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Sanjana Veni DVND
M.Sc. Scholar, Department of Plant Pathology, College of Agriculture, University of Agricultural Sciences, Dharwad, Karnataka, India

Rao MSL
Principal Scientist and Head, Department of Plant Pathology, AICRP on groundnut, Main Agricultural Research Station, Dharwad, Karnataka, India

Patil BR
Professor and Head, Department of Genetics and Plant Breeding, University of Agricultural Sciences, Dharwad, Karnataka, India

Kulkarni VR
Senior Scientist, Department of Plant Pathology, AICRP on Cotton, Agricultural Research Station, Dharwad, Karnataka, India

Corresponding Author:
Sanjana Veni DVND
M.Sc. Scholar, Department of Plant Pathology, College of Agriculture, University of Agricultural Sciences, Dharwad, Karnataka, India

Evaluation of efficacy of new fungicides and bio agents for the management of white rust of mustard

Sanjana Veni DVND, Rao MSL, Patil BR and Kulkarni VR

Abstract
Mustard (*Brassica juncea* L. Czern. and Coss) Is an important oilseed crop grown in India. White rust of mustard caused by *Albugo candida* (Pers.) Kuntze, is one of the major diseases of mustard causing significant yield loss. It's always better not to use same molecules for the management for long periods to avoid the probable development of resistance by the pathogens to the old fungicides. Accordingly, the current investigations were carried out to assess the efficacy of new molecules and bioagents both under lab and field conditions. The results of these investigations showed the efficacy of two fungicides Azoxystrobin 18.2% + Difenconazole 11.4% SC at 0.1 per cent concentration and Metalaxyl 8% + Mancozeb 64% at 0.2 per cent concentration in reducing white rust disease incidence with high returns (good quality pod and seed yield). It is suggested that these can be tested further under large-scale trials.

Keywords: Mustard, white rust, management, fungicides

1. Introduction
Mustard (*Brassica juncea* L. Czern. and Coss) is an important oilseed crop of the crucifer family which is grown as an annual or biennial crop in India. In Northern India, it is an important source of edible oil and is predominantly grown in *Rabi* season. The seeds contain approximately 38-46 per cent oil content (Kumar, 2012) [6]. In India, mustard is grown in an area of 7.9 million hectares with an annual production of 11.963 million tons and productivity of 1497 kg/ha (Indiaagristat, 2022) [11].
Despite its economic importance, mustard cultivation faces numerous challenges, including biotic and abiotic stresses. Among the biotic stresses, white rust disease, caused by the biotrophic oomycete pathogen *Albugo candida* (Pers.) Kuntze, is the most destructive and widely spread disease of mustard (Kolte, 1985). The yield losses range from 17-34 per cent in India (Yadav and Gupta, 2011; Pandey *et al*., 2013) [12, 8].
White rust is characterized by raised pustules on the underside of the leaves during flowering, later extending to stems, inflorescence and pods in severe cases. Systemic infection often leads to stag head formation (Meena *et al*., 2014) [7]. The combined infection results in yield loss of about 89.9 per cent (Godika *et al*., 2001) [4].
To prevent such high level of losses there is a need for implementation of effective disease management strategies. Fungicides such as metalaxyl 8 per cent + mancozeb 64 per cent, mancozeb, metalaxyl and copper oxychloride are being used for management of white rust (Saharan *et al*., 1984; Gairola and Tewari, 2019) [9, 3]. Because of the emergence of new races of the pathogen and the possibility of development of resistance to these fungicides by the pathogen, there is a need to evaluate some new fungicides for better management of the disease. Hence the present study was undertaken to evaluate some novel fungicides and bioagents against white rust of mustard.

2. Materials and methods
The evaluation of efficacy of selected fungicides and bioagents was done first under *in vitro* conditions against *A. Candida* in the Department of Plant Pathology, College of Agriculture, University of Agricultural Sciences, Dharwad, Karnataka. Among the fungicides and bioagents tested under *in vitro* conditions, few fungicides and bio agents which were found better were further evaluated under field conditions in the research plots of Main Agricultural Research Station, University of Agricultural Sciences, Dharwad.

Maintenance of white rust inoculum: As *A. candida* is an obligate pathogen, it was maintained on susceptible cultivar Varuna under glasshouse.

Whenever required, sporangial suspension was prepared by scraping pure white rust pustules containing sporangia using blade or brush and placing them in water and adjusting the concentration using haemocytometer.

2.1 *In vitro* evaluation of fungicides and bioagents by cavity slide method

The experiment was conducted in a two factor completely randomized design (CRD), with each treatment replicated four times. Five systemic, five combi product fungicides and four bioagents which were purchased from the local shop were evaluated using cavity slide technique at three different concentrations against *A. candida*. The required concentrations of fungicides were prepared by dissolving known quantity in sterile distilled water. Similarly, the required concentrations of bioagents, which were obtained from the Institute of Organic Farming (IOF), College of Agriculture, and Dharwad were prepared by dissolving in water under aseptic conditions.

The sporangial suspension was prepared separately in sterile distilled water with a concentration of 2.5×105 sporangia/ml and adjusted by haemocytometer 10µL concentration of each fungicide and bioagent was placed in separate cavity slide containing 10µL sporangial suspension and mixed well to get the desired concentration. The cavity slides with 10µL of distilled water and 10µL of sporangial suspension served as a check (Control). These slides were placed in a moist chamber and incubated at 10 ºC for 12 hours for sporangial germination, then observed under a compound microscope. Per cent sporangial germination was calculated using the following formula

$$\text{Per cent sporangial germination} = \frac{\text{No.of sporangia germinated}}{\text{Total number of sporangia}} \times 100$$

The per cent inhibition was calculated by the following formula (Vincent, 1947) [10].

$$\text{Per cent inhibition} = \frac{C-T}{C} \times 100$$

Where, C = Per cent germination in control
T = Per cent germination in treatment

2.2 Evaluation of fungicides and bioagents under field conditions

During *Rabi* 2022-23, the efficacy of two selected systemic fungicides, two combi product fungicides and one bioagent which showed effectiveness against white rust of mustard under *in vitro* conditions were evaluated in the field using the susceptible mustard variety NRCHB-101 in three replications at the Main Agricultural Research Station, Dharwad. Field trial was laid out in a randomized complete block design (RCBD) with 45 cm spacing between the rows and 10 cm in between the plants. Sowing was done on November 2, 2022. Fertilizers and irrigation were given as per recommendations.

The selected fungicides and bioagents were evaluated in 12 different combinations including control. Two sprays were given at a 7-day interval, starting from the onset of disease at 50 days after sowing. Based on the per cent leaf area infected, observations on white rust were recorded at regular intervals using 0-9 disease rating scale (AICRP-RM, 2012) [1].

Table 1: Disease scoring scale (0-9 grade) for white rust of mustard

Scale	Percent leaf area infected
0	No symptoms
1	<5%
3	6-10%
5	11-25%
7	26-50%
9	>50%

The per cent disease index (PDI) was calculated using the formula given by Wheeler (1969) [11].

$$\text{Per cent disease index (PDI)} = \frac{\text{Sum of all numerical ratings}}{\text{No.of leaves examined} \times \text{Maximum disease grade}} \times 100$$

Additionally, 15 days prior to harvest, the stag head incidence was noted. The percentage stag head incidence was calculated using the following formula.

$$\text{Staghead incidence} = \frac{\text{No.of plants showing stagheads/plot}}{\text{Total no.of plants/plot}} \times 100$$

3. Results and Discussion

3.1 *In vitro* evaluation of fungicides and bioagents by cavity slide method

Among the five systemic fungicides tested at three concentrations (0.025, 0.05 and 0.1%), maximum inhibition of sporangial germination was recorded in treatments involving Azoxystrobin 23% SC at all the three concentrations (81.33, 82.00 and 82.66%) which were on par with each other followed by Dimethomorph 50% WP with sporangial germination inhibition percentage of 74.30, 76.98 and 77.65 at 0.025, 0.05 and 0.1% concentrations respectively (Table 2).

Among the combo product fungicides tested at three concentrations (0.1, 0.2 and 0.3%), maximum inhibition of sporangial germination was noticed in treatments involving Azoxystrobin 18.25% + Difenconazole 11.4% SC at all the three concentrations (81.69, 87.28 and 87.95%) and it was significantly superior over rest of the treatments followed by Metalaxyl 4% + Mancozeb 64% WP at 0.1 per cent (80.68%), 0.2 per cent (82.26%) and at 0.3 per cent with 82.36% inhibition of sporangial germination (Table 3).

Among the four bioagents tested at three different concentrations (0.3, 0.5 and 1%), the maximum per cent inhibition was recorded in treatments involving Trichoderma harzianum at all the three concentrations (62.99, 67.00 and 68.28%) which was found significantly superior over rest of the treatments followed by Pseudomonas fluorescens at 0.3 per cent (46.48%), 0.5 per cent (51.09%) and at 1.0 per cent with 55.98 per cent inhibition of sporangial germination (Table 4).

Table 2: *In vitro* evaluation of systemic fungicides on inhibition of sporangial germination of *Albugo candida*

SL. No.	Fungicides	Per cent inhibition of sporangial germination			Mean
		Concentration (%)			
		0.025	0.05	0.1	
1	Fosetyl Aluminium 80% WP	57.98(49.30) *	70.77 (57.12)	74.80 (59.84)	67.85 (55.46)
2	Azoxystrobin 23% SC	81.33(64.49)	82.00(64.90)	82.66 (65.39)	82.00 (64.89)
3	Dimethomorph 50% WP	74.30 (59.71)	76.98 (61.10)	77.65 (61.79)	76.31 (60.87)
4	Propiconazole 25% EC	66.04 (54.36)	69.92 (56.74)	73.92 (59.29)	69.96 (56.76)
5	Tebuconazole 25.9% EC	60.65 (51.15)	64.55 (53.46)	67.43(55.20)	64.21 (53.26)
Mean		66.71 (54.76)	72.41 (58.31)	76.43 (60.95)	71.85 (57.96)
		S. Em. ±		C.D at 1%	
Fungicides (F)		1.13		3.30	
Concentrations (C)		0.88		2.53	
F × C		1.34		4.12	

*Figures in parentheses indicate arc sine values

Table 3: *In vitro* evaluation of combi product fungicides on inhibition of sporangial germination of *Albugo candida*

SL. No.	Fungicides	Per cent inhibition of sporangial germination			Mean
		Concentration (%)			
		0.1	0.2	0.3	
1.	(Metalaxyl 4% + Mancozeb 64%) 68% WP	80.68 (63.74) *	82.26 (65.09)	82.36 (65.17)	82.10 (64.97)
2.	(Azoxystrobin 18.2% + Difenconazole 11.4%) 29.65% SC	81.69 (64.56)	87.28 (69.40)	87.95 (69.69)	85.30 (67.46)
3.	(Cymoxanil 8% + Mancozeb 64%) 72% WP	63.12 (52.66)	66.95 (54.83)	74.13 (59.40)	68.07 (55.59)
4.	(Metiram 44% + Dimethomorph 9%) 53% WG	45.08(42.18)	54.18(47.47)	68.75(55.99)	56.00 (48.45)
5.	(Hexaconazole 4% + Zineb 68%) 72% WP	49.52 (44.72)	58.85 (49.88)	63.12 (57.16)	57.16 (49.12)
Mean		66.71(54.76)	72.41(58.31)	76.43(60.95)	71.85(57.96)
		S. Em. ±		C.D at 1%	
Fungicides (F)		0.38		1.09	
Concentrations (C)		0.24		0.67	
F × C		0.67		1.90	

*Figures in parentheses indicate arc sine values

Table 4: *In vitro* evaluation of bioagents on inhibition of sporangial germination of *Albugo candida*

SL. No.	Bioagents	Per cent inhibition of sporangial germination			Mean
		Concentration (%)			
		0.3	0.5	1.0	
1	*Trichoderma Harzianum*i of Strain	62.99 (52.53) *	67.00 (54.94)	68.28 (55.72)	66.09 (54.39)
2	*Pseudomonas fluorescens* IOF Strain	46.48 (42.98)	51.09 (45.62)	55.98 (48.43)	51.18 (45.68)
3	*Bacillus subtilis* IOF Strain	44.34 (41.75)	49.46 (44.69)	51.66 (45.95)	48.49 (44.13)
4	*Neofusicoccum parvum* IOF Strain	41.44 (40.07)	45.47(42.40)	48.29(44.02)	45.07(42.17)
Mean		48.81(44.32)	53.26(46.87)	56.05(48.47)	52.71(46.55)
		S.Em. ±		C.D at 1%	
Bioagents (B)		0.50		1.54	
Concentrations (C)		0.44		1.40	
B × C		0.87		2.62	

*Figures in parentheses indicate arc sine values

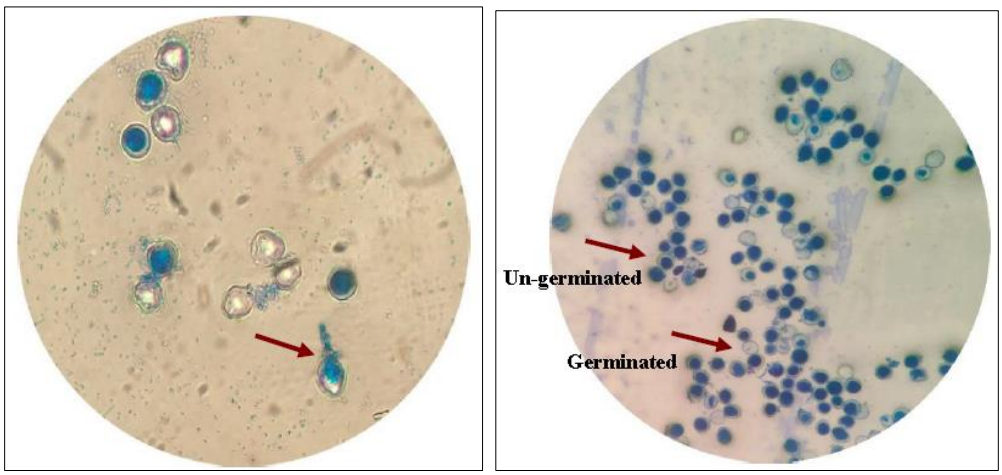

Plate 1: Microphotographs related to germination of sporangia, A) Germination of sporangia (release of zoospores), B) Germinated and unterminated sporangia

3.2 Evaluation of fungicides and bioagents under field conditions

The results of the field experiment showed significant difference among the treatments compared to control. Among the twelve treatments, T_4 involving two sprays of Azoxystrobin 18.2% + Difenconazole 11.4% SC @ 0.1 per cent concentration at 7 days interval recorded the lowest per cent disease index of 50.73 which was on par with T_3 involving two sprays of Metalaxyl 8% + Mancozeb 64% WP @ 0.2 per cent concentration (52.57 PDI). The next best treatment was T_8 involving one spray of Dimethomorph 50% WP @ 0.1 per cent concentration followed by second spray with *Trichoderma harzianum* @ 0.5 per cent concentration with per cent disease index of 67.64. The maximum PDI was observed in control (89.49) followed by T_9 (83.98). With respect to stag head incidence, the minimum stag head incidence was observed in T_4 (1.90%) followed by T_3 (6.30%) and T_9 (10.41). The maximum staghead incidence was observed in control (61.53%) followed by T_9 (46.69%). The highest seed yield was obtained in T_4 (6.95 t/ha) which was on par with T_3 (6.84 t/ha). The lowest seed yield was obtained in control (3.80 t/ha) followed by T_{11} (4.14 t/ha) (Table 5 and Figure 1).

In the current study, a new fungicide Azoxystrobin 18.2% + Difenconazole 11.4% SC @ 0.1 per cent concentration and Metalaxyl 8% + Mancozeb 64% WP @ 0.2 per cent concentration showed effectiveness in management of white rust in mustard. The efficacy of Azoxystrobin 18.2% + Difenconazole 11.4% SC is being reported for the first time whereas the efficacy of Metalaxyl 8% + Mancozeb 64% WP has been reported previously in similar studies (Gairola and Tewari, 2019; Yadav, 2019; Choudhary, 2021) [3, 13, 2].

Plate 2: Symptoms of white rust of mustard on different plant parts, A) White pustules on lower surface of leaves, B) Yellow spots on upper surface of leaves, C) on stem, D) on petiole, E) on siliquae, F) stag heads

Table 5: Field evaluation of fungicides and bioagents against white rust of mustard

Treatment No.	Treatment	Per cent disease incidence (PDI)				Staghead zncidence (%)	Seed yield (t/ha)
		Before spray	7 days after 1st spray	7 days after 2nd spray	14 days after 2nd spray		
1	Azoxystrobin 23% SC @ 0.1%	33.76 (35.50)*	46.45 (42.95)	70.83 (57.30)	77.59 (61.74)	17.65 (24.83)	5.83
2	Dimethomorph 50% WP @ 0.1%	33.56 (35.38)	51.83 (46.03)	74.58 (59.71)	76.45 (60.96)	32.44 (34.70)	4.83
3	Metalaxyl 8% + Mancozeb 64% WP @ 0.2%	33.67 (35.45)	38.66 (38.42)	50.73 (45.40)	52.57 (46.45)	6.30 (14.50)	6.84

4	Azoxystrobin 18.2% + Difenconazole 11.4% SC @ 0.1%	32.81 (34.93)	34.81 (36.14)	45.54 (42.42)	50.73 (45.40)	1.90 (7.86)	6.95
5	Azoxystrobin 23% SC @ 0.1% → Metalaxyl 8% + Mancozeb 64% WP @ 0.2%	33.54 (35.37)	46.22 (42.81)	77.44 (61.63)	80.61 (63.88)	46.25 (42.83)	4.64
6	Dimethomorph 50% WP @ 0.1% → Azoxystrobin 18.2% + Difenconazole 11.4% SC @ 0.1%	33.78 (35.52)	44.45 (41.8)	70.25 (56.93)	73.61 (59.07)	38.82 (38.52)	5.72
7	Azoxystrobin 23% SC @ 0.1% → *Trichoderma harzianum* @ 0.5%	33.89 (35.58)	48.85 (44.32)	74.38 (59.58)	79.37 (62.97)	22.00 (27.96)	5.48
8	Dimethomorph 50% WP @ 0.1% → *Trichoderma harzianum* @ 0.5%	33.75 (35.49)	40.63 (39.58)	59.22 (50.30)	67.64 (55.31)	10.41 (18.80)	4.91
9	Metalaxyl 8% + Mancozeb 64% WP @ 0.2% → *Trichoderma harzianum* @ 0.5%	33.91 (35.60)	55.71 (48.26)	81.54 (64.55)	83.98 (66.40)	46.69 (43.09)	5.31
10	Azoxystrobin 18.2% + Difenconazole 11.4% SC → *Trichoderma harzianum* @ 0.5%	33.38 (35.56)	44.47 (41.80)	74.19 (59.45)	78.5 (62.37)	18.63 (25.54)	4.26
11	*Trichoderma harzianum* @ 0.5%	33.67 (35.45)	52.48 (46.40)	70.66 (57.19)	75.63 (60.41)	41.65 (40.18)	4.14
12	Control (Untreated check)	33.86 (51.76)	69.66 (56.56)	86.64 (68.56)	89.49 (71.12)	61.53 (51.65)	3.80
S.Em. ±		NS	1.11	1.76	1.95	0.78	0.38
C.D. at 5%			3.37	5.29	5.87	2.34	1.25

NS-Non-Significant, *Figures in parenthesis indicate arcsine transformed values

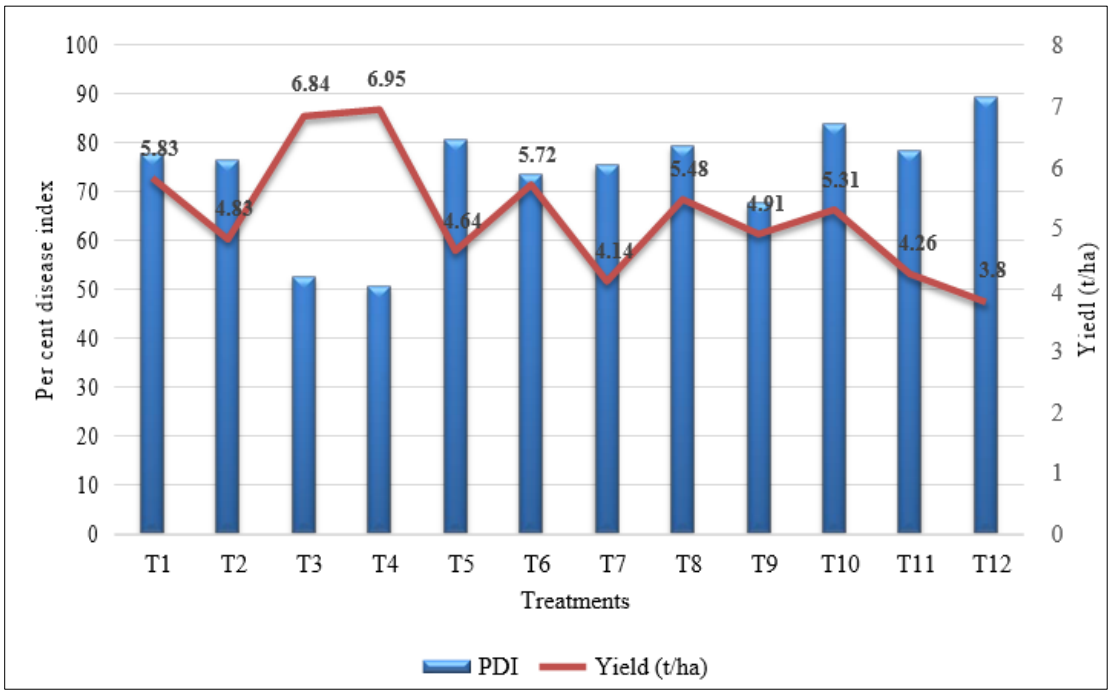

Fig 1: Effect of fungicide treatment on disease incidence and yield of mustard var. NRCHB-101

4. Conclusion

Among the different fungicides evaluated for their efficacy against white rust of mustard, Azoxystrobin 18.25% + Difenconazole 11.4% SC @ 0.1% and Metalaxyl 8% + Mancozeb 64% WP @ 0.2% were found effective in minimizing the white rust disease incidence with high seed yield.

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