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Evaluation of non-systemic fungicides in *invitro* condition for conidial germination inhibition of *Leveillula taurica* pathogen in cluster bean

Mehul Sangani[1*], Hardik Movaliya[2], Pratik Davariya[3] and Vishruta Babariya[1]

[1]Department of Plant Pathology, JAU, Junagadh;
[2]Main Oilseeds Research Station, JAU, Junagadh;
[3]Department of Entomology, JAU, Junagadh;
**Corresponding author's email: mdsangani@jau.in*
ORCID: https://orcid.org/0000-0003-0007-3771

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ABSTRACT

Cluster bean [*Cyamopsis tetragonoloba* (L.) Taub.] ranks among the economically important legumes grown across India, and its productivity is frequently limited by powdery mildew incited by *Leveillula taurica*. Six non-systemic fungicides, each tested at three concentrations, were screened *in vitro* by the poisoned food technique to identify candidates worth carrying forward for field evaluation. Every fungicide suppressed conidial germination of *L. taurica* to some degree, but wettable sulphur was clearly the standout, cutting germination by 83.47 per cent at its highest concentration (0.25%) and by 80.00 per cent at 0.20%. Dinocap ranked second, mancozeb, thiram and chlorothalonil gave broadly comparable, intermediate suppression, and zineb was the weakest performer among the fungicides tested against *L. taurica*.

Keywords: *Leveillula taurica*, *in vitro*, powdery mildew and cluster bean

INTRODUCTION

Cluster bean [*Cyamopsis tetragonoloba* (L.) Taub.] is a hardy, drought-tolerant legume with a long history of cultivation on the Indian subcontinent, grown today mainly across Rajasthan, Gujarat, Haryana, Uttar Pradesh and Punjab, which together account for the bulk of the country's guar acreage.

Yield in this crop is held back by a mix of biotic and abiotic pressures, among which fungal diseases such as anthracnose, angular leaf spot, root rot, rust and charcoal rot are particularly damaging, with nematodes, viral agents and phytoplasma adding to the burden. Powdery mildew caused by *Leveillula taurica* stands out among these for the regularity of its occurrence, favoured by the relatively cool, dry weather of December and January and it has repeatedly been recorded at moderate to severe levels in cluster bean fields around Junagadh district of Gujarat. Once the disease sets in, growers in the region typically resort to repeated fungicide sprays from about a month after sowing through to crop maturity, making chemical control the de facto first line of defence. Given how central fungicidal management has become for this disease, the present study was undertaken to screen a set of non-systemic fungicides for their inhibitory action against *L. taurica*.

MATERIAL AND METHODS

The inhibitory action of each agrochemical on spore germination of *L. taurica* was assessed *in vitro* using the poisoned food technique on Potato Dextrose Agar (PDA) following the approach of Bagchi and Das (1968). This experiment was conducted as a laboratory trial. For each treatment, the required quantity of the agrochemical was pipetted into autoclaved PDA before it set, and the medium was then dispensed in 5 ml aliquots into sterile Petri dishes to give a thin& even layer. Conidia lifted from young *L. taurica* colonies with a fine, sterile camel-hair brush were dusted aseptically onto the solidified medium and the inoculated plates were held at 23 ± 1°C for 48 hours with germination scored under a compound microscope at 12-hour intervals. Plates poured with plain PDAwithout any agrochemical served as the untreated control. A conidium was scored as germinated once its germ tube exceeded the spore's own width.

Percent inhibition of spore germination in each treatment was calculated using following formula (Bliss, 1934).

$$I = \frac{C - T}{C} \times 100$$

Where,

I = Percent inhibition
C = Number of germinated spore in control
T = Number of germinated spore in treatment
The six non-systemic fungicides selected for screening, along with their trade names, are listed in Table 1, and the three concentrations at which each was tested are set out in Table 2.

Table 1 : Non-systemic fungicides included in the *in vitro* screening

Common name	Chemical name	Trade name
Mancozeb	Manganese ethylene bis dithiocarbamate	Dithane M-45 75 % WP
Chlorothalonil	Tetrachloro isophthalonitrile	Kavach 75 % WP
Dinocap	A mixture of 2, 4 – di-nitro-6-octylphenyl crotonates & 2, 6-dinitro-4-octaylphenyl crotonates	Karathane48 % EC
Zineb	Zinc ethylenebisdithiocarbamate	Zineb 75 % WP
Thirum	Dimethylcarbamothioylsulfanyl N,N-dimethylcarbamodithioate	Thiram 75% WS
Wettable Sulphur	Sulphur	Sulfex 80% WP

Table 2: Concentrations of each fungicide evaluated against *L. taurica in vitro*

Sr. No.	Technical/activeIngredient	Concentration in ppm		
		1	2	3
1.	Mancozeb 75% WP	1500	2000	2500
2.	Thiram 75% WS	1500	2000	2500
3.	Chlorothalonil 75% WP	1500	2000	2500
4.	Zineb 75 % WP	1500	2000	2500
5.	Wett. sulphur 80% WP	1500	2000	2500
6.	Dinocap 48% EC	200	250	300
7.	Control	-	-	-



Advances of powdery mildew symptoms on cluster bean leaves

RESULTS AND DISCUSSION

Table 3 and Fig. 1 summarise the extent to which each fungicide suppressed spore germination of *L. taurica*. Every treatment reduced germination relative to the control, but wettable sulphur at 0.25 per cent was clearly the most inhibitory, cutting germination by 83.47 per cent, with its 0.20 per cent concentration close behind at 80.00 per cent.

Table 3 : Effect of non systemic fungicides on spore germination inhibition of *L. taurica in vitro*

Sr. No.	Fungicide	Concentration (%)	Per cent spore germination	Per cent inhibition over control	Mean per cent inhibition
1	Mancozeb 75% WP	0.15	20.04	59.98(50.74)*	
		0.20	16.70	66.67 (54.74)	65.56
		0.25	15.03	70.05 (56.83)	
2	Chlorothalonil 75% WP	0.15	23.38	53.39 (46.94)	
		0.20	20.01	60.07 (50.81)	57.85
		0.25	19.98	60.12 (50.84)	
3	Dinocap 48% EC	0.020	16.73	66.61 (54.70)	
		0.025	13.36	73.38 (58.94)	71.07
		0.030	13.39	73.34 (58.91)	
4	Wettable sulphur 80% WP	0.15	13.41	73.28 (58.88)	
		0.20	10.02	80.00 (63.44)	78.91
		0.25	8.35	83.47 (66.01)	
5	Thiram 75% WP	0.15	23.40	53.34 (46.92)	
		0.20	19.99	60.05 (50.80)	60.03
		0.25	16.67	66.71 (54.77)	
6	Zineb 75% WP	0.15	30.06	40.00 (39.23)	
		0.20	26.72	46.67 (43.09)	45.52
		0.25	25.05	49.90 (44.95)	
7	Control	-	50.00	-	
		Fungicide (F)	Concentration (C)	F x C	
	S. Em. ±	0.48	0.31	0.84	
	CD at 5%	1.42	0.93	2.47	
	CV %		2.63		

*Figures in parentheses show angular transformed values.

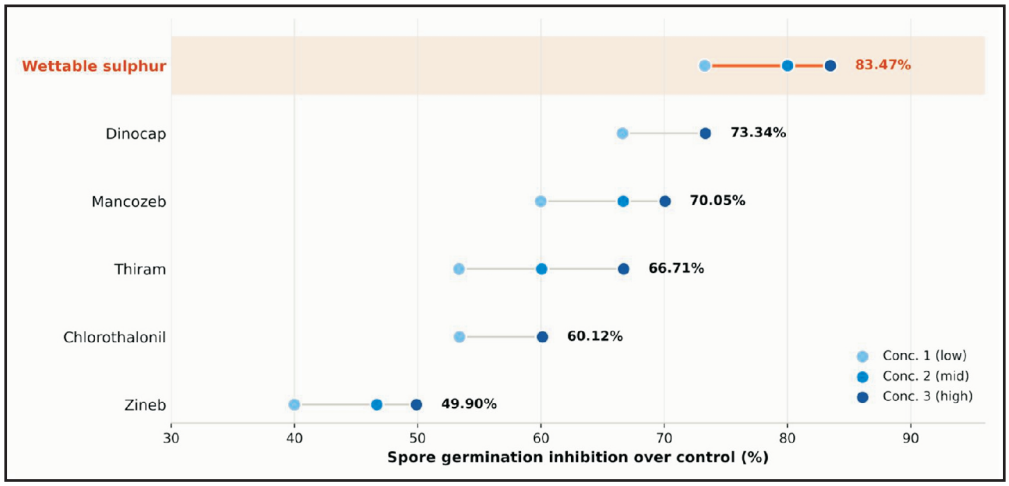


Figure 1:Per cent spore germination inhibition of *Leveillula taurica* by different non systemic fungicides *in vitro*

The mechanism by which elemental sulphur exerts its fungitoxic effect has been debated for decades. Early workers doubted that sulphur itself was the active agent. Sempio (1932) instead proposed that toxicity arose from sulphur derivatives generated during fungal metabolism and a subsequent hypothesis held that fungal spores reduce sulphur to hydrogen sulphide which was itself toxic. That view lost ground once colloidal sulphur was shown in 1953 to outperform hydrogen sulphide directly which shifted attention instead to oxidation products such as sulphur dioxide, sulphuric acid and thiosulphuric acid. The view that has since gained ground and the one adopted here is that elemental sulphur itself rather than any single derivative is the fungitoxic principle (Yang *et al.*, 2011).

The superior and consistent performance of wettable sulphur over the other fungicides evaluated can be attributed to its multi-site mode of action. Elemental sulphur is progressively oxidised/reduced at the spore surface to hydrogen sulphide and other reactive sulphur intermediates that interfere simultaneously with several respiratory enzymes of the fungal electron transport chain, uncoupling oxidative phosphorylation and depriving the germinating conidium of the ATP needed for germ-tube emergence (McCallan, 1949). Because this mode of action does not depend on a single and specific biochemical target unlike dinocap, mancozeb, thiram, chlorothalonil and zineb.Which explains the consistently higher and dosedependent inhibition (73.28 to 83.47 per cent across 0.15 to 0.25 per cent concentrations) recorded for wettable sulphur in the present study.

Dinocap at 0.025 and 0.030 per cent formed the next most effective group, giving 73.38 and 73.34 per cent inhibition respectively, values that were statistically at par with each other.

Mancozeb, thiram and chlorothalonil occupied an intermediate band of efficacy in the present trial while zineb consistently gave the weakest suppression of *L. taurica* spore germination among all six fungicides tested.

These results align with Sangani *et al.* (2018) who likewise found wettable sulphur to be the most inhibitory treatment against spore germination and germ-tube elongation of *L. taurica* on cluster bean in field condition. The effectiveness of wettable sulphur against the related pathogen *Erysiphe polygoni* has similarly been documented on fenugreek (Chovatiya, 2010), pea (Tripathi *et al.*, 2001), green gram (Kunkalikar, 1989) and black gram (Nawaz and Narayanasamy, 1983) pointing to a fairly consistent pattern of sulphur efficacy across powdery mildew pathogens of different legumes.

CONCLUSION

This *in vitro* screening of non-systemic fungicides against *L. taurica* identified wettable sulphur as the standout performer with a mean spore germination inhibition of 78.91 per cent across the three concentrations tested followed by dinocap at 71.07 per cent making these two fungicides the most promising candidates for against powdery mildew of cluster bean.

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