrol agents*: Based on the *in vitro* assays, the two best *T. asperellum* 6413 (T_{17}) and *T. asperellum* 8619 (T_2) isolates were selected and subjected to three different modes of application with two isolates of *T. indica* on Sonalika variety of wheat (Table 3). In addition, propiconazole 0.1% was used as a control. Pre-inoculation

Table 3 Effect of pre-inoculation, post-inoculation and pre-and post-inoculation treatments of fungal antagonists against Karnal bunt

Treatment	Ti12			Ti18		
	% Incidence	COI	Average percent disease control	% Incidence	COI	Average percent disease control
Pre-inoculation						
T_1, *T. asperellum* 8619	35.39^b	16.15^b	16.95^c	32.56^b	17.15^b	26.28^c
T_2, *T. asperellum* 6413	28.57^c	13.64^c	26.85^b	31.76^c	14.41^c	28.08^b
T_3, Propiconazole 0.1%	0^d	0^d	100^a	0^d	0^d	100^a
T_4, Control	42.60^a	24.13^a	0^d	44.17^a	23.13^a	0^d
CD at 5%	0.35	0.09	0.31	0.20	0.22	0.306
SEM	0.036	0.002	0.027	0.012	0.001	0.026
Post-inoculation						
T_1, *T. asperellum* 8619	37.37^b	21.97^b	12.29^c	34.25^a	19.17^b	22.46^c
T_2, *T. asperellum* 6413	33.85^c	15.77^c	20.57^b	33.70^c	14.32^c	23.68^b
T_3, Propiconazole 0.1%	0^d	0^d	100^a	0^d	0^d	100^a
T_4, Control	42.60^a	24.13^a	0^d	44.17^a	23.13^a	0^d
CD at 5%	0.305	0.302	0.179	0.285	0.325	0.094
SEM	0.026	0.026	0.009	0.023	0.030	0.002
Pre- and post-inoculation						
T_1, *T. asperellum* 8619	33.33^b	18.33^c	21.77^c	31.65^b	15.50^b	28.35^c
T_2, *T. asperellum* 6413	29.49^c	16.35^b	29.49^b	30.58^c	14.41^c	30.74^b
T_3, Propiconazole 0.1%	0^d	0^d	100^a	0^d	0^d	100^a
CD at 5%	0.170	0.415	0.148	0.251	0.048	0.083
SEM	0.008	0.049	0.006	0.018	0.001	0.002

treatment with biocontrol agents revealed that T_2 treatment (*T. asperellum* 6413) gave 26.85% disease control \against Ti12 and 28.08% against Ti18. Propiconazole exhibited 100% disease control. T_1 (*T. asperellum* 8619) and T_2 (*T. asperellum* 6413) were found significant. Post-inoculation application of biocontrol agents revealed that T_2 treatment (*T. asperellum* 6413) gave 20.57% disease control with Ti12 and 23.68% with Ti18. Propiconazole gave 100% disease control. T_1 (*T. asperellum* 8619) and T_2 (*T. asperellum* 6413) were found significant. The pre- and post-inoculation of *Trichoderma* antagonists, the highest per cent disease control found in T_2 treatment (*T. asperellum* 6413) with 29.49% and 30.75% against Ti12 and Ti18 respectively. T_1 treatment (*T. asperellum* 8619) showed per cent disease control of 29.49% with Ti12 and 30.75% with Ti18. Propiconazole gave 100% disease control. Both the T_1 (*T. asperellum* 6413) and T_2 (*T. asperellum* 8619) were statistically significant. In conclusion, T_2 treatment (*T. asperellum* 6413) showed significantly constant and good results in all three assays. In earlier reports suggested that combination of propiconazole with the *Trichoderma viride* was effective to inhibit the Karnal bunt pathogen (Iquebal *et al.* 2021). In addition, numerous *Trichoderma* spp. have potential to inhibit *Tilletia horrida* (Wang *et al.* 2022). Still, there is need to identify the potential antagonists which inhibit *T. indica* with better control efficiency. Further, wheat microbiome approach might be explored to identify the bacterial and fungal antagonists to manage the Karnal bunt of wheat.

REFERENCES

Amer G A M, Singh D V, Aggarwal R and Dureja P. 1998. Microbial antagonism of *Neovossia indica*, causing Karnal bunt of wheat. *Current Science* **75**: 1393–96.

Anonymous 2022–23. Annual report, 2022–23. Department of Agriculture and Cooperation; Farmers' Welfare Ministry of Agriculture and Farmers' Welfare Government of India Krishi Bhawan, New Delhi. www.agricoop.nic.in

Aujla S, Sharma I and Singh B. 1987. Physiologic specialization of Karnal bunt of wheat. *Indian Phytopathology* **40**(3): 333–36.

Aujla S, Sharma I and Singh B. 1989. Rating scale for identifying wheat varieties resistant to *Neovossia indica* (Mitra) Mundkur. *Indian Phytopathology* **42**(2): 161–62.

Calvo H, Mendiara I, Arias E, Gracia P, Blanco D and Venturini E. 2020. Antifungal activity of the volatile organic compounds produced by *Bacillus velezensis* strains against postharvest fungal pathogens. *Postharvest Biology and Technology* **166**: 111208.

Gurjar M, Mohan M, Singh J, Saharan S and Aggarwal R. 2021. *Tilletia indica*: Biology, variability, detection, genomics and future perspective. *Indian Phytopathology* **74**(1): 21–31.

Gurjar M S, Aggarwal R, Jogawat A, Kulshreshtha D, Sharma S, Solanke A U, Dubey H and Jain R K. 2019. De novo genome sequencing and secretome analysis of *Tilletia indica* inciting Karnal bunt of wheat provides pathogenesis-related genes. *3 Biotech* **9**(6): 219.

Iquebal M A, Mishra P, Maurya R, Jaiswal S, Rai A and Kumar D. 2021. Centenary of soil and air borne wheat Karnal bunt disease research: A review. *Biology* **10**(11): 1152.

Manakkatt H M, Gurjar M S, Saharan M S and Aggarwal R. 2024. Expression analysis of genes involved in teliospores germination of *Tilletia indica* inciting Karnal bunt of wheat. *Molecular Biology Reports* **51**: 726.

Magaldi S, Mata-Essayag S, Hartung C, Perez C, Colella M, Carolina O and Yudith O. 2004. Well diffusion for antifungal susceptibility testing. *International Journal of Infectious Diseases* **8**: 39–45.

Mitra M. 1931. A new bunt on wheat in India. *Annals of Applied Biology* **18**: 178–79.

Pandey V, Singh M, Pandey D and Kumar A. 2018. Integrated proteomics, genomics, metabolomics approaches reveal oxalic acid as pathogenicity factor in *Tilletia indica* inciting Karnal bunt disease of wheat. *Scientific Reports* **8**(1): 7826.

Panse V and Sukhatme P. 1967. *Statistical Methods for Agricultural Workers*. Indian Council of Agricultural Research, New Delhi, India.

Ruangwong O U, Wonglom P, Suwannarach N, Kumla J, Thaochan N, Chomnunti P, Pitija K and Sunpapao A. 2021. Volatile organic compound from *Trichoderma asperelloides* TSU1: Impact on plant pathogenic fungi. *Journal of Fungi* **7**(3): 187.

Shanmugaraj C, Kamil D, Kundu A, Singh P, Das A, Hussain Z, Gogoi R, Shashank P, Gangaraj R and Chaithra M. 2023. Exploring the potential biocontrol isolates of *Trichoderma asperellum* for management of collar rot disease in tomato. *Horticulture* **9**(10): 116.

Sharma I, Tyagi B S, Singh G, Venkatesh K and Gupta O P. 2015. Enhancing wheat production- A global perspective. *The Indian Journal of Agricultural Sciences* **85**(1): 3–13.

Singh S, Sehgal D, Kumar S, Arif R, Vikram P and Sansaloni P. 2020. GWAS revealed a novel resistance locus on chromosome 4D for the quarantine disease Karnal bunt in diverse wheat pre-breeding germplasm. *Scientific Reports* **10**: 5999.

Srinivasa N, Sriram S, Singh C and Shivashankar S. 2017. Secondary metabolites approach to study the bio-efficacy of *Trichoderma asperellum* isolates in India. *International Journal of Current Microbiology and Applied Sciences* **6**(5): 1105–23.

Tilocca B, Cao A and Migheli Q. 2020. Scent of a killer: Microbial volatilome and its role in the biological control of plant pathogens. *Frontiers in Microbiology* **11**: 41.

Vajpayee G, Asthana S and Sundaram S. 2015. Screening of antagonistic activity of bacteria against *Tilletia indica*. *Indian Journal of Natural Sciences* **5**(29): 4302–13.

Vincent J M. 1947. Distortion of fungal hyphae in the presence of certain inhibitors. *Nature* **159**(4051): 850.

Wang A, Shu X, Xu D, Jiang Y, Liang J, Yi X, Zhu J, Yang F, Jiao C, Zheng A and Yin D. 2022. Understanding the rice fungal pathogen *Tilletia horrida* from multiple perspectives. *Rice* **15**: 64.

Weisskopf L. 2013. The potential of bacterial volatiles for crop protection against phytopathogenic fungi. *Microbial Pathogens and Strategies for Combating Them: Science, Technology and Education* **2**: 1352–63.

Zadoks J, Chang T and Konzak C F. 1974. A decimal code for the growth stages of cereals. *Weed Research* **14**(5): 415–21.