



RESEARCH ARTICLE

Harnessing new molecule systemic fungicides for effective management of seed-borne diseases in groundnut

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Abstract

The new molecule fungicides viz. Azoxystrobin 18.2 % + Difenoconazole 11.4 % SC (Amistar top) at 0.05 % and 0.1 %, Azoxystrobin 11 % + Tebuconazole 18.3 % SC (Custodia) at 0.1 % and 0.2 %, Thiophanate methyl 45 % + Pyraclostrobin 5 % FS (Xelora) at 0.1 % and 0.2 %, Fluxapyroxad 2.5 % + Pyraclostrobin 2.5 % SC (Merivon) at 0.05 % and 0.1 %, Pyraclostrobin 13.3 % + Epoxyconazole 5 % FS (Opera) at 0.075 % and 0.15 %, Penflufen 13.28 % + Trifloxystrobin 13.2 % FS (Ever Golxtend) at 0.05 % and 0.1 %, Trifloxystrobin 25 % + Tebuconazole 50 % WG (Nativo) at 0.025 % and 0.05 %, Difenoconazole 25 % EC (Score) at 0.1 % and 0.2 %, Pyraclostrobin 10 % CS (Chowkidar) at 0.05 % and 0.1 %, Tebuconazole 2 % DS (Raxil) at 0.05 % and 0.1 %, Fluxapyroxad 33.3 % FS (Systiva) at 0.075 % and 0.15 %, Carboxin 37.5 % WS + Thiram 37.5 % WS (Vitavax Power) at 0.3 % and 0.6 % were evaluated against seed infection, germination and vigor of groundnut variety G7 under *in vitro* and *in vivo* conditions during 2021-2024. Based on their efficacy in reducing the *Aspergillus niger* infection and enhancing seed germination and seedling vigor under *in vitro* conditions during 2021-2022, Pyraclostrobin 13.3 % + Epoxyconazole 5 % FS at 0.075 %, Penflufen 13.28 % + Trifloxystrobin 13.2 % FS at 0.1 %, Thiophanate Methyl 45 % + Pyraclostrobin 5 % FS at 0.1 % and Carboxin 37.5 % WS + Thiram 37.5 % WS at 0.3 % were further evaluated under *in vitro* condition during 2022-2023 and pot and field conditions during 2023-2024. Of which, Pyraclostrobin 13.3 % + Epoxyconazole 5 % FS (Opera) at 0.075 % outperformed the chemical check, Carboxin 37.5 % WS + Thiram 37.5 % WS at 0.3 % in terms of field emergence, seedling mortality, seedling vigor and disease control, proving its effectiveness in managing collar rot incidence in groundnut.

Keywords: collar rot; field emergence; groundnut; new molecule fungicides; seedling mortality

Introduction

Groundnut (*Arachis hypogaea* L.) is being affected by many seed and soil-borne fungal pathogens under field conditions as well as during storage. The prevalence of *Aspergillus*, *Penicillium* and *Rhizopus* species on groundnut seeds adversely affect the viability and decrease the germination percentage of infected seeds (1). Although *Aspergillus flavus* and *Aspergillus niger* were the predominant mycoflora of groundnut (2). Despite causing diseases in groundnut, these mycoflora may tremendously affect the quality of the seed during storage by depleting the reserve food material stored in the seeds, reducing seedling vigor by producing hydrolytic enzymes, creating an unpleasant odour or flavour and rendering the seeds unfit for human consumption, ultimately by producing numerous mycotoxins along with a change in the chemical makeup of the seeds (3, 4)

Aflaroot disease in groundnut, caused by *A. flavus*, is characterized by yellowing and wilting of plants, rotting of roots and discolored, shriveled pods, leading to significant yield loss. In addition to causing disease in the field, *A. flavus* also contaminates stored groundnut seeds by producing hepatocarcinogenic aflatoxins

B1, B2, G1 and G2 (5). The *A. niger* van Tieghem causes up to 15 % loss due to pre-emergence rot and 2 % loss through post-emergence collar rot in groundnut (6, 7). In India, the losses account for 40 % to 50 % in terms of seedling mortality due to *A. niger* (8, 9), which necessitates serious attention to be taken to ward off this pathogen, otherwise the losses due to them will significantly impact total production and productivity (10, 11).

Seed is the basic and most critical input for sustainable agriculture. Seeds harbor several pathogens both externally and internally, which affects crop growth at either early or later stages (12, 13). Of the several management strategies, fungicides serve as an important and efficient method to control seed-borne mycoflora (14, 15). Thus, seed treatment with fungicides seems to be the best option to enhance field emergence and ensure sustainable groundnut production (16). Researchers have periodically tested the efficacy of different fungicides against *A. niger* from time to time and envisaged effective seed-dressing fungicides against collar rot disease in groundnut (17-21). Furthermore, the fungicides that have been used for seed treatment purposes for the past few decades in India are going to be banned in the near future by the Central Insecticides Board & Registration Committee (CIB&RC), Government

of India due to the development of resistance in fungi and residual toxicity in other crops and hence, there is a need to identify best-suited and cost-effective seed-dressing fungicides to protect groundnut seedlings from seed and seedling-associated pathogens (22-24). Keeping these aspects in view, the present study was undertaken to evaluate the efficacy of novel systemic fungicide formulations against seed germination, seedling vigor and control of seed-borne pathogens of groundnut, particularly *A. niger*, under *in vitro* and *in vivo* conditions.

Materials and Methods

Experiments were conducted at Pandit Jawaharlal Nehru College of Agriculture & Research Institute, Karaikal, UT of Puducherry, during 2021-2024 to evaluate the efficacy of the following new molecule fungicides listed in Table 1 against seed infection, germination and seedling vigor of groundnut variety G7.

In vitro evaluation of new molecule fungicides against seed-borne pathogens of groundnut during 2021-2022

1 kg of groundnut seeds (Cv. G7) was treated with the required doses of fungicides separately, maintaining 1 kg of untreated seeds as a control. A total of 100 treated seeds of each fungicide were evaluated in four replications, the day after treatment with the respective fungicidal dose for seed health and quality parameters by adapting the standard blotter method and paper towel method as per International Seed Testing Association (ISTA) (25).

The incubated seeds were examined under a stereoscopic-binocular microscope for associated type and frequency of fungi seven days after incubation and mortality (%) & seed infection (%) were worked out. The observations on the number of seeds germinated and seedling length (root length + shoot length) were recorded on ten days after incubation and categorization of normal seedlings was done as described by earlier research (26) and seedling dry weight, vigor index I and vigor index II were calculated using the formulae viz. Seed vigor index I = Standard Germination (%) X Seedling length (cm) and Seed vigor index II = Standard Germination (%) X Seedling dry weight (mg) as per earlier reports.

In vitro evaluation of promising fungicides against seed-borne pathogens of groundnut during 2022-2023

The fungicides outperformed under *in vitro* conditions during 2021-2022 including Penflufen 13.28 % + Trifloxystrobin 13.2 % FS (Ever Golxtend) at 1ml/kg of seed, Pyraclostrobin 13.3 % + Epoxiconazole 5 % SE (Opera)-0.75ml/kg, Thiophanate methyl 45 % +

Pyraclostrobin 5 % FS (Xelora) and Carboxin 37.5 % WS + Thiram 37.5 % WS (Vitavax Power) at 3g/kg of seed were evaluated the following day after seed treatment for seed health and quality parameters with four replications by adopting the standard blotter method and paper towel method as per ISTA, along with an untreated control (25). The biometric observations on type and frequency of fungi, seed germination (%), seed infection (%), seedling mortality (%) and seedling length (cm), were recorded and seedling dry weight, vigor index I and vigor index II were calculated.

In vivo evaluation of promising fungicides against seed-borne pathogens of groundnut during 2023-24

The groundnut seeds of cultivar G7 were inoculated with *A. niger* at 10^6 conidia/ml and allowed to air dry for 24 hr and then the seeds were treated separately with Penflufen 13.28 % + Trifloxystrobin 13.2 % FS (Ever Golxtend) at 1 ml/kg of seed; Pyraclostrobin 13.3 % + Epoxiconazole 5 % SE (Opera) at 0.75 ml/kg; Thiophanate methyl 45 % + Pyraclostrobin 5 % FS (Xelora) at 1 ml/kg and Carboxin 37.5 % WS + Thiram 37.5 % WS (Vitavax Power) at 3 g/kg of seed, allowed to dry under shaded conditions for 24 hr and then used for pot culture assay and field emergence studies.

In pot culture studies, the fungicide-treated groundnut seeds were sown in 5 kg capacity pots filled with sterilized soil, at 10 seeds per pot, by maintaining pathogen-treated and untreated controls and each treatment was replicated four times in a completely randomized block design.

The field emergence experiment was conducted by randomly selecting a hundred seeds from each treatment in four replications and sowing them at 4-5 cm depth in a well-prepared seedbed with adequate moisture content in a randomized block design under controlled conditions. The number of seedlings that emerged above the ground eight days after sowing was considered as normal seedlings. Field emergence was expressed as a percentage (25).

Field emergence (%) =

$$\frac{\text{Number of seedlings germinated on eight day}}{\text{Total number of seeds sown}} \times 100$$

The percentage seedling mortality and percentage disease incidence will be recorded at 15, 30 and 45 days after sowing. Shoot length (cm), root length (cm) and dry weight (g/plant) were recorded at 45 days after sowing under controlled conditions and vigor index I

Table 1: New molecule fungicides used in this study

Treatment	Fungicide	Trade Name	Dosages (%)	
			X	2X
T1	Azoxystrobin 18.2 % + Difenconazole 11.4 % SC	Amistar top	0.5	1.0
T2	Azoxystrobin 11 % + Tebuconazole 18.3 % SC	Custodia	1.0	2.0
T3	Thiophenate methyl 45 % + Pyraclostrobin 5 % FS	Xelora	1.0	2.0
T4	Fluxapyroxad 2.5 % + Pyraclostrobin 2.5 % SC	Merivon	0.5	1.0
T5	Pyraclostrobin 13.3 % + Epoxyconazole 5 % FS	Opera	0.75	1.5
T6	Penflufen 13.28 % + Trifloxystrobin 13.2 % FS	Ever Golxtend	0.5	1.0
T7	Trifloxystrobin 25 % + Tebuconazole 50 % WG	Nativo	0.25	0.5
T8	Difenconazole 25 % EC	Score	1.0	2.0
T9	Pyraclostrobin 10 % CS	Chowkidar	0.5	1.0
T10	Tebuconazole 2 % DS	Raxil	0.5	1.0
T11	Fluxapyroxad 33.3 % FS	Systiva	0.75	1.5
T12	Carboxin 37.5 % WS+Thiram37.5 % WS	Vitavax Power	3.0	6.0
T13	Control	-	-	-

and vigor index II were calculated.

Statistical Analysis

The data generated from various experiments of this study were statistically analysed (28). Data were statistically analyzed using analysis of variance (ANOVA) at a significance level ($p < 0.05$) using R software.

Results

In vitro evaluation of new molecule fungicides during 2021-2022

The *in vitro* evaluation of new molecule fungicides demonstrated significant variations in seed germination, seedling growth and seed infection. Among the tested fungicides, Pyraclostrobin 13.3 % + Epoxyconazole 5 % FS (T5) recorded the highest germination percentage (96.5 % at D1 and 97.0 % at D2), with superior seedling vigor indices (vigor index I: 3842.0 at D1 and 4128.6 at D2; vigor index II: 333.2 at D1 and 359.2 at D2). Additionally, this treatment showed complete inhibition of seed infection at both doses tested. Thiophanate methyl 45 % + Pyraclostrobin 5 % FS (T3) also exhibited high germination rates (93.5 % at D1 and 98.5 % at D2) and enhanced seedling growth, with root length measuring 16.07 cm at D1 and 16.31 cm at D2. The treated seeds were free from infection at D2. In contrast, the untreated seeds (T13) showed the lowest germination percentage (43.5 %) and significantly reduced shoot (15.76 cm) and root lengths (11.08 cm) with the highest seed infection percentage (70.0 %). Among the systemic fungicides, Azoxystrobin 18.2 % + Difenconazole 11.4 % SC (T1) and Fluxapyroxad 2.5 % + Pyraclostrobin 2.5 % SC (T4) exhibited moderate efficacy, improving shoot and root lengths along with seedling vigor indices. Seedling

dry weight was highest in treatments T3, T5 and T6, indicating improved seedling establishment (Table 2 & Fig. 1).

The Pyraclostrobin 13.3 % + Epoxyconazole 5 % FS (Opera) (T₅) and Thiophanate methyl 45 % + Pyraclostrobin 5 % FS (Xelora) (T₃), Penflufen 13.2 % + Trifloxystrobin 13.2 % FS (Ever Golxtend) (T₆) and Carboxin 37.5 % WS + Thiram 37.5 % WS (T₁₂) (Vitavax Power) treated seeds were free of fungal infection at both D1 and D2, highlighting their strong antifungal activity. Fluxapyroxad 33.3 % FS (Systiva) (T₁₁) recorded *A. flavus*, *Mucor*, *Rhizopus* and *Penicillium* sp. at both D1 and D2. In contrast, the untreated control (T13) exhibited the highest fungal diversity, with *A. flavus*, *A. niger*, *Mucor* spp., *Rhizopus* spp. and *Penicillium* spp., correlating with the lowest germination and seedling vigor. Overall, T3, T5, T6 and T12 were the most effective, while T13 demonstrated the highest fungal incidence (Table 3).

In vitro evaluation of promising fungicides during 2022-2023

The *in vitro* evaluation of selected new molecule fungicides demonstrated significant enhancements in seed germination, seedling growth and overall seed health parameters compared to the untreated control (Table 4). Among the treatments, Pyraclostrobin 13.3 % + Epoxiconazole 5 % SE (T2) (Opera) exhibited the highest germination percentage (86.0 %), followed closely by Thiophanate methyl 45 % + Pyraclostrobin 5 % FS (T₃) (Xelora) (84.5 %). In contrast, the untreated control (T5) recorded the lowest germination percentage at 55.0 %. Seedling growth parameters also varied among treatments, with Pyraclostrobin 13.3 % + Epoxiconazole 5 % SE (T2) (Opera) showing the greatest root length (14.08 cm), while Thiophanate methyl 45 % + Pyraclostrobin 5 % FS (T3) (Xelora) exhibited a root length of 13.01 cm, both significantly higher than the control (10.23 cm).

Table 2. *In vitro* evaluation of new molecule fungicides on seed and seedling vigour of groundnut during 2021-2022

Treatments	Shoot length (cm)*		Root length* (cm)		Seedling dry* weight (g)		Vigour index I*		Vigour index II	
	D1 (X Dosage)	D2 (2X Dosage)	D1	D2	D1	D2	D1	D2	D1	D2
T1 Azoxystrobin 18.2 % + Difenconazole 11.4 % SC (Amistar Top)	21.17	21.60	16.40	20.11	3.47	3.80	3528.6	3867.2	324.3	354.4
T2 Azoxystrobin 11 % + Tebuconazole 18.3 % SC (Custodia)	10.48	12.17	15.89	20.72	3.93	3.48	2390.5	3060.6	357.7	324.1
T3 Thiophanate methyl 45 % + Pyraclostrobin 5 % FS (Xelora)	17.79	14.20	16.07	16.31	3.54	3.64	3159.4	3005.5	331.4	357.9
T4 Fluxapyroxad 2.5 % + Pyraclostrobin 2.5 % SC (Merivon)	21.60	22.12	19.23	20.72	3.55	3.72	3743.1	4088.6	325.5	354.9
T5 Pyraclostrobin 13.3 % + Epoxyconazole 5 % FS (Opera)	20.89	21.59	18.87	20.99	3.46	3.72	3842.0	4128.6	333.2	359.2
T6 Penflufen 13.28 % + Trifloxystrobin 13.2 % FS (Ever Golxtend)	20.43	20.89	17.93	20.71	3.49	3.60	3563.4	3675.2	322.3	316.9
T7 Trifloxystrobin 25 % + Tebuconazole 50 % WG (Nativo)	8.63	10.30	16.10	19.58	3.79	3.81	2320.6	2737.8	356.7	349.2
T8 Difenconazole 25 % EC (Score)	19.28	20.53	15.42	17.27	3.09	3.82	2828.2	2704.3	252.5	271.6
T9 Pyraclostrobin 10 % CS (Chowkidar)	20.32	21.56	16.96	19.88	3.67	3.64	3232.5	3149.6	318.1	276.2
T10 Tebuconazole 2 % DS (Raxil)	15.33	17.52	17.71	20.27	3.29	3.37	2811.5	3559.3	280.0	314.2
T11 Fluxapyroxad 33.3 % FS (Systiva)	20.01	19.99	16.92	18.16	3.57	3.47	1167.6	3379.4	331.5	303.8
T12 Carboxin 37.5 % WS + Thiram 37.5 % WS (Vitavax Power)	20.63	22.17	17.73	20.84	3.65	3.88	3568.6	3872.0	339.1	350.0
T13 Untreated Control	15.76	15.76	11.08	11.08	3.01	3.01	3430.1	3430.1	130.7	130.7
Mean	17.9	18.5	16.6	19.0	3.50	3.61	3045.1	3261.2	307.9	312.5
SEd	0.82	0.60	1.38	1.51	0.20	0.05	255	261	26.31	28.07
CD (0.05)	1.66	1.21	2.79	3.05	0.41	0.09	516	529	53.22	56.78

*Mean of four replications.

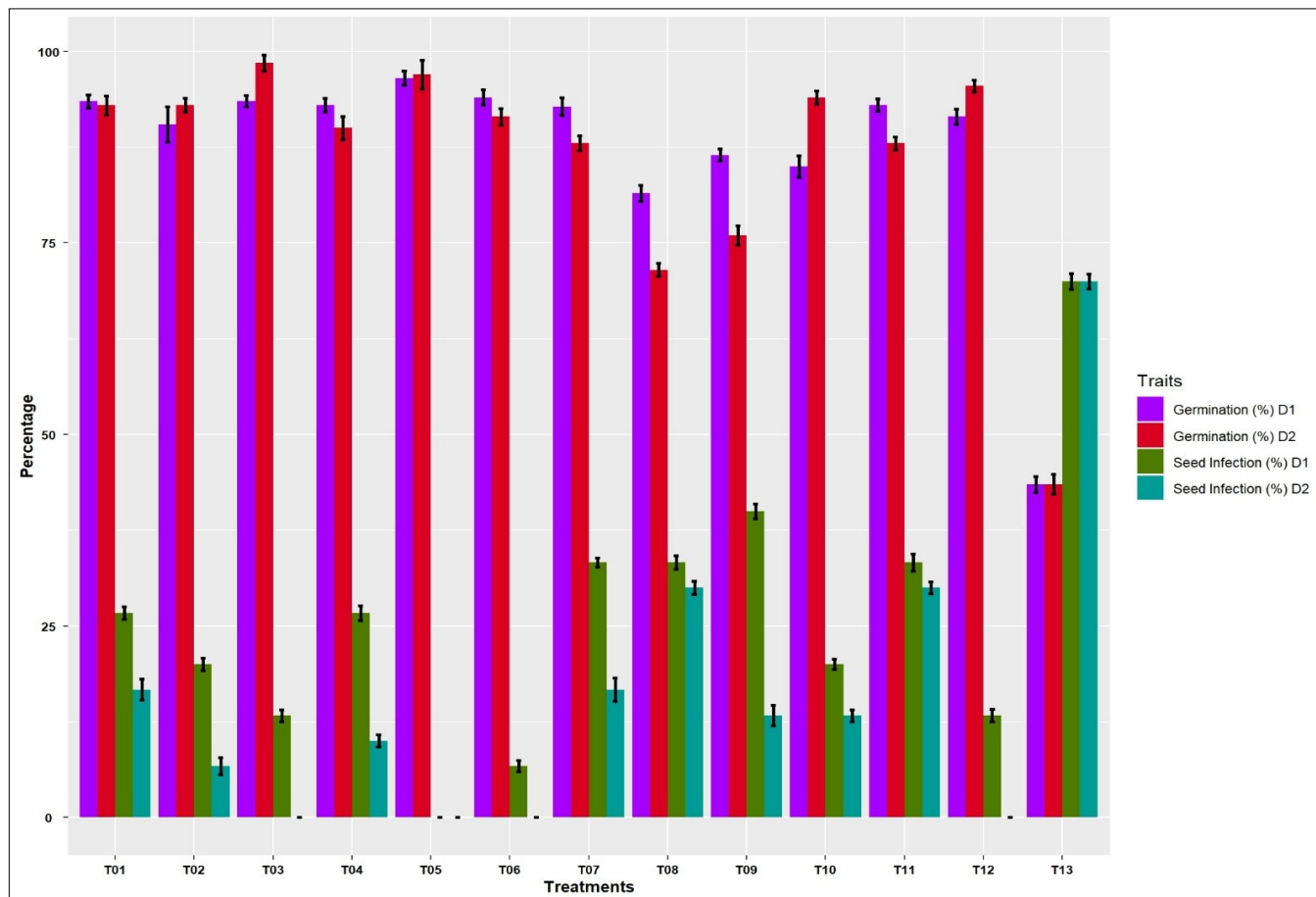


Fig. 1. *In vitro* evaluation of new molecule fungicides on germination and seed infection in groundnut.

T1-Azoxystrobin 18.2 % + Difenconazole 11.4 % SC, **T2**- Azoxystrobin 11 % + Tebuconazole 18.3 % SC, **T3**- Thiophenate methyl 45 % + Pyraclostrobin 5 % FS, **T4**- Fluxapyroxad 2.5 % + Pyraclostrobin 2.5 % SC, **T5**- Pyraclostrobin 13.3 % + Epoxyconazole 5 % FS, **T6**- Penflufen 13.28 % + Trifloxystrobin 13.2 % FS, **T7**- Trifloxystrobin 25 % + Tebuconazole 50 % WG, **T8**- Difenconazole 25 % EC, **T9**- Pyraclostrobin 10 % CS, **T10**-Tebuconazole 2 % DS, **T11**-Fluxapyroxad 33.3 % FS, **T12**-Carboxin 37.5 % WS + Thiram 37.5 % WS, **T13**-Control

Table 3. Effect of seed dressing fungicides on distribution of mycoflora in Groundnut during 2021-2022

Treatments	Dosages (%)		Dosages (%)	
	X	X	X	D2
T1 Azoxystrobin 18.2 % + Difenconazole 11.4 % SC (Amistar Top)	0.5	0.5	<i>A. flavus, A. niger and Rhizopus</i>	<i>Rhizopus and Mucor</i>
T2 Azoxystrobin 11 % + Tebuconazole 18.3 % SC (Custodia)	1.0	1.0	<i>A.niger and Rhizopus</i>	<i>Rhizopus</i>
T3 Thiophenate methyl 45 % + Pyraclostrobin 5 % FS (Xelora)	1.0	1.0	Nil	Nil
T4 Fluxapyroxad 2.5 % + Pyraclostrobin 2.5 % SC (Merivon)	0.5	0.5	<i>A. niger and Rhizopus</i>	Nil
T5 Pyraclostrobin 13.3 % + Epoxyconazole 5 % FS (Opera)	0.75	0.75	Nil	Nil
T6 Penflufen 13.28 % + Trifloxystrobin 13.2 % FS (Ever Golxtend)	0.5	0.5	Nil	Nil
T7 Trifloxystrobin 25 % + Tebuconazole 50 % WG (Nativo)	0.25	0.25	<i>A. niger, Mucor and Rhizopus</i>	<i>Rhizopus</i>
T8 Difenconazole 25 % EC (Score)	1.0	1.0	<i>A. niger, Mucor and Rhizopus</i>	<i>Rhizopus and Mucor</i>
T9 Pyraclostrobin 10 % CS (Chowkidar)	0.5	0.5	<i>A. niger, Mucor, Rhizopus and Penicillium</i>	<i>Rhizopus and Mucor</i>
T10 Tebuconazole 2 % DS (Raxil)	0.5	0.5	<i>A. flavus and Rhizopus</i>	Nil
T11 Fluxapyroxad 33.3 % FS (Systiva)	0.75	0.75	<i>A. flavus, Mucor, Rhizopus and Penicillium</i>	<i>A. flavus, Mucor, Rhizopus and Penicillium</i>
T12 Carboxin 37.5WS+Thiram37.5 %WS (Vitavax Power)	3.0	3.0	Nil	Nil
T13 Untreated Control	-	-	<i>A. niger, A. flavus, Mucor, Rhizopus and Penicillium</i>	<i>A. niger, A. flavus, Mucor, Rhizopus and Penicillium</i>

Table 4. *In vitro* evaluation of selected new molecule fungicides on seed germination, seedling vigour, seed infection and seedling associated pathogens of groundnut during 2022-2023

Treatments	Dosage (ml or g / kg of seed)	Germination (%) [*]	Shoot length (cm) [*]	Root length (cm) [*]	Seedling dry weight (g) [*]	Vigour index I [*]	Vigour index II [*]	Seed infection (%) [*]	Reduction in Seed infection over control (ROC) [*]	Types of fungi observed [*]
T1 Penflufen 13.2 % + Trifloxystrobin 13.2 % FS (Ever Golxtend)	1 ml	75.8 (60.53)	6.56	11.97	0.160	1402	12.14	0.00 (0.00)	100.0	-
T2 Pyraclostrobin 13.3 % + Epoxyconazole 5 % SE (Opera)	0.75 ml	86.0 (68.03)	7.26	14.08	0.177	1832	15.18	0.00 (0.00)	100.0	-
T3 Thiophanate methyl 45 % + Pyraclostrobin 5 % FS (Xelora)	1 ml	84.5 (66.82)	6.94	13.01	0.173	1684	14.59	0.00 (0.00)	100.0	-
T4 Carboxin 37.5 % WS + Thiram 37.5 % WS (Vitavax Power)	3 g	83.5 (66.03)	6.94	12.60	0.167	1631	13.94	3.33 (10.51)	96.0	<i>Rhizopus</i>
T5 Control		55.0 (47.87)	5.20	10.23	0.154	839	8.48	93.33 (75.3)	-	<i>A. niger</i> , <i>A. flavus</i> , <i>Mucor</i> , <i>Rhizopus</i> and <i>Penicillium</i>
Mean		77.0	6.58	12.38	0.166	1478	12.87	19.33		
SEd		1.08	0.11	0.16	0.005	20.07	0.18	1.36		
CD (0.05)		2.31	0.23	0.34	0.010	42.78	0.37	3.02		

* Mean of four replications. Values in parenthesis are arcsine transformed values

The assessment of seedling vigor indices revealed that Pyraclostrobin 13.3 % + Epoxyconazole 5 % SE (Opera) recorded the highest vigor index I (1832), followed by Thiophanate methyl 45 % + Pyraclostrobin 5 % FS (Xelora) (1684). Similarly, vigor index II was observed to be the highest in Pyraclostrobin 13.3 % + Epoxyconazole 5 % SE (Opera) (15.18), indicating its superior effect on seedling vigor. Furthermore, seed infection was completely inhibited (0.00 %) in seeds treated with Penflufen 13.2 % + Trifloxystrobin 13.2 % FS (Ever Golxtend), Pyraclostrobin 13.3 % + Epoxyconazole 5 % SE (Opera) and Thiophanate methyl 45 % + Pyraclostrobin 5 % FS (Xelora). The control treatment, however, exhibited a high seed infection percentage (93.33 %) with the presence of *A. flavus*, *A. niger*, *Mucor* sp., *Rhizopus* sp. and *Penicillium* sp. Meanwhile, seeds treated with Carboxin 37.5 % WS + Thiram 37.5 % WS (Vitavax Power) showed 3.33 % seed infection with the occurrence of *Rhizopus*, though it significantly reduced seed infection compared to the untreated control. These findings highlight the potential of these fungicide formulations in improving seed health and seedling vigor while effectively controlling seed-associated fungal infections.

The fungal infection frequency analysis revealed that seeds treated with Penflufen 13.2 % + Trifloxystrobin 13.2 % FS (Ever Golxtend), Pyraclostrobin 13.3 % + Epoxyconazole 5 % SE (Opera) and Thiophanate methyl 45 % + Pyraclostrobin 5 % FS (Xelora) exhibited complete suppression of *A. flavus*, *A. niger*, *Mucor* spp., *Rhizopus* spp., and *Penicillium* spp. with cent per cent free of infection. In contrast, the untreated control showed a high incidence of fungal pathogens, with 2.75 % *A. flavus*, 7.50 % *A. niger*, 2.50 % *Penicillium* spp., 1.75 % *Mucor* spp. and 2.00 % *Rhizopus* spp. Notably, Carboxin 37.5 % WS + Thiram 37.5 % WS (Vitavax Power) effectively suppressed all pathogens except *Rhizopus* spp. (0.25 %), indicating its relatively lower efficacy in complete pathogen inhibition compared to other treatments (Table 5).

***In vivo* evaluation of promising fungicides during 2023-24**

Among the tested fungicides, Pyraclostrobin 13.3 % + Epoxyconazole 5 % FS (Opera) (T2) recorded the highest germination percentage (86.0 %) and the lowest seedling mortality (26.7 %), followed by Thiophanate methyl 45 % + Pyraclostrobin 5 % FS (Xelora) (T3) with 82.3 % germination and 33.3 % mortality. Both treatments also resulted in reduced disease incidence of 13.92 % and 15.23 % respectively and superior shoot and root length, contributing to higher vigor indices and dry matter production. Penflufen 13.2 % + Trifloxystrobin 13.2 % FS (Ever Golxtend) (T₁) also showed considerable disease control with 18.09 % incidence, with improved seedling vigor and biomass. On the other hand, the untreated control (T5) and pathogen-treated seeds (T6) exhibited the lowest germination rates (71.0 % and 64.7 % respectively), with significantly higher disease incidence (18.32 % and 19.03 %) and seedling mortality (60.0 % and 61.8 %). These results highlight the role of fungicidal seed treatments in enhancing seedling establishment and reducing pathogen-induced mortality (Table 6 & Fig. 2).

In field emergence studies, Pyraclostrobin 13.3 % + Epoxyconazole 5 % FS (Opera) (T2) again outperformed other treatments, with the highest field emergence (83.0 %) and lowest disease incidence (5.8 %), resulting in improved shoot (32.40 cm) and root length (11.61 cm). Similarly, Thiophanate methyl 45 % + Pyraclostrobin 5 % FS (Xelora) (T3) showed notable improvement in emergence (77.5 %) and disease suppression (9.2 %). Penflufen 13.2 % + Trifloxystrobin 13.2 % FS (Ever Golxtend) (T1) provided moderate disease control (15.1 %) and improved plant vigor.

Table 5. Effect of seed dressing fungicides on distribution of mycoflora in Groundnut during 2022-2023

Treatment	Dosage (ml or g / kg of seed)	Frequency of distribution (%)*					Mean
		<i>Aspergillus flavus</i>	<i>Aspergillus niger</i>	<i>Penicillium spp.</i>	<i>Mucor spp.</i>	<i>Rhizopus spp.</i>	
T1 Penflufen 13.2 % + Trifloxystrobin13.2 % FS (Ever Golxtend)	1 ml	0.00	0.00	0.00	0.00	0.00	0.00
T2 Pyraclostrobin13.3 % + Epoxyconazole 5 % SE (Opera)	0.75 ml	0.00	0.00	0.00	0.00	0.00	0.00
T3 Thiophanate methyl 45 % + Pyraclostrobin 5 % FS (Xelora)	1 ml	0.00	0.00	0.00	0.00	0.00	0.00
T4 Carboxin 37.5 % WS + Thiram 37.5 % WS (Vitavax Power)	3 g	0.00	0.00	0.00	0.00	0.25	0.05
T5 Control		2.75	7.50	2.50	1.75	2.00	3.30
Mean		0.55	1.50	0.50	0.35	0.45	0.67

*Mean of four replications.

Table 6. Effect of seed dressing fungicides on seed and seedling associated pathogens of Groundnut cv. G7 in Pot culture and field emergence during 2023-2024

Treatment/ Fungicide	Dosage (ml or g / kg of seed)	Pot Culture*					Field emergence*				
		Shoot length (cm)	Root length (cm)	Vigour index I	Vigour index II	Dry matter production (g/plant)	Shoot length (cm)	Root length (cm)	Vigour index I	Vigour index II	Dry matter production (g/plant)
T1 Penflufen 13.2 % + Trifloxystrobin 13.2 % FS (Ever Golxtend)	1ml	18.09	12.79	2399	4.68	4.68	31.82	10.68	3222	481	6.35
T2 Pyraclostrobin 13.3 % + Epoxyconazole 5 % SE (Opera)	0.75ml	19.03	13.32	2783	5.30	5.30	32.40	11.61	3653	610	7.35
T3 Thiophanate methyl 45 % + Pyraclostrobin 5 % FS (Xelora)	1ml	18.32	11.19	2429	4.94	4.94	29.21	10.74	3096	560	7.22
T4 Carboxin 37.5 % WS + Thiram 37.5 % WS (Vitavax Power)	3g	16.81	11.42	2032	5.02	5.02	30.52	10.92	2802	396	5.86
T5 Untreated seed (Control)		15.23	10.62	1835	4.35	4.35	28.88	9.68	2445	371	5.85
T6 Pathogen treated seeds		13.92	9.87	1539	4.38	4.38	27.35	9.42	2195	313	5.25
Mean		16.90	11.50	2178	4.80	4.80	30.0	10.5	2902	455	6.32
SEd		0.37	0.17	42.66	0.07	0.07	0.09	0.60	52.88	8.05	0.05
CD at 5 %		0.82	0.37	90.93	0.15	0.15	0.21	1.34	112.7	17.16	0.12

*Mean of four replications.

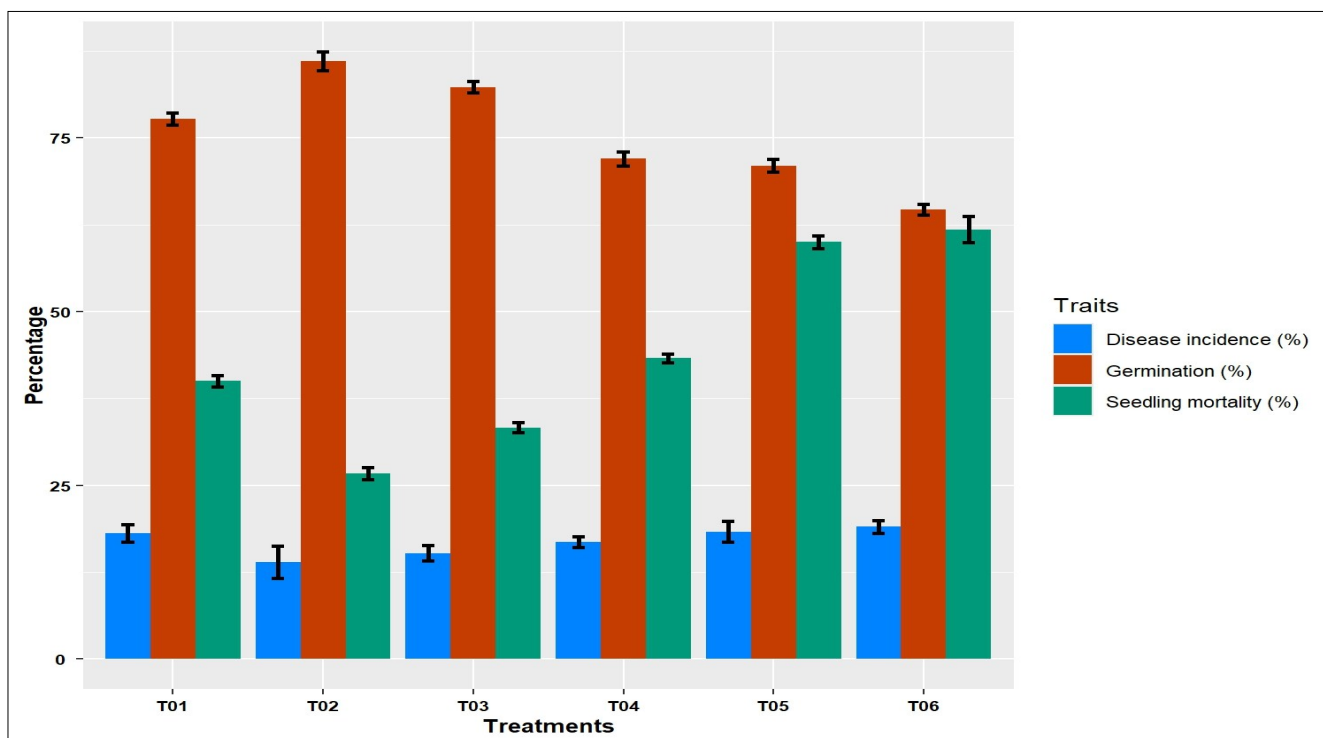


Fig. 2. Effect of seed dressing fungicides on seed germination, disease incidence and seedling mortality of Groundnut cv. G7 in Pot culture during 2023-2024.

Conversely, the untreated control (T5) and pathogen-inoculated seeds without fungicide treatment (T6) exhibited the poorest performance, with significantly lower emergence rates of 63.4 % and 59.7 % respectively. These treatments also recorded the highest disease incidence, at 21.6 % and 24.5 %, along with noticeably poor plant growth parameters, indicating severe impairment in seedling health and establishment.

In contrast, the fungicidal treatments Pyraclostrobin 13.3 % + Epoxyconazole 5 % FS (Opera) (T2) and Thiophanate methyl 45 % + Pyraclostrobin 5 % FS (Xelora) (T3) proved highly effective under both pot culture and field emergence conditions. These treatments consistently enhanced seedling vigor, significantly reduced disease incidence and supported robust plant growth and establishment. The results clearly demonstrate the superior performance of these formulations in managing seed-borne pathogens and improving groundnut crop health (Table 6 & Fig. 3).

Discussion

Fungicides play a crucial role in groundnut seed treatment by preventing the development of seed-borne pathogens, which can significantly reduce seed germination and crop yield (29). The application of fungicides helps in controlling fungal diseases caused by *Aspergillus* sp., *Mucor* sp., *Rhizopus* sp. and *Penicillium* sp., which are commonly associated with groundnut seeds (30, 31). By treating seeds with fungicides, the risk of disease transmission to seedlings is minimized, promoting healthy plant

growth and enhancing crop productivity (32, 33). This study aimed to assess the efficacy of novel systemic fungicides in managing seed-borne diseases in groundnut. The results revealed promising insights into the effectiveness of the selected fungicides in improving seed germination, seedling growth and mitigating seed infection under both *in vitro* and *in vivo* conditions. Pyraclostrobin 13.3 % + Epoxyconazole 5 % FS (Opera) emerged as the most effective fungicide in terms of seed germination and seedling vigor. This combination not only showed the highest germination rates (96.5 % at D1 and 97.0 % at D2) but also exhibited superior seedling vigor indices (vigor index I: 3842.0 at D1 and 4128.6 at D2). These results align with previous studies highlighting the efficacy of strobilurin-based fungicides in promoting plant growth and improving resistance to fungal infections (34-36). Furthermore, this treatment demonstrated complete inhibition of seed infection, which is crucial for ensuring healthy crop establishment, particularly under conditions prone to fungal contamination.

In addition to Pyraclostrobin 13.3 % + Epoxyconazole 5 % FS (Opera) (T2), Thiophanate methyl 45 % + Pyraclostrobin 5 % FS (Xelora) (T3) also showed significant improvements in seed germination and seedling growth. The enhancement in root length and seedling vigor, along with the reduction in seed infection, underscores the fungicide's potential in managing seed-borne diseases. For instance, earlier research demonstrated that treating seeds with carbendazim 12 % + mancozeb 63 % at 3.0 g/kg resulted in a remarkable 95.17 % germination rate, reduced disease incidence and the highest pod yield of 27.23 q/ha (37). A similar

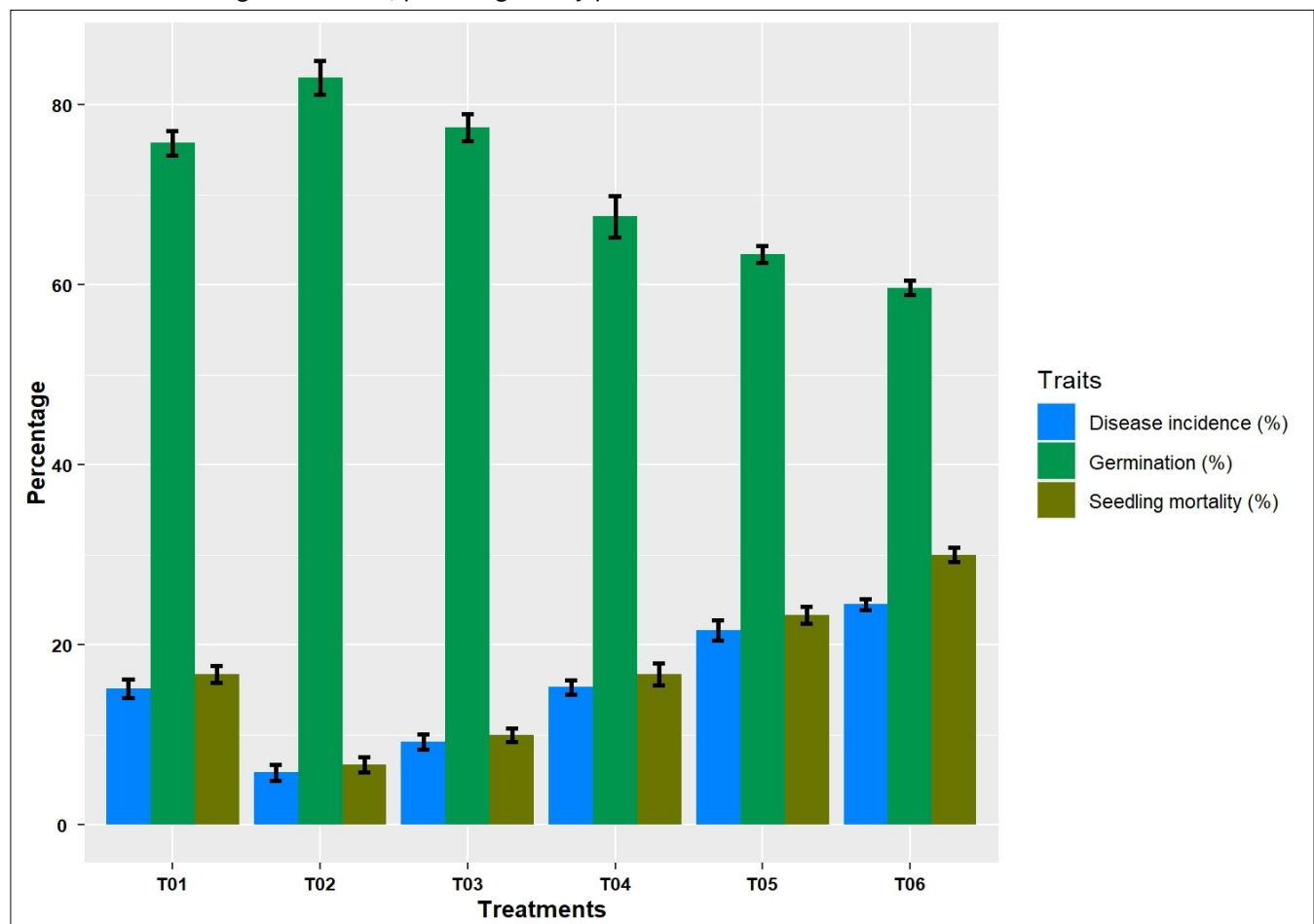


Fig. 3. Effect of seed dressing fungicides on seed germination, disease incidence and seedling mortality of Groundnut cv. G7 in field emergence during 2023-2024.

T1- Penflufen 13.2 % + Trifloxystrobin 13.2 % FS (Ever Golxtend), **T2-** Pyraclostrobin 13.3 % + Epoxyconazole 5 % SE (Opera), **T3-** Thiophanate methyl 45 % + Pyraclostrobin 5 % FS (Xelora), **T4-** Carboxin 37.5 % WS + Thiram 37.5 % WS (Vitavax Power), **T5-** Control.

outcome was observed at 2.5 g/kg, further confirming the potential of this combination to significantly improve groundnut productivity.

Notably, the *in vivo* evaluations conducted in pot culture and field conditions further validated the efficacy of the selected treatments. Groundnut seeds treated with Pyraclostrobin 13.3 % + Epoxyconazole 5 % FS (Opera) (T2) demonstrated excellent field emergence (83 %) and superior seedling growth, with minimal seedling mortality (6.7 %) and a lower incidence of collar rot (5.8 %). These findings align with earlier studies which reported that soil application of fungal bioagents combined with seed treatment fungicides significantly reduced collar rot incidence (8.15 %) in groundnut (19). Similarly, another earlier study clearly depicted the effectiveness of Pyraclostrobin seed treatment in soybean enhancing seedling emergence (95.29 %) and maximum pod yield (21.33 q/ha), emphasizing its potential as a key component in integrated disease management (38). In continuation with our results, previous reports highlighted the efficacy of strobilurin-based fungicides, particularly Tebuconazole 50 % + Trifloxystrobin 25 % 75WG, in effectively managing stem rot of groundnut (39). Their study demonstrated a significant reduction in disease incidence (3.5 %-4.4 %) and an increase in pod yield (2804-2746 kg/ha), emphasizing the potential of strobilurins in disease suppression.

The application of Penflufen 13.2 % + Trifloxystrobin 13.2 % FS (Ever Golxtend) was also effective in promoting seedling growth, with similar improvements in seed germination and vigor indices. However, its performance was slightly less pronounced compared to Pyraclostrobin 13.3 % + Epoxyconazole 5 % FS (Opera). This suggests that while Penflufen-based formulations can be beneficial for seed protection, further optimization of treatment concentrations and formulations may enhance their efficacy.

Overall, the results of this study support the use of new molecule fungicides as an effective means of managing seed-borne diseases in groundnut. The promising performance of Pyraclostrobin 13.3 % + Epoxyconazole 5 % FS (Opera) in both *in vitro* and *in vivo* conditions highlights its potential as a viable option for groundnut seed treatment. The reduction in seedling mortality, coupled with improved seedling vigor and growth, demonstrates the practical benefits of these fungicides in safeguarding crop health and enhancing productivity. Moreover, the continued evaluation of other fungicides like Pyraclostrobin 13.3 % + Epoxyconazole 5 % FS (Opera), Thiophanate methyl 45 % + Pyraclostrobin 5 % FS (Xelora) and Penflufen 13.2 % + Trifloxystrobin 13.2 % FS (Ever Golxtend) will further contribute to the development of integrated disease management strategies that can help to mitigate the impact of seed-borne fungal pathogens on groundnut production. These findings are in agreement with previous studies, where the combination of Pyraclostrobin and Epoxiconazole demonstrated over 85 % efficacy in controlling fungal pathogens under field conditions (40). The foliar application of Opera (Pyraclostrobin + Epoxiconazole) not only conferred protection against pathogens but also significantly improved physiological responses under abiotic stress. In soybean, Opera application (0.15 %) under water stress conditions enhanced leaf area by 42 %, photosynthesis rate (194 %) and nitrate reductase activity (68 %) at the flowering stage, while biomass accumulation and yield improved by 92 % and 119 % respectively at the pod filling stage (41). These results underscore the dual role of this fungicide in mitigating biotic as well as abiotic stress.

Conclusion

In conclusion, inclusion of these novel fungicides, Pyraclostrobin 13.3 % + Epoxyconazole 5 % FS (Opera), Thiophanate methyl 45 % + Pyraclostrobin 5 % FS (Xelora) and Penflufen 13.2 % + Trifloxystrobin 13.2 % FS (Ever Golxtend), into seed treatment protocols offers a promising avenue for enhancing seedling health and reducing loss due to seed-borne diseases like collar rot. The results from this study underscore the need for continuous research to identify cost-effective, environmentally sustainable fungicide formulations that can provide long-term protection for groundnut crops while minimizing the adverse impact of chemical treatments on the environment and human health.

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Authors' contributions

CJ contributed to writing, review and editing, supervision, funding acquisition and conceptualization. TR contributed to supervision, funding acquisition and conceptualization. DSP contributed to writing, review and editing and data curation. AK¹ contributed to formal analysis and data curation. AK² contributed to supervision and conceptualization. All authors read and approved the manuscript [AK¹ stands for A Kamatchi and AK² stands for Atul Kumar].

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