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Pathogenicity test of *Alternaria brassicae* (Berk.) Sacc. using artificial inoculation methods on common varieties of rapeseed-mustard in Punjab region

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Abstract

To develop the precision, sensitivity for pathogenicity testing, a comparison was carried out among three different artificial inoculation methods namely foliar spraying method, drop method and biocontrol+foliar spray method for pathogenesis studies. The pathogen *A. brassicae* was isolated and from infected mustard plants. The drop inoculation method was most ideal as this fixed the inoculum on the target site. Drop method produces significantly higher number of disease lesions than the other inoculation methods and has the advantage of being accurate and precise. The number of initial disease lesions in drop method was highest in all of the time intervals of observation namely 71 in *B. juncea* (Giriraj), 59 in *B. juncea* (RLC-3), 53 in *B. napus* (GSC-7), 73 in *B. nigra* (PC-6) at 120h after pathogen inoculation compared to other methods respectively. The results in this particular study indicates that two varieties *B. juncea* (RLC-3) and *B. napus* (GSC-7) showed some tolerance as compared to other two varieties *B. juncea* (Giriraj) and *B. nigra* (PC-6).The biocontrol agent used in particular study showed that the tolerance can be induced in the plants using *Trichoderma viride*. As the number of lesions and pathogenicity caused by *A. brassicae* was reduced considerably when the culture of *T. viride* was used.

Key words: *Alternaria brassicae, artificial inoculation, Brassica spp., slide culture technique, Trichoderma spp.*

Introduction

Oilseed brassicas, also known as rapeseed-mustard, constitute the second largest group of Oilseed crops that contributes 32 per cent of the total Oilseed production in India. In India rapeseed mustard occupy an area of 57.62 lac ha with a production of 68.22 lac tons and productivity of 1184 kg / ha (Anon, 2016). Brassica oilseed crops play an important role in the diversification in cropping system and also in providing the quality food by meeting the fat requirement to same extent. However, the productivity of these crops is relatively low, thus there is a significant shortfall in the supply of required quantity of fat per capita (presently available of required per capita), no doubt there are several other crops like groundnut, sunflower etc. therefore huge amount of foreign exchange is drained for imported the huge quantity of oilseeds to meet the national requirement.

In Punjab, the area under brassica oilseed is decreasing under rapeseed mustard for the last 10years. This is due to the fact that the alternate Rabi crop of wheat is more profitable as the brassica crops are more vulnerable to several biotic and abiotic stresses. Of these the attack of diseases like Alternaria blight is more concerning point it is most destructive and wide spread affecting all the cultivated species. Highest yield obtained from a particular crop and averages realized at the state and national levels. A major contributory factor to this gap is its unchallenged exposure to a number of biotic, mesobiotic and abiotic stresses. This disease is also called the black spot (Louvet, 1958) or grey spot (McDonald, 1959) based on the symptoms produced on the host. Alternaria blight causing pathogens can attack all the aerial parts of plant and can cause huge losses in yield. Alternaria blight caused by most *Alternaria brassicae* (Berk.) Sacc. has been reported from all the continents of the world affecting cruciferous crops. The disease appears annually during the cropping season (from October to February) in different parts of India and causes enormous loss to growers (Prasad *et al*., 2006). Environmental factors play an important role in the development of the disease. The environmental variables viz., temperature, humidity, rainfall and sunshine are the most critical factors. They affect the pathogen and host or host-pathogen interaction during pathogenesis.

The ideal and most economical mean of managing the Alternaria blight disease of rapeseed-mustard would be the use of resistant varieties. Most of the cost effective and eco-friendly management strategies for evolving selecting the genotypes possessing the resistant/ tolerance reaction against the diseases. Information on resistance source is not available, although some sort of tolerance may be available (Shah *et al.,* 2005). Biocontrol agents can be effective to induce tolerance against *A.brassicae* and *A. brassicola.* Reshu and khan (2012) reported *Trichoderma viride* isolated from mustard leaf showed fungicidal activity against *A. brassicae*. Pathogenicity testing using artificial inoculation is easy and less time consuming technique to study the various aspects of plant pathology, including epidemiology, etiology, disease resistance, host-parasite interaction and disease control (Xu and Ko, 1998).

Detached leaf inoculation method has been found to be the elementary and swift method. The conventional method like spraying has the disadvantage of causing considerable variation in spore distribution (Tuite, 1969). Although the accuracy and precision were improved by applying a drop of inoculum with a modified hypodermic needle (Lapwood and Mckee, 1966)capillary pipette (Toussoun *et al*., 1960). In this report, to develop precision, sensitivity and for testing a comparison was carried out among three different artificial inoculation methods namely foliar spray method, drop method and biocontrol+ spray method for pathogenesis studies of *A. brassicae* on *B. juncea* (Indian mustard), *B. napus* (Gobhi Sarson) and *B. nigra* (Banarasi Rai).

Materials and Methods

Isolation: *A. brassicae* (Berk.) Sacc.was isolated from a diseased leaf of *B. juncea, B. napus* and *B. nigra* from Guru Kashi University Research Farm Talwandi Sabo, Bathinda (Punjab), India. The pathogen was isolated, purified by single spore technique (Toussoun and Nelson, 1976), and maintained on potato dextrose agar (PDA) media at 25°±1°C.

Identification using slide culture technique: The culture exhibiting maximum production was identified by both conventional methods (morphological and microscopic methods). Slide culturing was done to determine the microscopic features for morphological characterization according to standard taxonomic key (Singh et al. 2012; Singh et al. 2013.)

Inoculum preparation: Seven-day old *Alternaria* cultures grown on PDA plates were flooded with distilled water and spores were released by agitation with a sterile brush. The spores in this suspension were counted using a haemocytometer and the concentration was adjusted to10^4 spores/ml.

Detached leaf inoculation: Leaves were detached from the *B. juncea* (2 varieties- 'Giriraj' and 'RLC-3'), *B. napus* (1 variety- 'GSC-7') and *B.nigra* (1 variety- 'PC-6') washed under running tap water followed by a wash with autoclaved water and then surface was wiped off with 70% ethanol.

Pathogenicity test were carried out with foliar spray, drop method and biocontrol (*Trichoderma viride)*+ spray method on detached leaves of *B. juncea, B. napus* and *B. nigra.*In the spray method, inoculum was sprayed on detached leaves with the help of atomizer. In drop method; inoculum was placed on each detached leaf in the form of drop. In Biocontrol+spray method, inoculum was sprayed on leaf followed by (*Trichoderma viride*1.5% W.P) 1mg/ml suspension and go on with 10^4 spores/ml. Detached leaves were kept in sealed plastic bags with moistened filter paper and placed in BOD incubator at 25°±1°C and 70% relative humidity. Wetness of filter paper was maintained by spraying autoclaved distilled water. The plastic bags were observed for *A. brassicae* initial symptoms at intervals of 24 h after pathogen inoculation and the infected leaves were examined up to 120 h after pathogen inoculation. The numbers of disease lesions were counted on the detached leaves in all the inoculation methods at 24, 48, 72, 96 and 120 h after inoculation of pathogen while the control remains free from severe symptoms.

Results and Discussion

The fungus was identified according to standard taxonomic key including colony colour, and the morphology of hyphae and conidia. Colonies were fast growing, black to olivaceous-black or greyish colour when incubated on PDA plates at 30 °C for 7 days (Fig. 1a). Septate hyphae, condiophore structure and development of condia was seen under the microscope (Fig. 1b,c). Conidia were found in chains with a conspicuous beak, smooth, greyish-olive, with 7-8 cross septa and longitudinal ones, slightly constricted at the septa (Fig. 1d).

In this scrutiny, four varieties of *Brassica* spp. were taken as the criteria to find the numbers of lesions on the detached leaves in an incubation hours of 24, 48, 72, 96, 120 with different types of artificial inoculation methods namely spray, drop and biocontrol+spray methods. The best artificial inoculation method was analysed for the pathogenesis studies.

The *B. juncea* (1^{st} variety- Giriraj) at 48 h after inoculation of pathogen, drop method showed highest number of initial lesions that is (39) lesions, followed by spray (15) lesions and biocontrol+spray (1). Maximum number of lesions i.e. 71, 37 and 3 were appear on leaves of *B. juncea* using drop, spray and biocontrol+spray method,

1a. Slide culture 1b. Conidiophore 1c. Sepate hyphae 1d. Chain of conidia

Fig.1. a) Colony morphology. b) Structure of condiophore and development of conidia. c) Septate Hyphae. d) Chain of conidia.

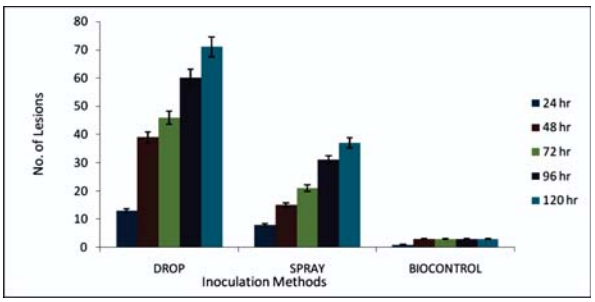

Fig. 2. Number of lesions on the detached leaf of *B. juncea* (variety-Giriraj) using different pathogen inoculation methods (drop, spray and biocontrol+ spray) at different time intervals (24, 48, 72, 96 and 120 h) after pathogen inoculation.

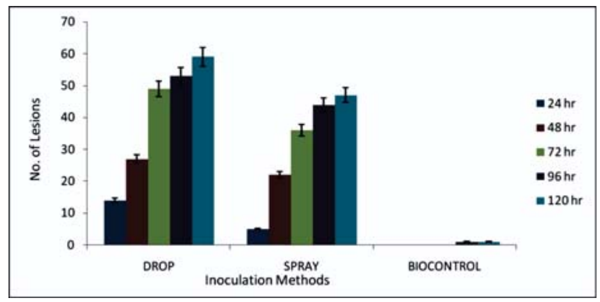

Fig. 4. Number of lesions on the detached leaves of *B. juncea* (variety- RLC-3) using different pathogen inoculation methods (drop, spray and biocontrol+ spray) at different time intervals (24, 48, 72, 96 and 120 h) after pathogen inoculation.

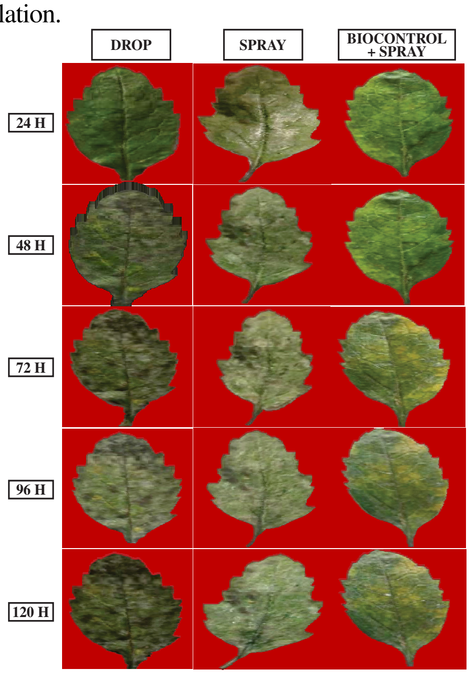

Fig 3. Development of initial black/brown disease lesions on the detached leaves of *B. juncea* variety Giriraj using different pathogen inoculation methods (drop, spray and biocontrol+ spray) at different time intervals (24, 48, 72, 96 and 120 h) after pathogen inoculation.

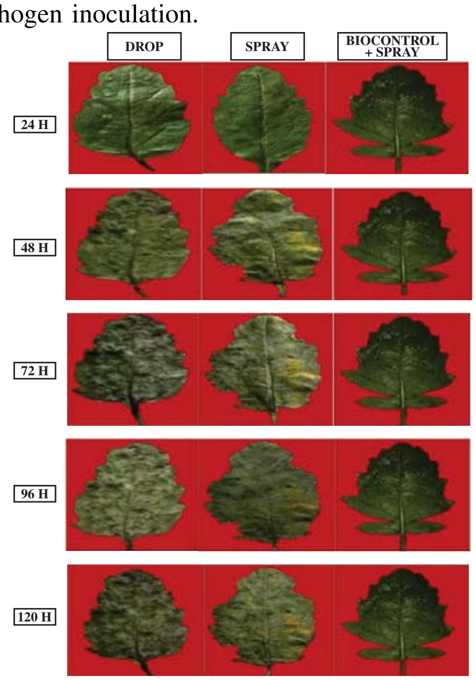

Fig. 5. Development of initial black/brown disease lesions on the detached leaves of *B. juncea* variety RLC-3 using different pathogen inoculation methods (drop, spray and biocontrol+ spray) at different time intervals (24, 48, 72, 96 and 120 h) after pathogen inoculation.

respectively after 120 hrs (Fig. 2). The drop method showed the highest number of initial lesions followed by spray (Fig. 3).

The *B. juncea* (2nd variety- RLC-3) at 24 h after inoculation of pathogen, drop method showed highest number of initial lesions that is (14) lesions, followed by spray (5) lesions and biocontrol+spray (0). Maximum number of lesions i.e. 59, 47 and 1 were appear on leaves of *B. juncea* using drop, spray and biocontrol+spray method, respectively after 120 hrs (Fig. 4). The drop method showed the highest number of initial lesions followed by spray (Fig. 5).

In *B. napus* (Variety- 'GSC-7') at 24 h after inoculation of pathogen, drop method showed highest number of initial lesions that is (10) lesions, followed by spray (6) and biocontrol+ spray (0). Maximum number of lesions i.e. 53, 35 and 2 were appear on leaves of *B. napus* using drop, spray and biocontrol+spray method, respectively after 120 hrs (Fig. 6). The drop method showed the highest number of initial lesions followed by spray (Fig. 7).

The *B. nigra* (Variety- 'PC-6') at 72 h after inoculation of pathogen, drop method showed highest number of initial lesions that is (44) lesions, followed by spray (19) lesions and biocontrol+spray (3). Maximum number of lesions i.e. 73, 55 and 4 were appear on leaves of *B. nigra* using drop, spray and biocontrol+spray method, respectively after 120hrs (Fig. 8). At 48, 72, 96, and 120 h after inoculation of pathogen, thereby the drop method showed the highest number of initial lesions followed by spray (Fig. 9).

Results revealed that out of three inoculation methods used, spore suspension drop inoculation method was most ideal as this fixed the inoculum on the target site. With this technique, a single conidiophore of *A. brassicae* was able to cause local lesions on leaves. This was also reported by Xu and Ko (1998), that even a single conidium containing drop were able to induce the development of local lesion in black mustard leaves. It also produces significantly higher number of disease lesions than the other inoculation methods and has the advantage of being accurate and precise. It is also easy to handle the inoculated plants. The results indicated that two varieties *B. juncea* (RLC-3) and *B. napus* (GSC-7) showed some tolerance as compared to varities *B. juncea* (Giriraj) and *B. nigra* (PC-6). The biocontrol agent used in particular study showed that the tolerance can be induced in the plants using *T. viride*. As the number of lesions and pathogenicity caused by *A. brassicae* was reduced considerably when the culture of *T. viride* was used.

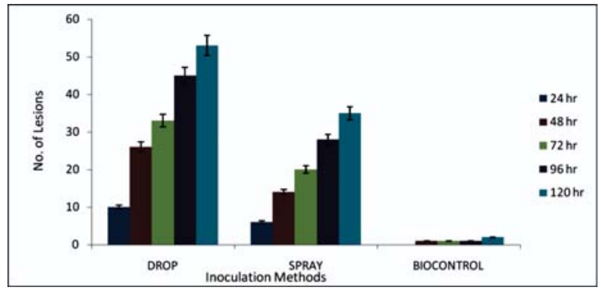

Fig. 6. Number of lesions on the detached leaves of *B. napus* (variety-GSC-7) using different pathogen inoculation methods (drop, spray and biocontrol+ spray) at different time intervals (24, 48, 72, 96 and 120 h) after pathogen inoculation.

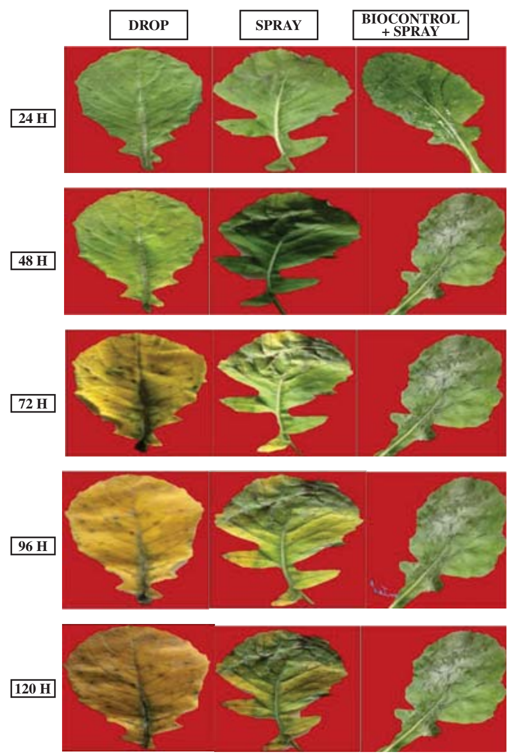

Fig. 7. Development of initial black/brown disease lesions on the detached leaves of *B. napus* variety GSC-7 using different pathogen inoculation methods (drop, spray and biocontrol+ spray) at different time intervals (24, 48, 72, 96 and 120 h) after pathogen inoculation.

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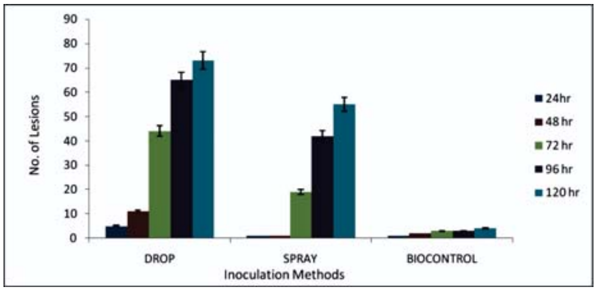

Fig. 8.Number of lesions on the detached leaves of *B. nigra* (variety-PC-6) using different pathogen inoculation methods (drop, spray and biocontrol+ spray) at different time intervals (24, 48, 72, 96 and 120 h) after pathogen inoculation.

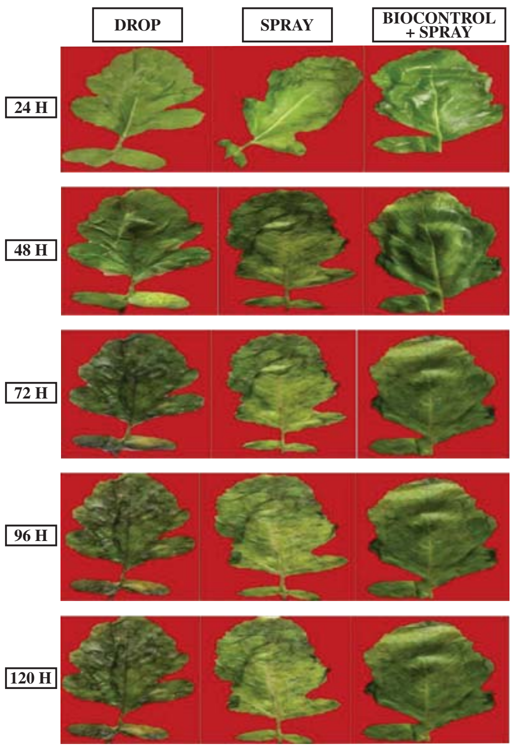

Fig 9.Development of initial black/brown disease lesions on the detached leaves of *B. nigra* variety PC-6 using different pathogen inoculation methods (drop, spray and biocontrol+ spray) at different time intervals (24, 48, 72, 96 and 120 h). after pathogen inoculation.

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