



TOXIC EFFECT OF FUNGAL FILTRATE ON YOUNG SHOOTS OF RICE (*ORYZA SATIVA* LINN.) IN GONDIA DISTRICT OF EAST VIDARBHA, INDIA.

¹Rane V.I., ²Suryawanshi B.G., ³Bhagat G.K.

Jagat Arts, Commerce & Indiraben Hariharbhai Patel Science College, Goregaon, Dist: - Gondia (M.S.), India. 441801.

Email-vijay1968rane@gmail.com¹, Email-bhagatgajadhar@gmail.com³

Abstract- The term “toxin” has been adopted in the plant pathological literature to describe a toxic substance produced by microorganism or microorganism-host interaction, which acts directly on living host protoplast to influence either the course of disease development or symptom expression. Soil is one of the most varied environments for microbial biodiversity including fungal microorganisms. The fungal population is isolated maximum close to the soil surface and with soil depth as rhizoplane or rhizosphereregion of ricefield. Many disorders due to various fungi have been found in the physiology of rice plant, which are responsible to produce various mycotoxins like *Aspergillus flavus* and *Aspergillus oryzae* produce aflatoxin B₁, *Aspergillus versicolor* and *A. sydowii* produce sterigmatocystin, *Fusarium graminearum*, *F. oxysporum*, *F. moniliforme* etc. produce zearalenone, many species of *Penicillium* produce patulin, *Aspergillus ochraceus*, *A. sulphureus* etc. produce ochratoxin A, many species of *Penicillium* produce penicillin and *Fusarium solani*, *F. roseum* produce T₂- toxin. Significant difference was observed in wilting of young shoots (bearing 4-6 leaves) in toxic culture filtrate of 20 fungi during bioassay study. Climate of the Gondia district is tropical hot and favorable for the growth of fungi with suitable soil for rice cropping.

Key words: Toxin, Fungal filtrate, Bioassay, Wilting.

INTRODUCTION

Rice is the staple food crop of over half of the world population. More than 40% population of the countries like China, Japan, Philippines, Malaya, Shrilanka, and Mexico using rice as a primary food. India is the largest rice growing nation in the world and has highest consumers. Rice not only forms main part of the food of majority of the people but also bears a large influence on their life and economic conditions. Some of the rice varieties are used for making rice flakes, rice pops, puffed rice and in some countries for distillation of beverages. Rice straw is used as fodder, manure, roofing and strawboards material whereas, rice bran is used in manufacturing of soaps and extraction of rice bran oil [1]. Rice husk is also used in processing of bricks and in power station to generate electricity recently [2].

Gauman states “Microorganism are pathogenic only if they are toxigenic: in other words, the agents responsible for disease can damage their hosts only if they form toxins microbial poisons that penetrate into the host tissues” [3]. The strains of the fungus varied in their ability to produce toxin and high toxin production was associated with high pathogenicity [4]. The fungi harvest a large number of toxins called mycotoxins is group of highly toxic secondary metabolites of the fungi. Because of their powerful toxic nature and fairly common occurrence under natural conditions mycotoxins have fascinated worldwide attention in the current years. Mycotoxins are natural secondary metabolites produced by fungi which grow on a variety of agricultural products including cereals, grains, nuts, spices, apples, dried fruits,

and coffee beans. It is projected that 20–25% of food crops worldwide encompass mycotoxin contamination [5]. Many disorders have been found in the physiology of rice by causing various fungal organisms, which are responsible to produce various mycotoxins like *Aspergillus flavus* and *Aspergillus oryzae* produce aflatoxin B₁, *Aspergillus versicolor* and *A. sydowii* produce sterigmatocystin, *Fusarium graminearum*, *F. oxysporum*, *F. moniliforme* etc. produce zearalenone, many species of *Penicillium* produce patulin, *Aspergillus ochraceus*, *A. sulphureus* etc. produce ochratoxin A, many species of *Penicillium* produce penicillin and *Fusarium solani*, *F. roseum* etc. produce T₂-toxin (Fig-1).

MATERIALS AND METHOD

Preparation of culture filtrate:

The production of toxins by various fungi was studied by growing it on Czapek's Dox Agar (CZA) broth. Initially 250 ml broth was taken in to series of Erlenmeyer flasks (100 ml). The fungal spots from seven-day-old subculture were inoculated into the broth and incubated at 25 ± 2°C for a period of seven days. After incubation period, the culture was filtered through Whatman No. 1 filter paper, thus obtained culture filtrate. This culture filtrate was used for further toxin studies. The production of toxin and wilting of young shoots was assayed by following bioassay methods.

Wilting of young shoot:

Shoots were taken from field bearing 4-6 leaves were immersed in vials containing 5 ml of test solution (filtrate). Vials were incubated for 24 hours at room temperature. Symptoms were observed using a scale from 0 to 4 (0 = no damage, 1 = stem drooping, 2=leaves collapse, 3=leaves water soaked and 4= leaves dry and brittle).

RESULT

Significant difference was observed in wilting of young shoots (bearing 4-6 leaves) in toxic culture filtrate of 20 fungi during bioassay. Wilting effects noted after 24 hours at room temperature in table-1. Two-three young shoots were immersed in each culture filtrates and observations were narrated about symptoms using a scale from 0-4 (0 = no damage, 1 = stem drooping, 2 = leaves collapse, 3 = leaves water soaked and 4 = leaves dry and brittle).

Wilting of seedling in culture filtrates of *Aspergillus ustus*, *Penicillium* sp., *Curvularia lunata* and *Nigrospora oryzae* was observed as stem drooping; in culture filtrates of *Aspergillus sulphureus*, *A. ochraceus*, *A. sydowii*, *Trichoderma harzianum*, *T. viride*, *Alternaria tenuissima*, *Fusarium oxysporum*, and *F. moniliforme* as leaves collapse; in culture filtrates of *Aspergillus niger*, *A. wentii*, *Alternaria humicola* and *Fusarium avenaceas* leaves water soaked, similarly in culture filtrates of *Phytophthora cyamopsis*, *Helminthosporium oryzae*, *Staphylotrichum coccospoprum* and *Fusarium poaeas* leaves dry and brittle. Whereas, incontrol, there was no damage occurs in seedling (Table-1 and Plate-1).

Table 1: Effect of toxins on young shoots

S N	Treatment	Symptoms of wilting				
		0	1	2	3	4
1	Control (aqueous)	+	-	-	-	-
2	<i>Phyphthora cyamopsis</i>	-	-	-	-	+
3	<i>Aspergillus niger</i>	-	-	-	+	-
4	<i>Aspergillus ochraceus</i>	-	-	+	-	-
5	<i>Aspergillus sulphureus</i>	-	-	+	-	-
6	<i>Aspergillus sydowii</i>	-	-	+	-	-
7	<i>Aspergillus ustus</i>	-	+	-	-	-
8	<i>Aspergillus wentii</i>	-	-	-	+	-
9	<i>Penicillium</i> sp.	-	+	-	-	-
10	<i>Trichoderma harzianum</i>	-	-	+	-	-
11	<i>Trichoderma viride</i>	-	-	+	-	-
12	<i>Alternaria humicola</i>	-	-	-	+	-
13	<i>Alternaria tenuissima</i>	-	-	+	-	-
14	<i>Curvularia lunata</i>	-	+	-	-	-
15	<i>Helminthosporium oryzae</i>	-	-	-	-	+
16	<i>Staphylotrichum coccosporum</i>	-	-	-	-	+
17	<i>Nigrospora oryzae</i>	-	+	-	-	-
18	<i>Fusarium oxysporum</i>	-	-	+	-	-
19	<i>Fusarium poae</i>	-	-	-	-	+
20	<i>Fusarium moniliforme</i>	-	-	+	-	-
21	<i>Fusarium avenacea</i>	-	-	-	+	-

(0 = no damage, 1 = stem drooping, 2 = leaves collapse, 3 = leaves water soaked and 4 = leaves dry and brittle)

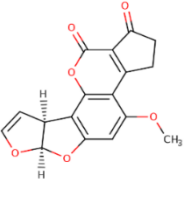
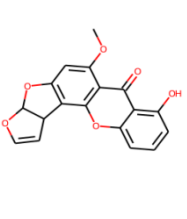
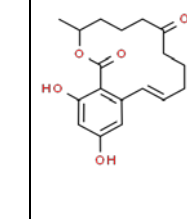
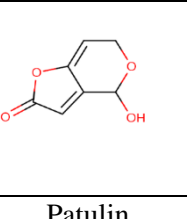
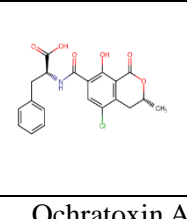
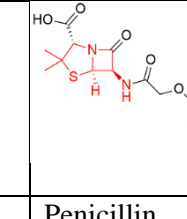
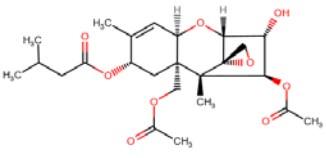
		
Aflatoxin B ₁	Sterigmatocystin	Zearalenone
		
Patulin	Ochratoxin A	Penicillin
		
T ₂ -toxin		

Fig-1 Various toxins secrets by fungal isolates

Source:

https://in.images.search.yahoo.com/search/images?_ylt=AwrX.vkssd1ljJsJjA.7HAX.;_ylu=Y29sbwNzZzMEcG9zAzEEdnRpZAMEc2VjA3Nj?p=chemical+structures+of+fungal+toxins+images&fr=mcafee

Wilting of seedling in culture filtrates of 04 fungal organisms was observed as stem drooping; in culture filtrates of 08 fungal organisms was observed as leaves collapse; in culture filtrates of 04 fungal organisms was observed as leaves water soaked, similarly in culture filtrates of 04 fungal organisms was observed as leaves dry and brittle. Whereas, not a single filtrate remains without any kind of damage related to wilting (Table-1). *Helminthosporium oryzae*, *Curvularia lunata*, *Nigrospora oryzae*, *Fusarium oxysporum* and *F. moniliforme* reported previously as pathogenic to rice and their culture filtrates might be toxic, which affect the seed germination, growth of root as well as shoot, causes various wiltings and leaf lesions significantly during bioassay. Some other, which are not reported as pathogen of rice but showed all above activities significantly during bioassay.

DISCUSSION

Mycotoxins are group of extremely toxic secondary metabolites of the fungi. Rice is one of the important cereals, which favors mycotoxin production. Rice is recurrently harvested with moisture content ranging from 18-22 percent, a moisture level high enough to support fungal growth and toxin production. Natural occurrence of aflatoxin and aflatoxin producing fungi in rice has been reported from various parts of the world [6]. *Helminthosporium oryzae*, *Curvularia lunata*, *Nigrospora oryzae*, *Fusarium oxysporum* and *F. moniliforme* informed previously as pathogenic to rice and their culture filtrates might be toxic, which affect the seed germination, growth of root as well as shoot, causes various wiltings and leaf lesions significantly during bioassay.

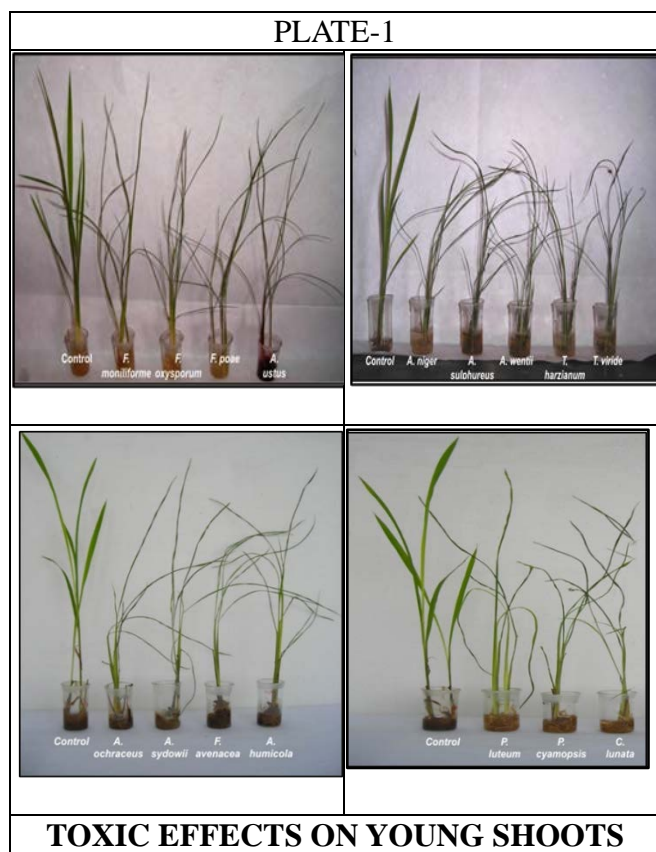
When present these toxins causes a number of serious diseases such as estrus syndrome by zearalenone in swine, cattle and other laboratory animals [7]. The storage fungi include *Aspergillus*, *Penicillium*, *Absidia*, *Mucor*, *Rhizopus*, *Chaetomium*, *Paecilomyces*, *Neurospora*, *Nigrospora*, *Sporendonema* etc produce mycotoxins in different food grains like wheat, rice, maize, bajra and ragi causes different disorders to the crop.

The production of toxin by the pathogen was assayed by adopting seed germination of paddy, ragi, green gram, black gram, black gram, and tomato and groundnuts bioassay[8]. Where, they got only 20.50 % germination in culture filtrate of *Ceratocystis paradoxa*. Whereas, in control recorded more root length (3.60cm). Present study is agreed with this investigation. Various aspects of toxicity influenced of fungal organism were mentioned by many workers of India and abroad [9]; [10]; [11] and [12].

CONCLUSION

Seven days incubation period was found to be sufficient for the production of toxins. Total 20 organisms were tested for production of toxin by bioassay methods. Fungal forms found to be toxic to the rice and their effects are found to be as follows.

Toxicity of culture filtrates influence the wilting of young shoots of rice stem was observed as **stem drooping** in culture filtrates of *Penicillium sp.* *Curvularia lunata* and *Nigrospora oryzae*. Filtrates influence the wilting was observed as **leaves collapse** in



Fusarium moniliforme, *F. oxysporum*, *A. sulphureus*, *Trichoderma harzianum*, *Aspergillus ochraceus*, *A. sydowii*, and *Alternaria alternata*. Filtrates influence the wilting was observed as **leaves water-soaked** in *Aspergillus niger*, *A. wentii*, *Fusarium avenacea* and *Alternaria humicola*. Filtrates influence the wilting was observed as **dry and brittle leaves** in *Fusarium poae*, *Phytophthora cyamopsis*, *Helminthosporium oryzae* and *Staphylotrichum coccosporum*. Whereas, there was **no damage** of young shoot in control.

Helminthosporium oryzae, *Curvularia lunata*, *Nigrospora oryzae*, *Fusarium oxysporum* and *F. moniliforme* reported previously as pathogenic to rice and their culture filtrates might be toxic, which affect the wiltings of young shoots significantly.

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REFERENCES

[1] Umar Garba, Riantong Singanusong, Sudarat Jiamyangyuen and Tipawan

- Thongsook 2017, Extraction and utilization of rice bran oil: A review. The 4th International Conference on Rice Bran Oil.1-12.
- [2] Sutas, J. Mana A. Pitak, L. 2012, "Effect of Rice Husk and Rice Husk Ash to Properties of Brick," *Procedia Engineering*. 32, pp. 1061-1067, 2012.
- [3] E. Gaumann, "Principles of plant infection," Crosby Lockwood and Son Ltd. London. 543. 1950
- [4] Rhys Brown, Emily Priest, Julian R. Naglik, and Jonathan P. Richardson, "Fungal Toxins and Host Immune Responses," *Front Microbiol.* 12, pp. 643-649, April 2021.
- [5] M. Eskola, G. Kos, C. T. Elliott, J. Hajslova, S. Mayar, R. Krska, "Worldwide contamination of food-crops with mycotoxins: validity of the Widely Cited 'FAO Estimate' of 25%," *Crit. Rev. Food Sci. Nutr.* 60, PP. 2773–2789, September 2020.
- [6] R.V. Bhat, V. Nagarjan, P. G. Tulpule, Health Hazards of mycotoxins in India. ICMR, New Delhi. 58, 1978.
- [7] K. Chang, H. Kurtz and C. J. Mirocha, C.J., "Effects of mycotoxins on swine production," *Am. J. Vet. Res.* 40, pp. 1260-1267, September 1979.
- [8] K.B. Yadahalli, S. S. Adiver and S. Kulkarni, "Ceratocystis paradoxa Associated mycotoxin-detering bud germination in sugarcane. *Indian Phytopath.* 60(2), 194-197, 2007.
- [9] R. A. Ludwig, "Toxin production by *Helminthosporium sativum* and its significance in disease development," *Can. J. Bot.* 35, pp. 291-303, May 1957.
- [10] J. D. Miller, "Aspect of the ecology of *Fusarium* toxins in cereals," *Netherlands; Kluwer Academic Publishers* spp. 19-27, 2002.
- [11] K. Narasimha Rao, G. Shyam Prasad, S. Girisham and S. M. Raddy, "Factors influencing Zearalenone production by *Fusarium moniliforme*" *Indian Phytopath.* 60(1), pp. 123–125, 2007.
- [12] Shikha Dixit and Singh, S. 2007, "Fumosis production by *Fusarium moniliforme* isolates from maize grains," *Indian Phytopath.* 60(3), pp. 380-382, 2007.