International Journal of Biotechnology and Biochemistry
ISSN 0973-2691 Volume 15, Number 2 (2019) pp. 73-79

http://www.ripublication.com

Effect of $KMnO_4$ on Seed- Borne Fungi of *Brassica Campestris* (Mustard)

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Abstract

The health of seed is very important for the crop production because seeds are considered as highly effective means for transporting plant pathogens over long distances. Pathogen free healthy seeds are required to reduce enormous losses. In our study we had selected mustard seeds. We prepared three different series of $KMnO_4$ i.e. 0.005%, 0.010% and 0.015% for the treatment. In the present investigation five sp. of fungi were observed during experiment of determination of seed mycoflora. The observed species was *Aspergillus niger, A. flavus, A. fumigates, A. luchuansis* and *Rhizopus nigricans.* PDA media was used for culture of fungi associated with mustard seeds. Minimum fungal infection was exhibited by seeds treated with varying concentration of KMn04Such as 5mg, l0mg and 15mg The results were highly promising of the seed mycoflora, whereas the growth of fungus is inhibited by ($KMnO_4$) compared to control. The result indicates that possible application of $KMnO_4$ as disinfectant on mustard seed that reduces the seed fungal infection. Results in this study showed that mustard seeds were strongly infected. As it was not reported earlier by anyone, it is a new research on seed borne fungi of mustard.

Keywords: pathogen, mycoflora, PDA, disinfectant.

INTRODUCTION

The crop *Brassicas* have been very important as food crops in the form of vegetables, oilseeds, feed and fodder, green manure, and condiments and have played a great role in the human history by contributing a good share of food in one form or another. *Brassica* $ belongs to family *Brassicaceae Brassica rapa* and *brassica campestris* were first described as two species by Linnaeus, *B. rapa* being the turnip form of and *B. campestris* , the wild weedy form. Metgzer in 1883 concluded that this were the same species and combined the taxa under the name *B. rapa* (Toxeopus et al., 1984). Mustard seeds are an excellent source of essential B-complex vitamins such as folates, niacin, thiamin, riboflavin, pyridoxine (vitaminB-6), pantothenic acid. These B-

complex groups of vitamins help in enzyme synthesis, nervous system function and regulating body metabolism.

Seed is the most important input for crop production. Pathogen free healthy seed is urgently needed for desired plant populations and good harvest. Many plant pathogens are seed-borne, which can cause enormous crop losses; reduction in plant growth and productivity of crops (Williams and McDonald, 1983; Kubiak and Korbas, 1999; Dawson and Bateman, 2001; Islam et al., 2009)

Seed-borne fungi include all fungal types contaminating the surface of seeds or infecting seed tissues. Seed- transmitted fungi are those that cause no infection to a seed itself but infect seedlings in the nursery or field (Neergaard 1979). The major fungal diseases that attack *Brassica* species in India include Alternaria blight caused by *Alternaria brassicae/Alternaria brassicicola* (Berk) Sacc., white rust caused by *Albugo Candida* (Pers.) Kunzee and downy mildew caused by *Peronospora parasitica* (Pers. Ex Fr.).

Potassium Permanganate ($KMnO_4$) is a chemical compound of manganese, potassium and oxygen. The permanganate ion is a strong oxidizing agent. It dissolves in water to give deep purple solution, evaporation of which gives prismatic purple-black glistening crystals. The effect of $KMnO_4$ for controlling of seed borne disease as disinfectant as well as the fungicide.

Materials and Methods

Collection of Materials

Seed lots were collected from the field of Meerut locality in 2015-16. Alcohol cleaned polythene bags were used for the collection of seed samples.

External inspection of seed

The seeds of each sample were examined by naked eye and then under the low magnification of compound microscope, with magnification upto 40X and under good light the dry seed were examined for impurities.

Seed washing test

Fifty seeds were taken of each sample and mixed with sterile water in a culture tube and it was shaken on an automatic shaker for 5-10 seconds. The suspension was examined under microscope for the presence of spores or fungal hyphal bits.

Moisture content

The initial weight noted and dried at 120°C for 8-12 hour, the moisture content

W - W

de^i—Til x 100

$^{W}1$

Wj = Weight of sample

W_2 = different between initial weight of sample after drying torment in initial weight

and weight after drying of seed.

Viability test

Hundred seeds were taken from the collected sample longitudinally, with a scalpel so that the embryo is exposed to the Tetrazolium chloride solution. One half of this seed is used for the test and other half is discarded.

Seed treatment and determination of seed mycoflora

To determine the effect of treatment on seed-borne fungi of mustard 0.05%, 0.10%, 0.15% solution of potassium permanganate was used. The seeds were treated with $KMnO_4$ solution. For treatment of seeds, 0.005%, 0.010%, 0.015 % solution of potassium permanganate was prepared by adding 5, 10, 15mg $KMnO_4$ into 100 ml of distilled water. The surface sterilized seeds were taken and soaked in aqueous solution of potassium permanganate for 12, 18, 24 hours. Then the seeds were plated on agar medium at a fixed distance according to the size of the seeds. Sterilized petriplates are used for this purpose. The mild hot agar medium is poured in each petriplate and seeds were plated carefully under sterilized conditions. The plates were incubated for 6 to 7 days at 26 ±2 degree Celsius in a chamber having alternating cycles of NUV light and darkness. The plates then observed, and they were soaked in water for 24 hours at room for the effect of seed treatment with $KMnO_4$ on internal temperature. Next day seeds are then dissected seed mycoflora.

RESULTS AND DISCUSSION

The present investigation is amid at analysis of the effect of potassium permanganate (disinfectant agents) on seed-borne mycoflora of mustard. The internal mycoflora of mustard seed was isolated during September 2016 to October 2016 by using agar plate method in which potato dextrose agar medium was used. All the experiments were carried out in Mycology laboratory of Botany Department, Chaudhary Charan Singh University, Meerut.

Seed Viability Percentage

The test was done on two samples (healthy and unhealthy) containing 50 seeds each. By counting the number of coloured seeds the viability was determined and expressed in percent as-

$$\text{Viability} = \frac{\text{Number of viable seeds}}{\text{Total number of seeds}} \times 100$$

The viability percentage was calculated for each replicate by using the above formula

S. No.	Types of Seeds	Total Number of Seeds	Total Number of Viable Seeds	Viability (%)
1	Healthy	50	38	76%
2	Unhealthy	50	26	52%

The study of the internally seed borne mycoflora of the samples revealed the presence of four species of the *Aspergillus niger, Aspergillus flavus, A.fumigates, A. luchuansis* one species of *Rhizopus nigricans,* species.

Evaluation of Seed Health testing Methods

Seed samples of mustard were used for evaluation of seed health testing. The results are presented in the table PDA medium was found to be suitable for isolation of *A. niger, A. flavus, A. fumigates, A. luchuansis and Rhizopus nigricans.* Totally five fungi including both saprophytic as well as pathogenic were recorded. The results of this study indicated that dominance of *Aspergillus niger, A. flavus, A. fumigates, A. luchuansis and Rhizopus nigricans.* After Seven days of incubation the highest number of contaminated seeds were predicted.

Seed treatment and determination of seed mycoflora

To determine the effect of treatment on seed-borne fungi of mustard 0.005%, 0.010%, 0.015% solution of potassium permanganate was used. The seeds were treated with $KMnO_4$ solution. For treatment of seeds, 0.005%, 0.010%, 0.015% conc. solution of potassium permanganate was prepared by adding 5, 10, 15mg into 100 ml of distilled water. The surface sterilized seeds were taken and soaked in aqueous solution of potassium permanganate for 12, 18, 24 hours. Then, the seeds were placed on agar medium at a fixed distance according to the size of the seeds. Seeds were placed on potato agar dextrose (PDA) media.

Frequency of fungal infection

Fungal infection Frequency (FIF) was calculated to determine the most susceptibility of mustard seed of Meerut locality to various fungus infections. The total Infection % = infection percentages of the component plating test were calculi by usrng following forn^ae:

$$\frac{Number\ of\ seeds\ infected\ by\ pathogen}{Total\ number\ of\ seeds} \times 100$$

Table- Showing the number of infected seed frequency observed

S. No.	Cone. of $KMnO_4$	Total no. of Infected Seeds Frequency Observed		
		12 hrs	18 hrs	24 hrs
Control	-	80%	90%	60%
1.	0.005%	80%	100%	30%
2.	0.010%	70%	90%	40%
3.	0.015%	80%	60%	20%

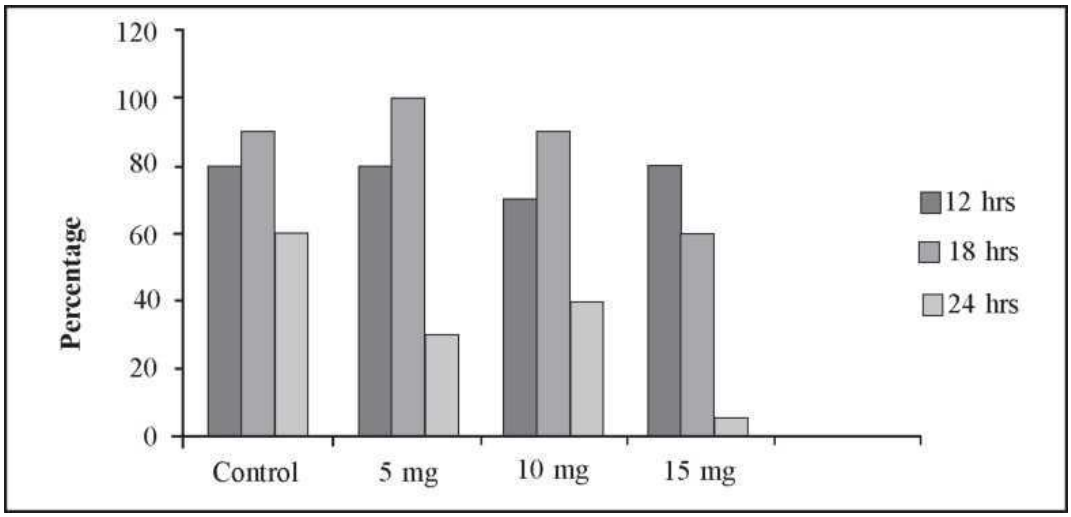

Figure- Effect of $KMnO_4$ on seed Borne Fungi on Mustard at different interval

Several species of *Aspergillus* such as *A. niger, A. flavus, A. fumigates A. luchuansis* and one species of *Rhizopus nigricans* were isolated from the mustard seeds selected for present research work.

PDA media was used for culture of fungi associated with mustard seeds. Seed lots were collected from the field of Meerut. Minimum fungal infection was exhibited by seeds treated with varying concentration of $KMnO_4$ such as 0.005%, 0.010% and 0.015%. Thus $KMnO_4$ acts as disinfectant against seed borne fungi of mustard. As reported by the Webber and Posselt, (1972), the primary mode of pathogen inactivation by Potassium permanganate is direct oxidation of cell material or specific enzyme destruction. Potassium permanganate is a powerful oxidizing agent in alkaline or acidic solution.

The same result was also reported by the, John R. Edwardson (1996). Potassium permanganate killing and fixing techniques have been made that give excellent preservation of virus particles and aggregates, as well as host cell contents, in ultra

sections of plant tissues. A Bradbury and meek (1960) reported that $KMnO_4$ caused general swelling that its rate of penetration was slow, and that RNA and histones were removed and presumably leached out after permanganate fixation. Shalla (1961) was unable to find TMV particles in infected leaves fixed in $KMnO_4$ and, furthermore, presented evidence that isolated particles of TMV were degraded by exposure to $KMnO_4$.

It was observed that 0.015% conc. showing minimum percentage of infected seed as compared to other treatment as well as control after 24 hours of treatment. It was also observed that 0.005% conc. showing maximum percentage of infected seed as compared to other treatment as well as control after 18 hours of treatment.

The results were highly promising of the seed mycoflora, whereas the growth of fungus. is inhibited by ($KMnO_4$) compared to control. The result indicates that possible application of $KMnO_4$ as disinfectant on mustard seed that reduces the seed fungal infection.

CONCLUSION

After complete observation of all the experimental data it is concluded that $KMnO_4$ work as good sterilizing agent for seed borne fungi. $KMnO_4$ being a cheap material may be used as a surface sterilizer for mycoflora. Very low concentration of $KMnO_4$ such as 0.015% exhibited the best result against seed borne fungi as compared to all other treatment as well as control.

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