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Early detection and management of white uust disease (*Albugo candida*) in rapeseed-mustard

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Abstract

The early detection of *Albugo candida* was done by PCR-based assay and light microscopy. In PCR based assay the primers ITS1 (3'-GAGGGACTTTTGGGTAATCA-5') and Short ITS JV34 (3'-CGCCATTTAGAGGAAGGTGA-5') and JV37 (3'-GTCAAGCAAAACAT-5') were used to amplify the ITS region of *A. candida* and *Alternaria brassicae*. PCR amplification of *A. candida* from inoculated symptomatic and asymptomatic leaves yielded PCR products of 1200 bp and 600 bp of ITS1 and Short ITS primers, respectively whereas no bands were amplified in *A. brassicae*. This confirmed the presence of *A. candida* in asymptomatic inoculated leaves at early stages i.e. 1, 2, 3, 4, 5 and 6 days after inoculation (DAI). In light microscopy the presence of pathogen structures were observed from inoculated symptomatic and asymptomatic inoculated leaves. The presence of pathogen structure viz. mycelium and sporangia was observed in asymptomatic leaves at early stages i.e. at 6,7,8 and 9 DAI ,in symptomatic leaves at 10 and 11 DAI and no fungal structures in healthy mustard leaves after staining with 1 percent cotton blue in lacto phenol and 0.4% trypan blue. During evaluation of rapeseed-mustard germplasm it has been observed that some *Brassica* germplasm escaped from the white rust disease in field found susceptible in glasshouse at both the stages (EC-399299) or only at true leaf stage (Katili local, *Eruca sativa*, Basanti and Banarasi rai, PWR-14-8, PWR-14-9, PWR-14-10, PWR-14-11, RMT-1-10-1, IC 597942 and IC265495). The study revealed that for the confirmation of resistant sources, it is very essential to evaluate *Brassica* germplasm first in field and then in glasshouse at both the stages i.e. at cotyledonary and true leaf stage under high disease pressure. Among various fungicides Metalaxyl 8% + Mancozeb 64% (Ridomil MZ @ 0.25%) and a biological origin Azoxystrobin (Amistar 25 EC @ 0.1%) were found highly effective in inhibiting sporangial germination *in-vitro* and in controlling white rust disease (no occurrence of disease) in glasshouse and in field.

Keywords: *Albugo candida, disease reaction, early detection, fungicides, white rust*

Introduction

White rust caused by *Albugo candida* is considered as one of the major consequences in low productivity of rapeseed mustard due to its destructive nature, wide distribution and grain yield losses. In India yield loss due to this disease was 17- 34 per cent in Indian mustard (Saharan *et al*., 1984; Kolte, 1985; Yadava *et al.,* 2011) and 34 per cent in Toria (Kolte *et al*., 1981). Considering the problem it is very essential to recognize the infection as early as possible before appearance of white rust symptoms. Early detection could be helpful in the decision making of preventive foliar applications at appropriate time i.e. before development of pathogen in the host for the cost effective management **(**Sankarana *et al*., 2010). The early detection technique was reported by the various workers in rapeseed mustard using PCR amplifications of *A. candida* specific primers (Choi *et al*., 2006; Armstrong, 2007) and by light microscopy of infected leaf samples after staining for the presence of pathogen structures (Giri *et al*., 2003). Resistant genotypes of rapeseed-mustard against white rust has been reported by most of the workers based on field studies and some based on glasshouse and few on both i.e. field as well glasshouse. It has been observed from earlier studies that the some Brassica genotypes which showed resistant reaction in field were found susceptible when tested in glasshouse. Therefore, there is a need to evaluate the rapeseed mustard genotypes first in the field then in glasshouse at cotyledonary and true leaf stage under artificially epiphytotic condition for the confirmation of resistance sources in rapeseed mustard. Since mid 90's control of white rust disease has been reported by various workers by the use of fungicides with varying degree of success (Shivpuri and Gupta, 2001; Mehta *et al*., 2005; Biswas *et al*., 2007). But still there is need to evaluate new fungicides for the cost effective management of white rust disease. The present investigation was under taken with the objectives of early detection of *A. candida,* the cause of white rust disease in infected leaves of rapeseed-

mustard; Evaluation of rapeseed-mustard genotypes in field and in glasshouse (at cotyledonary and true leaf stage) under artificial epiphytotic condition; and Evaluation of fungicides for the effective management of the disease.

Materials and Methods

Isolation, purification and maintenance of white rust inoculum

A highly virulent *A. candida* isolate (Pantnagar) collected from mustard was isolated, purified and maintained on susceptible variety Varuna. The *A. candida* was purified by single pustule inoculation and was confirmed under microscope by studying morphological characteristics.

Effect of different suspension media on sporangial germination and disease development

Treatments i.e. Glucose (2 %), *Brassica* leaf extract and Sterilized distilled water were taken to see their effect on sporangial germination and development of white rust symptoms. The sporangial suspension concentration i.e. 2.5×10^5sporangia/ml was adjusted in all treatments. The test plant *B. juncea* cv. Varuna was inoculated at cotyledonary stage (7-8 DAS) with sporangial suspension containing zoospores. The observations on incubation period and latent period were recorded at 10-12 DAI at cotyledonary stage. The sporangial germination i.e. release of zoospores in cavity slide was recorded under microscope after 8 hrs of incubation at 10p C. The number of germinated sporangia was counted at 40 X in per microscopic field. The per cent sporangial germination was calculated using following formula:

$$\text{Percent sporangial germination} = \frac{\text{Number of sporangia germinated}}{\text{Total number of sporangia}} \times 100$$

Early detection of *A. candida* in asymptomatic inoculated leaves of rapeseed-mustard

Early detection of *A.candida* from the asymptomatic inoculated leaves of highly susceptible *B. juncea* cv. Varuna was carried out by PCR based assay of ITS (internal transcribed spacer) region and short-ITS of the ribosomal DNA (rDNA) of *A. candida*. Samples were collected from asymptomatic inoculated leaves at 1, 2, 3, 4, 5, and 6 day after Inoculation (DAI), symptomatic plants at 10 and 12 DAI grown under controlled artificial epiphytotic condition and from healthy (un-inoculated) plants at 8 and 9 day after sowing (DAS) grown in a separate chamber under glasshouse.

Genomic DNA extraction from white rust pustules, pure culture of *A. brassicae* and healthy leaves of mustard cv. Varuna

Isolation of high molecular weight genomic DNA is a prerequisite for molecular analysis. The isolation procedure described by Choi *et al*. (2006) was used for isolation of *A.candida* DNA. CTAB procedure (Doyle and Doyle,1990) was used for the isolation of DNA of *Alternaria brassicae* and *Brassica* leaf.

Purification and quantification of genomic DNA

Purification was done by RNase (5 µl, 10mg/ml) treatment. The quantification of genomic DNA was done by taking the absorbance on UV spectrophotometer. The optical density was measured at 260 nm and 280 nm. The concentration of DNA in the sample was related to optical density and calculated by the following formula:

Concentration of DNA (µg/ml) = A_{260}nm x 50 x dilution factor/1000

PCR amplification

DNA polymerase chain reaction procedure described by Choi *et al*. (2006) was used by reaction mixture (20 ìl) which consist of 1.25ìl of 10x PCR buffer, 0.37ìl of 25 mM $MgCl_2$, 2.0 ìl dNTPs mix (10 mM each of dATP, dCTP, dGTP, dTTP), 0.1 ìl Taq DNA polymerase, 0.8 ìl of forward and reverse primer, 2 ìl of genomic DNA and 14.18 ìl of sterilized double-distilled water. The reaction mixture was vortexed and centrifuged at 12000 rpm for 2 min. Amplifications were performed in Biored Master Cycler gradient. Amplified products were separated on 1.2 per cent agarose gel in 1X TAE buffer at 100V. The gel was stained with 0.5µg/ml Ethidium bromide solution and visualized by illumination under UV light in gel doc system (Alpha Innotech, Alpha-Imager EC). The size of amplification products were determined by comparison to low range DNA Ruler plus marker.

Observations

All the amplified bands ITS (1200 bp) and Short ITS (600 bp) were marked as present or absent for *A. candida* in each leaves sample. These specific bands of *A. candida* were confirmed with the amplification of *A. brassicae* and *B. juncea* cv. Varuna DNA.

Light microscopy

Early detection of *A. candida* using light microscopy was done by microscopic examination of the samples collected from asymptomatic inoculated leaves (1, 2, 3, 4, 5, 6, 7, 8 and 9 DAI), symptomatic leaves (10 and 11 DAI) of *A.*

candida and healthy leaves (8 and 9 DAS) for the presence or absence of pathogen structure i.e. mycelium and sporangia after staining with specific fungal stain.

Evaluation of rapeseed-mustard germplasm at cotyledonary and true leaf stage for the confirmation of resistance under artificial epiphytotic field and glass house condition

Field studies

Seventy *B. juncea* germplasm, 89 Brassica germplasm from UDN (Uniform disease nursery) and 77 Brassica germplasm from NDN (National disease nursery) were evaluated in field under artificial epiphytotic condition for the resistant sources. Two rows of 3m length of each Brassica germplasm were sown in 5-10 cm spacing. A susceptible check (Varuna) as infector was sown after each two rows. 10 plants were randomly selected from each row of each genotype and marked to record observations. Each row of Brassica genotypes was artificially sprayed with sporangial suspension (2.5×10^5 sporangia/ml) of *A. candida* at 45 DAS and the observations on disease severity were recorded at 70, 80 and 90 DAS using 0-9 rating scale (Anonymous, 2011)and infection rate and AUDPC were calculated.

Glasshouse studies

Brassica genotypes evaluated in field were further tested in glasshouse for the confirmation of resistant sources against white rust disease. 10 seeds of each *Brassica* genotypes to be tested were sown. Two separate set of experiments were conducted. The set of plant were inoculated with sporangial suspension of *A. candida* at cotyledonary stage (10 DAS), while other at true leaf stage (15 DAS). The observations on incubation period (days) i.e. just after appearance of symptoms, latent period (days) i.e. just after development of symptoms (white rust pustules), size of the pustules (mm), phenotypic disease reactions (0-7 rating scale, Leekie *et al.*, 1996) and percent disease severity (0-6 rating scale, Conn *et al*., 1990) at cotyledonary stage (10 DAI) and at true leaf stage (15 DAI) were recorded.

The Percent disease index (PDI) was calculated by using following formula:

$$\text{Disease index}\,(\%) = \frac{\text{Sum of all numerical ratings}}{\text{Number of leaves examined} \times \text{Maximum grade}} \times 100$$

Evaluation of fungicides and garlic extract for management of white rust disease

Thirteen fungicides and one garlic extract were evaluated against sporangial germination of *A. candida in-vitro* and in reducing white rust disease in glasshouse and in field to identify their efficacy in managing disease.

In-vitro studies

The sporangial suspension (2.5×10^5 sporangia/ml) of *A. candida* along with fungicides/ garlic extract was placed in cavity slide. The cavity slide without any treatment (sporangial suspension) served as check. The cavity slides placed in moist chamber were incubated at 10^0C for 12 hrs. The observations on number of germinated and un-germinated sporangia were recorded in each treatment after 12 hrs by placing the cavity slide under compound microscope and percent sporangial germination and percent sporangial germination inhibition was calculated.

$$\text{Percent sporangial Germination}\,(\%) = \frac{\text{Number of sporangia germinated}}{\text{Total number of sporangia}} \times 100$$

Per cent inhibition = (C – T) / C × 100

Where,

C = Per cent sporangial germination in control

T = Per cent sporangial germination in treatment

Glasshouse studies

Experiment was conducted in glasshouse in two successive years (2015-16 and 2016-17). The fungicides and garlic extract (Table 7) were 1st sprayed on plants grown in pots at 15 DAS then after 24 hrs plants were sprayed with sporangial suspension of *A. candida*. The plants sprayed only with sporangial suspension were kept as check. The inoculated plants were kept in glasshouse under favorable conditions for the development of disease. The observations on incubation period (days), latent period (days) percent disease severity were recorded at 20 days after inoculation (DAI).

The percent disease inhibition was calculated using following formula:

Percent Disease Inhibition = (C – T) / C × 100

Where,

C = Percent Disease Index in control

T = Percent Disease Index in treatment

Field studies

Table 1: *In-vitro* effect of different suspension media on sporangial germination, incubation period, latent period and disease index of *A. candida*

Suspension media	Sporangial germination	Incubationperiod	Latentperiod	Disease index (%)
	8hr	days	days	
Sterilized distilled water	74.3 (59.6)	9.7	11.0	26.0 (30.7)
Brassica Leaf extract	85.9 (68.0)	5.7	8.3	32.3 (34.6)
Glucose 2%	83.1 (65.7)	8.7	10.3	29.3 (32.8)
CD 5%	1.23	1.26	1.40	0.96
CV	3.96	6.87	4.84	8.47

Values in parentheses are angular transform value

Table 2: Early detection of *A.candida* in asymptomatic inoculated leaves of cv. Varuna by PCR and light microscopy PCR amplification

Asymptomatic inoculated leaves						Symptomatic inoculated leaves		Healthy leaves	
1DAI	2DAI	3DAI	4DAI	5DAI	6DAI	10DAI	12DAI	8DAS	9DAS
+	+	+	+	+	+	+	+	-	-
Light microscopy									
Asymptomatic inoculated leaves						Symptomatic inoculated leaves		Healthy leaves	
Stained with 1 % cotton blue in lacto-phenol									
3DAI	5DAI	6DAI	7DAI	8DAI	9DAI	10DAI	11DAI	8DAS	9DAS
-	-	+	+	+	+	+	+	-	-
Stained with 0.4 % trypan blue									
3DAI	5DAI	6DAI	7DAI	8DAI	9DAI	10DAI	11DAI	8DAS	9DAS
-	-	+	+	+	+	+	+	-	-

+ Presence of *A.candida*
- Absence of

The experiment was conducted in field in two successive years (2015-16 and 2016-17). Each plant in each row was artificially inoculated by spraying with sporangial suspension of *A. candida*. The each plant was sprayed separately by desired concentrations of fungicides and garlic extract. 10 plants were randomly selected in each plot and marked to record observations. The observations on disease severity, Infection rate and AUDPC were recorded at 50, 60, 70, 80, and 90 DAS and stag head incidence at 100 DAS.

Results and Discussion

Effect of different suspension media on the sporangial germination and disease development

Among three suspension media viz. Brassica leaf extract, glucose (2%) and distilled water, maximum sporangial germination (85.9 %), minimum incubation and latent period (5.7 & 8.3 days) and maximum per cent disease index (32.3%) was observed in Brassica leaf extract which was at par to 2% glucose (83.1 % & 8.7; 10.3 days & 29.3 %) respectively (Table 1).

Early detection of *A. candida*, in infected leaves of rapeseed-mustard

Polymerase chain reaction (PCR)

The primers ITS1 (3'-GAGGGACTTTTGGGTAATCA-5') and Short ITS JV34 (3'-CGCCATTTAGAGGAAGGTGA-5') and JV37 (3'-GTCAAGCAAAACAT-5') were used to amplify the ITS region of *A. candida* and *A. brassicae*. PCR amplification of *A. candida* from inoculated symptomatic and asymptomatic leaves yielded PCR products of 1200 bp and 600 bp of ITS1 and Short ITS primers, respectively whereas no bands were amplified in

Table 3: Disease reactions of *A.candida* on different *B.juncea* lines tested in field* (Pooled data 2015-16 & 2016-17) and glasshouse** (2015-2016)

PRD lines	Disease reactions			PAB lines	Disease reactions			PWR lines	Disease reactions		
		In Glasshouse		lines		In Glass house		lines		In Glasshouse	
	In Field	Coty. stage	True leaf		In Field	Coty. stage	True leaf		In Field	Coty. stage	True leaf
PRD-14-1	MR	S	S	PAB-14-1	S	MS	MS	PWR-14-1	I	S	MR
PRD-14-2	MR	S	S	PAB-14-2	S	S	MS	PWR-14-2	I	MS	MR
PRD-14-3	MR	S	S	PAB-14-3	S	S	S	PWR-14-3	I	R	MR
PRD-14-4	MR	MS	MS	PAB-14-4	S	S	S	PWR-14-4	I	R	MR
PRD-14-5	MR	S	MS	PAB-14-5	MR	MS	MS	PWR-14-5	MR	R	MR
PRD-14-6	MR	S	MS	PAB-14-6	S	S	S	PWR-14-6	I	R	MR
PRD-14-7	S	S	S	PAB-14-7	MR	S	MS	PWR-14-7	I	R	MR
PRD-14-8	MR	S	MS	PAB-14-8	MR	MS	S	PWR-14-8	I	I	R
PRD-14-9	MR	S	MS	PAB-14-9	MR	MS	MS	PWR-14-9	I	I	R
PRD-14-10	S	S	S	PAB-14-10	MR	S	MS	PWR-14-10	I	I	HR
PRD-14-11	MR	MS	MS	PAB-14-11	S	S	S	PWR-14-11	I	I	R
PRD-14-12	MR	S	MS	PAB-14-12	S	S	S	PWR-14-12	I	S	MR
PRD-14-13	MR	MS	MS	PAB-14-13	MR	S	S	PWR-14-13	MR	R	MR
PRD-14-14	MR	MS	S	PAB-14-14	MR	S	MS	PWR-14-14	MR	MS	MR
PRD-14-15	MR	S	MS	PAB-14-15	MR	S	MS				
PRD-14-16	MR	S	MS	PAB-14-16	MR	MS	S				
PRD-14-17	MR	S	MS	PAB-14-17	MR	MS	MS				
PRD-14-18	MR	MS	S	PAB-14-18	MR	S	S				
PRD-14-19	S	S	S	PAB-14-19	HS	HS	HS				
PRD-14-20	S	S	S	PAB-14-20	MR	MS	S				
PRD-14-21	MR	S	MS	PAB-14-21	MR	MS	S				
PRD-14-22	MR	MS	S	PAB-14-22	S	S	S				
PRD-14-23	MR	S	S	PAB-14-23	MR	S	MS				
PRD-14-24	S	S	S	PAB-14-24	MR	S	MS				
PRD-14-25	S	S	S	PAB-14-25	MR	S	MS				
PRD-14-26	MR	MS	MS	PAB-14-26	S	S	S				
PRD-14-27	MR	S	S	PAB-14-27	S	S	S				
PRD-14-28	MR	S	S								
PRD-14-29	MR	S	S								

* 0-9 rating scale, **0-6 rating scale

I=Immune, R= Resistant, MR= moderately resistant, MS=moderately susceptible, S=Susceptible,

Table 4: Disease reaction of *A. candida* on different Brassica germplasm under UDN tested in *field and **glasshouse (2015-16 and 2016-17)

UDN (2015-16)	Disease reactions			UDN (2016-17)	Disease reactions		
	In Field	In Glass house			In Field	In Glass house	
		Cotyldonary leaf	True leaf			Cotyledonary leaf	True leaf
Rohini	MS	MS	MS	PRD 2013-9	S	MS	MS
PHR-2	MR	MS	S	NPJ-177	S	MR	MR
DLSC-1	I	I	I	DRMR-40	S	MR	S
EC 399299	MR	S	S	RMM-09-01-1	MS	MS	MS
RMM 09-01-1	MR	MS	MS	PRD 2013-6	MS	S	MS
DRMR 32	MS	S	S	PDZ-1	MR	MR	MR
PRD 2014-21	S	S	S	PAB-14-3	MS	S	S
PRD 2013-3	S	MS	MS	PRD 2014-22	MS	S	MS
RMWR 09-5-1	R	MS	MS	DRMR-73	MS	MR	MR
DRMRMJA 35	MR	MR	MS	PDZ-3	MS	MS	MS
DRMR 2035	R	MR	MR	PAB-14-26	MS	S	MS
ABS(3)-21	MR	MS	MS	PRE-2011-15	MS	MR	MR
DRMR-73	MS	MR	MS	PAB-14-7	MS	MS	MR
RMM 10-1-1	S	MS	S	PDZ-2	MS	MS	MS
PRD 2013-8	MS	MS	HS	PRD 2014-26	MS	MS	MS
DRMR-2019	MR	MS	S	DRMR-7	S	MS	HS
DRMR-72	MR	S	HS	RMM 10-12-1	S	MS	MS
ABS(3)-16	S	S	MS	PAB-14-11	MS	MR	MS
DRMR-312	I	I	I	DRMR-2019	MS	S	S
NPJ-177	MR	S	MS	PRD 2014-27	MS	S	S
PRD 2013-6	HS	MS	MS	PRD 2013-8	MS	S	S
PRD 2013-9	HS	MS	MS	RMM-09-04	MS	MS	MS
PRD 2013-2	S	MS	MR	DRMRIJ 13-38	MS	S	S
DRMR-40	S	MS	MS	RMM 09-6-1	S	MS	MS
ABS(3)-15	HS	MS	MS	PRD 2014-21	MS	S	HS
RMM 09-1-1-2	S	MS	MS	PDZ-4	S	MS	HS
RMM-09-04	I	MS	S	PRD 2013-3	S	MS	MS
DRMR-316	S	MS	MS	PAB-14-25	MS	MS	MS
PRE-2011-15	HS	S	MS	DRMR-72	S	S	S
RH-1212	HS	S	S	PAB-14-22	S	S	S
DRMR-7	S	MS	MS	DRMR-32	S	MS	MS

-	-	-	-	ABS(3)-15	MR	MS	MS
-	-	-	-	RMT-10-9-1	MR	S	MS
-	-	-	-	DRMR-100	I	I	I
-	-	-	-	RMT-1-10-1	I	MS	MS
-	-	-	-	RH-1212	MR	MS	MS
-	-	-	-	PRD 2014-16	S	MR	MS
-	-	-	-	RMWR-09-5-1	MR	S	S
-	-	-	-	ABS(3)-44	S	MR	MS
-	-	-	-	RMM 09-1-1-2	S	HS	S
-	-	-	-	PRD 2014-1	MR	MS	MS
-	-	-	-	ABS(3)-16	S	S	S
-	-	-	-	DRMR-316	I	I	I
-	-	-	-	DRMR-312	I	I	I
-	-	-	-	Rohini	MS	S	MS
-	-	-	-	EC 399299	MR	MS	S
-	-	-	-	PHR 2	MS	MS	S
-	-	-	-	GSL 1	I	MR	MR
-	-	-	-	DLSC 1	I	I	I
-	-	-	-	YSB 9	MS	MS	S
-	-	-	-	PT 303	MS	MR	MR
-	-	-	-	RTM 314	MR	MS	MR
-	-	-	-	DRMR 1-5	MR	S	S
				DRMR 2-11	MR	MS	MS

* 0-9 rating scale, **0-6 rating scale

I=Immune, R= Resistant, MR= moderately resistant, MS=moderately susceptible, S=Susceptible

Table 5: Disease reaction of *A. candida* on Brassica germplasm under NDN tested in *field and in **glasshouse (2015-16 and 2016-17)

NDN	Disease reactions			NDN	Disease reactions		
	In Field	In Glass house			In Field	In Glass house	
		Cotyldonary leaf	True leaf			Cotyledonary leaf	True leaf
Bio-YSR	R	MR	MR	DRMRIJ 12-39	MR	MR	MR
Rohini	MR	MS	MS	DRMRIJ 12-43	MR	HS	MS
DRMRIJ 12-51	S	MS	S	DRMRIJ 12-51	MS	MR	MR
DRMRIJ 12-02	S	S	MS	DRMRIJ 12-65	MR	MR	MR
DRMRIJ 12-27	MR	MS	MS	DRMR-2035	MR	S	MR
RH 1231	MR	S	MS	DRMRIJ 12-21	HS	MS	MS
DRMRIJ 12-41	R	MS	S	DRMRIJ 12-27	MR	MS	MR
DRMRIJ 12-14	MR	S	S	ABS (3)-21	MR	S	MR
DRMRIJ 12-39	MR	S	S	DRMRIJ 12-48	I	MR	MR
DRMR-2035	MR	S	S	DRMRIJ 12-14	MR	MR	MR
DRMRIJ 12-48	MR	MS	MS	DRMRIJ 12-52	MR	MS	MR
DRMRIJ 12-06	MR	S	S	DRMRAB-7-13	MR	MS	MR
RMWR-09-05-01	S	S	MS	DRMRIJ 15-66	HS	HS	S
DRMRJA 35	R	S	S	DRMRIJ 12-06	HS	S	MR
DRMRIJ 12-44	MR	MR	MR	DRMRIJ 12-26	MR	MR	MR
DRMRIJ 12-03	R	MS	MS	DRMRIJ 12-41	HS	MS	MR
RH 305	MR	S	MS	DRMRIJ 12-37	HS	MR	MR
DRMRIJ 12-21	S	S	S	DRMRAB-7-53	MS	MR	MS
DRMRIJ 12-37	I	S	S	DRMRIJ 12-40	HS	S	S
DRMRIJ 12-26	S	S	MS	DRMRAB-7-233	MS	MR	MS
DRMR-2019	MR	S	S	DRMRIJ 12-28	MR	S	MS
RH 1234	I	MR	MR	DRMRIJ 12-50	MR	MR	MR
DRMRIJ 12-28	MS	MS	MS	DRMR-2019	MS	MR	MR
NDRE-08-14-01	I	S	MS	DRMRIJ 12-44	HS	MR	MR
DRMRIJ 12-65	I	S	S	DRMRIJ 15-85	MR	S	MS
DRMRIJ 12-43	I	S	S	DRMRMJA 35	S	MS	MR
DRMRIJ 12-40	I	S	S	PWR-15-8	MR	MS	MS
-	-	-	-	RMWR-09-5-1	MR	MR	MS

-	-	-	-	PDZ-6	MR	MR	MR
-	-	-	-	NPJ 181	MS	MS	MR
-	-	-	-	RH1234	MR	MR	MR
-	-	-	-	NDRE-08-14-1	MR	MS	MR
-	-	-	-	RLC-5	MR	MR	MR
-	-	-	-	PRD-2013-2	MR	MS	MS
-	-	-	-	RRN-871	MR	MS	MR
-	-	-	-	Rohini	MR	MR	MR
-	-	-	-	RH305	MR	S	S
-	-	-	-	RMWR-09-5	MR	MS	MS
-	-	-	-	RH1231	MR	MR	MR
-	-	-	-	RMM-09-10	MR	MR	MR
-	-	-	-	RL-JEB-52	MR	MR	MS
-	-	-	-	RH-1209	MR	MR	MS
-	-	-	-	PDZ-5	MR	MR	MR
-	-	-	-	Rohini	MR	MS	MS
-	-	-	-	Bio-YSR	MR	MR	MR

* 0-9 rating scale, **0-6 rating scale

I=Immune, R= Resistant, MR= moderately resistant, MS=moderately susceptible, S=Susceptible

Table 6: *In vitro* effect of fungicides and garlic bulb extract on sporangial germination of *A.candida* (Pooled data 2015-16 and 2016-17)

Treatment	Concentration							
	25 ppm		50 ppm		75 ppm		100 ppm	
	Germination (%)	Inhibition (%)	Germination (%)	Inhibition (%)	Germination (%)	Inhibition (%)	Germination (%)	Inhibition (%)
Metalaxyl 64%+ Mancozeb 8% WP	17.0(24.4)	77.0(61.3)	13.8(21.8)	81.4(64.5)	11.7(20.0)	80.1(63.6)	7.8(16.2)	87.8(69.6)
Mancozeb 75% WP	37.6(37.8)	49.8(44.9)	34.1(35.7)	53.3(46.9)	30.0(33.2)	46.5(42.9)	22.8(28.6)	63.2(52.7)
Propiconazole 25 % EC	27.6(31.7)	62.2(52.0)	27.0(31.3)	62.5(52.2)	24.3(29.5)	58.9(50.2)	20.7(27.1)	67.8(55.5)
Tebuconazole 50% Trifloxystrobin 25% WG	28.1(32.0)	53.3(46.9)	27.0(31.3)	62.5(52.3)	23.7(29.1)	51.9(46.1)	20.9(27.2)	62.7(52.5)
Difenoconazole 25% SC	40.8(39.7)	45.0(42.1)	36.1(36.9)	50.4(45.2)	31.6(34.2)	46.2(42.8)	25.5(30.3)	58.9(50.2)
Tebuconazole 25.9 % EC	41.6(40.2)	40.6(39.6)	36.0(36.4)	50.8(45.2)	31.7(34.2)	55.9(48.4)	26.2(30.8)	64.1(53.2)
Hexaconazole 5% SC	42.9(40.9)	39.9(39.1)	37.8(38.9)	48.2(44.0)	32.7(34.9)	54.1(47.4)	26.8(31.2)	63.8(53.0)
Azoxystrobin 25 % SC	17.8(25.0)	79.3(62.9)	16.4(23.9)	80.0(62.0)	13.4(21.5)	83.2(65.8)	7.3(15.7)	86.9(68.7)
Trifloxystrobin 50% WG	19.4(26.1)	72.5(58.4)	15.1(22.9)	78.9(62.6)	12.2(20.4)	83.1(65.7)	10.8(19.1)	84.7(67.0)
Kresosim methyl 44.3% SC	21.4(27.5)	64.7 (53.5)	19.2(26.0)	74.7(59.9)	15.9(23.5)	78.9(62.7)	13.4(21.4)	79.7(63.3)
Cymoxanil 8% + Mancozeb 64% WP	49.1(44.5)	28.1(32.0)	47.1(43.3)	35.5(36.6)	44.5(41.8)	38.5(38.4)	45.5(42.4)	37.6(37.8)
Fosetyl-aluminum 80 % WDG	49.3(44.6)	28.2 (31.8)	48.1(43.9)	34.1(35.7)	47.7(43.7)	34.4(35.9)	45.8(42.6)	36.1(36.9)
Dimethomorph 50% WP	49.9(45.0)	27.9(32.1)	48.4(44.1)	33.6(35.4)	47.4(43.5)	32.8(36.1)	46.8(43.1)	35.8(36.7)
	100 ppm		**200 ppm**		**300 ppm**		**400 ppm**	
Garlic	39.1(38.7)	42.0(40.3)	35.6(36.6)	51.3(45.8)	31.0(33.8)	56.7(48.8)	24.5(29.7)	66.8(54.8)
Check	75.0(60.0)	0.0(0.0)	73.2(58.3)	0.0(0.0)	74.4(61.0)	0.0(0.0)	75.8(60.6)	0.0(0.0)
CD 5%	1.79	7.78	2.20	2.09	1.55	10.91	2.20	6.47
CV	2.27	8.59	6.92	5.14	8.17	11.08	7.32	6.06

Values in parentheses are angular transform value

Observations were recorded 12 hrs after incubation at 10^0C

Table 7: Effect of fungicides and garlic extract on incubation period, latent period and per cent disease of *A. candida* in glasshouse (Pooled data of 2015-16 and 2016-17)

Treatment	IP (Days)	LP (Days)	Per cent disease index Days after inoculation (DAI)			% disease inhibition
			10	15	20	20 DAI
Metalaxyl 64%+ Mancozeb 8% 72 (0.25)%	0.0	0.0	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	100.0 (90.0)
Mancozeb (0.2)%	11.7	12.7	0.0 (0.0)	11.8 (19.5)	12.9 (21.3)	77.2 (66.1)
Propiconazole (0.1)%	19.7	21.7	0.0 (0.0)	0.0 (0.0)	6.2 (14.0)	95.4 (82.8)
Tebuconazole 50% Trifloxystrobin 25% (0.1)%	18.7	22.7	0.0 (0.0)	0.0 (0.0)	5.2 (12.7)	97.1 (84.3)
Tebuconazole (0.1)%	9.6	11.7	0.0 (0.0)	20.6 (26.6)	22.8 (29.2)	42.1 (40.4)
Kresosim methyl (0.1)%	19.7	20.7	0.0 (0.0)	0.0 (0.0)	6.9 (14.5)	54.5 (47.6)
Hexaconazole (0.1)%	9.7	11.7	0.0 (0.0)	26.9 (31.6)	36.8 (38.2)	31.1 (33.3)
Azoxystrobin (0.1)%	0.0	0.0	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	100.0 (90.0)
Trifloxystrobin (0.1)%	19.7	22.7	0.0 (0.0)	0.0 (0.0)	6.0 (14.1)	92.3 (80.4)
Difenoconazole (0.1)%	10.7	12.7	0.0 (0.0)	15.8 (22.0)	29.7 (32.0)	44.2 (42.3)
Dimethomorph (0.1)%	11.7	13.7	0.0 (0.0)	18.7 (27.3)	28.0 (33.3)	43.2 (41.1)
Fosetyl-aluminum 80 % WDG (0.1)%	11.7	12.5	0.0 (0.0)	20.4 (30.7)	31.4 (37.6)	25.3 (30.0)
Cymoxanil 8% + Mancozeb 64% 72 WP (0.1)%	9.7	11.7	0.0 (0.0)	19.4 (26.8)	31.0 (34.1)	31.5 (33.9)
Garlic (2%)	17.7	19.0	0.0 (0.0)	12.0 (22.0)	14.9 (24.9)	65.5 (54.1)
Check (water spray)	6.69	8.67	25.3 (30.4)	34.4 (36.3)	43.7 (42.0)	0.0 (0.0)
CD 5%	1.48	0.89	8.87	0.67	1.08	8.37
CV	5.14	4.30	12.19	6.67	7.95	15.75

Values in parentheses are angular transform value
0-6 rating scale

A. brassicae. This confirmed the presence of *A. candida* in asymptomatic inoculated leaves at early stage i.e. 1, 2, 3, 4, 5 and 6 DAI (Table 2). The PCR based assays was also used by earlier workers to detect the pathogen from asymptomatic infected plant tissue (Lovic *et al*., 1995; Jacobson *et al*., 1996) as well as obligate pathogens (Mutasa *et al*., 1995; Tham *et al*., 1994).

Light microscopy

The presence of pathogen structure i.e. mycelium and sporangia in inoculated symptomatic and asymptomatic leaves were observed. In inoculated asymptomatic leaves the pathogen structures were observed at 6 days after inoculation, in inoculated symptomatic leaves at 10 and 11 days after inoculation where as no fungal structure was observed in healthy mustard leaves after staining with 1 percent cotton blue in lacto phenol and 0.4% trypan blue (Table 2). Still there is the need to standardize the staining techniques for the early detection through light microscopy. The detection of pathogen structure using light microscopy from asymptomatic infected leaves in rapeseed-mustard plants were also observed after staining (Giri *et al*., 2003 and Massand *et al*., 2010).

Evaluation of rapeseed-mustard genotypes at cotyledonary and true leaf stage for resistance under artificial epiphytotic field and glasshouse condition in Field

Evaluation of Brassica materials for resistance sources

In field among various Brassica materials PWR-14-1, PWR-14-2, PWR-14-3, PWR-14-4, PWR-14-6, PWR-14-7, PWR-14-8, PWR-14-9, PWR-14-10, PWR-14-11, PWR-14-12 (*B. juncea* lines), DLSC 1, DRMR-312, RMM-09-04, DRMR-316, DRMR-100, RMT-1-10-1, GSL 1, DRMRIJ 12-37, RH 1234, NDRE-08-14-01 and DRMR-IJ 1 were found free from the white rust disease (Table 3,4 &5). Based on field studies the Brassica genotypes viz. HC-l, PCC-l (*B. carinata*), GSL-1501 (*B. napus*), EC-129126-1 and Shiva (Saharan and Krishnia, 2001); *B. napus* genotypes viz. EC-338997, PBN-2001, EC-339000, PBN-2002, and DGS-1 and *B.carinata* cv. PBC-9921 (Gupta *et al*., 2002) and *B. juncea* genotypes viz. CBJ-001, CBJ-003 and CBJ-004 (China) JM06011 (Australia) (Kumar and Kalha, 2005; Li *et al*., 2008, 2009; DRMR, 2011; Meena *et al*., 2011) were reported free from white rust disease.

Glasshouse studies

In glasshouse among various *Brassica* genotypes including DLSC-1, DRMR-312, DRMR-316, DRMR-100, RH 1234 and NDRE-08-14-01, NBPGR-15, NBPGR-353, NBPGR-354, NBPGR-355, IC 313379, IC 317528, IC 298024 and IC 420528, EC-399299 were free from the disease at both the stages i.e. at cotyledonary and true leaf stage. Katili local, *E. sativus*, Basanti and Banarasi rai, PWR-14-8, PWR-14-9, PWR-14-10, PWR-14-11 and RMT-1-10-1 were free from the disease only at cotyledonary stage but susceptible at true leaf stage (Table 3, 4 & 5). On the basis of glasshouse studies at cotyledonary stage earlier workers reported *B. juncea* viz. CBJ-001, CBJ-003, CBJ-004, JM06011, JM06010, JM06021, JM06004, and JM06013 (*B. juncea*) as resistant sources (Li *et al*. 2008). EC 399301 and EC 399299 showed resistant reaction only at true leaf stage (Mishra *et al*., 2009) while *B. juncea* accessions viz. RESJ-1033 and RESJ-1051 showed resistant reaction at the cotyledonary stage but susceptible at true leaf stage (Awasthi *et al*., 2012).

Evaluation of fungicides and garlic extract for management of white rust disease

Among the tested fungicides and garlic extract, Metalaxyl 8%+ Mancozeb 64% (0.25%) and a new fungicide which is a biological origin i.e. Azoxystrobin 25 EC (0.1%) were highly effective in controlling white rust disease (no occurrence of disease) followed by Propiconazole, Tebuconazole 50% + Trifloxystrobin 25%, Trifloxystrobin, Kresosim methyl (each with 0.1%) and Garlic extract (2%). However, Cymoxanil+Mancozeb, Hexaconazole, Difenoconazole, Dimethomorph and Fosetyl-aluminum (each with 0.1%) were found least effective in managing white rust disease *in vitro*, glasshouse as well in field (Table 6). Metalaxyl 8%+Mancozeb 64% (Ridomil MZ @ 0.25%) also reported as most effective fungicides by earlier workers in managing white rust disease in field (Bhatia and Gangopadhyay, 2002; Ahmed and Srivastava, 2003; Mehta *et al*., 2005; Biswas *et al*., 2007; Meena *et al*., 2011). In the present investigation garlic bulb extracts was also found effective in managing white rust disease which is in agreement with the work of Chattopadhyay *et al*. (2005), who reported garlic bulbs extract a better choice than fungicides in the management of oilseed crops diseases including white rust.

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