RESEARCH ARTICLE

PATHOGENICITY TEST BY USING ARTIFICIAL INOCULATION METHODS AND ECO-FRIENDLY MANAGEMENT OF *ALBUGO CANDIDA* ON *BRASSICA JUNCEA* UNDER PUNJAB REGION

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Abstract: The oilseed crops especially rapeseed-mustard play a vital role in agricultural economy of the world. Diseases of plant pathogens are considered an important biotic constraint, which leads to significant yield losses of crop world-wide. Of all the agricultural pests and diseases that threaten mustard crop, white rust is one of the most devastating. White rust of mustard is caused by an obligate biotrophic fungus *Albugo candida*. Under technique of detached leaf inoculation, three method were used viz., spore suspension solution spray method, directly spores picking method and inoculation with infected leaf method under laboratory conditions. Directly spores picking method has found to be finest and swift method. The conventional technique like spraying method has the disadvantage of causing considerable variation in spores distribution. *Trichoderma viride* was found most effective in Pre Treatment than Treatment After Disease Infection while neem oil was less effective in Pre Treatment.

Keywords: *Albugo candida, Brassica juncea*, Eco-friendly management, Pathogenicity, *Trichoderma viride*

INTRODUCTION

Rapeseed- mustard (Brassica species) is the third most important oilseed crop after soybean and groundnut, contributing nearly about 20-25 percent of the total oilseed production in the country. In India, rapeseed-mustard occupy approximately 22.2 % of total oilseeds cultivated area and approximately 32 % of the country's total oilseed production, with an area of 6.00mha, production of 8.04mt as well as yield of 1339 kg/ha during 2017-18 (Anonymous, 2018). In India rapeseed- mustard is one of the most important crop and major source of edible oil and oil content varying from 35-49 percent. Biotic stresses are diseases and pests and abiotic stresses are environmental factors like temperature, humidity and rainfall.

Indian mustard (*B. juncea*) is a major oilseed crop of Punjab. Rapeseed and mustard were grown on 30.5 thousand hectares during 2017-18 with production of 45.7 thousand tonnes and an average yield of 14.98 quintals per hectare (PAU Rabi Package Practices 2019-20). In Punjab, the area under cultivation of rapeseed- mustard has been decreased from the last 10 years. This is due to the fact that the alternate rabi season crop of wheat is more profitable because of brassica crops are more vulnerable to numerous biotic and abiotic stresses. Diseases of plant pathogens are considered an important biotic constraint, which leads to significant yield losses of crop world-wide. White rust [*A. candida* (lev.) Kuntze] have been reported to be most wide spread and destructive fungus diseases of mustard all over the world (Kolte, 1985). Bal and Kumar (2014) reported that, the average loss of seed yield due to white rust was estimated to be 36.88 per cent.

White rust of mustard is caused by an obligate biotrophic fungus *A. candida* has been referred to as white blister rust (Holub *et al.*, 1995 and Kaur *et al*., 2011). Singh and Singh (1983) maximum disease intensity was obtained when mustard plant was inoculated with sporangia germinated at 10°C and applied to the surface of lower leaf and the plants kept covered. Kumar *et al*., (1995) studied factors involved in the development of white rust disease of mustard under Hisar (Haryana) conditions and their reports revealed that older leaves were more susceptible than younger leaves. Older leaves showed symptoms 4 days after inoculation with the disease intensity of 11.2 per cent compared with younger leaves, which showed symptoms 6 days after inoculation with disease intensity of 5.7 per cent. On lower surface, the symptoms appeared 3 days after inoculation while on upper surface in 5 days after. Detached leaf inoculation method has been found to be the finest and swift method. The conventional technique like spraying method has the disadvantage of causing considerable variation in spores distribution (Tuite, 1969).

The obligate biotrophic fungus (*A. candida*) highly variable nature of the pathogen poses a challenge for the eco-friendly management of the disease. The toxin substances obtained from different species of plant, manage a different fungal diseases of crop plants (Raghav, 2003 and Shakywar *et al.*, 2012). Seed treatment with *T. viride* (1%) + foliar spray of copper oxychloride (0.25%) was found best for management of white rust disease and also increase the seed yield (Gopi *et al.*, 2016). In this report, to develop precision, sensitivity and for testing a comparison was carried out among three different artificial inoculation methods under the technique of detached leaf

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inoculation namely spore suspension solution spray method, directly spores picking method and inoculation with infected leaf method under laboratory conditions for Pathogenicity studies of *A. candida* on *B. juncea* (Indian mustard) and its eco-friendly management.

MATERIALS AND METHODS

Isolation and proving Pathogenicity: To confirm the pathogenicity of the fungus causes white rust on rapeseed-mustard crop. "Koch postulates" was employed for proving the pathogenicity of *A. candida* by different methods such as in screen house conditions in pots and artificial detached leaf inoculation method under laboratory conditions at Guru Kashi University Talwandi Sabo, Bathinda (Punjab), India.

Pathogenicity test in Screen house:

Seeds of susceptible mustard cultivar varuna were sown (@15 seeds / pot) in twelve (12) earthen pots (30 cm dia.) filled with steam sterilized potting mixture of soil: sand: FYM (2:1:1). Nine to ten healthy growing mustard seedlings /pot were maintained, watered regularly and kept in screen house for further growth. Mustard leaves showing typical symptoms of white rust disease were brought from the field to the laboratory and spore suspension was made in sterilized petri dishes containing sterilized water by crushing 10 fully infected leaves of white rust in 20 ml of sterilized water. The zoospore suspension (2.5×10^5 /ml) of each isolate was adjusted with the help of hemocytometer and sprayed. The obtained spore suspension was inoculated by spraying on the healthy plants of varuna with the help of hand atomizer during evening hours, spores were also dusted with Camel hair brush and also infected leaf with white rust disease was directly stapled (cello tape method) on lower side of the experimentally tested plant. The healthy plants of the varuna cultivar sprayed with water were maintained as control. All the artificially inoculated plants were covered with polythene bags to maintain humidity. Next day morning pots were watered. This practice of watering continued for a week. These plants were observed daily till, the typical symptoms were developed of white rust disease on experimentally treated plants.

Detached leaf inoculation: In laboratory experiment, leaves were detached from the susceptible cultivar of mustard washed under running tap water followed by a wash with autoclaved water and then surface was wiped off with 70% ethanol. Under technique of detached leaf inoculation, three method were used viz., spore suspension solution spray method, directly spores picking method and inoculation with infected leaf method under laboratory conditions. Twenty five times, minute injury create on healthy leaves with the help of sterilized blade and spore suspension solution was sprayed on detached leaves with the help of atomizer in spore suspension solution spray method. In inoculation with infected leaf method, twenty five times, minute injury create on healthy leaves with the help of sterilized blade and infected leaves of white rust placed on upper side of injured leaves. First step was same above two methods and followed by pick the spores from infected leaves of white rust with the help of inoculating needle and spores are placed on injured portion of healthy leaves in directly spores picking method. Detached leaves were kept in petri dishes with moistened filter paper and placed in BOD incubator at 10°C and 70% relative humidity for the sporangial germination. Wetness of filter paper was maintained with spraying autoclaved distilled water. The petri dishes were observed for *A. candida* initial symptoms at intervals of 12 hrs after pathogen inoculation, after that petri dishes of the artificially treated detached leaves remove from the BOD incubator and placed at Ambient temperature. Artificially infected leaves were examined up to 24 hrs after pathogen inoculation. The numbers of germinated disease spores were counted on the detached leaves in all the inoculation methods at third day after inoculation of pathogen while the control remains free from severe symptoms. The obtained symptoms were compared with the infected leaf for confirmation. Directly spores picking method also applied on lower surface of older and younger leaves of mustard placed on wet paper under the petri dishes.

Eco-friendly management of white rust caused by *A. candida*

In vitro, directly spores picking method is used to check the antifungal activity of biocontrol agent (*T.viride*) and botanical neem oil (Azadirachtin 0.03%), at different concentrations (125, 250, 500, 1000 ppm) on sporangial germination of *A. candida* under two type of treatments, nine petri dishes were selected for pre treatment (eight for treatment and one for control) four petri dishes were selected for each treatment, one petri dishes for one concentration and control was same in all treatments. In treatment after disease infection nine petri dishes (eight for treatment and one for control) were also selected in 25 isolated petri dishes on the basis of nearly same percentage of sporangial germination to find better result.

PT - Pre treatment (Management of before disease infection / before sporangial germination)

TADI - Treatment After Disease Infection (Management of after sporangial germination).

Percent of Sporangial germination and Percent of Inhibition in each treatment was calculated by using following formula:

Percent sporangial germination (%) =

$$\frac{\text{Number of sporangia inhibition}}{\text{Total number of sporangia}} \times 100$$

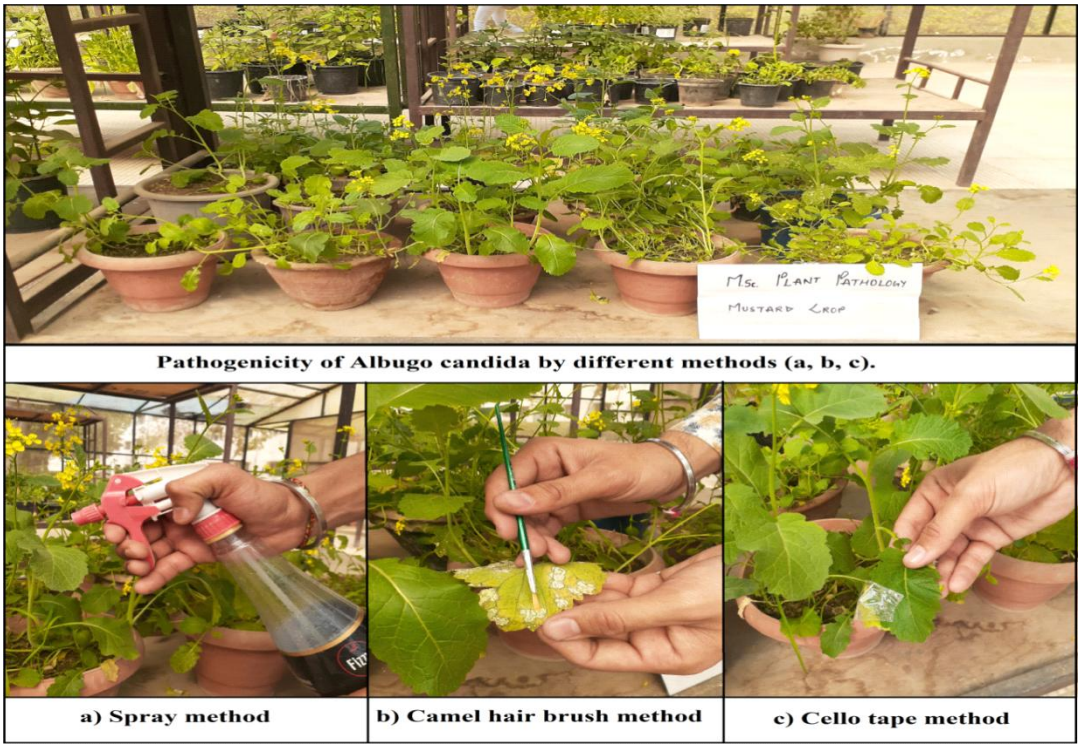

Fig. 1 Pathogenicity of *A. candida* by different methods

RESULTS AND DISCUSSION

Pathogenicity of *A. candida* was proved on the susceptible cv. Varuna of mustard under controlled conditions in the screen house. The typical symptoms of small, round whitish spots on lower surface of the leaves were observed that were similar to those occurring on naturally infected plants in one week while the control remains free from severe symptoms. Thus, pathogenicity of the test pathogen was proved, based on the typical symptoms observed on naturally and artificially infected mustard plants and microscopic observations of test pathogen it was identified and confirmed as *A. candida*. Microphotograph of spores of *A. candida* taken from typical symptoms observed on naturally infected plants are presented in (Fig. 2).

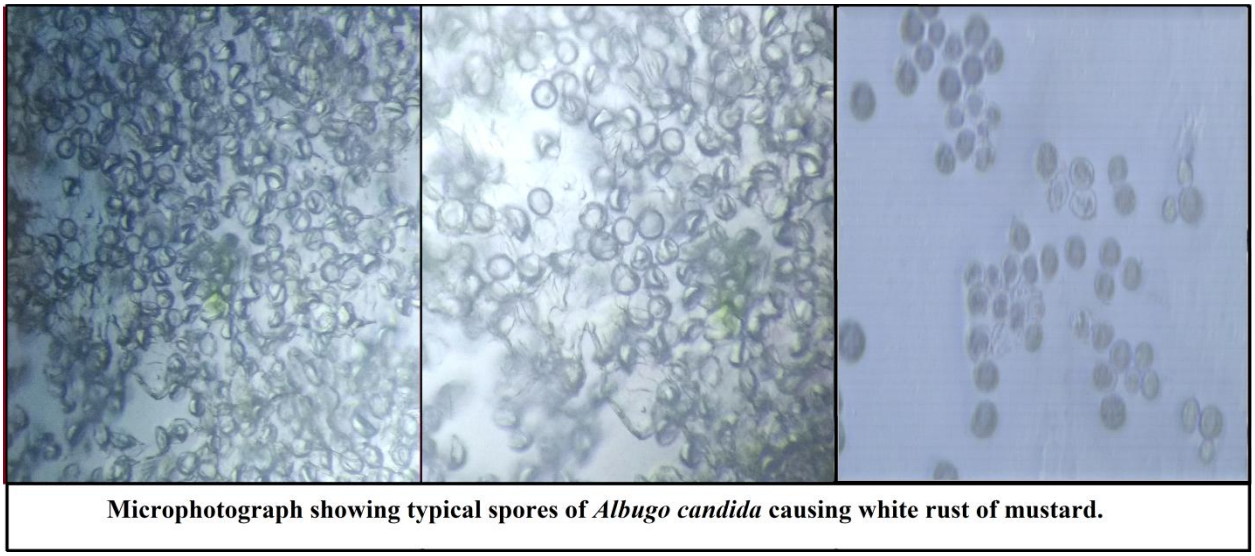

Fig. 2 Microphotograph showing typical spores of *A. candida* causing white rust of mustard

Artificial detached leaf inoculation**,** leaves were detached from the susceptible cultivar (varuna) of mustard washed under running tap water followed by a wash with autoclaved water and then surface was wiped off with 70% ethanol, three methods are used viz., spore suspension solution spray method, directly spores picking method and inoculation with infected leaf method under laboratory conditions. Results (fig.

3) revealed that, directly spores picking method has been found to be the best and swift method for sporangial germination (56%) while both spore suspension solution spray method and inoculation with infected leaf method sporangial germination (28%). Directly spore picking method also applied on lower surface of older and younger leaves of mustard and (fig. 4) found that older leaves were more susceptible than younger leaves. Older leaves with the (73.33%) sporangial germination compared with younger leaves (62.67 %) sporangial germination. Similar results regarding pathogenicity were earlier reported by Pandey *et al.*, (2013) and kumar *et al.*, (1995).

Fig. 3 Artificial inoculation of *A. candida* on detached leaves by different methods

Fig. 4 Directly spores picking method on younger and older leaves of mustard

Biocontrol agent (*T. viride*) result in pre treatment overall mean (61%), treatment after disease infection overall mean of inhibition (40.24%) and botanical (Neem oil) result in pre treatment overall mean (26%), treatment after disease infection overall mean of inhibition (32.97%) were evaluated. *T. viride* was found most effective in pre treatment than treatment after disease infection while neem oil was less effective in pre treatment. The data is summarized in Table- 1, 2 and Fig. 5.

Table 1 Effect of bio-agent and botanical on sporangial germination of *Albugo candid*a in Pre Treatment

S. No.	Treatments	Pre Treatment of disease infection (% inhibition of sporangial germination)				Overall mean %
		Concentration (in ppm)				
		125	250	500	1000	
1	*Trichoderma viride*	44	60	64	76	**61**
2	Neem oil	16	20	28	40	**26**
3	Control	0				0

Table 2 Effect of bio-agent and botanical on sporangial germination of *A. candid*a in Treatment After Disease Infection

S. No.	Treatments	Treatment After Disease Infection (% inhibition of sporangial germination)				Overall mean %
		Concentration (in ppm)				
		125	250	500	1000	
1	*Trichoderma viride*	14.29	26.67	46.67	73.33	**40.24**
2	Neem oil	13.33	21.42	40	57.14	**32.97**
3	Control	0				0

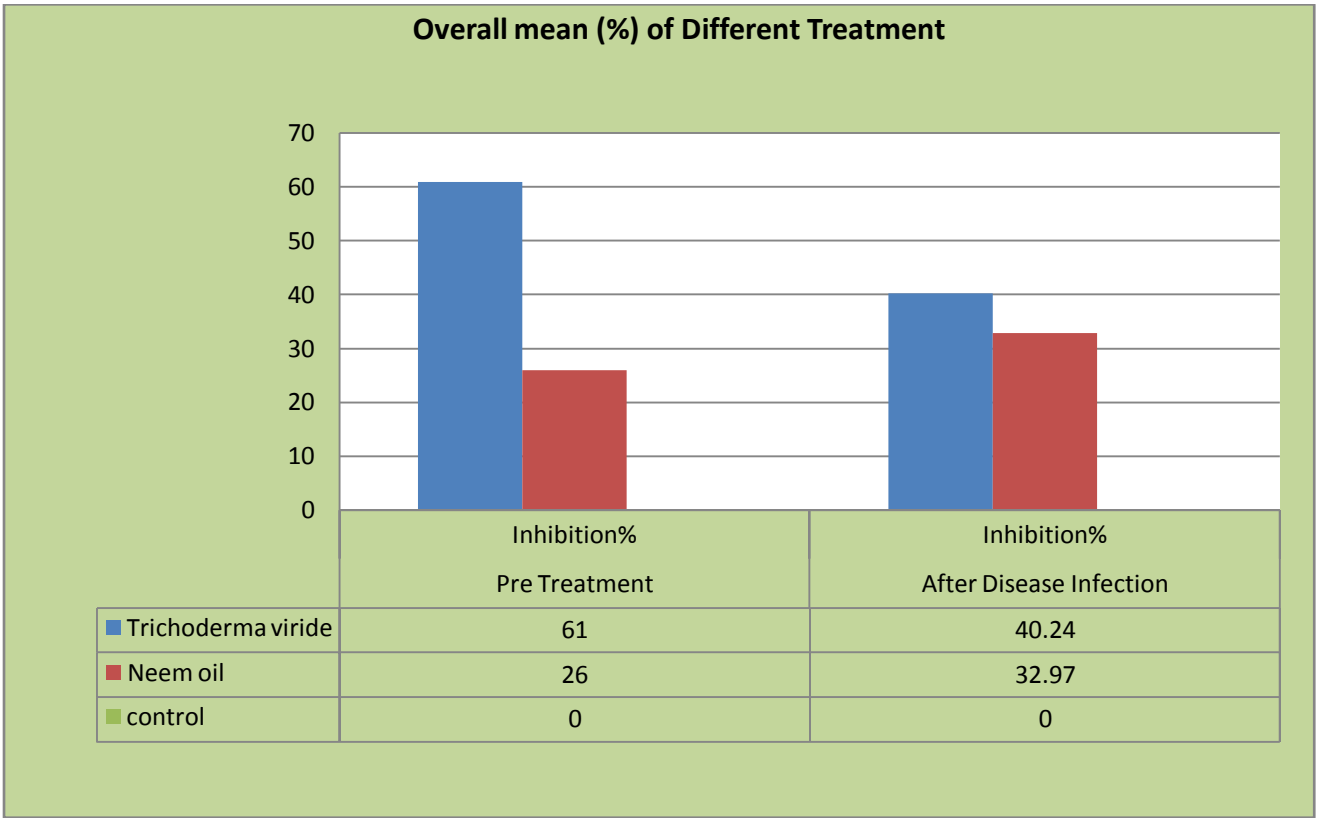

Fig. 5. Effect of bio-agent and botanical on sporangial germination of *A. candid*a in Pre Treatment of disease infection and Treatment after Disease Infection.

CONCLUSION

Detached leaf inoculation method has been found to be the finest and swift method. The conventional technique like spraying method has the disadvantage of causing considerable variation in spores distribution. After Green Revolution, the use of chemical fertilizers and pesticides are increase by Indian farmers to maintain and enhanced crop productivity. However, excess use of pesticide chemicals during the last few decades in controlling pests and diseases resulted in negative impacts on the environment, producing inferior quality and harming consumer health. Degradation of environment due to pesticides is a worldwide problem and they have contaminated almost every component of our environment. In recent times, diverse approaches are being used to mitigate and manage a variety of plant pathogens for control of diseases. *T. viride* was found most effective in pre treatment than treatment after disease infection treatment while neem oil was less effective in pre treatment. Use of biological agents and botanicals is the alternative approach for management of disease that is eco-friendly and reduces the harmful impact on environment.

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