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EVALUATION OF DIFFERENT FUNGICIDES AGAINST *ASPERGILUS FLAVUS* AND THEIR COMPARATIVE EFFICACY UPON GERMINATION OF INFECTED RICE SEEDS

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ABSTRACT. *Aspergilus flavus* is the most common seed born fungus that deteriorates the seed quality and minimizes the export value of the rice all over the world. Fungicides are the most successful and commonly used way to manage any fungi but more use of fungicides have resulted evolution in the seed born fungi so efforts are required off and on to stay ahead of the fungal races. Keeping in view, the present research work was conducted to evaluate different fungicides against *A. flavus* and their comparative efficacy upon the infected rice seeds. Experiment was laid out in completely randomize design with varying concentrations of fungicides (20, 40, 60 and 80 ppm) under laboratory conditions. Statistical results shown significant reduction in mycelial growth and improved the seed germination as well. The results were significantly better when the fungicides were used at 80 ppm, as compared to low concentrations. Regarding mycelial growth, Kumulus-DF and Cabrio-Top were comparative to each other, followed by Trimiltox-Forte, Cordate and Copper oxychloride, while for the other attribute of infected grain germination Kumulus-DF proven better in comparison with Trimiltox-forte and Cabrio-Top, followed by Cordate and Copper oxychloride.

Keywords: Kumulus-DF; Cabrio-Top; Trimiltox-Forte; Cordate; Copper oxychloride.

INTRODUCTION

Most of the Asian countries consume rice (*Oryzae sativa* L.) as staple food as it is highly nutritive (Zafar *et al.,* 2004). Economy of Pakistan is highly dependent upon rice export to other countries (Zahid *et al.,* 2005). Recently, in Pakistan, the particular commodity was harvested from 2850 thousand hectares by

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recording the total production as 10351 metric tons (USDA, 2015). More than 40 diverse viral, bacterial and fungal diseases attack this highly nutritive crop resulting in adverse yield losses (Khan *et al*., 1990; Rehman *et al.,* 2013; Islam *et al.,* 2016a; Islam *et al.,* 2017a, d). Among most of the fungi, rice quality is being deteriorated by seed born fungus *A. flavus*, as it is most common and is isolated by several researchers (Arshad *et al*., 2009; Habib *et al*., 2012; Ishfaq *et al*., 2015; Islam *et al.,* 2016b; Islam & Ahmed, 2016). The infested seeds may fail to germinate, transmit disease from seed to emerging seedlings and from those seedlings to growing plants (Fakir *et al.,* 2002). The particular fungus is also involved in association with many seed born fungus to result in complex diseases. The other fungus, that are involved with this, are *Pyricluaria, Alternaria, Bipolaris, Curvularia, Rhizopus, Mucor, Schlerotenia, Fusarium* etc., and all these play vital role in qualitative, as well as quantitative losses of rice crop (Wahid *et al.,* 2001; Habib *et al*., 2012). In absence of the long lasting resistance, the management of the seed born fungi is commonly dealt with seed dressing fungicides. Although the fungicides are hazardous (Islam & Ahmed, 2016; Islam *et al.,* 2016c; Islam *et al.,* 2017b,c; Islam, 2017), but they have been proved as successful solution to manage different crop infecting fungi (Latif *et al.,* 2006; Shakahwat, 2009; Habib *et al*., 2007, 2012; Rehman *et al*., 2013), but due to their excessive usage, the pathogen undergoes evolution against the chemicals so time to time evaluation of the new compounds is a dire need of time to stay one step ahead of the pathogen. So, the present study was carried out to evaluate some fungicides against the most common seed born fungus *A. flavus* and their comparative efficacy was also tested upon the infected seed germination percentage.

MATERIALS AND METHODS

Isolation, purification and identification of *Aspergilus flavus*

Infected grain samples were collected, immersed in 70% ethanol solution for 1 min, rinsed twice in sterilized water, dried on blotter paper, placed on PDA (Potato starch = 20 g; Agar Agar = 20 g; Dextrose = 20 g; Distilled water = 1 L) in Petri plates and then placed in incubator at 27±2°C for almost 5-6 days (Nghiep & Gaur, 2005). The Petri plates were observed under low power stereo microscope and identification of *A. flavus* was done by colony growth, color, sporulation type and available literature (Barnett & Hunter, 1990; Mathur & Kongsdal, 2003).

In vitro* evaluation of different fungicides against *Aspergillus flavus

Laboratory evaluations of different fungicides were conducted under completely randomized design (CRD) by using four replications. Random applications of following treatments were applied to each Petri plate, where T1 = Copper oxychloride (50% WP); T2 = Kumulus-DF (80% Sulphur); T3 = Cabrio-Top (250 g WP); T4 = Cordate (4 WP); T5 = Trimiltox-Forte (41% WP) and T0 = Control.

All the fungicides were processed for *in vitro* evaluations against the *A. flavus* colony growth by application of poisoned food technique with variable concentrations (20, 40, 60, 80 ppm). Before pouring the potato dextrose media in the plates, 1 ml from each fungicidal concentration was added to the Petri plates. Afterwards, pouring of approximately 12-15 ml of sterilized medium in each sterilized Petri plate (9 cm) was performed and was allowed for solidification leading to incubation by addition of 0.7 cm discs of 10 days old *A. flavus* culture. Four replications of all the concentrations kept for each fungicide along with the control. Incubation process was done at 27 ± 2^0C and data regarding colony diameter was recorded after a week (Rao & Lalithakumari, 1987). The results were recorded for all the attributes, as well as control check. Fungal radial growth inhibition was recorded on the basis of colony diameter by following formula (Sundar *et al*., 1995).

$$\text{Percent inhibition } (I) = \frac{X - Y}{X} \times 100 ,$$

where, X = Growth of test fungus in control in cm and Y = Growth of test fungus in treatment in cm.

Evaluation of fungicides for germination test

All the five fungicides were also tested for their comparative efficacy upon the *A. flavus* infested rice seeds to check the improvement in the germ-ability of the seeds. The fungal suspension from the isolated culture of *A. flavus* was prepared (1.50×10^5/ml) as described by Ma *et al*., (2008) and seeds were dipped into the suspension (*Fig. 1*).

Figure 1 - Preparation of *Aspergilus* suspension and dipping of rice seeds

Four concentration (20, 40, 60 and 80 ppm) were prepared for seed dressing purposes and 200 fungal infested rice seeds were placed in fungicides for 24 hrs while keeping the untreated infested seeds as control check. Afterwards, the soaked seeds from each fungicidal treatment were sown by using standard blotter paper technique in separate petri plates (ISTA, 1985). Each Petri plate containing 50 seeds was labeled while using four Petri plates for each fungicidal treatment. Percentage germination of seeds was drawn by following formula:

$$\% \text{ Age germination} = \frac{\text{Number of seeds germinated}}{\text{Number of seed sown}} \times 100$$

Statistical analysis

The data regarding colony inhibition of *A. flavus* by varying fungicidal treatments and infested seeds germination was recorded and subject to statistical analysis, where all possible interactions were determined by analysis of variance and comparison of means of all the data was done by LSD test at 5% level of probability (Steel *et al*., 1997).

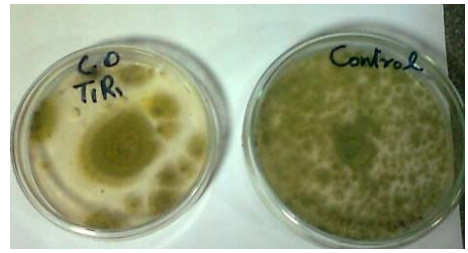
RESULTS

***In vitro* evaluation of various fungicides against *Aspergillus flavus* by poisoned food technique**

Our results exhibited that all the fungicides decreased the mycelial growth at application of varying dozes, as compared to control. But, relatively higher concentrations of all the fungicides were proved better as inhibition of colony growth of fungus was directly proportional to the concentrations of fungicides applied. Average inhibition decreased with the decrease in fungicidal concentrations, i.e. 80 ppm concentration (46.90%), 60 ppm (33.31%), 40 ppm (18.23%) and then at 20 ppm (8.50%). All the fungicides performed different at different concentration (*Fig. 2*). On average basis, highest significant mycelia inhibition was recorded by Kumulus-DF (44.040%), followed by Cabrio-Top (43.38%), Trimaltox-Forte (41.8%), Copper oxychloride (40.87%) and Cordate (37.05%). Effect of different fungicides on the colony growth with respect to concentration applied showed significant relationship. Result indicated that with increased concentrations of fungicides, colony growth decreased. For example, *Fig. 3* shows significant growth inhibition at 80 ppm by Kumulus-DF (52.20%) and low significant growth inhibition by Cordate (39.02%), as compared to control (100%). For 60 ppm, Kumulus-DF recorded growth inhibition as 38.83%, while Cordate proved to be least effective (25.73%). For 40 ppm attribute, Kumulus-DF exhibited 17.97% growth inhibition and lower significant growth inhibition was shown by Cordate (14.00%). At 20 ppm, Kumulus-DF resulted 11.20% inhibition, while Cordate recorded 7.0%, as compared to control (100%) (*Fig. 4*).

Figure 2 - Stock solutions of various fungicides

Cabrio-Top at 80 ppm



Copper Oxychloride at 80 ppm

Kumulus-DF at 80 ppm

Trimaltox-Forte at 80 ppm

Cordate at 80 ppm

Figure 3 - Germination percentage at 80 ppm concentration of fungicides

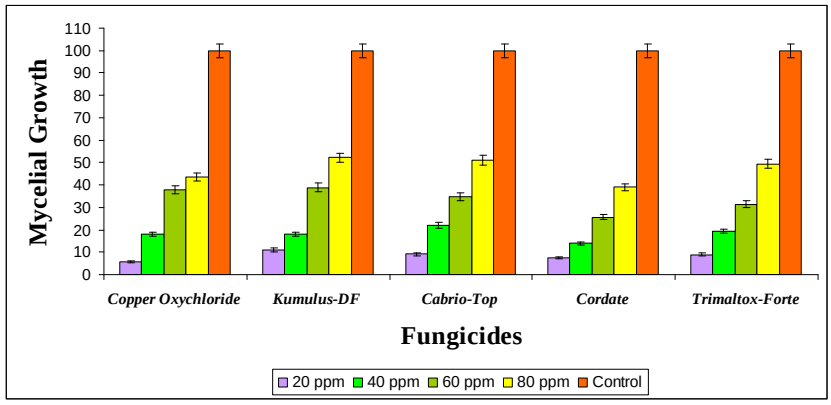


Figure 4 - Evaluation of fungicides against *Aspergillus flavus* by poisoned food technique

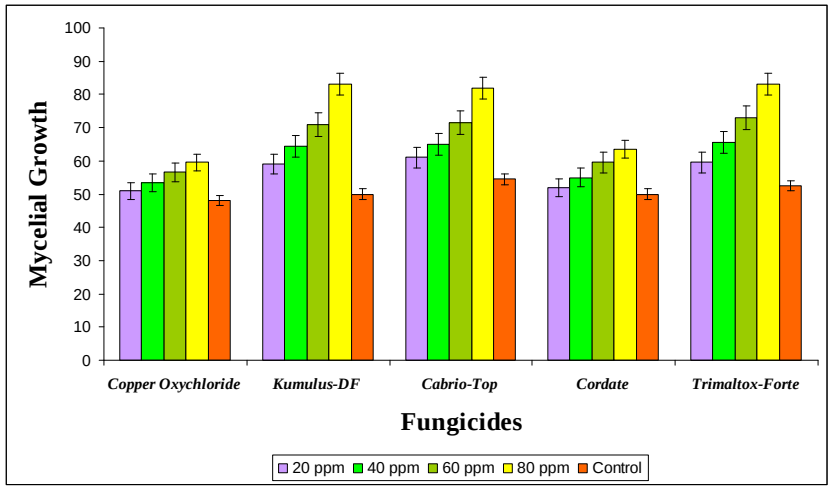


Figure 5 - Efficacy of various fungicides on germination percentage of rice seeds by using standard blotter paper technique

The efficacy of various fungicides on germination percentage of rice seeds by using standard blotter paper technique

Fig. 5 revealed that seed dressing of infested rice seeds by different fungicides improved the germination percentage, as compared to control. Germination percentage was also directly proportional to the applied fungicidal concentrations as at 80 ppm concentration average germination percentage for all the fungicides were 82.7%, followed by 60 ppm (71.5%), 40 ppm (65%) and 20 ppm (59.8%). Germination percentage for all the fungicides varied significantly at all these concentrations**.** The germination percentage was recorded improved at higher concentrations of fungicides, as at 80 ppm Kumulus-DF and Cabrio-Top exhibited results 83.00% and 82.00%, while Copper oxychloride and Cordate performed below power by recording the results as 59.50% and 63.50%, respectively. The concentrations of fungicides applied upon infested grains proved directly proportional to the improvement in germination percentage of the seed as reduced concentration, i.e. 20 ppm exhibited minimized germination of seeds and results recorded were 59.00%, 61.00%, 59.5% for Kumulus-DF, Cabrio-Top, Trimaltox-Forte and 51.00%, 52.00% for Copper oxychloride and Cordate, respectively (*Fig. 5*).

DISCUSSION

A. flavus is associated with many other seed born fungi and results in yield and quality losses of rice (Khan *et al*., 1990). In the absence of long lasting resistance, the cheapest and

good method of controlling the disease is through seed treatment with fungicides. The use of fungicides has been credited successful against the target fungi by many researchers previously (Latif *et al.,* 2006; Shakahwat, 2009; Habib, 2007,2012; Rehman, 2013). We found that 80 ppm of various fungicides proved lethal and successfully inhibited the colony growth of *A. flavus*. Our results are nearing to the results of (Ahmad *et al.,* 2002), who documented the successful use of different fungicides approximately similar concentrations against seed born pathogens including *A. flavus*. Sitara & Hasan (2011) were also in agreement with our results as they isolated the *A. flavus* from discolored rice seeds and documented the successful management of discolored rice grains by using fungicides. Similar in results was (Arshad *et al*., 2009), who isolated eight seed born fungi including *A. flavus* and evaluated four fungicides (Topsin-M, Dithane M-45, Ridomil, and Carbendazim) and categorized Redomil as best. We found that due to excessive use of fungicides again and again, least concentrations of fungicides did not prove more lethal against the fungus as 20 ppm concentrations of all the fungicides exhibited least colony growth inhibition. These findings are in line with findings of Habib *et al.* (2012), who evaluated Copper oxychloride along with six other fungicides and reported low concentration of fungicides as less successful against the seed born mycoflora. The results of Ashwini *et al.* (2015) are also encouraging as he also successfully managed the *A. flavus* and other seed borne fungi *via* fungicides.

We further tested the fungicides for seed dressing purposes by checking the percentage germination of infested seeds and found that the germination percentage of seeds was improved by fungicidal dressing. We recorded seed germination more than 80% on average basis, which is encouraging by the results of Nghiep & Gaur (2005), who reported 69.5% increase in the germination percentage of *A. flavus* affected seeds. In context to that Ridomyl Gold was found to be effective against seed borne mycoflora, which also encourage our results (Sitara & Akhter, 2007). The results strengthen by the reports that explained about more than 71% germination in seeds dressed by different fungicides (Ibiam *et al.,* 2006).

CONCLUSION

Our experimental findings reveal that fungicides reduced the mycelial growth and also improved the seed germination of infested seeds. The results were significantly better when the fungicides were used at 80 ppm, as compared to low concentrations. Regarding mycelial growth, Kumulus-DF and Cabrio-Top were comparative to each other, followed by Trimiltox-Forte, Cordate and Copper oxychloride, while for the other attribute of infected grain germination

Kumulus-DF proven better in comparison with Trimiltox-Forte and Cabrio-Top, followed by Cordate and Copper oxychloride. So, we recommend the higher concentration of fungicides for the better management of *A. flavus.*

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