The Pharma Innovation Journal 2021; 10(12): 2150-2154

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2021; 10(12): 2150-2154

www.thepharmajournal.com
Received: 01-09-2021
Accepted: 08-10-2021

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In vitro efficacy of Fungicides against stem rot of groundnut (*Arachis hypogaea* L.) caused by *Sclerotium rolfsii* Sacc

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Abstract
In vitro experiment was carried out to test efficacy of nine different fungicides *viz*., three systemic, three non-systemic and three combi fungicides against *Sclerotium rolfsii* Sacc. The results of the experiment revealed that among the systemic fungicide *viz*., Tebuconazole (92.97% @ 500 ppm and 94.36% @ 1000 ppm), Non-systemic fungicide Mancozeb (94.00% and 94.44%), Combi-fungicide Carboxin 37.5% + Thiram 37.5% (94.35% and 94.44%) at 1500 ppm and 2000 ppm recorded significantly highest average mycelial inhibition of the test pathogen. least average mycelial inhibition recorded with systemic fungicide Azoxystrobin (8.18% @ 500 ppm and 39.19% @ 1000 ppm)Non-systemic fungicideZineb (6.00% and 37.40%) and Combi-fungicide Metalaxyl 8% + Mancozeb 64%(45.22% and 73.93%) at 1500 ppm and 2000 ppm.

Keywords: *Sclerotium rolfsii*, groundnut, *in vitro*, fungicides

Introduction
Groundnut (*Arachis hypogaea* L.) is most important food and cash crops commonly cultivated in tropical and subtropical regions. Groundnut is reported to have originated from South America and then its cultivation spread to other regions. There are two subspecies of cultivated groundnut, hypogaea and fastigiata, However, most of the commercially cultivated varieties belongs to the hypogaea, fastigiata (Valencia), and vulgaris (Spanish) species. Groundnut is the 13th most important food crop and 3rd most important oilseed crop of the world. Groundnut seed can be consumed raw, boiled or roasted or crushed for edible oil.Asia accounts for about 50% of the global area and 60% of production. India accounts for about 25% of global area and contributes 19% to world groundnut production.
In India, groundnut is grown in four seasons, namely, *Kharif* (85%), *Rabi* (10%), summer (4%) and spring (less than 1%). *Kharif* groundnut is sown from June to November mostly in the states of Gujarat, Andhra Pradesh, Tamil Nadu, Karnataka, Maharashtra, Rajasthan, Madhya Pradesh and Uttar Pradesh. *Rabi* groundnut is grown from November to April mostly in Central, Eastern and Southern parts of the country. Groundnut is prone to many diseases among them Collar rot / seedling blight (*Aspergillus niger*van Tieghem), stem rot / Sclerotium wilt (*Sclerotium rolfsii* Sacc.), dry wilt or dry root rot (*Macrophominaphaseolina* (Tassi) Goid., *Rhizoctonia bataticola* (Taub., Butler) are considered as economically important diseases.In India, stem rot occurs in all groundnut growing states, particularly more severe in Gujarat, Maharashtra, Madhya Pradesh, Odisha and Tamil Nadu. About 27% or more yield loss due to this disease has been reported from India (Chohan, 1974). Mayee and Datar (1988) have reported yield losses of over 25% in Maharashtra. The indirect losses such as reduction in dry weight and oil content are also reported.

Materials and Methods
The isolation was done in two ways
A. Direct isolation
A pointed needle duly sterilized was used and the fungus growth/sclerotia from infected stem was directly transferred in to plates containing PDA media under aseptic condition and plates were incubated at 26 ± 2 ºC for optimum growth.

B. Tissue isolation method
Repeated isolations were carried out aseptically from groundnut plant showing typical stem rot

symptoms. After washing thoroughly with tap water, the infected stem part was cut in to small bits, surface sterilized with 0.1% $HgCl_2$ (1g/lit.) followed by three subsequent washing with sterilized distilled water. The sterilized pieces then transferred aseptically under laminar airflow on sterilized Petri plates containing 20 ml Potato Dextrose Agar (PDA) medium. The Petri plates were incubated in Biological Oxygen Demand (B.O.D.) incubated at 26 ± 2 ºC temperature for optimum growth.

The fungal hyphae developing from the infected tissues were sub-cultured aseptically on PDA media containing in Petri plates. Thus, pure culture was obtained by hyphal tip method and microscopically examined for identification and it was further purified by using single sclerotial body. The culture was maintained on PDA for further investigations.

Poisoned food technique

Potato dextrose agar (PDA) was prepared and sterilized in an autoclave. Three systemic, three non-systemic and three combi-fungicides were used in the experiment. For 100 ml of sterilized and cooled PDA medium, systemic fungicides were added @ 0.005 ml and 0.01 ml for 500 ppm and 1000 ppm concentrations respectively, non-systemic and combi-fungicides were added @ 0.015 ml and 0.020 ml for making 1500 ppm and 2000 ppm concentrations respectively. Of each prepared concentrations 20 ml of PDA was poured into sterilized petri plates under aseptic conditions and allowed to solidify for 5–10 min. From seven days old culture plates, 5 mm discs were cut from outer margin of vigorously growing test pathogen were placed at the centre of plates containing poisoned medium and untreated plates. The petri plates then incubated in an incubator at 26 ± 2 ºC. The diameter of the colony was measured after seven days of incubation.

Percent inhibition of test pathogen was recorded as per Vincent (1947) formula.

$$R = \{(C - T) / C\} \times 100$$

Where

R = Per cent inhibition

C = Radial growth of pathogen colony in control

T = Radial growth of pathogen colony in treatment

Table 1: Details of Systemic, Non-Systemic and Combi fungicides used in experiment

Sr. No.	Name of chemicals	Active ingredients	Concentration Tested
Systemic fungicides			
1	Tebuconazole 250 EC	250 EC	500 ppm, 1000 ppm
2	Azoxystrobin 23 SC	23 SC	500 ppm, 1000 ppm
3	Thiophanate Methyl 70 WP	70 WP	500 ppm, 1000 ppm
Non-Systemic fungicides			
4	Zineb 75% WP	75 WP	1500 ppm, 2000 ppm
5	Mancozeb 75% WP	75 WP	1500 ppm, 2000 ppm
6	Captan 50% WP	50 WP	1500 ppm, 2000 ppm
Combi fungicides			
7	Carboxin 37.5% +Thiram 37.5% WP	75 WP	1500 ppm, 2000 ppm
8	Metalaxyl 8% +Mancozeb 64% WP	72 WP	1500 ppm, 2000 ppm
9	Carbendazim 12% + Mancozeb 63% WP	75 WP	1500 ppm, 2000 ppm
10	Control	-----	-----

Results and Discussion

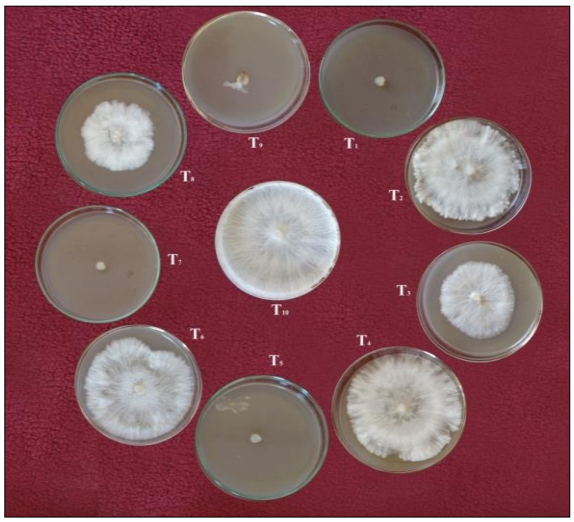
The results obtained from the present study as well as discussions have been summarized under following heads:

Systemic fungicides

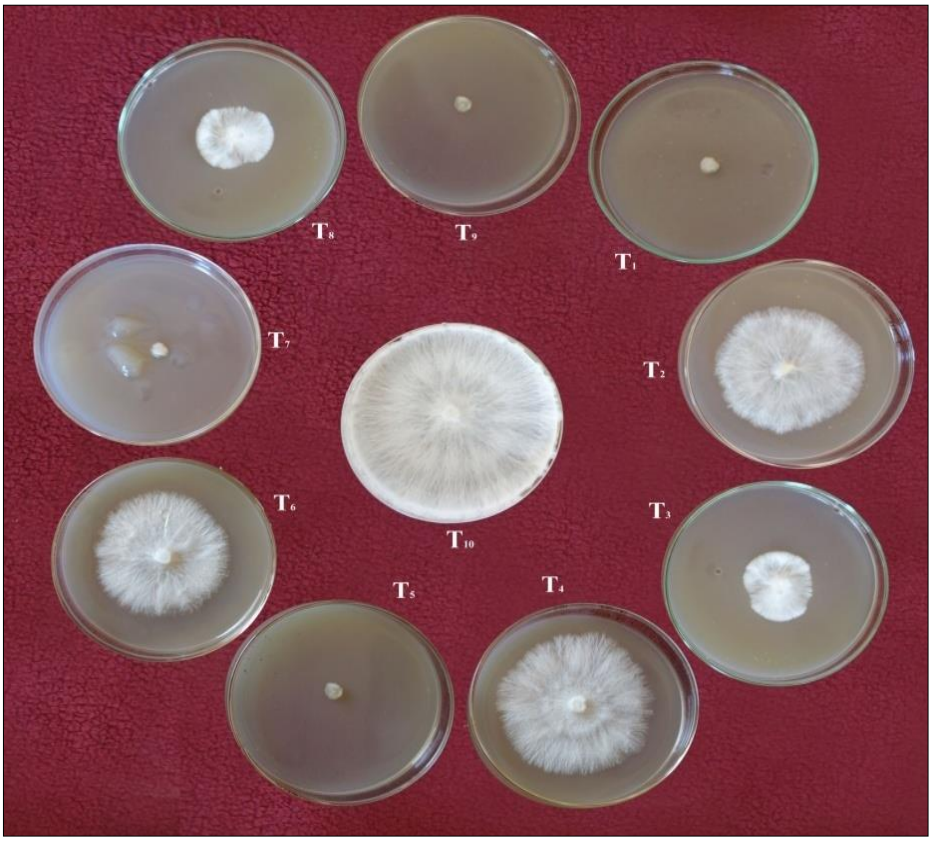
Results revealed that all the three systemic fungicides tested (@ 500 ppm and 1000 ppm each) significantly inhibitedmycelial growth of *Sclerotium rolfsii* Sacc. over untreated control. Further, the percent mycelial growth inhibition was increased with increase in concentrations of the fungicides tested.

The results of systemic fungicides at 500 ppm conc. revealed that percent mycelial growth inhibition of the test pathogen was ranged from 8.18% (Azoxystrobin) to 92.97% (Tebuconazole). However, Tebuconazole (92.97%) was significantly superior over Thiophanate methyl (42.22%) and Azoxystrobin (8.18%) which was found least effective.

At 1000 ppm conc., percent mycelial growth inhibition was increased compared to 500 ppm and it was ranged from 39.19% (Azoxystrobin) to 94.36% (Tebuconazole). However, significantly highest mycelial inhibition was recorded with the fungicide Tebuconazole (94.36%) which was signicantly superior over Thiophanate methyl (67.03%) and Azoxystrobin (39.19%) with least mycelia growth inhibition.



(A) Systemic (500 ppm), non-systemic (1500 ppm) and combi fungicides (1500 ppm)



(B) *S*ystemic (1000 ppm), non-systemic (2000 ppm) and combi fungicides (2000 ppm)

T_1	Tebuconazole 250 EC	T_6	Captan 50% WP
T_2	Azoxystrobin 23 SC	T_7	Carboxin 37.5% + Thiram 37.5%
T_3	Thiophanate Methyl 70 WP	T_8	Metalaxyl 8% + Mancozeb 64%
T_4	Zineb 75% WP	T_9	Carbendazim 12% + Mancozeb 63%
T_5	Mancozeb 75% WP	T_{10}	Control

Plate 1: *In vitro* efficacy of fungicides against mycelial growth and inhibition of *Sclerotium rolfsii* Sacc**.**

Table 2: *In vitro* efficacy of fungicides against mycelial growth and inhibition of *Sclerotium rolfsii* Sacc**.**

Tr. No.	Treatments	Col. dia. *(mm) at Conc.		% Inhibition*	
Systemic fungicides		**500 ppm**	**1000 ppm**	**500 ppm**	**1000 ppm**
T_1	Tebuconazole 250 EC	6.33	5.08	92.97 (74.62)	94.36 (76.26)
T_2	Azoxystrobin 23 SC	82.64	54.73	8.18 (16.62)	39.19 (38.75)
T_3	Thiophanate Methyl 70 WP	52.00	29.67	42.16 (40.52)	67.03 (54.96)
Non-Systemic fungicides		1500 ppm	2000 ppm	1500 ppm	2000 Ppm
T_4	Zineb 75% WP	84.60	56.34	6.00 (14.18)	37.40 (37.70)
T_5	Mancozeb 75% WP	5.40	5.00	94.00 (75.82)	94.44 (76.36)
T_6	Captan 50% WP	77.63	56.56	13.74 (21.76)	37.49 (37.76)
Combi fungicides		1500 ppm	2000 Ppm	1500 ppm	2000 ppm
T_7	Carboxin 37.5%+ Thiram 37.5%	5.20	5.00	94.35 (76.25)	94.44 (76.36)
T_8	Metalaxyl 8% + Mancozeb 64%	49.30	23.46	45.22 (42.26)	73.93 (59.30)
T_9	Carbendazim 12% + Mancozeb 63%	7.80	5.67	91.33 (72.88)	93.70 (75.46)
T_{10}	Control	90.00	90.00	00 (00.00)	00 (00.00)
S.E.±		0.32	0.42	0.36	0.52
C.D. (P=0.01)		0.95	1.24	1.06	1.55

*Mean of three replications, Col= Colony, Dia.= Diameter, Conc. = Concentration,
Figures in parenthesis are arc sine transformed values

Non systemic fungicides

Non systemic fungicides at 1500 ppm conc. were evaluated and results revealed that all the three non-systemic fungicides significantly inhibitedmycelial growth of *Sclerotium rolfsii* Sacc. over untreated control. Least percent mycelial growth inhibition of the test pathogen obtained was 6.00% (Zineb) and highest 94.00% (Mancozeb). However, significantly highest mycelial inhibition was recorded with the fungicide Mancozeb (94.00%) which was found significantly superior over Captan (13.74%) and Zineb (6.00%) with least percent mycelial growth inhibition.

The three Non systemic fungicides at 2000 ppm conc. were tested and results revealed that least perecent mycelial growth inhibition of the test pathogen obtained 37.40% with Zineb. however, significantly highest percent mycelial growth inhibition was recorded with the fungicide Mancozeb (94.44%) followed by Captan (37.49%).

Combi fungicides

The results of three Combi fungicides at 1500 ppm conc. srevealed that percent mycelial growth inhibition of the test pathogen ranged from 45.22% (Metalaxyl 8% + Mancozeb 64%) to 94.35% (Carboxin 37.5% + Thiram 37.5%). However, significantly highest mycelial inhibition was recorded with the fungicides Carboxin 37.5% + Thiram 37.5% (94.35%) which was found significantly superior over Carbendazim 12% + Mancozeb 63% (91.33%) and Metalaxyl 8% + Mancozeb 64% (45.22%) with least percent mycelial growth inhibition.

Combi fungicides at 2000 ppm conc were recorded percent mycelial growth inhibition of the test pathogen 73.93% (Metalaxyl 8% + Mancozeb 64%) to 94.44% (Carboxin 37.5% + Thiram 37.5%). However, significantly highest mycelial inhibition was recorded with the fungicides Carboxin 37.5% + Thiram 37.5% (94.44%) which was at par with Carbendazim 12% + Mancozeb 63% (93.70%) and significantly superior over Metalaxyl 8% + Mancozeb 64% (73.93%) with leastpercent mycelial growth inhibition.

Conclusion

All the tested fungicides found to inhibit mycelial growth of the test pathogen however the results revealed that among the systemic fungicide *viz*., Tebuconazole (92.97% @ 500 ppm and 94.36% @ 1000 ppm), Non-systemic fungicide Mancozeb (94.00% and 94.44%), Combi-fungicide Carboxin 37.5% + Thiram 37.5% (94.35% and 94.44%) at 1500 ppm and 2000 ppm recorded significantly highest average mycelial inhibition of the test pathogen. Whereas, least average mycelial inhibition recorded with systemic fungicide Azoxystrobin (8.18% @ 500 ppm and 39.19% @ 1000 ppm) Non-systemic fungicide Zineb (6.00% and 37.40%) and Combi-fungicide Metalaxyl 8% + Mancozeb 64% (45.22% and 73.93%) at 1500 ppm and 2000 ppm.

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