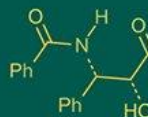


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In-vitro evaluation of bio-agents and ready-mix fungicides against collar rot of groundnut caused by *Aspergillus niger* van Tieghem

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Abstract

Collar rot of groundnut (*Arachis hypogaea* L.) caused by *Aspergillus niger* van Tieghem is a major soil-borne disease causing significant pre and post-emergence losses in semi-arid groundnut growing regions of India. The present investigation was undertaken to evaluate the efficacy of six bioagents and eight ready-mix fungicides against *A. niger* under *in-vitro* conditions. Antagonistic activity of bioagents was assessed by the dual culture technique, while fungicidal efficacy was evaluated by the poisoned food technique at 100 and 200 ppm concentrations. Among the bioagents, *Trichoderma asperellum* exhibited the highest mycelial growth inhibition of 68.61 percent, followed by *Trichoderma viride* (61.39%) and *Trichoderma virens* (49.17%), while *Bacillus subtilis* was the least effective (32.50%). Among the ready-mix fungicides evaluated, Tebuconazole 50% + Trifloxystrobin 25% WG at 200 ppm was the most effective, recording near-complete inhibition of 99.63 percent with a minimum colony diameter of 0.33 mm, followed by the same combination at 100 ppm (99.26%). Mancozeb 50% + Thiophanate methyl 25% WG (72.96% at 200 ppm) and Azoxystrobin 18.2% + Difenconazole 11.4% SC (68.15% at 200 ppm) were the next most effective treatments. Azoxystrobin 2.5% + Thiophanate methyl 11.25% + Thiamethoxam 25% FS was the least effective (6.30% at 100 ppm). These results highlight the potential of *T. asperellum* based biocontrol and tebuconazole + trifloxystrobin-based fungicide formulations as key components of an integrated management strategy for collar rot of groundnut.

Keywords: *Aspergillus niger*, collar rot, groundnut, biocontrol, *Trichoderma asperellum*, dual culture, poisoned food technique, tebuconazole, trifloxystrobin, *in-vitro*

Introduction

Groundnut (*Arachis hypogaea* L.) is one of the most economically important oilseed crops of the world, cultivated across an area of approximately 5 million hectares with an annual production of around 11.29 million tonnes (2025). Gujarat ranks among the leading groundnut-producing states, In Gujarat, it is grown on about 2.20 million hectares with a production of 5.99 million tons and average productivity of 2728.80 kg/ha in *Kharif* 2025-26. The crop, however, faces severe yield losses due to a variety of fungal diseases throughout its growth period. Among these, collar rot caused by *Aspergillus niger* van Tieghem is considered one of the most destructive seed borne diseases in warm and semi-arid production environments, causing pre-emergence damping-off, post-emergence wilting, and plant mortality ranging from 10 to 50 percent under favourable conditions.

Chemical fungicides have traditionally been the primary tool for managing collar rot, but their indiscriminate use has led to concerns over fungicide resistance development, disruption of beneficial soil microbiota, environmental contamination, and human health risks. These challenges have stimulated interest in biological control agents, particularly *Trichoderma* species and plant growth promoting rhizobacteria, as environmentally benign and sustainable alternatives. Bioagents exert their antagonistic effects through multiple mechanisms including mycoparasitism, competition for nutrients and space, production of cell wall degrading enzymes and elaboration of volatile and non-volatile antifungal secondary metabolites.

Ready-mix fungicide formulations combining two or more active ingredients with complementary modes of action offer several advantages over single ingredient products,

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including broader spectrum activity, reduced likelihood of resistance development and improved efficacy at lower individual active ingredient doses. Several such combinations including triazole + strobilurin, dithiocarbamate + benzimidazole, and carboxamide + dithiocarbamate combinations have shown promise against *Aspergillus* diseases of oilseeds; however, systematic *in-vitro* evaluation of the newer generation of multi component seed treatment formulations against *A. niger* under Indian conditions remains limited.

Materials and Methods

Both bioagent and fungicide trials were conducted in the Post-Graduate Laboratory, Department of Plant Pathology, B. A. College of Agriculture, Anand Agricultural University (AAU), Anand, Gujarat. All experiments were laid out in a Completely Randomized Design (CRD). The bioagent experiment comprised seven treatments (six bioagents and one control) with four replications. The fungicide experiment comprised 17 treatments (eight fungicides at two concentrations each, plus an untreated control) with three replications. The test pathogen, *A. niger* was isolated from collar rot affected groundnut plants and pathogenicity was confirmed by Koch's postulates prior to use in the experiments. Virulent cultures were maintained on Potato Dextrose Agar (PDA) at 27±1 °C.

Six bioagents were evaluated: *Trichoderma asperellum*, *Trichoderma viride*, *Trichoderma harzianum*, *Trichoderma virens*, *Pseudomonas fluorescens*, and *Bacillus subtilis*. Standard cultures of all bioagents were obtained from authenticated sources and maintained on PDA (for fungal bioagents) and Nutrient Agar (NA) (for bacterial bioagents) at 27±1 °C. Fresh sub cultures were prepared seven days prior to experimentation to ensure active and vigorous growth.

The antagonistic activity of bioagents against *A. niger* was assessed by the dual culture technique of Dennis and Webster (1971) [4]. Sterile Petri plates (90 mm diameter) containing approximately 20 ml of sterilized PDA were used. After medium solidification, a 5 mm mycelial disc cut from the actively growing margin of a seven day old culture of *A. niger* using a sterile cork borer was placed at one edge of the Petri plate, approximately 1 cm from the margin. An equal-sized disc (5 mm) of the antagonist culture was placed diametrically opposite to the pathogen disc at an equal distance from the margin. For bacterial antagonists (*P. fluorescens* and *B. subtilis*), a loopful of fresh 48 hour old broth culture was streaked in a zig-zag pattern approximately 3 cm away from the pathogen disc. Plates inoculated with the pathogen disc alone served as the untreated control. All plates were incubated at 27±1 °C. Each treatment was replicated four times. Radial growth of the pathogen was recorded every 24 hours until the control plates showed complete mycelial growth. percent growth inhibition (PGI) was calculated using the formula of Vincent (1947) [15]: The percentage inhibition of mycelial growth over control was calculated using the formula given by Vincent (1947) [15]:

$$\text{PGI} = \frac{\text{DC}-\text{DT}}{\text{DC}} \times 100$$

Where;

PGI = percent growth reduction of test pathogen

DC = Radial growth of test pathogen in control (mm)

DT = Radial growth of test pathogen in treatment (mm)

Eight ready-mix fungicide formulations were evaluated at two concentrations (100 and 200 ppm) against *A. niger* by the poisoned food technique (Nene and Thapliyal, 1993) [9]. Required quantities of each fungicide were calculated and incorporated into sterilized, lukewarm PDA to obtain the desired concentrations. After thorough mixing to ensure uniform distribution, 20 ml of poisoned medium was poured aseptically into sterile Petri plates (90 mm diameter) and allowed to solidify. A 5 mm mycelial disc from a seven day old culture of *A. niger* was placed centrally on each poisoned plate under aseptic conditions. Plates with unamended PDA served as the untreated control. Inoculated plates were incubated at 27±1 °C and radial mycelial growth was recorded every 24 hours until full growth was observed in the control. Three replications were maintained per treatment. PGI was calculated using Vincent's (1947) [15] formula as described above.

Results and Discussion

Biological control

The results of the *in-vitro* evaluation of bioagents against *Aspergillus niger* by the dual culture technique are presented in Table 1. All tested bioagents significantly suppressed mycelial growth of *A. niger* compared to the untreated control (90.00 mm growth), confirming their antagonistic potential against the pathogen.

Among the six bioagents evaluated, *Trichoderma asperellum* (T₄) recorded the highest percent growth inhibition of 68.61 percent with the minimum radial mycelial growth of 28.25 mm after seven days of incubation. This was followed by *Trichoderma viride* (T₁), which exhibited 61.39 percent inhibition (34.75 mm growth), and *Trichoderma virens* (T₃), which showed 49.17 percent inhibition (45.75 mm growth). *Trichoderma harzianum* (T₂), *Pseudomonas fluorescens* (T₅), and *Bacillus subtilis* (T₆) exhibited moderate to lower levels of inhibition (33.06, 38.61, and 32.50%, respectively) (Figure 1).

The present findings corroborate earlier reports on the *in-vitro* antagonism of *Trichoderma* species against *A. niger*. Mahadevi *et al.* (2019) reported that *Trichoderma viride* isolate Tv-3 exhibited 77.7% inhibition of *A. niger*, while Pudake *et al.* (2019) [10] documented that *T. harzianum* recorded the highest inhibition (99.93%) against *A. niger* in dual culture. Saran *et al.* (2021) [13] found *T. harzianum* to be the most effective bioagent (77.67% inhibition) against *A. niger* under *in-vitro* conditions, followed by *T. viride* (72.92%). Bhushan *et al.* (2021) [3] reported 88.64% inhibition of *A. niger* by a superior *Trichoderma* isolate (GT₂) in dual culture. Rani *et al.* (2023) [11] observed that *T. harzianum* MBNRT-1 inhibited *A. niger* by 72.9%, while the bacterial bioagent *Bacillus amyloliquefaciens* MBNRB-3 recorded 63.0% inhibition. The relatively lower performance of *B. subtilis* compared to *Trichoderma* spp. in the present study is consistent with the general trend observed across multiple studies, wherein fungal bioagents belonging to the genus *Trichoderma* outperform bacterial antagonists in *in-vitro* assays against *A. niger*, possibly due to the direct mycoparasitic interaction possible between two fungal organisms.

The present results also align with Alamene (2015) [1], who reported that *Trichoderma* strains (*T. harzianum* T-22, *T.*

asperellum, and *T. viride*) exhibited stronger antagonism against *A. niger* than bacterial bioagents (*Pseudomonas chlororaphis* spp. *aureofaciens* and *Bacillus amyloliquefaciens*) under *in-vitro* conditions. Vineela *et al.* (2020) ^[16] documented that *T. hamatum* was the most effective *Trichoderma* species against *A. niger* among the three species tested, reinforcing the importance of species level and isolate level variation in biocontrol efficacy. The present study identifies *T. asperellum* as a superior bioagent for managing collar rot of groundnut, and its further evaluation under pot and field conditions is recommended.

Chemical control

The *in-vitro* efficacy of eight ready mix fungicide formulations at 100 and 200 ppm against *Aspergillus niger* evaluated by the poisoned food technique is presented in Table 2. All fungicide treatments significantly suppressed mycelial growth of *A. niger* compared to the untreated control (90.00 mm), demonstrating their antifungal potential at the concentrations tested.

Tebuconazole 50% + Trifloxystrobin 25% WG (T₉-T₁₀) emerged as the most effective fungicide combination, recording near complete inhibition at both concentrations: 99.26 percent inhibition (0.67 mm colony diameter) at 100 ppm and 99.63 percent inhibition (0.33 mm) at 200 ppm. This near complete suppression of *A. niger* growth underscores the potent synergistic antifungal activity of the triazole-strobilurin combination. The dual and complementary modes of action of this combination likely account for its outstanding efficacy and also reduce the probability of resistance development by the pathogen.

The second most effective treatment was Mancozeb 50% + Thiophanate methyl 25% WG, which recorded 58.52 percent inhibition at 100 ppm and 72.96 percent inhibition (24.33 mm colony diameter) at 200 ppm. Azoxystrobin 18.2% + Difenconazole 11.4% SC showed 64.07 percent inhibition at 100 ppm and 68.15 percent at 200 ppm, while Carboxin 37.5% + Thiram 37.5% WP recorded 54.07 and 58.52 percent inhibition at 100 and 200 ppm, respectively, ranking as the third and fourth most effective treatments in the trial.

In contrast, Azoxystrobin 2.5% + Thiophanate methyl 11.25% + Thiamethoxam 25% FS (T₁-T₂) and Pyraclostrobin 3.5% + Thiram 15% + Clothianidin 22.5% FS (T₃-T₄) were the least effective, recording only 6.30-7.41% and 7.04-8.15% inhibition, respectively, at both concentrations (Figure 1).

The present findings are in close agreement with earlier reports on the chemical management of collar rot of groundnut. Nath and Patel (2022) ^[8] reported that tebuconazole completely inhibited *A. niger* growth under *in-vitro* conditions, consistent with the near-complete inhibition observed in the present study. Sekhon *et al.* (2019) ^[14] similarly documented complete inhibition of *A. niger* by tebuconazole at 25-200 ppm and by azoxystrobin at 100-200 ppm, and 94.38% inhibition by carbendazim + mancozeb at 200 ppm. Saharan *et al.* (2016) ^[12] reported 100% inhibition of *A. niger* by propiconazole, carbendazim, and carboxin at higher concentrations (200-1000 ppm). Lora and Begum (2019) ^[6] identified mancozeb and carbendazim as the most effective fungicides against *A. niger*, while Anand *et al.* (2010) ^[2] reported carbendazim and hexaconazole as the most effective systemic fungicides against the pathogen. The present results add to this body of evidence by demonstrating that triazole-strobilurin combinations (tebuconazole + trifloxystrobin) represent the most effective class of ready-mix fungicide formulations currently available for managing *A. niger* in groundnut.

Table 1: Effect of bioagents on mycelial growth inhibition of *Aspergillus niger* under *in-vitro* conditions

Tr. No.	Bioagent	Mycelial growth (mm)	Growth inhibition (%)
T ₁	<i>Trichoderma viride</i>	34.75	61.39
T ₂	<i>Trichoderma harzianum</i>	60.25	33.06
T ₃	<i>Trichoderma virens</i>	45.75	49.17
T ₄	<i>Trichoderma asperellum</i>	28.25	68.61
T ₅	<i>Pseudomonas fluorescens</i>	55.25	38.61
T ₆	<i>Bacillus subtilis</i>	60.75	32.50
T ₇	Control (Pathogen only)	90.00	-
	S.Em.±	1.58	
	C.D. at 5%	4.63	
	C.V. (%)	5.88	

Table 2: Effect of ready-mix fungicides on mycelial growth inhibition of *Aspergillus niger* under *in-vitro* conditions

Tr.	Fungicide	Conc. (ppm)	Mycelial growth (mm)	Growth inhibition (%)
T ₁	Azoxystrobin 2.5% + Thiophanate methyl 11.25% + Thiamethoxam 25% FS	100	84.33	6.30
T ₂		200	83.33	7.41
T ₃	Pyraclostrobin 3.5% + Thiram 15% + Clothianidin 22.5% FS	100	83.67	7.04
T ₄		200	82.67	8.15
T ₅	Mancozeb 50% + Thiophanate methyl 25% WG	100	37.33	58.52
T ₆		200	24.33	72.96
T ₇	Hexaconazole 5% + Captan 70% WP	100	73.33	18.52
T ₈		200	68.33	24.07
T ₉	Tebuconazole 50% + Trifloxystrobin 25% WG	100	0.67	99.26
T ₁₀		200	0.33	99.63
T ₁₁	Carboxin 37.5% + Thiram 37.5% WP	100	41.33	54.07
T ₁₂		200	37.33	58.52
T ₁₃	Azoxystrobin 18.2% + Difenconazole 11.4% SC	100	32.33	64.07
T ₁₄		200	28.67	68.15
T ₁₅	Chlorothalonil 75% WP	100	61.00	32.22
T ₁₆		200	58.67	34.81
T ₁₇	Control (Pathogen only)	-	90.00	-
	S.Em.±		1.32	
	C.D. at 5%		3.80	
	C.V. (%)		4.38	

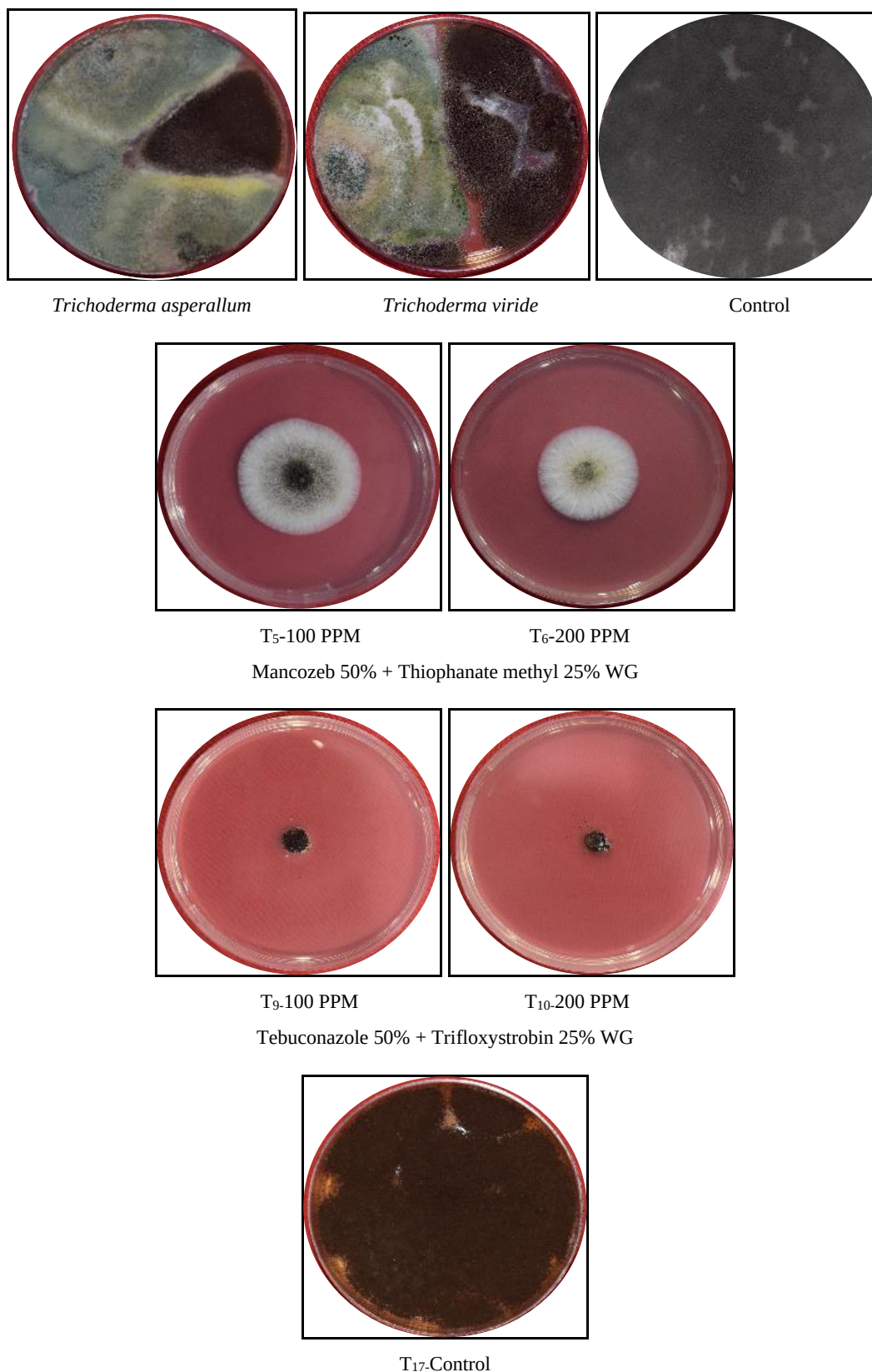


Fig 1: Effect of bio-agents on mycelial growth inhibition of *Aspergillus niger*

Conclusion

The *in-vitro* evaluation of bioagents and fungicides against *Aspergillus niger*, the causal organism of collar rot of groundnut, demonstrated the clear superiority of specific treatments within each category. Among the six bioagents evaluated, *Trichoderma asperellum* was the most effective, recording 68.61 percent mycelial growth inhibition, followed by *Trichoderma viride* (61.39%) and *Trichoderma*

virens (49.17%). The differential efficacy among *Trichoderma* species and bacterial bioagents underscores the importance of species selection and strain characterisation for optimising biocontrol programmes.

Among the eight ready-mix fungicide formulations evaluated, Tebuconazole 50% + Trifloxystrobin 25% WG was the outstanding performer, recording near complete suppression of *A. niger* growth (99.63%) at 200 ppm and

99.26 percent at 100 ppm, by virtue of its dual, complementary mode of action targeting both ergosterol biosynthesis and mitochondrial respiration. Mancozeb 50% + Thiophanate methyl 25% WG and Azoxystrobin 18.2% + Difenconazole 11.4% SC ranked next in efficacy.

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