Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2019; 8(2) 2265-2267
Received: 21-01-2019
Accepted: 25-02-2019

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Effect of artificial inoculation methods for disease development of downy mildew and white rust in *Brassica juncea* (L.)

S Ravi and RP Awasthi

Abstract
Per cent disease index DM: for downy mildew, soil+spray treatment was most effective method given highest per cent disease index (48.33 %) lowest in seed (27.67 %). PDI (WR): For white rust disease index was highest in soil+spray (57.67%) at 21 DAI (days after inoculation) and minimum seed + soil (48.33 %). In case of soil+spray maximum (44.67 %) at 14 DAI, and minimum seed + soil (35.33 %). It was found that combination treatments were effective at both 14 &21 DAI. Incubation period (IP), was most effective in combination soil+ spray treatment for both DM and WR (most effective for disease appearance) and was followed by lesser for in other seed +spray treatment compared to soil +spray. Alone spray treatment incubation period is most effective and followed by soil than seed treatment had much (IP) and found least effective. WR pustule size: At 14 DAI, the pustule size were small (S:0.5-1.0 mm) in alone treatments viz., seed, soil and spray; medium in soil+spray, seed+spray and seed+soil mixed treatments. At 21 DAI, the pustule size went to medium(M: 1-3mm) in seed, soil and spray and became large (L: 3-4mm) in case of mixed viz., soil+spray, seed +spray and seed+soil indicated as effective treatments to increase disease indices.

Keywords: mustard, *Brassica juncea*, *Hyaloperonospora brassicae*, *Albugo candida*

Introduction
Rapeseed-mustard are important oilseed crops throughout the world which rank third after oil palm and soybean in production of vegetable oils while second in the production of oilseed proteins after soybean (USDA, 2011) [15]. The characteristic symptoms as white to cream yellow raised pustules by white rust (WR) and downy growth with necrotic lesions by downy mildew (DM) on the abaxial surface of leaf. These two diseases associated for causing severe infection on rapeseed-mustard. Severe infection at flowering stage, often in association with *Albugo candida* and *Hyaloperonospora brassicae* causes extensive distortion, hypertrophy, hyperplasia and sterility resulting inflorescence malformation called "staghead" (Awasthi *et al.*, 1997 b) [3]. In toria loss due to white rust is more if associated with downy mildew (Kolte, 1985a) [9]. Yield losses caused by WR or a mixture of WR and DM, range between 17% to 60%, (Harper and Pittman, 1974; and Kolte *et al.*, 1981) [7, 8]. Depending on the severity of both foliar and staghead phase of the disease, the per cent yield losses ranging from 23-89.8 % in Indian mustard [*B. juncea* (L.) Czern and Coss] in India (Bains and Jhooty, 1979; Lakra and Saharan, 1989a) [4, 10]. Identified several genotypes with stable resistance, but very few have been utilised for developing WR resistant cultivar. Thus, understanding of *Brassica* genotypes by *A. candida* interactions is of vital importance in identifying resistant genotypes for specific adaptability. GSL-1, EC-414299 and EC-399299 showed additive gene for horizontal resistance to WR which can prove good donors in further genetic improvement programmes. Varuna, JMM 07-2, JMM 027-1 and JYM 10 had non-additive gene action for pathogenicity to WR. PBC- 9221, GSL 1, EC- 414299 and EC- 399299 were very similar in genetic make-up for disease resistance while Varuna showed maximum divergence in genetic constitution from these strains (AICRPRM, 2009) [1].

Materials and Methods
The experiment was conducted at Oil seed laboratory and Glass house- Plant Pathology, College of Agriculture, GBPUA&T, Pantnagar. Suitable methods of inoculation for test pathogen, an inoculation was prepared, infected staghead samples were collected from *B. juncea* plants grown at NEB-CRC, Pantnagar. For inoculations materials were prepared alone: seed, soil and spray, in combination: seed+ soil, soil+spray, seed+spray and including check.

Artificial inoculation through oospores from staghead for pathogenicity

1. **Inoculum preparation:** In order to determine the suitable method of inoculation for test pathogens, an inoculum was prepared as per Singh *et al*. (1999) [14] and Armstrong (2007) [2]. Heavily infected sporulating inflorescences (Staghead) were collected from mature *B. juncea* cv. Varuna. Infected material was stored in sealed plastic bags at 4^0C before use. The sporulating material (1gm) was macerated in sterile distilled water (50ml) using a household blender to from an aqueous suspension and collected in Erlenmeyer flask. It was further filtered through double layered muslin cloth. The resulting suspension was used for inoculation after incubating it for 1 week at 16^o C and vigorously shaking it at frequent intervals.
2. **Inoculation methods:** For seed inoculation, 10 ml of spore suspension was mixed with a few gm of seed then placed in filter paper disc, inside vacuum filter apparatus. After the water dries up in the paper disc, the inoculated seeds were sown about 1 cm deep in autoclaved soil placed in 5 cm diameter pots as per treatment. For soil inoculation, the suspension was added to the soil (10 ml/100gm soil). For spray inoculation, the suspension was sprayed on the surface of each cotyledon (7 DAS) to run off with an atomizer. Mixture treatments were applied similarly and an untreated control without any inoculation was used as check. Three replications were maintained for each treatment. The potted plants were kept in humid chamber for 3 days and then maintained under controlled at glasshouse conditions at 95 % relative humidity.
3. **Observations**

a) Per cent disease index(PDI): At 10 days after inoculation (DAI) (for downy mildew) and 14 and 21 DAI (for white rust) the disease rating on cotyledonary and true leaves were recorded on the basis of (0-9) rating scale for both *H. brassicae* and *A. candida* as described by Nashaat and Awasthi (1995) [12].

The disease index was calculated as:

$$\text{Disease index} = \frac{\text{Sum of all numerical rating}}{\text{Number of leaves examined} \times \text{Maximum grade (9)}} \times 100$$

b) Incubation period: Plants were observed daily to record disease reaction. The incubation period was considered as the time period in days between inoculation to the first appearance of DM/WR symptoms on cotyledons.

c) Pustule size (WR): The size of pustules on cotyledonary leaves were recorded based on observations of at least five such pustules from each treatment. These were further represented as S (Small=0.5-1.0 mm); M (Medium=1-3mm); L (Large=3-4mm) and PP/N (Pin pointed or Necrotic fleck-if pustule is not evident).

Statistical analysis, the data obtained under glass house condition were analysed using completely randomized design (CRD).

Results and Discussion

Artificial inoculation through oospore from staghead for pathogenicity: Various inoculation methods were presented in (Table 1. and Fig1.).

Per cent disease index(DM): For Downy mildew, soil+spray treatment was most effective method given highest per cent disease index (48.33 per cent) for DM followed by seed +spray (43.67 per cent) and minimum in Seed (27.67 per cent).

Per cent disease index (WR): For white rust disease index was highest in case of soil+spray (57.67 per cent) at 21 DAI (days after inoculation). Seed+spray (51.67 per cent) at 21 DAI and seed + soil (48.33 per cent) at 21 DAI. In case of soil+spray (44.67 per cent) at 14 DAI, followed by seed+spray (39.33 per cent) and seed + soil (35.33 per cent) treatments. It was found that white rust PDI combination treatments were effective at both 14 &21 DAI.

Incubation period(IP): Incubation period, was most effective in case of soil+ spray treatment for both DM and WR most effective for disease appearance and was followed by lesser for in seed +spray treatment compared to soil +spray. Alone spray treatment incubation period was effective and followed by soil and seed treatments had much IP and found least effective.

WR pustule size: At 14 DAI, the pustule size were small (S:0.5-1.0 mm) in alone treatments viz., seed, soil and spray; in medium soil+spray, seed+spray and seed+soil mixed treatments. At 21 DAI, the pustule size went to medium(M: 1-3mm) in seed, soil and spray treatments and became large (L: 3-4mm) in case of mixed treatments viz., soil+spray, seed +spray and seed+soil indicated them as effective treatments to increase disease indices.

Table 1: Effect of artificial inoculation through (oospore) on white rust (WR) and downy mildew (DM)

Treatments	Incubation Period		Per cent Disease Index (PDI)			WR Pustule Size	
	DM	WR	DM (*10 DAI)	WR (*14 DAI)	WR (*21 DAI)	14 DAI	21 DAI
Seed	11	12	27.67(31.73)	26.33(30.87)	31.33(34.04)	S	M
Soil	11	12	28.33(32.16)	26.67(31.09)	35.33(36.47)	S	M
Spray	9	11	30.33(33.42)	28.33(32.16)	38.33(38.25)	S	M
Seed+Soil	10	11	40.33(39.43)	35.33(36.47)	48.3344.04)	M	L
Soil+Spray	10	12	48.33(44.04)	44.67(41.94)	57.67(49.41)	M	L
Seed+Spray	11	11	43.67(41.36)	39.33(38.84)	51.6745.96)	M	L
Check	_	_	0.00(0.00)	0.00(0.00)	0.00(0.00)	_	_
CD at 5 %	Between different treatment		0.54 (0.33)				
	Between DAI		0.35 (0.21)				
	Interaction		0.95 (0.57)				

- *Means of three replication; DAI: Days after inoculation;
- L=Large (3-4mm); M=Medium (1-3mm); S=Small (0.5-1.0mm).
- () Values in parentheses are angular transformed.

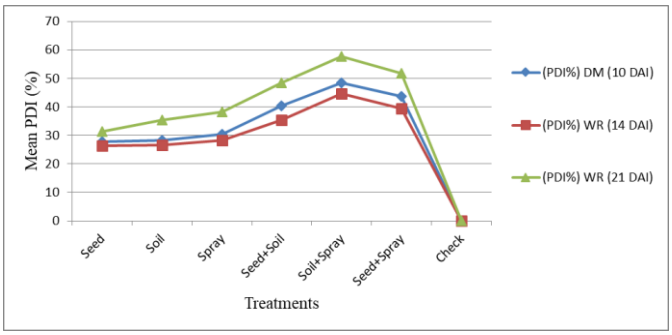

Fig 1: Effect of artificial inoculation through (oospore) on white rust and downy mildew

Petrie and Verma (1974) [13] described a reliable and reproducible technique for inducing maximum germination of *Albugo candida* oospores. They found that maximum germination of *A. candida* oospores was 88 per cent at 13˚C from hypertrophied inflorescences of *B. campestris* and were able to germinate 71 per cent of the oospore within two weeks of collection from the field. Verma and Bhowmik (1988) [16] observed that treatment of oospores with 200 ppm $KMnO_4$ for 10 minutes induces increased germination. Lakra and Saharan (1989b) [11] located and estimated amount of oospore of *A. candida* in infected tissues after grinding and suspending 1 g of infected material in known quantity of water. They found that oospores were formed in only mature hypertrophied cup shaped leaves contained (8.75×10^5) oospores whereas they were more in hypertrophied staghead portions (21.85×10^5). Armstrong (2007) [2] also used oospores for inoculation after macerating and blending sporulating material to form an aqueous suspension to study *A. candida* Pathology in *L. oleraceaum.* Goyal *et al*. (1996) [6] also done artificial inoculation sites of *A. candida* in *Brassica juncea*; based on plant age and incubation condition for staghead formation. Bhatt (2012) [5] also observed that disease index in combination: soil+spray for DM maximum at 10 DAI and for WR 14 DAI and 21 DAI; for DM minimum seed+soil 10 DAI, for WR seed+spray 14 DAI and seed+soil 21 DAI. In alone: disease index minimum in soil treatment for DM 10 DAI; for WR minimum in seed at 14 and 21 DAI. For DM maximum in spray treatment 10 DAI and WR spray 14 and 21 DAI.

Acknowledgement
Our special thanks to the oilseed lab- and Officer in charge, glass house- Department of Plant Pathology, College of Agriculture, G. B. Pant University of Agriculture and Technology, Pantnagar, U.S. Nagar, Uttarakhand.

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