ISSN : 2350-0743

www.ijramr.com

*International Journal of Recent Advances in Multidisciplinary Research*
*Vol. 05, Issue 09, pp.4078-4080, September, 2018*

# RESEARCH ARTICLE

## SEASONAL VARIATION OF MYCOFLORA ASSOCIATED WITH DISEASED AND HEALTHY LEAVES OF RAPESEED –MUSTARD VARIETIES UNDER ORGANIC FARMING SYSTEM IN MANIPUR

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**ARTICLE INFO**

***Article History:***
Received 14th June, 2018
Received in revised form
27th July, 2018
Accepted 12th August, 2018
Published online 30th September, 2018

***Keywords:***
Mycoflora, Organic farming,
*Aspergillus clavatus*, White rust.

**ABSTRACT**

The seasonal occurrences of fungi were varied in different months starting from December to March in both the years (*rabi* 2014-15 and 2015-16). In the infected plant (leaves) the pooled number of seasonal mycoflora was found to be highest in March (22.50 $cfug^{-1}$ leaf x 10) and the lowest in December (15.00 $cfug^{-1}$ leaf x 10). In healthy plant the highest occurrence of mycoflora was found in March (16.50 $cfug^{-1}$ leaf x 10) and the lowest in January (11.50 $cfug^{-1}$ leaf x 10). March was found congenial for abundant occurrence of mycoflora for both diseased and infected leaves.The prominent genera in this season were *Aspergillus clavatus, A. niger, Penicilium sp. Fusarium sp*. However, other fungi were also found in less abundance.

## INTRODUCTION

Rapeseed – mustard is one of the most important cruciferous crops basically cultivated for oils and vegetables during *rabi* season across the world. Rapeseed and mustard are the third most important edible oilseed crops of the world after soybean and oil palm. While in India rapeseed-mustard is the second most important oilseed crop after groundnut both in area and production (Kumar, 2012; Kumar and Chopra, 2014). In Manipur about 25% of the total edible oil requirements are met from domestic productions of oilseed crops such as groundnut, soybean, rapeseed and mustard (Singh *et al.,* 2013). The phylloplane is a natural habitat on leaf surface which supports heterogenous population comprising both pathogen and non-pathogens. The phylloplane microbes cover a wide variety of micro-organisms including yeasts, filamentous fungi, actinomycetes, blue green algae and even ferns (Aneja, 2003). Antagonism is the promising mechanism for biological control of pathogens on leaf surface of vegetable crops. The advantages of a biological approach to control disease include to reduce environmental damage, reduced human health risks and improved soil conditions and agricultural sustainability (Nakkeeran *et al*., 2002). So, some organisms are friends and foes to the plant. However, such work is very rare in the study site. Hence, the present investigation was carried out to study the seasonal variation of mycoflora associated with diseased and healthy leaves of rapeseed –mustard varieties under organic farming system in Manipur

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## MATERIALS AND METHODS

Field experiments were conducted at Kakching, an agricultural hub of diverse crops, located about 45 km away from Imphal during *rabi* seasons (2014-15 and 2015-16). The experimental varieties used were two cultivars of mustard namely *Brassica juncea* (L.) Czern. and Coss. cv. Local Yella ($V_1$) and *B. juncea* Czern. and Coss. cv. *Lamtachabi* ($V_2$) and two varieties of rapeseed namely *B. rapa* (L.) var. *M27* ($V_3$) and *B. rapa* (L.) var. *ragini* ($V_4$). The crop was sown in last week of October in plots [(2.2 x 1.3) $m^2$] by keeping 5 cm as border lines and replicated three times. Plant seedlings (5-6 numbers) were raised by broadcasting method and only one plant was kept by thinning after two weeks. The experiment was laid out in RBD. A spacing of (30 x 10) $cm^2$ between row to row and plant to plant was maintained. Locally available FYM (farm yard manure) was applied locally to the crop. Normal agricultural practices such as irrigation and weeding were also done from time to time to raise the crop as usual.

**Sampling:** A monthly sampling was conducted starting from December at 45 DAS (days after sowing) after appearance of disease on the crop. Sampling was done randomly in the experimental plots to collect sample diseased leaves. Twenty leaf samples of white rust were collected for each of the four varieties of rapeseed - mustard. These samples were brought to the laboratory using sterile polythene bags for further experiment. Samples of healthy leaves were collected by following the method as cited above.

**Isolation of leaf surface mycoflora:** Isolation of leaf surface mycoflora (fungi) from infected and healthy leaves was

**Table 1. Seasonal variation of fungal population on diseased leaf surface of Rapeseed-Mustard during two consecutive crop seasons: *Rabi* 2014 & 2015**

| Fungi | *Rabi* 2014 ( Fungi: cfug $^{-1}$ leaf x 10 ) | | | | *Rabi* 2015 ( Fungi: cfug $^{-1}$ leaf x 10 ) | | | |
|---|---|---|---|---|---|---|---|---|
| | December | January | February | March | December | January | February | March |
| *Aspergillus niger* | 2 | 3 | 4 | 5 | 1 | 2 | 3 | 4 |
| *Rhizopus sp.* | 1 | 1 | 2 | 1 | 1 | 2 | 1 | 2 |
| *Mucor sp.* | 1 | 1 | 1 | 1 | 2 | 2 | 1 | - |
| *Aspergillus clavatus* | 1 | 3 | 2 | 4 | 1 | 1 | 2 | 2 |
| *Trichoderma viride* | 2 | 1 | 1 | 1 | 1 | 2 | 1 | 1 |
| *Trichoderma harzianum* | 1 | 1 | 1 | 1 | 1 | - | 1 | 2 |
| *Penicillium sp.* | 2 | 3 | 3 | 1 | 3 | 1 | 3 | 3 |
| *Nigrospora sp.* | 1 | 1 | - | - | 1 | 2 | - | 1 |
| *Verticillium sp.* | - | - | - | 1 | 1 | - | 1 | 1 |
| *Curvularia sp.* | - | - | 1 | 1 | - | - | 1 | 2 |
| *Fusarium sp.* | 2 | 2 | 2 | 3 | 1 | 2 | 2 | 3 |
| White sterile mycelium | - | - | 1 | 1 | 1 | - | - | 1 |
| *Albugo candida* | 1 | 1 | 1 | 2 | 2 | 1 | 2 | 1 |
| Total | 14 | 17 | 19 | 22 | 16 | 15 | 18 | 23 |
| Pooled Mean | | | | | 15.00 | 16.00 | 18.50 | 22.50 |

**Table 2. Seasonal variation of fungal population on the leaf surface of healthy Rapeseed-Mustard during two consecutive crop seasons: *Rabi* 2014 & 2015**

| Fungi | Rabi 2014 ( Fungi: cfug $^{-1}$ leaf x 10 ) | | | | Rabi 2015 ( Fungi: cfug $^{-1}$ leafl x 10 ) | | | |
|---|---|---|---|---|---|---|---|---|
| | December | January | February | March | December | January | February | March |
| *Aspergillus niger* | 2 | - | 1 | 2 | 1 | 2 | 3 | 1 |
| *Rhizopus sp.* | 1 | 1 | 2 | 1 | 3 | 2 | 1 | 2 |
| *Mucor sp.* | 1 | 1 | 1 | 1 | 2 | 2 | 1 | - |
| *Aspergillus clavatus* | 1 | 2 | 2 | 4 | 1 | 1 | 2 | 2 |
| *Trichoderma viride* | 2 | 1 | 1 | 1 | 1 | - | 1 | 1 |
| *Trichoderma harzianum* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 2 |
| *Penicillium sp.* | 2 | 3 | - | 1 | 3 | 1 | 1 | 1 |
| *Nigrospora sp.* | 1 | 1 | - | - | 1 | - | - | - |
| *Verticillium sp.* | - | - | - | 1 | 1 | - | 1 | 1 |
| *Curvularia sp.* | - | - | 1 | 1 | - | - | 1 | 2 |
| *Fusarium sp.* | 2 | 2 | 1 | 3 | 3 | 2 | 2 | 3 |
| White sterile mycelium | - | - | 1 | 1 | 1 | - | - | 1 |
| *Albugo candida* | - | - | - | - | - | - | - | - |
| Total | 13 | 12 | 11 | 17 | 18 | 11 | 14 | 16 |
| Pooled | | | | | 15.50 | 11.50 | 12.50 | 16.50 |

conducted separately by following dilution plate technique (Singh and Rai, 1980). 5mm diameter leaf discs (total 100 discs) from twenty white rust infected leaves were collected for isolation and were transferred into a 250 ml conical flask containing 100 ml distilled water. The flasks were shaken mechanically for 20 minutes to obtain a homogenous suspension. The suspension was further diluted (1:10) by mixing a 10 ml of aliquot with 90 ml of sterile distilled water. From the stock solution ($10^{-1}$ concentration) 1ml suspension per plate was inoculated in petriplates containing BNPRA (approx. 20ml) where streptomycin (30µg/ml) was also added after autoclaving and solidification at about 38 $\pm 1^0$C for protecting bacterial contamination. A gentle shaking was done to obtain a homogenous suspension of the medium in the petriplates. Three replications were maintained. The same method and procedure as cited above was followed for isolation of fungi from healthy plant leaves. The whole experimental set up was kept inside incubator at 25 $\pm 1^0$C for 5 days.

**Identification of isolated leaf surface mycoflora:** The isolated leaf surface fungi were examined under microscope. Their identification was done by comparing the vegetative and reproductive structures using standard literatures (Barnett and Hunter, 1972; Singh, 2009 and IMA website: www.mycobank.org).

**Calculation of CFU (colony forming unit):** The colony forming unit/s per gram of leaf was calculated by using the formula given by Aneja, 2003 with slight modification as follows:

$$\text{CFU g}^{-1}\text{ leaf} = \frac{\text{Number of colonies per plate x dilution factor}}{\text{Weight of the sample taken}}$$

## RESULTS AND DISCUSSION

The seasonal occurrences of fungi were varied in different months starting from December to March in both the years (*rabi* 2014-15 and 2015-16). In the infected plant (leaves) the pooled number of seasonal mycoflora was found to be highest in March (22.50 cfug$^{-1}$ leaf x 10) and the lowest in December (15.00 cfug$^{-1}$ leaf x 10). The prominent genera in this season were *Aspergillus clavatus*, *A. niger*, *Penicilium* sp. *Fusarium* sp. However, other fungi were also found in less abundance. In healthy plant the highest occurrence of mycoflora was found in March (16.50 cfug$^{-1}$ leaf x 10) and the lowest in January (11.50 cfug$^{-1}$ leaf x 10). It may be due to the congenial temperature, RH and rainfall etc. of the habitat. In the present study higher fungal population was found in diseased plants than healthy (table 1 and 2). The workers (Singh *et al*., 1986) found that infected leaves harboured larger fungal population / cm$^2$ leaf surface and more fungal species than healthy leaves. Similarly, Sadavism and Prasad (Sadavism and Prasad, 1973) compared the phyllosphere population of diseased and healthy leaves and noticed that fungal population increased manifold on the diseased leaves than healthy ones in tapioca.

**Conclusion**

March was found congenial for abundant occurrence of mycoflora for both diseased and infected leaves. The prominent genera in this season were *Aspergillus clavatus*, *A. niger*, *Penicilium* sp. *Fusarium* sp. However, other fungi were also found in less abundance. In the present study fungal population was higher in the diseased plants than healthy plants.

**Acknowledgement**

The author is very grateful to the fellowship rendered by Manipur University,Canchipur, Imphal, India. The warm gratitude also goes to the Head, Centre of Advanced Study in Life Sciences, Manipur University for providing laboratory facilities.

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