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Isolation, purification and management of *Alternaria brassicae* through cow by product *in vitro*

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Abstract
Background: Alternaria blight of mustard caused by *Alternaria brassicae* and *Alternaria brassicicola* is most widespread and destructive disease of rapeseed-mustard causing major yield losses that may range from 15 to 71 per cent in productivity and 14 to 36 per cent in oil content. Therefore, in the present investigation, our main emphasis was to find out some new cow by product for management of Alternaria leaf spot of mustard.
Method: Cow by products like cow dung, cow urine, butter milk and panchgavaya were tested at three concentrations *viz.*, 5, 10 and 15 per cent against *A. brassicae* under *in vitro* conditions.
Results: Inhibition of mycelial growth of *A. brassicae* was highest by panchgavaya followed by coe urine in *in vitro* conditions.

Keywords: Mustard, *Alternaria brassicae*, leaf spot, cow by products

Introduction
Mustard crop play an important role in agricultural economy of India. Oilseed brassicas also called rapeseed-mustard placed in family brassicaceae. This family is one of the most economically important plant families. According to ancient scripture and literature, mustard has been cultivated from 5000 BC (Kumar *et al*., 2014) [6]. Mustard are the third most important oil seed crop in terms of area and production after soybean and palm in the world and in India it occupy second position after groundnut (Kumar and Chopra, 2014) [7].
Among oilseeds, Indian mustard is one of the most important which contribute about 85 per cent of total rapeseed-mustard produced in India (Kumar and Chauhan, 2005) [5].
Mustard growing major states in India are Rajasthan, Madhya Pradesh, UP, Haryana, Punjab and Gujarat. Mustard is often grown under some non-traditional areas of South India like Karnataka, Tamilnadu and Andhrapradesh. Contribution of India in global rapeseed-mustard production is 6.33 million tonnes with an area of 6.41 million hectares (Anonymous, 2017-18).
Alternaria blight of mustard caused by *Alternaria brassicae* and *Alternaria brassicicola* is most widespread and destructive disease of rapeseed-mustard causing major yield losses that may range from 15 to 71 per cent in productivity and 14 to 36 per cent in oil content (Meena *et al*., 2010). Besides losses in yield and oil content, it also adversely affects seed quality causing reduction in seed size and leading to discoloration (Prasad and Lallu, 2006) [9].
Keeping in view the seriousness of the disease that cause heavy losses in yield as well as quality of the seed, present investigation was planned to elucidate an effective management strategy for Alternaria leaf spot of mustard.

Material and Method
1. Isolation, purification and pathogenicity test of *Alternaria brassicae*
Cleaning and sterilization of material
The materials used in this study were washed thoroughly in tap water and rinsed with double distilled water. The plastic wares were air-dried and the glasswares were dried in hot air oven. The cleaned and dried materials were wrapped in aluminium foil, covered with paper and autoclave at 121 °C at 15 PSI for 15 minutes.

Isolation and purification of *Alternaria brassicae*
Collection of leaf spot infected samples and isolation of *Alternaria brassicae* from infested mustard plants were carried out from mustard field. The infested leaf samples were gently washed in tap water for removing the soil particles adhering on leaf surface.

The washed leaf parts were cut into small pieces and surface sterilized in 0.1% sodium hypochloride solution in Petri dishes for 1-2 minutes followed by repeated washing in sterilized distilled water. The surface sterilized pieces were transferred aseptically on potato dextrose agar (PDA) medium in Petri dishes and kept in BOD incubator for 7 days at 28±1 °C for growth of the pathogen.

In order to obtain the pure cultures of *Alternaria brassicae*, single hyphal tip method was used. The hyphal suspension of the isolate was prepared in sterilized distilled water so as to obtain 5-6 hypha per microscopic field (10x). The hyphal suspension was spread over the surface of sterilized 2 per cent plain agar medium in Petri dishes and incubated at 28±1 °C for 18 - 22 hours. The single hyphal piece was observed under low power objective and cut through dummy objective. Such pieces were transferred separately on potato dextrose agar (PDA) slants with the help of an inoculating needle and incubated at 28±1 °C for seven days. These cultures were observed under microscope and slants stock of cultures were prepared and kept in refrigerator for further studies.

Pathogenicity test under pot condition

Pathogenicity of *A. brassicae* was proved under pot condition, pathogenicity test was conducted on mustard var. Pusa bold by atomizing the distilled water containing mycelial bits of *A. brassicae*. After treatment, the inoculated plants were covered with a polythene cover for 5 days to maintain high humidity of > 90 per cent. Uninoculated plant served as control. The inoculated plants were examined for the development of leaf spot symptoms (Wagh *et al*. 2013) [10]. The symptoms appeared within 10 days and the fungus was re-isolated from the infected leaves and the culture obtained was compared with the original to confirm the identity.

2. To test the efficacy of cow by products *against Alternaria brassicae in vitro*

Cow by product

In this experiment, different plant extracts & cow by products were tested for their efficacy against *Alternaria brassicae* in *in vitro* condition.

Preparation of cow by products

To study the antifungal property of plant extracts, the poisoned food technique was adopted. The stock solutions of different cow by products (5, 10 and 15%) were mixed with 95, 90, and 85 ml of PDA media, respectively, so as to get 5, 10 and 15 per cent concentrations and sterilized. Twenty ml of such medium was poured under aseptic conditions into sterile Petri plates allowed to solidify. Mycelial discs (5mm) were cut out using sterile cork borer from periphery of actively growing culture of *A. brassicae* and one such disc was placed on the centre of each Petri plate. The treatments were replicated thrice. Control was maintained by growing the pathogen on PDA plates without plant extracts. Plates were incubated at temperature of 28+2 °C for 7 days and radial growth was taken at the time when maximum growth occurred in the control plates.

Table 1: Following cow by products were tested for their effect on pathogen

Sn	Treatment	Concentration (%)		
T_1	Cow Dung	5	10	15
T_2	Cow Urine	5	10	15
T_3	Butter Milk	5	10	15
T_4	Panchgavaya	5	10	15
T_5	Control			

Cow by products were tested for its effect on growth of *Alternaria brassicae* using poisoned food technique suggested by Nene and Thapliyal, (1973) [8] with different concentrations 5%, 10%, 15%.The effect of extracts of locally available cow by products were tested for growth inhibition of *A. brassicae* and per cent inhibition of fungal growth for each treatment and concentration were calculated by the formula.

Per cent inhibition of the fungal growth by cow by products were calculated by the following formula.

Formula

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

C = Mycelial growth of M. phaseolina in control (mm)

T = Mycelial growth of M. phaseolina in presence of plant extracts (mm)

Result and Discussion

1. Isolation, purification and pathogenicity test of *Alternaria brassicae*

Collection of disease sample

The sample of diseased leaves of mustard were collected from the College Instructional farm, Swami Keshwanand Rajasthan Agricultural University, Bikaner. The diseased leaf sample was used to isolate the pathogen and other studies.

Isolation and purification of *Alternaria brassicae*

Standard tissue segment method was followed to obtain *Alternaria brassicae* from the diseased mustard leaves showing the typical Alternaria leaf spot symptoms. Fungus was isolated in Petri dishes using PDA medium. To obtain pure culture of *Alternaria brassicae* single spore technique was followed, Petri dishes and slants of fungus with full growth were incubated at 25±1 °C for 15 days.

Proving the Pathogenicity of *Alternaria brassicae*

Pathogenicity test was proved by atomizing the distilled water having mycelial bits of *Alternaria brassicae* on the leaves of mustard plants in pot condition. After the inoculation, the symptoms is started occur on inoculated leaves. The symptoms observed on leaves of plants in pot were similar to observed in the field conditions. The reisolated culture from inoculated leaves was confirmed as *Alternaria brassicae.* The similar results was observed by Duhan and Suhag (1989) [4], Bhatti *et al*. (2000) [2], Thippeswamy *et al*. (2006) [9] and Chauhan *et al*. (2009) [3].

Plate 1: Pathogenecity test of Alternaria leaf spot of mustard

2. Efficacy of cow by products

Four cow by products *viz*., cow urine, cow dung, cow milk and butter milk were tested at 5, 10 and 15% concentration against *A. brassicae* using Poisoned Food Technique *in vitro.* fig 1 and plate - 2 shows the effectiveness of all the tested the fungus.

Among the tested cow by products at 5% concentration, the maximum inhibition of the growth of fungus was observed in panchgavaya (60.16%) followed by cow urine (47.53%).

At 10% and 15% concentration, the maximum inhibition of the growth of fungus was observed in panchgavaya followed by cow urine. Data shows that effect of different cow by products at this concentration was more than the 5% concentration.

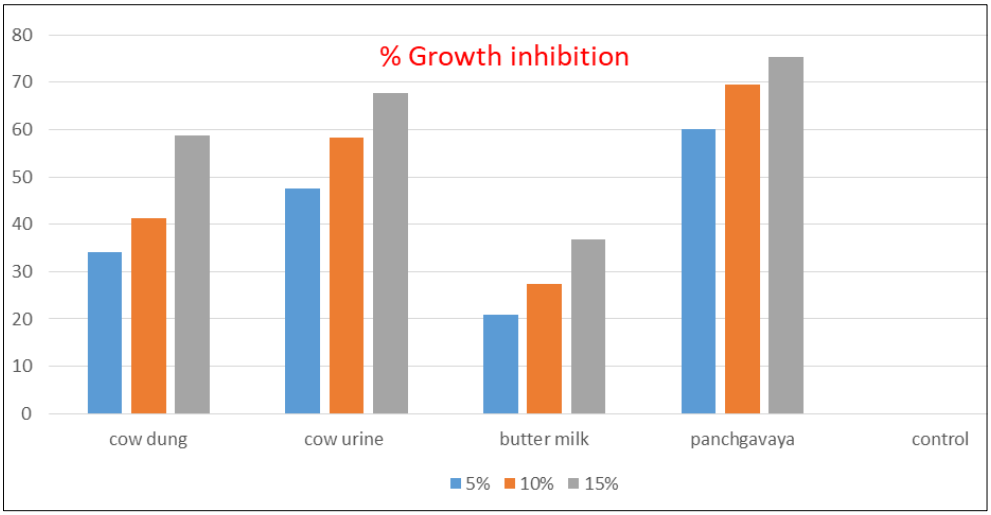

Fig 1: Effect of cow by products on mycelial growth of *A. brassicae* in *in vitro*

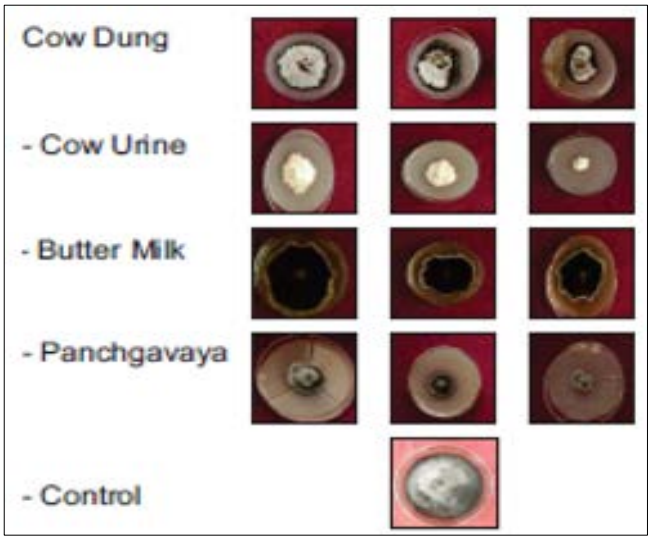

Plate 2: Effect of cow by products on mycelial growth of *A. brassicae* in *in vitro*

Conclusion

It can be concluded unequivocally considering the results that among the tested cow by products, panchgavaya was found most effective in *in vitro* conditions and inhibited 75.28% mycelial growth of *A. brassicae* at 15% concentration.

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