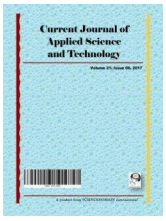

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# Effect of Fungicides on Sclerotia of Aflatoxigenic *Aspergillus flavus*

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***Authors' contributions***

*This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.*

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## ABSTRACT

The opportunistic pathogen *Aspergillus flavus* infects a vast range of agricultural crops and products, before and during harvest, handling, shipment and storage and can produce aflatoxins out of which Aflatoxin B1 was classified as group I human carcinogens by International Agency for Research on Cancer. This mitotic fungus produces sclerotia which can resist unfavourable environmental conditions by remaining dormant for long periods and may contain a considerable quantity of aflatoxins. Subsequently, sclerotia possess threat to contaminate the food chain and spreading of infection by adhering to crops in the field or during transportation. Thus suitable fungicides need to be explored for the management of sclerotia and *Aspergillus flavus*. Hence in the current investigation, the effect of three commercial fungicides indicated as CD, MZ and CH having active compositions Carbendazim 50%, Mancozeb 75% and Copper Hydroxide 77% respectively, was tested on sclerotia of aflatoxigenic *Aspergillus flavus* and the subsequent inhibition of mycelia growth and aflatoxin production was observed for two generations. Variation in colony characteristics could not be observed in either generation. Carbendazim and Mancozeb were found

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better than Copper Hydroxide fungicide for the inhibition of the germination of sclerotia but Mancozeb inhibited mycelia growth. Carbendazim and Copper Hydroxide had reduced the ability of the fungus to produce aflatoxin B1 whereas Mancozeb elevated the aflatoxin B1 production in the first generation. *A. flavus* cultures had no difference in Aflatoxin production in the second generation. Both mycelia growth and aflatoxin B1 production could not be inhibited by the same fungicide used in this study. Considering the deleterious effect of aflatoxins on human, animal health and agriculture, strategies need to be devised for the careful selection of fungicides to control *Aspergillus flavus*.

*Keywords: Aspergillus; mycotoxin; aflatoxin; mancozeb; carbendazim; sclerotia.*

## 1. INTRODUCTION

The ubiquitous fungus *Aspergillus flavus* is mainly known as an opportunistic pathogen and abundantly found mostly in tropics where hot and humid climate exit in the most part of the year. Apart from causing invasive Aspergillosis in humans, mycoses in animals and many diseases in insects, the awful part of this fungus is that it infects a vast range of agricultural crops and products before and during harvest, handling, shipment and storage [1,2]. In case of acute infestation under poor storage conditions, it can produce aflatoxins in many crops like maize, wheat, sorghum, oilseeds, cotton seeds, fresh and dried vegetables, spices, cattle feed and rice. Aflatoxins are a group of mycotoxins which include aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1) and aflatoxin G2 (AFG2) are considered as highly toxic and carcinogenic compounds that cause disease in livestock and humans. The International Agency for Research on Cancer (IARC) has clarified AFB1 in the group I as human carcinogens [3-5].

Furthermore, this mitotic fungus produces specialized resistant structures known as sclerotia which are pigmented, compact aggregates of specialized hyphae derived from cleistothecia and may represent a vestige of ascospore production. Sclerotia are important survival structures, stored with reserve food materials hence can resist unfavourable environmental conditions by remaining dormant for long periods [6-10]. This makes the control of this fungus very difficult by various means like the application of chemical fungicides. Further, the dormant sclerotia may adhere to the surface of seeds and grains and may cross geographical boundaries during national and international transport. In addition to that, the sclerotia may also contain a considerable quantity of aflatoxins which may contaminate the food chain [7].

Many commercial fungicides synthesised from various chemical constituents like Carbendazim, Copper Hydroxide, Mancozeb, Tridemorph, Triadimenol, Difenoconazole, Miconazole and Fenpropimorph etc. are available in the market and they have the potential to control the vegetative growth, spore germination and aflatoxin production by *Aspergillus flavus* and other fungi [11-14]. At the same time, many of these fungicides under sublethal or sub-inhibitory dosage might cause morphological as well as physiological alterations in pathogenic fungi. Subsequently, these alterations might lead to the increased growth, enhanced production of aflatoxins [15-18] and other mycotoxic secondary metabolites. Even emergence of fungicide-resistant strains was observed [19-24]. Some fungicides can induce mutations in pathogens which are extremely dangerous environmental factors that could lead to the emergence of pathogens with increased aggressiveness and adaptability to agrocenosis conditions [25].

However, information regarding the effect of these fungicides on sclerotia germination and subsequent deliberations is lacking. Hence, an attempt was made in the current investigation to study the preliminary effect and efficiency of three commercial fungicides symbolised as CD, MZ and CH to reduce and restrict the germination of sclerotia of an aflatoxigenic *Aspergillus flavus*. The active ingredients of the fungicides were Carbendazim, Mancozeb and Copper Hydroxide respectively. The subsequent germination & mycelia growth of fungicide-treated sclerotia on culture media and aflatoxin production were also studied to observe the long-term effect.

## 2. MATERIALS AND METHODS

### 2.1 Isolation of *Aspergillus flavus* (A28) Isolate and Sclerotia

The *Aspergillus flavus* (A28) culture was collected from Dr. (Mrs.) U. Dhua's laboratory, Crop Protection Division, National (previously

Central) Rice Research Institute, Cuttack, India. The *A. flavus* was isolated from indoor air of farmer's house around the paddy storage area. This *A. flavus* isolate was chosen since it already proved to produce high amounts of aflatoxins (>10 µg/ml) and sclerotia. For the current study, sclerotia were harvested using a small paint brush from mature culture. After washing with sterilised water to remove spores, the sclerotia were then blot dried and finally completely dried using vacuum pump. The dried sclerotia were used for interaction with fungicides *in vitro*.

## 2.2 Interaction of Sclerotia of A-28 with Fungicides

Three commercial fungicides which were commonly used by farmers and available in the market were used in the current study. The aim of the current study was never to defame the makers of the fungicides hence the trade names have not been revealed. The fungicides were indicated by their active ingredients which are as follows: CD (50% Carbendazim w/w), MZ (Mancozeb 75% w/w) and CH (Copper Hydroxide 77% w/w). Based on the composition of active ingredient two concentrations were prepared such as 0.1% and 0.15% for MZ and CH. For CD 0.1% and 0.05% were taken. The concentrations of the fungicides were selected based on the recommendations which farmers usually follow to spray in their fields to control fungal diseases. All fungicidal solutions were prepared with sterilised distilled water. After dissolving, the fungicide solutions were kept in 60mm sterile Petri plates and exposed to UV for 30 minutes inside laminar air flow hood to avoid possible microbial contamination and then filter sterilised using a vacuum pump. An aliquot of 25µl from this solution was taken in cavity slides and then 20 numbers of uniformly sized sclerotia were kept in the solution. Sclerotia were also put in sterilised water to be considered as untreated control. The cavity slides were kept in humid chambers at 28°C and allowed to interact with fungicides for 24 hours without exposing the humid chamber to open air. The germination of sclerotia and formation of germ tubes were observed under 4X objective 15X eyepiece of an Inverted tissue culture microscope (Radical). Microscopic photography was done with a digital camera (Sony Cyber shot). Germination and growth of individual sclerotia were observed and scored with 1 to 5 evaluation scale as: 1- Zero (no growth), 2- Poor, 3- Moderate, 4-Good and 5-Very good. The average mode was taken into consideration for the evaluation of sclerotia germination for each treatment and control.

The fungicide-treated sclerotia were then inoculated in Petri plates containing β-Cyclodextrine-Potato Dextrose Agar media (β-CD-PDA) and incubated at 28°C. Composition β-CD-PDA /Litre: Peeled potato-250 g, Dextrose-20 g (Hi-media), β-Cyclodextrine-6 g (Hi-media) and Agar-15g. Untreated sclerotia were inoculated in the same media as a control. Observations were taken for comparison of mycelia growth and aflatoxin production with untreated control. Inhibition of mycelia growth was calculated as below:

$$\frac{\text{Colony area of Control} - \text{Colony area of treatment}}{\text{Colony area of control}} \times 100$$

$$\text{Colony area} = \pi r^2$$

The cultures raised by this process were considered as a fungicidal interacted first generation. From these plates, toxin extraction was also done after ten days. Sclerotia from first generation isolates were again interacted in the same fashion with the same concentration of fungicides to raise the second generation. Inhibition of mycelia growth and aflatoxin production was also observed for the second generation. Statistical calculations for significance were done using ANOVA of the MS Excel format.

## 2.3 Extraction of Aflatoxin

Toxin extraction was done as follows: Mycelia along with media and sclerotia were ground properly with 25 ml of extraction buffer (60% Methanol containing 0.5% KCl) in a sterilised mortar and pestle [26]. The solution was vortexed for proper mixing and then centrifuged at 12000 rpm for 10 minutes at 4°C. After filtration the supernatant was vacuum evaporated to 10 ml and then used for detection of aflatoxin B1 by Thin Layer Chromatography (TLC).

## 2.4 Detection of Aflatoxin B1 by TLC

20 g of Silica gel G (Hi-media) mixed with 40ml of distilled water and applied manually on a clean glass plate (22×21 cm) to make a thin and uniform gel. After drying properly it was kept inside oven at 121°C for one hour and then

cooled at room temperature. An aliquot of 20 µl each from aflatoxin B1 standard (Hi-Media, India) and sample was spotted at 4 cm above the bottom of the plate. Development of plate was done with 100 ml of developing solvent containing Chloroform to Acetone at 90:10 proportions. The distance travelled by the solvent front was observed. After development, the plate was dried and spots were visualised and documented under 360 nm on UV Transilluminator (G-Box).

## 3. RESULTS

The effect of three commercial fungicides indicated as CD, MZ and CH having active compositions Carbendazim 50%, Mancozeb 75% and Copper Hydroxide 77% respectively, were tested on sclerotia of aflatoxigenic *Aspergillus flavus* and the subsequent inhibition of mycelia growth and aflatoxin production were observed for two generations. Carbendazim and Mancozeb were found to be very effective in all concentrations to inhibit the germination of sclerotia *in vitro* as no germ tubes were observed under the microscope and thus germination evaluated with average mode of 1 in a 1 to 5 scale (Table 1 & Fig. 1). However, germ tubes have been formed by sclerotia treated with 0.1% and 0.15% of Copper Hydroxide fungicide though the germination was poor with the evaluated average mode of 2. The range of germination was poor to moderate (mode value 2-3) in this case. Moderate germination (average mode 3) was observed in untreated control i.e. sclerotia kept in sterilised water.

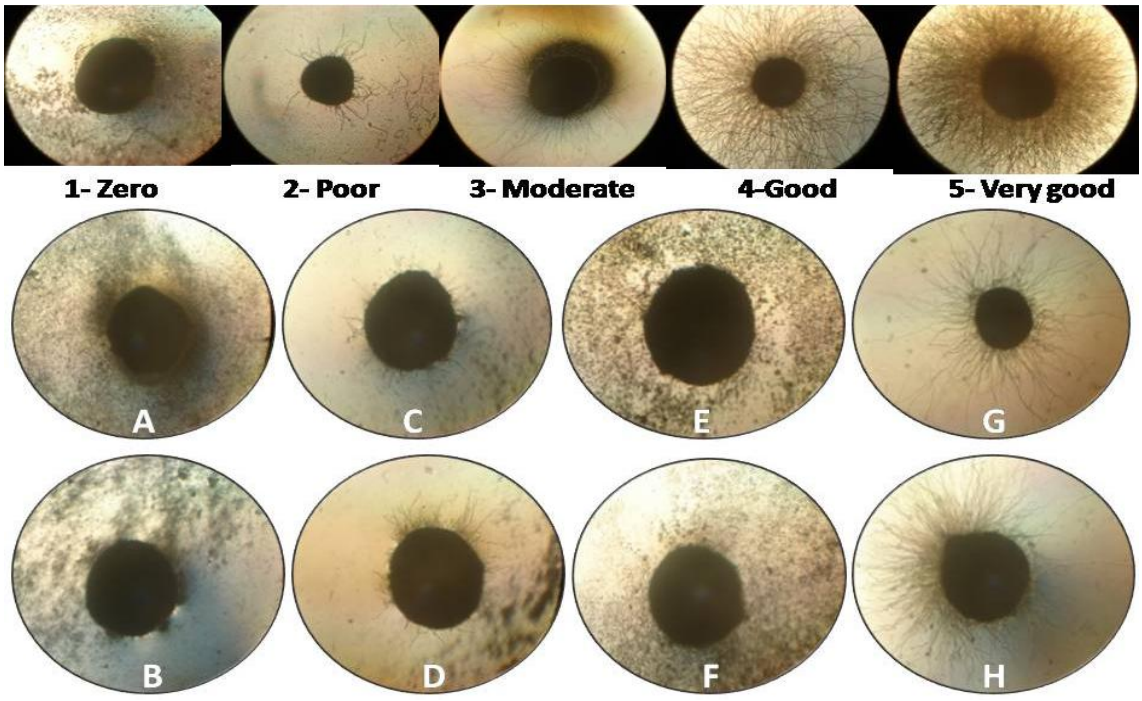
The fungicide-treated and untreated sclerotia were then inoculated in β-CD-PDA media to examine any possible effect on mycelia growth and aflatoxin production in the first generation. Inhibition of periodic mycelia growth was found to be more in sclerotia treated with Mancozeb. The colony areas of the culture, in this case, were inhibited up to 19.5% and 43.7% by 0.1% and 0.15% of Mancozeb respectively in the third day after inoculation in media (Table 2). Inhibition of colony area in cultures developed from sclerotia treated with other fungicides in all concentrations was much less and not significant. Culture characters were not significantly varied between treatments and control on the third day, however, pro-sclerotia were observed more in cultures developed from Copper Hydroxide and Mancozeb treated sclerotia (Fig. 2). As far as aflatoxin B1 production is concerned Carbendazim and Copper Hydroxide were found to be effective in comparison to Mancozeb. Cultures developed from sclerotia treated with 0.1% and 0.05% Carbendazim produced less than and equal to 2 µg/ml of aflatoxin B1 respectively in comparison with untreated control where the amount of aflatoxin produced was equivalent to 10 µg/ml. Cultures developed from sclerotia treated with 0.1% and 0.15% of Copper Hydroxide fungicide produced less than 10 µg/ml aflatoxin B1. However, in case of Mancozeb treatment aflatoxin B1 production was found to be more or equivalent to 10 µg/ml in first generation interaction (Fig. 3).

Sclerotia from first generation cultures were harvested and treated with same fungicides in the same fashion to raise second generation cultures. Inhibition of colony area followed the pattern as of the first generation where inhibition was found in cultures raised from sclerotia treated with Mancozeb. The inhibition was 21.1% and 24.8% by 0.1% and 0.15% of Mancozeb respectively on the third day of incubation.

**Table 1. Effect of fungicides on germination of *A. flavus* sclerotia**

| Sl. No. | Fungicides tested | Concentrations of fungicide tested % | *In 1-5 scale evaluation of germination/growth of sclerotia (Average mode) | *In 1-5 scale range | Remarks |
|---|---|---|---|---|---|
| 1. | CD | 0.05 | 1 | 1 | No germination |
| | | 0.1 | 1 | 1 | No germination |
| 2. | CH | 0.1 | 2 | 2-3 | Poor germination |
| | | 0.15 | 2 | 1-3 | Poor germination |
| 3. | MZ | 0.1 | 1 | 1 | No germination |
| | | 0.15 | 1 | 1 | No germination |
| 4. | Water (control) | - | 3 | 3-4 | Moderate germination |

** In 1-5 scale, evaluation of germination/ growth of A. flavus sclerotia*




**A**- MZ 0.1%
**B**- MZ 0.15%
**C**- CH 0.1%
**D**- CH 0.15%
**E**- CD 0.05%
**F**- CD 0.1%
**G & H**- untreated control

**Fig. 1. Formation of germ tubes in fungicide-treated sclerotia in cavity slides**

Negligible inhibition in mycelia growth was observed by treatments with other fungicides (Table 3). Culture morphology also didn't show drastic variation between control and fungicide treatment. However, the formation of pro sclerotia was more in cultures raised from sclerotia treated with Mancozeb (Fig. 4). Again in case of Mancozeb treatment, aflatoxin B1 production was found to be more than the untreated control (Fig. 5).

## 4. DISCUSSION

Although bio-pesticides are slowly replacing the chemical pesticides, a complete global look at the scenario indicates that chemical pesticides like fungicides are still applied as a major approach to control pathogens and diseases of crops. Infection of crops by aflatoxigenic strains of *Aspergillus flavus* may commence in the agricultural field hence fungicides are necessarily to be used [1,2]. Reports regarding the overall evidence concerning the effectiveness of fungicides are contradictory and in certain cases somewhat unexpected. Sarita et al. [11] found Carbendazim to be most effective in reducing the seed borne mycoflora and enhancing the germination percentage of mung bean (*Phaseolus aureus* Roxb.). Similarly, Rathod et al. [13] found Carbendazim and Mancozeb to be more repressive than other fungicides in control of seed-borne fungi of Groundnut. Mycotoxigenic fungi like *Aspergillus flavus* and other *Aspergillus* species have been very sensitive to Carbendazim even in a concentration of as low as 0.25% [14]. However, the effect of fungicides on sclerotia of aflatoxigenic *A. flavus* has not been demonstrated in similar studies. In the current investigation three chemical fungicides indicated as CD (Carbendazim), CH (Copper Hydroxide) and MZ (Mancozeb) (not revealing the trade names of fungicides) have been found to be effective in controlling the germination of sclerotia in vitro whereas some growth was observed in sclerotia treated with CH. Under experimental purpose, fungicides are mixed directly in growth media to observe the effect on the formation of mycelia, spores and aflatoxin production. But the subsequent changes in the pathogen strain treated with fungicides also need to be ascertained. The reason behind this is the availability of reports in the literature about the enhancement of growth and production of aflatoxin due to some fungicides. In the present

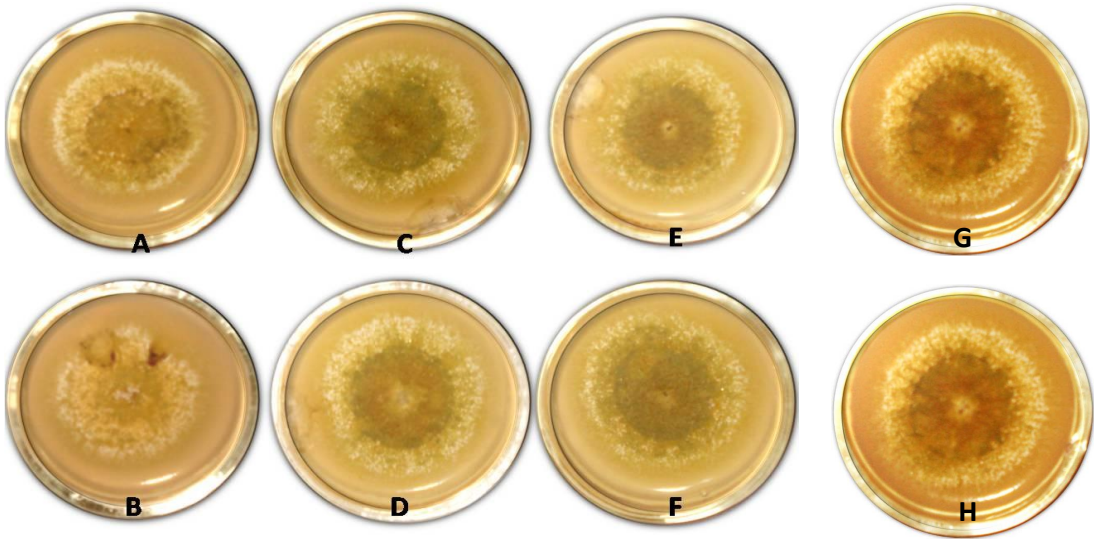
study, the fungicide-treated sclerotia were grown on culture media to examine any possible consequences in mycelia growth and aflatoxin production of *A. flavus* in two successive generations. Overall it was witnessed that Mancozeb was most effective in the inhibition of mycelia growth and Carbendazim was effective in inhibition of aflatoxin production by *A. flavus* in successive two generations. Mancozeb in both generations increased the production of aflatoxin B1 and sclerotia formation. The rapid decrease of the effect of MZ from first to the second generation might be due to the possible appearance of a rapid resistance mechanism which opened the door for further vigorous studies.

The use of fungicides can be very attractive to varying degrees in reducing mycotoxigenic fungal load, but one must be aware that under sublethal and lower concentrations these may increase fungal biomass and also stimulate the mycotoxin production and may alter the relative proportions of the component aflatoxins [18]. Even the previously discussed Carbendazim at lower concentrations (25-100 ppm) stimulated the growth of *Aspergillus flavus*, *A. ochraceus* and *A. versicolor* and aflatoxin B1 production, whereas at higher concentrations (2000-3000 ppm) growth and toxin production were completely inhibited [16]. In a classic case of study by Bayman and Cotty [15], up to 80% inhibition in radial growth of aflatoxigenic *A. flavus* was obtained by 100ppm of fungicide Triadimenol but at the same time maximum of 700 fold increase in aflatoxin production was also observed. Similarly, treatment of wheat crop with Mancozeb was found to elevate the production of aflatoxin B2 while AFB1 and deoxynivalenol, on the contrary, were reduced [17].

**Table 2. Periodic inhibition of mycelia growth developed from sclerotia of *Aspergillus flavus* (A-28) treated with fungicides. Mycelia growth on β-CD-PDA is shown for first generation interaction**

| Fungicide treatments | Periodic inhibition percentage* | | |
|---|---|---|---|
| | Day-1 | Day-2 | Day-3 |
| Mancozeb (0.1%) | 39.7 | 35.7 | 19.5 |
| Mancozeb (0.15%) | 64.4 | 60.5 | 43.7 |
| Copper hydroxide (0.1%) | 0.0 | 2.1 | 1.5 |
| Copper hydroxide (0.15%) | 9.0 | 18.7 | 11.6 |
| Carbendazim (0.05%) | 3.0 | 1.0 | 0.0 |
| Carbendazim (0.1%) | 15.7 | 12.8 | 5.0 |

** Inhibition in terms of colony area is shown here, LSD at p<0.05 is 7.32, LSD at p<0.01 is 9.8*




**A**- MZ 0.1%
**B**- MZ 0.15%
**C**- CH 0.1%
**D**-CH 0.15%
**E**-CD 0.05%
**F**-CD 0.1%
**G & H**- untreated control

**Fig. 2. Colony morphology of cultures developed from fungicide treated sclerotia and untreated control in first generation**

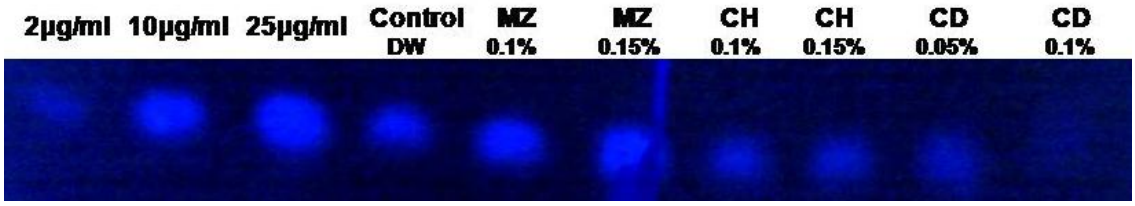



**Fig. 3. Aflatoxin B1 production by *A. flavus* cultures in the first generation developed from sclerotia treated with fungicides and untreated control. The intensity of blue illuminated spots corresponds to the quantity of aflatoxin B1**

**Table 3. Periodic inhibition of mycelia growth developed from sclerotia of *Aspergillus flavus* (A-28) treated with fungicides. Mycelia growth on β-CD-PDA is shown for second-generation interaction**

| Fungicide treatments | Periodic inhibition percentage* | | |
|---|---|---|---|
| | Day-1 | Day-2 | Day-3 |
| Mancozeb (0.1%) | 6.8 | 7.7 | 21.1 |
| Mancozeb (0.15%) | 6.8 | 8.0 | 24.8 |
| Copper hydroxide (0.1%) | 0.0 | 0.0 | 3.0 |
| Copper hydroxide (0.15%) | 0.0 | 0.0 | 3.2 |
| Carbendazim (0.05%) | 0.0 | 1.0 | 1.8 |
| Carbendazim (0.1%) | 0.0 | 1.0 | 2.5 |

** Inhibition in terms of colony area is shown here, LSD at p<0.05 is 1.57, LSD at p<0.01 is 2.15*

Apart from *Aspergillus flavus* and aflatoxins many other studies have also reported the hike in production of other mycotoxins by mycotoxigenic fungi caused by fungicidal treatments. Hysek et al. [19] observed an increase in the production of Deoxynivalenol (DON) in Spring Barley after treatment with the Fungicides Azoxystrobin and Tebuconazole. Carbendazim has been found to reduce fungal biota, but stimulated Ochratoxin A (OTA) production, while Fenhexamid, Mancozeb, and Copper Hydroxide plus copper also enhanced infection and OTA production in grapes [20]. Miguel et al. [23] found that treatment of *Fusarium verticillioides* with fludioxonil + metalaxyl-M reduced the mycelia growth effectively but a 10 fold increase in sporulation and 3.5 fold increase in fumonisin production was observed simultaneously.

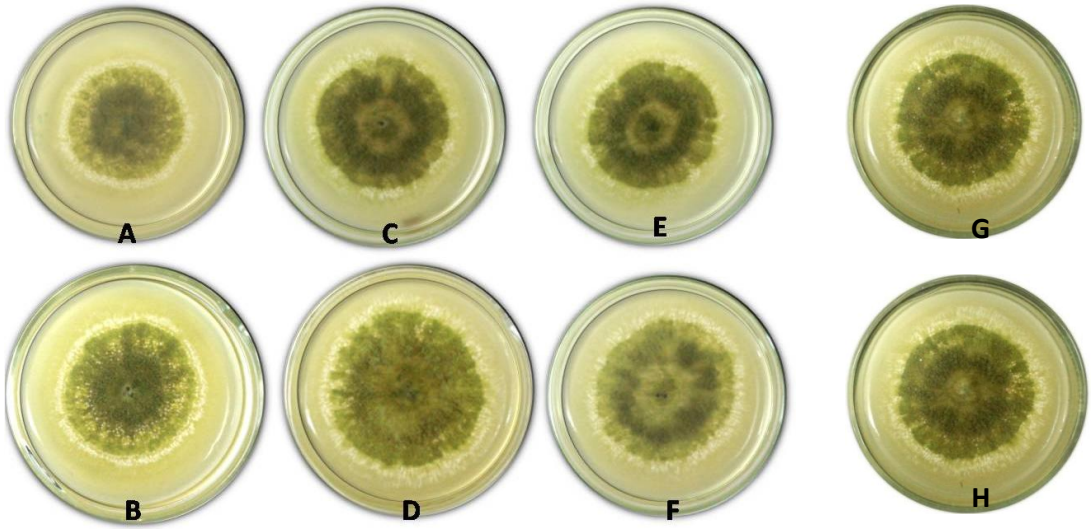



**Fig. 4. Colony morphology of cultures developed from fungicide treated sclerotia and untreated control in second generation**

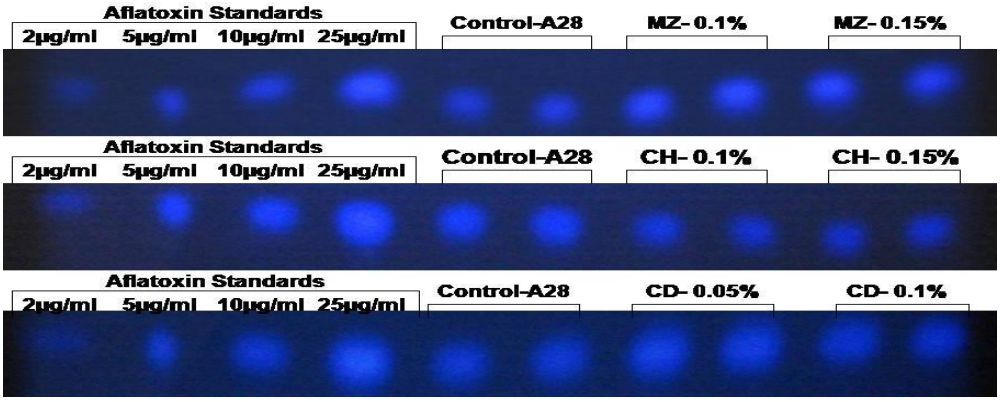



**Fig. 5. Aflatoxin B1 production by *A. flavus* cultures in the second generation developed from sclerotia treated with fungicides and untreated control. The intensity of blue illuminated spots corresponds to the quantity of aflatoxin B1**

Suboptimal doses of triazole fungicides transcriptionally activated the trichodiene synthase gene in *F. graminearum*. The accumulated results of Popiel et al. [24] showed that mycotoxin production by surviving mycelium could be enhanced when effective fungicides like Carbendazim was applied to a full-grown pathogen. It could thus be concluded that the expression of mycotoxin biosynthetic genes is strongly influenced not only by the amount or the type of antifungal compound but also the timing of fungicide exposition relative to mycelium growth. Beyond all these observations, the induction of mutations in many pathogens (*Botrytis cinerea*) by the fungicides resulted in the evolution of resistant strains which is an alarming situation [22].

Sclerotia are resistant structures formed by the aflatoxigenic fungi which can stay dormant during unfavorable conditions containing aflatoxin in some cases. Further, those may adhere to crops and crop residues and may cross geographical boundaries thus spreading the infection. Hence inactivation of these defiant structures turns necessary by careful selection of fungicides.

## 5. CONCLUSION

The increase in global human population put pressure on farming communities to yield more agricultural crops which in turn intensified the use of agrochemicals like fertilizers, pesticides, hormones etc. The appearance of new crop diseases and unprecedented increase in pathogen population lead to the inordinate use of antimicrobial chemicals like fungicides. As in the line of literature, in the current study also, no fungicide used was found to be effective in controlling both mycelia as well as aflatoxin production. Therefore careful selection should be made while opting for chemical fungicides to inhibit mycotoxic fungi like *Aspergillus flavus*. Though these chemical fungicides have been broadly effective in controlling many of crop diseases but simultaneously raised many environmental as well as health issues. Hence, the use of alternative management perspectives like bio-pesticides, microbial pesticides, botanicals etc. may be tried for sustainable agriculture and better future of world health.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

## REFERENCES

1. Fakruddin M, Chowdhury A, Hossain MN, Ahmed MM. Characterization of aflatoxin producing *Aspergillus flavus* from food and feed samples. Springer Plus. 2015;4(1): 159.
   DOI: 10.1186/s40064-015-0947-1
2. Klich MA. *Aspergillus flavus*: The major producer of aflatoxin. Mol Plant Pathol. 2007;8(6):713-22.
   DOI: 10.1111/J.1364-3703.2007.00436.X
3. IARC. Food items and constituents, heterocyclic aromatic amines and mycotoxins. In: IARC monographs on the evaluation of carcinogenic risks to humans. International Agency for Research on Cancer. 1993;489-521.

4. Lai X, Zhang H, Liu R, Liu C. Potential for aflatoxin B 1 and B 2 production by *Aspergillus flavus* strains isolated from rice samples. Saudi j Biol Sci. 2015;22(2):176-80.
DOI:http://dx.doi.org/10.1016/j.sjbs.2014.09.013
5. Tola M, Kebede B. Occurrence, importance and control of mycotoxins: A review. Cog Food Agriculture. 2016;2(1): 1191103.
DOI:http://dx.doi.org/10.1080/23311932.2016.1191103
6. Cotty PJ. Aflatoxin and sclerotial production by *Aspergillus flavus*: Influence of pH. Phytopathol. 1988;78:1250-53.
DOI: 10.1094/Phyto-78-1250.
7. Wicklow DT, Shotwell, L. Intrafungal distribution of aflatoxins among conidia and sclerotia of *Aspergillus flavus* and *Aspergillus parasiticus*. Can J Microbiol. 1983;29:1-5.
DOI: https://doi.org/10.1139/m83-001.
8. Rollins JA, Dickman MA. Increase in endogenous and exogenous cyclic AMP levels inhibits sclerotial development in *Sclerotinia sclerotiorum*. Appl Environ Microbiol. 1998;64:2539-2544.
9. Willetts HJ, Bullock S. Developmental biology of sclerotia. Mycol Res. 1992;96: 801-816.
DOI:https://doi.org/10.1016/S0953-7562(09)81027-7
10. McAlpin CE. An *Aspergillus flavus* mutant producing stipitate sclerotia and synnemata. Mycol. 2001;93:552-565.
DOI: 10.2307/3761740.
11. Sarita, Buts AK, and Singh R. Seed borne mycoflora of mung bean (*Phaseolus aureus* Roxb.) and its control by fungicides. Adv Appl Sci Res. 2014;5(6): 8-10.
12. Santos L, Marín S, Sanchis V, Ramos AJ. In vitro effect of some fungicides on growth and aflatoxins production by *Aspergillus flavus* isolated from Capsicum powder, Food Add Cont: Part A. 2011;28:1:98-106.
DOI: 10.1080/19440049.2010.529622.
13. Rathod LR, Jadhav MD, Kanse DS, Patil DP, Gulhane SD, Deshmukh PS. Effects of Fungicides on Seed Borne Pathogen of Groundnut. Int J Adv Biotechnol Rese. 2010;1(1):17-20.
14. Aggarwal A, Sharma D, Parkash V, Sharma S, Gupta A. Effect of bavistin and dithane M-45 on the mycorrhizae and rhizosphere microbes of sunflower. Helia. 2005;28(42):75-88.
DOI:http://dx.doi.org/10.2298/HEL0542075A
15. Bayman P, Cotty PJ. Triadimenol stimulates aflatoxin production by *Aspergillus flavus in vitro*. Mycol Res. 1990;94(8):1023-5.
DOI: https://doi.org/10.1016/S0953-7562(09)81327-0
16. El-Kady IA, El-Maraghy SS, Abdel-Mallek AY, Hasan HA. Effect of four pesticides on aflatoxin production by *Aspergillus flavus* IMI 89717. Zentralblatt für Mikrobiologie. 1993;148(8):549-57.
DOI:https://doi.org/10.1016/S0232-4393(11)80219-2
17. Da Luz SR, Pazdiora PC, Dallagnol LJ, Dors GC, Chaves FC. Mycotoxin and fungicide residues in wheat grains from fungicide-treated plants measured by a validated LC-MS method. Food chem. 2017;220:510-6.
DOI:http://dx.doi.org/10.1016/j.foodchem.2016.09.180
18. D'mello JF, Macdonald AM, Postel D, Dijksma WT, Dujardin A, Placinta CM. Pesticide use and mycotoxin production in *Fusarium* and *Aspergillus* phytopathogens. European J Plant Pathol. 1998;104(8): 741-51.
DOI:https://doi.org/10.1023/A:1008621505708
19. Hysek J, Vanova M, Hajslova J, Brozova J, Sychrova EL, Radova-Sypecka ZU, Sip V, Sykorova S, Chrpova JA, Tvaruzek L. Variation in the production of trichothecene mycotoxin deoxynivalenol (DON) in spring barley varieties after treatment with the fungicides azoxystrobin and tebuconazole. Plant Prot Sci. 2005;41(2):58.
20. Varga J, Kocsube S, Péteri Z, Vágvölgyi C, Tóth B. Chemical, physical and biological approaches to prevent ochratoxin induced toxicoses in humans and animals. Toxins. 2010;2(7):1718-50.
DOI: 10.3390/toxins2071718
21. Magan N, Medina A, Aldred D. Possible climate-change effects on mycotoxin contamination of food crops pre-and postharvest. Plant Pathology. 2011;60(1):150-63.
22. Hahn M. The rising threat of fungicide resistance in plant pathogenic fungi:

*Botrytis* as a case study. J Chem Biol. 2014;7(4):133-41.
DOI: 10.1007/s12154-014-0113-1

23. Miguel TD, Bordini JG, Saito GH, Andrade CG, Ono MA, Hirooka EY, Vizoni É, Ono E. Effect of fungicide on *Fusarium verticillioides* mycelial morphology and fumonisin B1 production. Brazilian J Microbiol. 2015;46(1):293-9.
DOI: http://dx.doi.org/10.1590/S1517-838246120120383

24. Popiel D na, Dawidziuk A, Koczyk G. The role of fungicides, their infl uence on expression of key effl ux and mycotoxin biosynthetic genes in the control of resistant *Fusarium* strains. 37th Mycotoxin Workshop. June 1-3, 2015, Bratislava, Slovakia.
Available:http://www.mykotoxin.de/upload/docs/Bratislava_Conference_abstracts_2015.pdf

25. Patyka V, Buletsa N, Pasichnyk L, Zhitkevich N, Kalinichenko A, Gnatiuk T, Butsenko L. Specifics of pesticides effects on the phytopathogenic bacteria. Ecol Chem Eng S. 2016;23(2):311-31.
DOI: 10.1515/eces-2016-0022

26. Waliyar F, Reddy SV. Training manual on "*Aspergillus flavus* seed infection and aflatoxin estimation by ELISA" and aflatoxin management options in groundnut. ICRISAT International Crops Research Institute for the Semi-Arid Tropics Patancheru 502 324 Andhra Pradesh, India; 2009.
Available:http://oar.icrisat.org/1983/1/Aflatoxin_Training_course_manual.pdf



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