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Josiya Joy

M.Sc. (Ag.) Student, Department of Plant Pathology, College of Agriculture, Dapoli, Dr. B.S.K.K.V. Dapoli, Maharashtra, India

RR Rathod

Assistant Professor, Department of Plant Pathology, College of Agriculture, Dapoli, Dr. B.S.K.K.V. Dapoli, Maharashtra, India

AP Suryawanshi

Head, Department of Plant Pathology, College of Agriculture, Dapoli, Dr. B.S.K.K.V. Dapoli, Maharashtra, India

In vitro efficacy of various fungicides against *A. candida* causing mustard white rust disease

Josiya Joy, RR Rathod and AP Suryawanshi

Abstract

Among seven systemic fungicides evaluated *in vitro* against *A. candida*, Metalaxyl 35% SD was most effective at both 500 and 750 ppm, with significantly highest sporangial germination inhibition of 72.62% and 76.94%, followed by Hexaconazole 5% EC (70.60% and 74.20%, respectively) and Difenoconazole 25% EC (68.52% and 71.92%, respectively). However, Thiophanate methyl 75% WP and Fosetyl-Al 80% WP were the least effective. Among the contact and combi-fungicides evaluated *in vitro*, Metalaxyl 4% + Mancozeb 68% WP resulted with highest sporangial germination inhibitions of 84.84% and 86.79%, respectively at 2000 ppm and 2500 ppm. The contact fungicide, Chlorothalonil 75% WP resulted with highest sporangial germination inhibition of 81.61% and 85.10%, respectively at 2000 ppm and 2500 ppm. Rest of the contact and combi-fungicides showed considerable inhibition of sporangial germination, over untreated control.

Keywords: Triclosan, TCS, determination, detection, sensor

Introduction

Rapeseed-mustard is an inevitable component of India's traditional culinary system that can be used as a source of edible oil, raw material for industrial products and as a spice. It is the third major oil seed crop of the world, after soybean and oil palm (Abhishek *et al.*, 2017) [1]. During 2017-18, the estimated area, production and productivity of rapeseed-mustard in the world was 36.68 mha, 72.42 mt and 1974 kg/ha respectively, of which India accounts for 19.8% (4.85 mha) and 9.8% (8.32 mt) of the total acreage and production respectively. Among the various oil seeds cultivated in India, rapeseed and mustard own 23.13% and 24.98% of the total area and production respectively. In Maharashtra, rapeseed-mustard was cultivated in 4398600 ha area from which the yield obtained was 5369400 t, having a productivity of 1221 kg/ha during 2016-17 (Anonymous, 2018) [3].

Major constraints in mustard production are the pests and diseases that cause a high level deflation and challenge the existing oil seed economy of the country. Prevalence of diseases such as, leaf blight (*Alternaria brassicae* (Berk.) Sacc.), white rust (*Albugo candida* (Pers.) Kuntze), downy mildew (*Peronospora parasitica* (Pers.) ex. Fr.), powdery mildew (*Erysiphe cruciferarum*), bacterial blight (*Xanthomonas campestris* pv. *Campestris*), sclerotinia stem rot (*Sclerotinia sclerotiorum*), club root (*Plasmodiophora brassicae*) etc. constitute the principal cause of instability in production and reduced yields of rapeseed-mustard. White rust is the major and widely prevalent disease of rapeseed and mustard, in India. It is caused by an oomycotic fungi *Albugo candida*, which appears in an epiphytic form, inducing serious damage to the cruciferous crops (Kolte, 1985) [7].

Albugo spp. are obligate parasites that reproduce asexually by means of the sporangia/zoospores and sexually by thick walled oospores. The sporangia are colourless, nearly spherical to rectangular borne on short, 12-18 µm diameter, club-shaped stalks (Sporangiophores), each of which produces a chain of spores, in basipetal succession with distinct thickening between the sporangia. As sporangia are produced they become tightly packed and eventually rupture the host epidermis. After release, the sporangia are disseminated by air currents, splashing rain, farm implements, workers, and insects. With cool temperatures (below 20°C) and free water on the host tissue, each sporangium can germinate directly by producing a germ tube or, more commonly, by forming 4 to 18 motile zoospores. The swarm spores swim about in a water film for a few minutes with the aid of two whiplash flagella. The zoospores soon come to rest, become spherical, form a cell wall, lose their flagella, and produce a germ tube. The germ tubes grow and penetrate leaf or other host tissue

Correspondence**Josiya Joy**

M.Sc. (Ag.) Student, Department of Plant Pathology, College of Agriculture, Dapoli, Dr. B.S.K.K.V. Dapoli, Maharashtra, India

through stomata (Meena *et al.*, 2014) [8].

A sporangium was considered germinated if it lacked cytoplasm and empty (Verma, 1987 and Anonymous, 2012) [11, 2]. Many fungicides are reported to have the ability to inhibit the sporangial germination of *A. candida*. Ability to inhibit the sporangial germination under *in vitro* conditions, points towards the fungicidal activity against the disease under natural epiphytotic conditions. Hence *in vitro* efficacy of various fungicides (systemic, contact, and combi fungicides) was evaluated in this present study.

Materials and Methods

Various fungicides (systemic, contact and combi fungicides), phytoextracts and essential oils were evaluated *in vitro* by applying sporangial germination test (glass cavity slides). Sporangial suspension of *A. candida* was prepared in sterile distilled water and used fresh (within 15 mins. of preparation) for sporangial germination technique. The double strength solutions of each test fungicide, phyto extracts and essential oils were prepared separately and evaluated their efficacy for inhibition of sporangial germination of the test pathogen. For the purpose, lid and bottom disc of the sterilized glass Petri plates were lined with moistened blotter paper and kept ready to hold the cavity slides. One drop of each i.e., test fungicide / phyto extract / essential oil and sporangial suspension were dispensed in the cavity slides with three cavities in three replications and were placed in bottom disc of glass Petri plates, lined with moistened blotter paper and closed immediately with the lid lined with moistened blotter paper. These Petri plates were then incubated at 25°C. Simultaneously, alone sporangial suspension in sterile distilled water of test pathogen placed in cavity slide with three cavities and kept in glass Petri plate lined with moistened blotter and incubated at 25 °C were maintained as untreated control.

Observations on spore germination were recorded at 24hrs after incubation, under Research microscope at 40X magnifications, under five different microscopic fields. A sporangium was considered germinated if it lacked cytoplasm and empty (Verma, 1987 and Anonymous, 2012) [11, 2].

The per cent sporangial germination was calculated by following formula (Vincent, 1927) [12]:

$$\text{Per cent Germination (PG)} = \frac{A}{B} \times 100$$

PG- Per cent germination

A- Number of sporangia germinated

B- Number of sporangia observed

The per cent inhibition was calculated by the following formula given by Vincent (1927) [12]

$$\text{Per cent Inhibition of sporangial germination (I)} = \frac{C - T}{C} \times 100$$

C- Germination of sporangia in control

T- Germination of sporangia in treatment.

3.2.2.1 *In vitro* evaluation of systemic fungicides (each @ 500, 750 ppm)

A total of seven each systemic, and contact/ combi fungicides (As detailed under treatments) were evaluated *in vitro* by applying sporangial germination test (Cavity slides with three cavities).

Experimental details: Systemic fungicides (each @ 500, 750ppm)

Design: CRD

Replications: Three

Treatments: Eight

Treatment details

Tr. No.	Treatments
T ₁	Metalaxyl 35% SD
T ₂	Difenoconazole 25% EC
T ₃	Hexaconazole 5% EC
T ₄	Azoxystrobin 25% SC
T ₅	Carbendazim 50% WP
T ₆	Fosetyl-Al 80% WP
T ₇	Thiophanate methyl 75% WP
T ₈	Control (untreated)

3.2.2.2. *In vitro* evaluation of contact and combi fungicides (each @ 2000, 2500ppm)

Design: CRD

Replications: Three

Treatments: Eight

Treatment details

Tr. No.	Treatments
T ₁	Metalaxyl 4% + Mancozeb 68% WP
T ₂	Azoxystrobin 11% + Tebuconazole 18.3% SC
T ₃	Zineb 75% WP
T ₄	Copper oxychloride 50% WP
T ₅	Mancozeb 75% WP
T ₆	Chlorothalonil 75% WP
T ₇	Propineb 70% WP
T ₈	Control (untreated)

Results and Discussion

The results (Table 1, Fig. 1) revealed that all of the seven systemic fungicides evaluated (each @ 500 ppm and 750 ppm) significantly influenced *A. candida* sporangial germination and its corresponding inhibition. At 500 ppm, Metalaxyl 35% SD (PLATE V) resulted with significantly least sporangial germination (16.37%) and their highest inhibition (72.62%) over untreated control, followed by Hexaconazole 5% EC (17.58% and 70.60% respectively), Difenoconazole 25% EC (18.82% and 68.52%, respectively), Azoxystrobin 25% SC (17.58%) and 66.98%, respectively), Carbendazim 50% WP (20.35% and 65.96%, respectively), Thiophanate methyl 75% WP (22.23% and 62.82%, respectively), and Fosetyl-Al 80% WP (22.68% and 62.08%, respectively). In respect of sporangial inhibition, the fungicides found on par to each other were Difenoconazole 25%EC and Azoxystrobin 25% SC, Azoxystrobin 25% SC and Carbendazim 50% WP (65.96%), Thiophanate methyl 75% WP and Fosetyl-Al 80% WP.

Table 1: *In vitro* efficacy of systemic fungicides against *A. candida*, causing white rust of mustard

Tr. No	Treatments	500 ppm		750 ppm	
		SG *(%)	Inhibition (%)	SG* (%)	Inhibition (%)
T ₁	Metalaxyl 35% SD	16.37 (23.87)	72.62 (58.5)	14.64 (22.50)	76.94 (61.30)
T ₂	Difenoconazole 25% EC	18.82 (25.71)	68.52 (55.87)	17.83 (24.98)	71.92 (58.00)
T ₃	Hexaconazole 5% EC	17.58 (24.79)	70.60 (57.17)	16.37 (23.87)	74.20 (59.47)
T ₄	Azoxystrobin 25% SC	19.74 (26.38)	66.98 (54.93)	18.76 (25.67)	70.44 (57.06)
T ₅	Carbendazim 50% WP	20.35 (26.81)	65.96 (54.31)	20.00 (26.57)	68.49 (55.85)
T ₆	Fosetyl-Al 80% WP	22.68 (28.44)	62.08 (51.99)	22.22 (28.12)	64.99 (53.72)
T ₇	Thiophanate methyl 75% WP	22.23 (28.13)	62.82 (52.43)	20.75 (27.10)	67.30 (55.12)
T ₈	Control (untreated)	59.79 (50.65)	0.00 (0.00)	63.48 (52.82)	0.00 (0.00)
S.E. \pm		0.27	0.41	0.23	0.39
C.D. (P = 0.01)		1.13	1.73	0.99	1.61

*Mean of three replications, SG: Sporangial Germination
Figures in parenthesis are arc sine transformed values

Similar trend in respect of sporangial germination inhibition was observed with the test systemic fungicides @ 750 ppm. Metalaxyl 35% SD resulted with significantly lowest sporangial germination (14.64%) and their highest inhibition (76.94%), over untreated control, followed by Hexaconazole 5% EC (16.37%, 74.20%, respectively), Difenoconazole 25% EC (17.83%, 71.92%, respectively), Azoxystrobin 25% SC (18.76%, 70.44%, respectively), Carbendazim 50% WP (20.00%, 68.49%, respectively), Thiophanate methyl 75% WP (20.75%, 67.30%, respectively) and Fosetyl-Al 80% WP (22.22%, 64.99%, respectively). In case of inhibition of sporangial germinations over control, the fungicides found on par to each other were Difenoconazole 25% EC and Azoxystrobin 25% SC, Carbendazim 50% WP and Thiophanate methyl 75% WP.

Thus these results revealed that Metalaxyl 35% SD resulted with maximum sporangial inhibition, at both 500 ppm and 750 ppm, followed by Hexaconazole 5%EC, Difenoconazole 25% EC, Azoxystrobin 25% SC, Carbendazim 50% WP, Thiophanate methyl 75% WP and Fosetyl-Al 80% WP. Further, inhibition was directly proportional to the concentration of the test fungicides.

A very few works have reported *in vitro* efficacy of systemic fungicides against *A. candida*, Khunti *et al.* (2001) [6] reported Metalaxyl 35% SD @ 0.02%, as the most effective fungicide *in vivo*, with minimum white rust disease incidence (26.6%) in mustard. Verma (1987) [11] recorded that Fosetyl-Al 80% WP as the least effective among all the treatments evaluated

in vitro with only 26.85% sporangial germination inhibition. Talukder *et al.* (2012) [10] reported the efficacy of Hexaconazole 25% EC @ 0.1% with 40.52% disease control of mustard white rust disease.

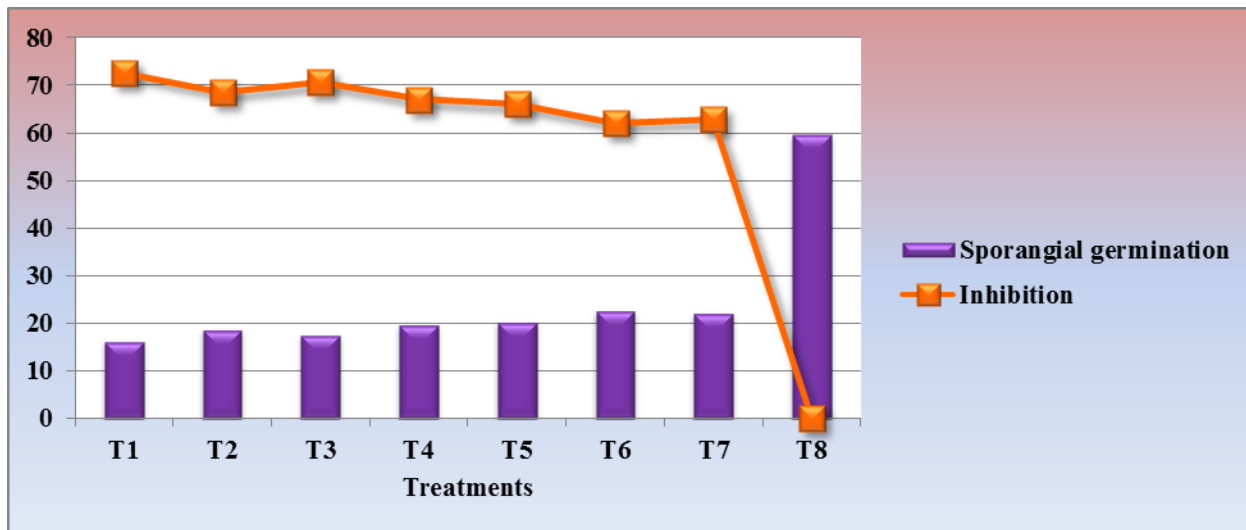
4.3.2 *In vitro* efficacy of contact and combi-fungicides

The results (Table 2, Fig. 2) indicated that the test contact and combi-fungicides exhibited a wide range of *A. candida* sporangial germination which decreased steadily and their corresponding inhibition increased with increase in concentrations of the test fungicides. At 2000 ppm, Metalaxyl 4% + Mancozeb 68% WP (PLATE V) resulted significantly least sporangial germination (9.70%) and their highest inhibition (84.84%) over untreated control, followed by Chlorothalonil 75% WP (11.76%, 81.61%, respectively), Mancozeb 75% WP (12.50%, 80.46%, respectively), Azoxystrobin 11% + Tebuconazole 18.3% SC (13.39%, 79.07%, respectively), Copper oxychloride 50% WP (14.29%, 77.67%, respectively), Propineb 70% WP (15.52%, 75.74%, respectively) and Zineb 75% WP (21.50%, 66.39%, respectively). In respect of sporangial inhibition, the fungicides found on par among them themselves were Chlorothalonil 75% WP and Mancozeb 75% WP, Mancozeb 75% WP and Azoxystrobin 11% + Tebuconazole 18.3% SC, Azoxystrobin 11% + Tebuconazole 18.3% SC and Copper oxychloride 50% WP, Copperoxychloride 50% WP and Propineb 70% WP.

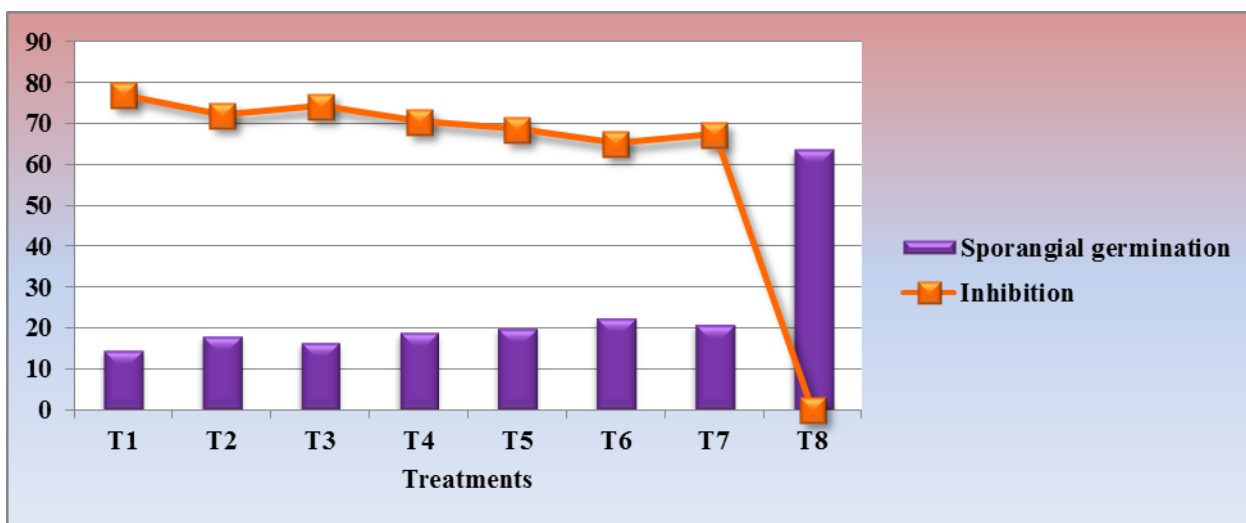
Table 2: *In vitro* efficacy of contact and combi-fungicides against *A. candida*, causing white rust of mustard

Tr. No	Treatments	2000 ppm		2500 ppm	
		SG* (%)	Inhibition (%)	SG* (%)	Inhibition (%)
Combi fungicides					
T ₁	Metalaxyl 4%+ Mancozeb 68% WP	9.70 (18.15)	84.84 (67.09)	8.19 (16.63)	86.79 (68.89)
T ₂	Azoxystrobin 11%+ Tebuconazole 18.3% SC	13.39 (21.46)	79.07 (62.77)	11.33 (19.67)	81.72 (64.69)
Contact fungicides					
T ₃	Zineb 75% WP	21.50 (27.62)	66.39 (54.57)	15.26 (22.99)	75.38 (60.25)
T ₄	Copper oxychloride 50% WP	14.29 (22.21)	77.67 (61.80)	12.50 (20.70)	79.83 (63.31)
T ₅	Mancozeb 75% WP	12.50 (20.70)	80.46 (63.77)	10.41 (18.82)	83.19 (65.80)
T ₆	Chlorothalonil 75% WP	11.76 (20.06)	81.61 (64.61)	9.24 (17.70)	85.10 (67.29)
T ₇	Propineb 70% WP	15.52 (23.20)	75.74 (60.49)	13.85 (21.85)	77.64 (61.78)
T ₈	Control (untreated)	63.97 (53.11)	0.00 (0.00)	61.98 (51.93)	0.00 (0.00)
S.E. \pm		0.35	0.52	0.32	0.51
C.D. (P = 0.01)		1.45	2.13	1.30	2.12

*Mean of three replications, SG: Sporangial Germination
Figures in parenthesis are arc sine transformed values



(a) @ 500 ppm



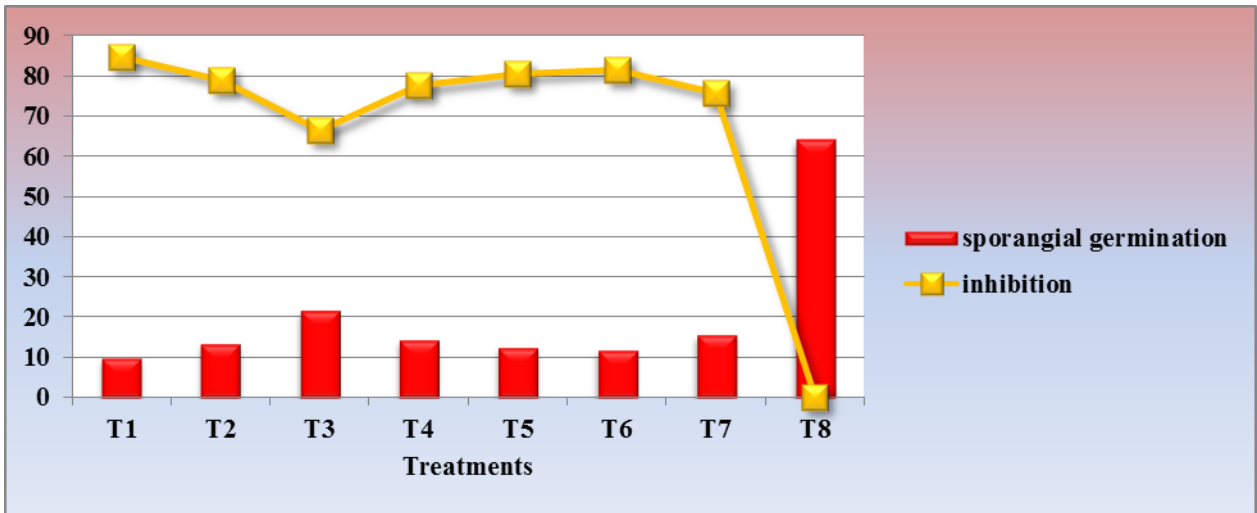
(b) @ 750 ppm

Fig 2: *In vitro* efficacy of systemic fungicides against *A. candida* @ 500 ppm (A) and 750 ppm (B)

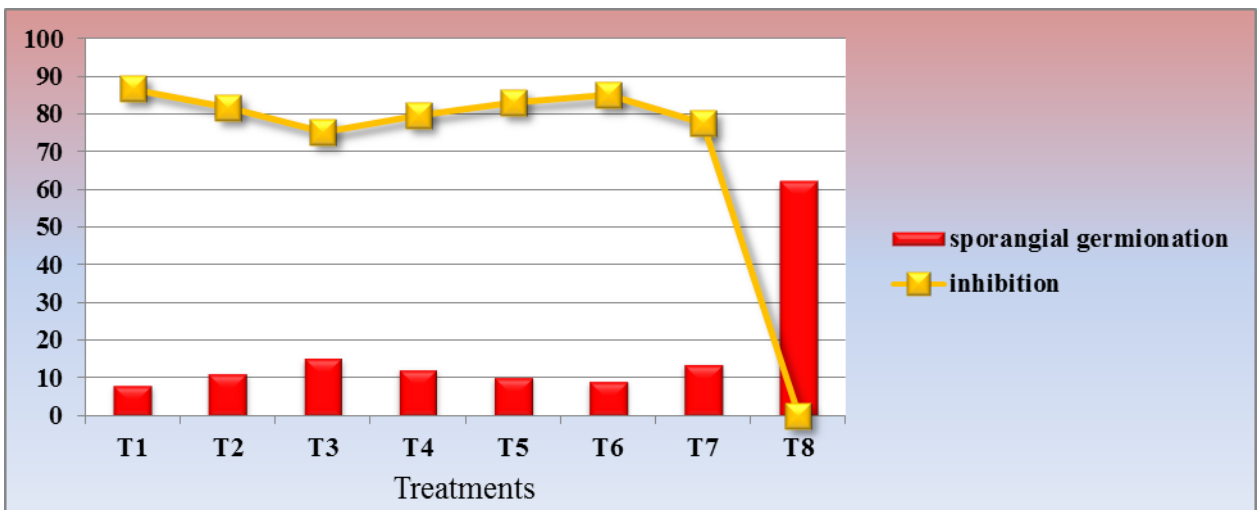
At 2500 ppm similarly, Metalaxyl 4% + Mancozeb 68% WP resulted with significantly least sporangial germination (8.19%) and their highest inhibition (86.79%), followed by Chlorothalonil 75% WP (9.24%, 85.10%, respectively), Mancozeb 75% WP (10.41%, 83.19%, respectively), Azoxystrobin 11% + Tebuconazole 18.3% SC (11.33%, 81.72%, respectively), Copper oxychloride 50% WP (12.50%, 79.83%, respectively), Propineb 70% WP (13.85%, 77.64%, respectively) and zineb 75% WP (15.26%, 75.38%, respectively), as against highest sporangial germination (61.98%) in untreated control. Chlorothalonil 75% WP and Mancozeb 75% WP, Copper oxychloride 50% WP and Propineb 70% WP were found at par with each other, in respect of inhibition of sporangial germination.

Thus the most effective fungicides with maximum sporangial inhibition were Metalaxyl 4% + Mancozeb 68% WP and Chlorothalonil 75% WP, which were further used for field evaluation, followed by Mancozeb 75% WP, Azoxystrobin 11% + Tebuconazole 18.3% SC and Copper oxychloride 50% WP.

These results are in harmony with the earlier findings of Verma (1987) ^[11] who reported Metalaxyl 4% + Mancozeb 64% WP @ 250 ppm as most effective with highest sporangial germination inhibition (72.33%) of *A. candida*, causing mustard white rust. Kalpana (2017) ^[5] reported Metalaxyl 8% + Mancozeb 64% WP @ 100 ppm as most effective with minimum sporangial germination (7.75%) and maximum sporangial inhibition (87.84%), followed by Azoxystrobin 25% EC @ 100 ppm (7.28% and 86.85%). Bhargava *et al.* (1997) ^[4] reported the superiority of Chlorothalonil 75% WOP @ 0.2% foliar sprays in controlling white rust of mustard. Similarly, Talukder *et al.* (2012) ^[10] reported Metalaxyl 4% + Mancozeb 64% WP @ 0.2%, as the most effective against white rust disease (*A. candida*) of mustard, which resulted with highest disease control (70.28%), followed by Copper oxychloride 50% WP @ 0.2% (47.23%). Muhammad *et al.* (2017) also reported Metalaxyl 8% + Mancozeb 64% WP @ 0.05% as most prominent against the white rust of mustard disease, with minimum disease intensity (24.09%).

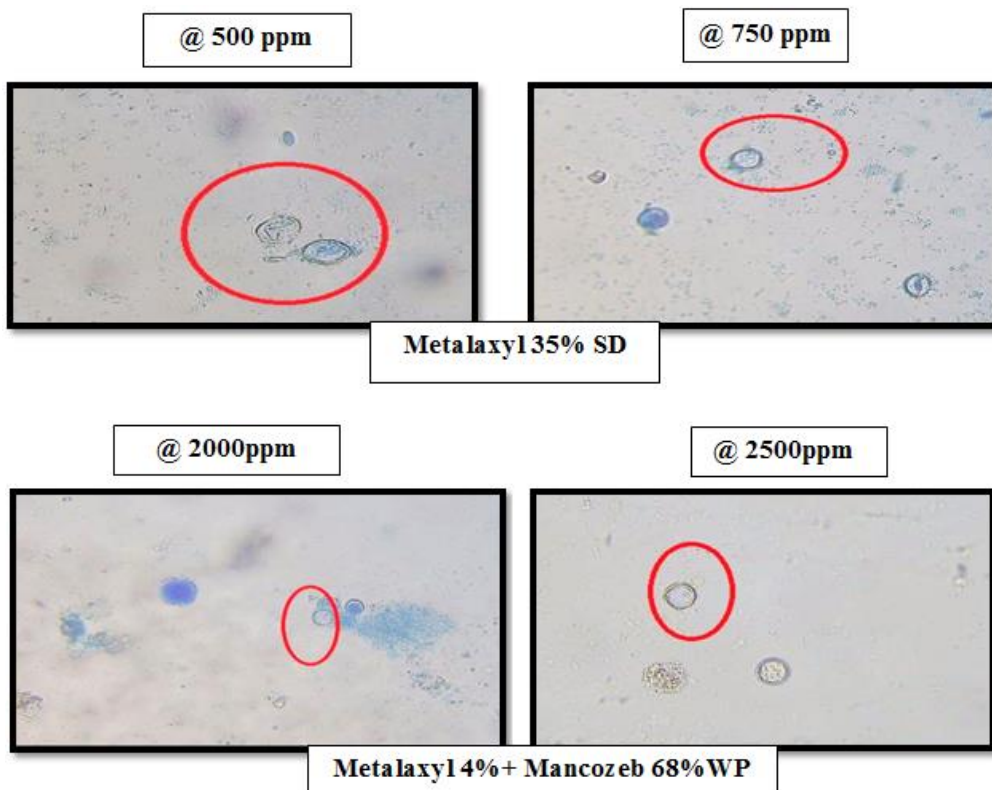


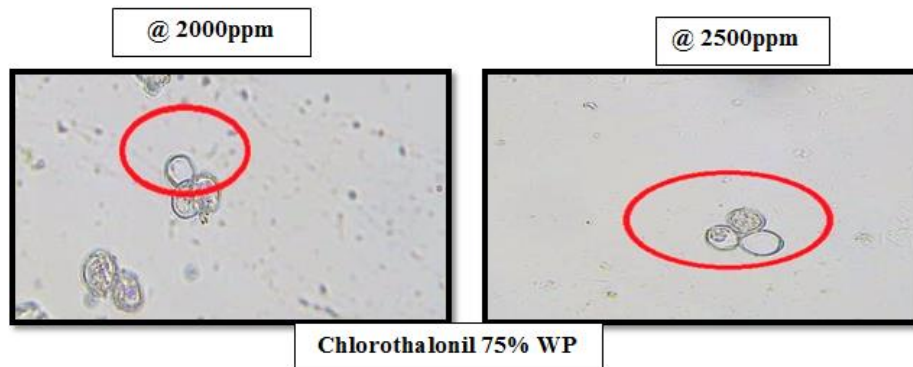
(a) @2000 ppm



(b) @2500 ppm

Fig 3: In vitro efficacy of contact and combi fungicides against *A. candida* @ 2000 ppm (A) and 2500 ppm (B)





In vitro inhibition of *A. candida* sporangial germination by most effective systemic, contact and combi-fungicides

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