`BADEGGI JOURNAL OF AGRICULTURAL RESEARCH AND ENVIRONMENT, 2024, 06(01), 47– 53

NCRI Press

Available online: www.ncribjare.org
ISSN: 2695-2122, e-ISSN: 2695-2114
DOI: https://doi.org/10.35849/BJARE202401/52/006
Journal homepage: www.ncribjare.org

Research Article

Grain Discolouration Disease Complex of Rice: Assessment and Management of Infested Grains Using Fungicides

Maji, E. A[1]., Imolehin, E. D[2]., Ocean, H. O[1] and Olapade, R. T[1]
[1]College of Biosciences, Salem University, Lokoja, Kogi State, Nigeria
[2]National Cereals Research Institute Badeggi, Niger State, Nigeria
Correspondence e-mail: adejoh_ojone@yahoo.com

Abstract
Strategic fungicide management is a crucial component in the fight against Grain Discolouration Disease (GDD) in rice. This silent killer has been plaguing rice farmers for decades, causing significant yield losses and threatening food security in many countries. Thus, samples of discoloured grains were collected and different fungi were isolated. Isolated fungi were identified based on their morphological characters. Also, the purified culture of each fungus was prepared. Pathogenicity test was carried out in-vitro and in-vivo using the percentage incidence calculation to confirm the involvement of fungi. Statistical results illustrated that the homogenous mixture of spore suspension contains fungal spores of *Fusarium* spp, *Tricoderma*, *Aspergillus flavus*, *Aspergilus niger*, *Helmithosporium*, *Cercospora*, *Alternaria*, *Bipolaris* and Curvularia of rice proven most lethal in showing the maximum symptoms in-vitro and in-vivo. Statistical data for seed germination exhibited that Carbendazim and Diathene-M45 were comparatively better and improved percentage seed germination up to considerable levels followed by Redomil, Topsin-M and Cordate respectively. The study justified that there are involvement of different fungal species in disease establishment and seed discolouration. Also, germination percentage of discoloured rice grains can be improved using various seed dressing fungicides.

Keywords: *Carbendazim, Diathene-M45, Disease complex, Grain discolouration, Redomil*

Introduction
Rice (Oryzae sativa L.) is a staple crop for millions of people around the world, providing a crucial source of nutrition and livelihood (Coaza et al. 2021). However, the threat of grain discolouration disease continues to plague rice farmers, causing significant yield losses and economic impact. This silent killer, also known as rice blast or rice brown spot, is caused by a fungal pathogen and can significantly reduce the quality and market value of rice (Yu et al. 2022).

Grain discolouration disease is caused by the fungus Pyricularia grisea, commonly known as rice blast. It can affect rice plants at all stages of growth, from seedling to maturity, and can cause significant damage to leaves, stems, and grains (Coaza et al. 2021). The disease is characterized by yellow or brownish spots on the leaves, which can eventually turn into dark brown or black lesions. As the disease progresses, the lesions can spread to the stems and grains, causing them to shrivel and turn black, reducing their market value.

In the case of unpredictable climatic conditions across different ecological zones, severity of the disease also ranges from minor to major (Yu et al. 2022). The shape and size of the grain are affected by grain discoloration which further leads to a

Received on 16th February, 2024; Revised 18th March,2024; Accepted 29th March, 2024 and Published on 27th April, 2024

significant lower yield of the crop. This malady also causes weight loss in paddy grain which also affects the post-harvest processing of the rice seeds and cooking quality. Strategies such as better utilization of genetic resources, precise pathogen identification and improved agricultural practices should be developed and suggested to help cope up against this malady (Kini *et al.* 2019).

The current study underlines the effectiveness of fungicide treatment, especially with respect to reducing contamination with the trichothecenes DON, DON3G, and NIV, as well as CUL and its derivatives, 5-hydroxy-CUL and 15-hydroxy-CUL, and AURO. All these metabolites were significantly reduced as a result of fungicide treatment in the years 2018, 2020, and 2021 according to both or at least one of the data analysis schemes (Del et al. 2022).

Interestingly, the fungicide-induced decrease of *Fusarium* metabolites was accompanied by a significant increase of the *Alternaria* metabolite IP and the metabolite TRYP (Leslie et al. 2021). Furthermore, correlation analysis indicated a negative correlation of both IP and TRYP concentrations with concentrations of *Fusarium* metabolite BEA and *Penicillium* metabolite N-benzoyl-phenylalanine (Eskola et al. 2020). Similarly, Drakopoulos et al., (2021) reported a negative correlation between the occurrence of IP and a number of *Fusarium* metabolites (e.g., AURO, CUL, 5-hydroxy-CUL, 15-hydroxy-CUL, DON, ENNs, MON, ZEN,) in the grain and straw of barley samples.

A negative correlation between metabolites produced by *Fusarium* spp., *Alternaria* spp., and *Penicillium* spp. could be due to competition between these fungi. This hypothesis is supported by the results of a recent study, which found a reduction of the concentration of Alternaria toxins, including IP, and the metabolite TRYP, following *Fusarium* inoculation of wheat (Del et al. 2022). It could be interesting to further investigate the effect of fungicide treatment on the ecological relationship between different fungal populations in future studies (Matumba et al. 2021). Stored rice commodities are continuously threatened by stored grain pest which also results in economic losses (Islam *et al.,* 2016a, 2017a; Azizi *et al.* 2020). Forty different types of viral, bacterial and fungal diseases stress the crop at its different stages (Islam and Ahmad, 2016; Islam *et al.,* 2017b; Yu *et al.* 2022). But as the crop is grown in the irrigated area and the conditions become humid in the crop ecosystem, so the conditions become favourable for the attack of different fungi (Rehman *et al.,* 2013 and Coaza *et al.* 2021). The complex fungal association may leads towards the attack of grain discolouration disease complex of rice, as several fungi are considered associated with the disease (Khan *et al.,* 2000; Arshad *et al.,* 2009; Habib *et al.,* 2012; Ashfaq *et al.,* 2015 and Azizi *et al.* 2020). Common symptoms include brown to black colour dark glumes or kernels (Ou, 1985). The infected seeds show reduced germination percentage and become a leading cause to transmit the disease to the seedlings and ultimately infecting the plants. From such seeds in Nigeria, commonly the fungi that have been isolated and reported are *Alternaria, Aspergillus, Curvularia, Rhizopus, Helminthosporium maydis, Penicillium* and *Trichoderma* (Islam and ahmad, 2016). The disease is associated with the qualitative as well as quantitative losses in Nigeria. So it is particularly important to identify the different fungi associated with the disease so that the better management practices can be followed to control the disease. As the quick and effective solution to manage different fungi are use of fungicides (Rehman *et al.,* 2013) so it is very important to find the fungicides that are effective and can be used to improve the germination percentage of the infested rice seeds.

Materials and Methods

The collection of discoloured and healthy grains was carried out from plant pathology research area, National Cereals Research Institute, Badeggi, Niger State, Nigeria. Preparation of PDA media was done weighing 20 grams potato starch, 20 grams agar agar, 20 grams dextrose and one litre of distilled water. Dextrose and potato starch was dissolved in 500 ml of water in one flask and adding agar agar in other 500 ml water in another flask (Ashfaq *et al.,* 2015). Then contents from both of the flasks were mixed to generate a final volume of one litre. The media was then autoclaved for twenty minutes at $121^{o}C$

and 15 psi. About 12- 15 ml of the autoclaved media was poured into the pre sterilized petri plates of 9 cm diameter and was placed in refrigerator after solidification.
The isolation of fungi was done from infected grain samples by immersing the grain samples in 70% ethanol solution for 1 minute. Afterwards, samples were rinsed in sterilized water twice, dried, and then placed upon the petri plates containing PDA. Hygienic conditions were maintained to reduce the chances of contamination. Incubation of all the petri plates was done at 27±2°C for 5-6 days (Nghiep and Gaur, 2005).

Purified culture from different isolated fungi was prepared and pathogenicity test was performed for confirmatory purposes. For this, surface sterilization of healthy rice grains was done with 70% and thorough washing was carried out with double distilled water. Inoculation of these healthy grains was performed by dipping them in fungal suspensions of 1.50×10^{5}/ml (Ma *et al.*, 2008) by using four petri plates while untreated grains were kept as control. Afterwards standard blotter paper method was followed leading to the incubation of petri plates for a week at 27°C. The developed fungal species were examined under different microscopes and data for percent incidence was recorded using the formula below (Mathur and Kongsdal, 2003).

$$\text{Percentage Incidence} = \frac{\text{Number of infested seed}}{\text{Number of plated seed}} \times 100$$

Inoculum of isolated fungi was prepared by picking the pure colonies grown on PDA media, placing them in 500 ml sterilized water separately in beakers, agitating and stirring upon mechanical stirrer for one hour and diluting it to make uniform fungal suspension of spores. Inoculation of healthy rice panicles was done by spraying the spore suspension while the untreated panicles were considered as a control. Observations upon the panicles were recorded on daily basis and fungi were re-isolated for confirmation purposes. Data for percentage incidence was recorded for two years using the formula below and mean data for the two years were used.

$$\text{Percentage Incidence} = \frac{\text{Number of infested panicles}}{\text{Number of healthy panicles}} \times 100$$

Efficacy of fungicides upon germination of discoloured grains was carried out using five fungicides namely; Cordate, Diathane M-45, Carbendazim, Redomil, and Topsin-M, were evaluated regarding their comparative efficacy upon fungal infested discoloured grains for two years to examine the comparative improvement in their germination percentage. For this purpose standard blotter paper technique was used using a completely randomized experimental design. The fungal suspension from the isolated cultures of all the fungi was prepared (1.50×10^{5}/ml) as described by Ma *et al.* (2008) and seeds were dipped into the suspension. Four different concentrations (20, 40, 60 and 80 ppm) of all the fungicides were prepared for seed dressing purposes and 200 fungal infested rice seeds were placed in fungicides for 24 hours 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 labelled while using 4 petri plates for each fungicidal treatment. Percentage germination of seeds two years was determined using the formula.

$$\text{Percentage Germination} = \frac{\text{Number of germinated seed}}{\text{Number of sown seed}} \times 100$$

The particular pathogenicity test was performed in the field in a randomized complete block design (RCBD) experiment involving three replications (Steel *et al*. 1997). Combinations of the following treatment were applied randomly to each block. F1= *Fusarium oxysporum* ; F2= *Aspergillus flavus* ; F3= *Aspergillus niger* ; F4= *Fusarium monilliforme* ; F5= *Helminthosprium oryzae* ; F6= *Curvularialunta*; F7=*Cercospora oryzae* ; F8= *Rhizoctonia solani* ; F9= *Bipoloris oryzae*. The treatments were

Variety = Basmati-2000 T1= F1+F2+F3
T2=F4+F5+F6
T3=F7+F8+F9
T4=F1+F2+F3+F4
T5=F5+F6+F7+F8
T6=F1+F2+F3+F4+F5
T7=F5+F6+F7+F8+F9

T8=F1+F2+F3+F4+F5+F6+F7
T9=F1+F2+F3+F4+F5+F6+F7+F8+F9
T10= To/Control

Results and Discussion

Recorded results indicated that the symptoms began to appear after ten days on all the seeds. Figure 1 indicates that seeds inoculated with fungal suspension exhibit the percentage incidence as 79.25 % as compared to untreated check (12.25 %). The study is in agreement to the identification efforts from 225 samples of rice which were collected from different states. Using blotter paper method and agar plate method, it was found to be contaminated with species of 16 fungal genera *Alternaria*, *Helminthosporiumspp, Curvularia*, *Fusarium* and *Aspergillus* spp (Riaz *et al.*, 1995). In agreement with our findings were also the findings of Guiterrez 2002 and Islam *et al.* 2017a who detected 16 fungi such as *Alternaria alternate, Aspergillus spp, Curvularia* spp, *Epicoccum* spp, *Fusarium graminearum, Fusarium moniliforme, Nakataeasigmoidea* and *Microdochium oryzae*. Javed *et al.* (2002) conducted a similar work upon 873 paddy seed samples during 1994-2002 for routine health test by Standard blotter paper method and detected *Alternaria, Cercospora, Fusarium, Aspergillus* spp, *Rhizopus, Chaetomium, Clletotrichum, Fusarium, Penicillium, Phoma, Pyricularia,* and *Sclerotium*. The fungal species identified in this study are also similar with the findings of Arshad *et al.* (2009).

The results interestingly revealed a significant effect in terms of increase in percentage incidence as compared to the control. Treatment T9 gave the mean percentage incidence for treated panicle as (77.667%) whi le treatment T1 that was including only three representative fungi was competitively showing the higher percentage (36.00%) than T2 & T3 (Figure 2). The mean percentage incidence in control was (11.667%). The treatment T3 was including *Fusarium* and *Aspergilus* spp. that exhibit that these play vital role in discolouration, Eskola et al. (2020) the efficacy of various fungicides on germination percentage of rice seeds by using standard blotter paper technique. Figure 3 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 at 80ppm. Concentration average germination percentages of all the fungicides were 77.8% followed by 60ppm (67.2%), 40ppm (62.6%) and 20ppm (57%). Whereas control attribute shown 49.9% seed germination. Germination percentage for all the fungicides varied significantly at all these concentrations (Matumba et al. 2021). The germination percentage as recorded, improved at higher concentrations of fungicides at 80 ppm. Carbendazim and Diathene-M45 exhibited results 83.00% and 81.00% while Redomil, Topsin-M and Cordate performed below power by recording the results as 79.00%, 77.00% and 69.00% 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 63.00%, 62.00%, 55.00% for Carbendazim, Diathene-M45, Redomil and 53.00%, 52.00% for Topsin-M and Cordate respectively (Figure 3).

Grain discolouration disease of rice is an emerging threat to rice crop in Nigeria. Researchers consider that grain discolouration disease complex is associated with many fungi such as *Fusarium, Phoma, Curvularia, Tricoderma, Aspergillus* spp, *Helmithosporium, Cercospora,* and *Rhizoctonia*.

We further tested five 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. Seed germination was recorded more than 80% on average basis regarding fungicide dressed infested seeds which is encouraging by the results of Nghiep and Gaur, (2005), Islam and Manzoor, (2017) and Leslie et al. (2021). They both reported 69.5% increase in the germination percentage of *Aspergillus Flavus* affected seeds. In context to that Ridomyl- Gold was found to be effective against seed borne mycoflora which also encourage our results (Sitara and Shahida, 2007; Del et al. 2022).

Conclusion

The experimental efforts in field as well as in laboratory justified that there are involvement of different fungal species in disease establishment and seed discolouration. This study further concludes that germination percentage of discoloured rice grains can be improved using various seed dressing fungicides.

References

Arshad HMI, Khan JA, Naz S, Khan SN, Akram M (2009). Grain discolouration disease complex a new threat for rice crop and its management. Pak. J. Phytopathol., 21(1): 31-36.

Ashfaq M, Shaukat MS, Akhter M, Haider MS , Mubashar U, Hussain SB (2015). Comparison of fungal diversity of local and exotic rice (Oryza Sativa L.) Germplasm for their seed health. J. Animal & Plant Sci., 25(5): 1349- 1357

Azizi MMF, Ismail IS, Ina-Salwany MY, Hata EM, Zulperi D. (2020). The emergence of Pantoea Del Puerto, O.; Gonçalves, N.P.F.; Medana, C.; Prevot, A.B.; Roslev, P. Attenuation of toxicity and occurrence of degradation products of the fungicide tebuconazole after combined vacuum UV and UVC treatment of drinking water. *Environ. Sci. Pollut. Res. Int.* 2022, *29*, 58312–58325.

Coaza A, Caiza RB, Garrido A, Moreno C, Guevara JL, Regalado H, Ramos L, Flores F, Garrido P. (2021). First report of Pantoea ananatis causing leaf spot disease of maize in Ecuador. Plant Dis. 105(10):3286.

Del Puerto, O., Gonçalves, N.P.F., Medana, C., Prevot, A.B.; Roslev, P. (2022). Attenuation of toxicity and occurrence of degradation products of the fungicide tebuconazole after combined vacuum UV and UVC treatment of drinking water, *Environ. Sci. Pollut. Res. Int.* 2022, *29*, 58312–58325.

Drakopoulos, D.; Sulyok, M.; Krska, R.; Logrieco, A.F.; Vogelgsang, S. (2021). Raised Concerns about the Safety of Barley Grains and Straw: A Swiss Survey Reveals a High Diversity of Mycotoxins and Other Fungal Metabolites. *Food Control* 2021, *125*, 107919

Eskola, M.; Kos, G.; Elliott, C.T.; Hajšlová, J.; Mayar, S.; Krska, R.(2020). Worldwide Contamination of Food-Crops with Mycotoxins: Validity of the Widely Cited "FAO Estimate" of 25. *Crit. Rev. Food Sci. Nutr.* 2020, *60*, 2773–2789.

Gutierrez SA, Mazzanti-de-Castanon MA, Cundamm MA (2002). Fungi isolated from rice seed in Argentina. Phytopathol., 37(2):156-163.

Habib A, Javed N, Sahi ST, Waheed M (2012). Detection of seed borne mycoflora of different coarse and fine rice varieties and their management through seed Treatments. Pak. J. Phytopathol., 24(2):133-136.

International Seed Testing Association (ISTA) (1985). International Rules for seeds testing. Seed Sci. Technol., 3:299-335.

Islam W, Rasool A, Wu Z (2016a). Inhibitory effects of medicinal plant extracts against Triboliumcastaneum (Herbst.) (Coleoptera: Tenebrionidae). Mayfeb J. Agric. Sci., 3: 15-20.

Islam W, Awais M, Noman A, Wu Z (2016b). Success of bio products against bacterial leaf blight disease of rice caused by Xanthomonas oryzaepv. oryzae. PSM Microbiol., 01(2): 50- 55.

Islam W, Ahmed M (2016). Inhibitory effects of organic extracts against Aspergilus flavus and their comparative efficacy upon germination of infested rice seeds. PSM Microbiol., 01(2): 79- 84.

Islam W, Nazir I, Noman A, Zaynab M, and Wu Z (2017). Identification of Different Fungi Associated with Grain Discolouration Complex Disease of Rice and Management of Infested Grains throughFungicides. International Journal of Scientific Research in Agricultural Sciences, 4(2), pp. 030-035,

Islam W, Nazir I, Noman A, Zaynab M, Wu Z (2017a). Inhibitory effect of different plant extracts on Trogoderma granarium (everts) (coleoptera: dermestidae). Int. J. Agri and Env. Res., 3(1): 121-130.

Islam W, Zhang J, Adnan M, Noman A, Zaynab M, Wu Z (2017b). Plant virus ecology: A glimpse of recent accomplishments. Appl.

Ecol. Env. Res., 15(1): 691-705.

Javed MS, Wahid A, Idrees M, Gill MA, Saleem A (2002). Seed mycoflora studies in rice. Pak. J. Physiol., 14(2): 132-134.

Khan JA, Jamil FF, Gill MA (2000). Screening of rice varieties/ lines against bakanae and baterial leaf blight (BLB). Pak. J. Phytopathol., 12: 6–11.

Kini K, Dossa R, Dossou B, Mariko M, Koebnik R, Silué D. (2019). A semi-selective medium to isolate and identify bacteria of the genus. Pantoea J Gen Plant Pathol. 85(6):424–427.

Leslie, J.F.; Moretti, A.; Mesterházy, Á.; Ameye, M.; Audenaert, K.; Singh, P.K.; Richard-Forget, F.; Chulze, S.N.; Ponte, E.M.D.; Chala, A.; et al. (2021). Key Global Actions for Mycotoxin Management in Wheat and Other Small Grains. *Toxins* 2021, *13*, 725.

Mathur SB, Kongsdal O (2003). Common laboratory seed health testing methods for detection fungi, Published by the ISTA, Switzerland, 1: 393- 399.

Ma LY, Ji Z, Bao J, Mu X, Li X, Zhuang J, Yang C, Xia Y (2008). Responses of rice genotypes carrying different dwarf genes to Fusarium moniliform and Gibberellic acid, Pl. Prod. Sci., 11: 134-138.

Matumba, L.; Namaumbo, S.; Ngoma, T.; Meleke, N.; De Boevre, M.; Logrieco, A.F.; De Saeger, S.(2021). Five Keys to Prevention and Control of Mycotoxins in Grains: A Proposal. *Glob. Food Sec.* 2021, *30*, 100562.

Nghiep HV, Gaur A (2005). Efficacy of seed treatment in improving Seed quality in rice. Oman Rice., 13: 42-51.

Ou SH (1985). Rice Diseases. CAB International Mycological Institute, Kew, Surrey, U.K.

Raiz M, Nayer MN, Anayetullah M (1995). Worked on seeds of 225 samples of rice, collected from the provinces of Pakistan. Pak. J. Sci. Ind. Res., 27(11): 389-392.

Rehman A, Mehboob S, Islam W, Khan NA (2013). Reaction of gram (Cicer Arietinum L.) varieties against gram blight disease (DidymellaRabiei (Kovatsch.) Arx) and its management through foliar fungicides in rain fed areas of Pakistan. Pak. J. Phytopathol., 25(01): 07-14.

Sitara U, Akhter S (2007). Efficacy of fungicides, sodium hypochlorite and neem seed powder to control seed borne pathogens. Pak. J. Bot., 39(1): 285-292.

Steel RGD, Torrie GH, Dickey (1997). Principles and Procedures of Statistics: A Biochemical Approach, 3rd ed. Mc Graw-Hill, New York, U.S.A.

Yu L, Yang C, Ji Z, Zeng Y, Liang Y, Hou Y. (2022). First report of new bacterial leaf blight of rice caused by Pantoea ananatis in southeast China. Plant Dis. doi:10.1094/PDIS-05-21.

Zahid MA, Akhtar M, Sabir M, Anwar M, Jamal A (2005). Genotypic and phenotypic correlation and path analysis in coarse grain rice. Proceeding of the International seminar in rice crop. Oct 2-3. Rice Research Institute, Kala Shah Kaku. 29.

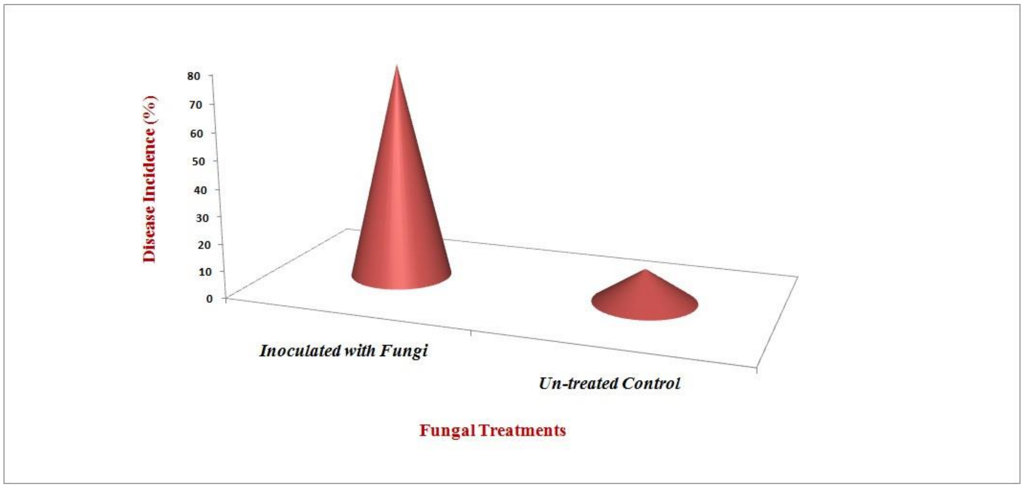

Figure 1: Mean **co**mparative percentage incidence data for two years under laboratory conditions

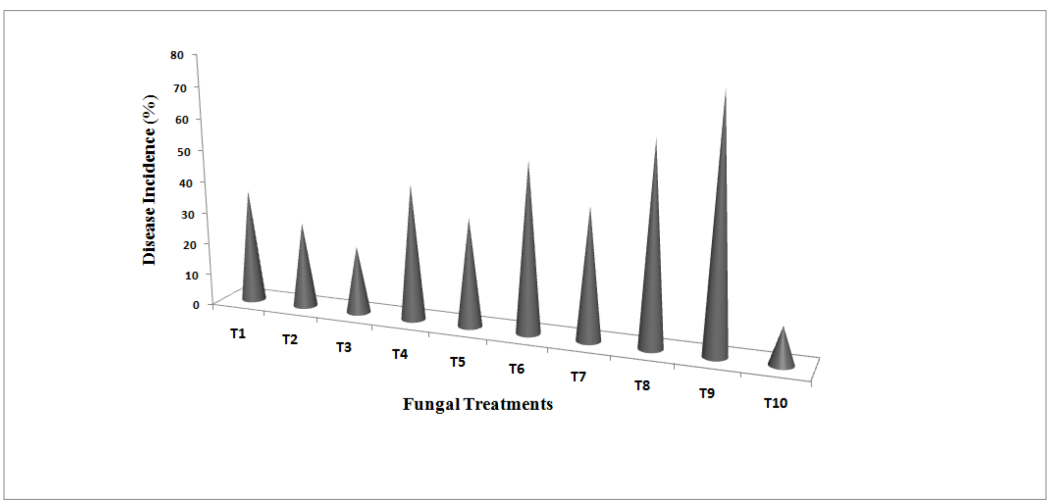

Figure 2: Mean comparative percentage incidence data for two years under Field conditions

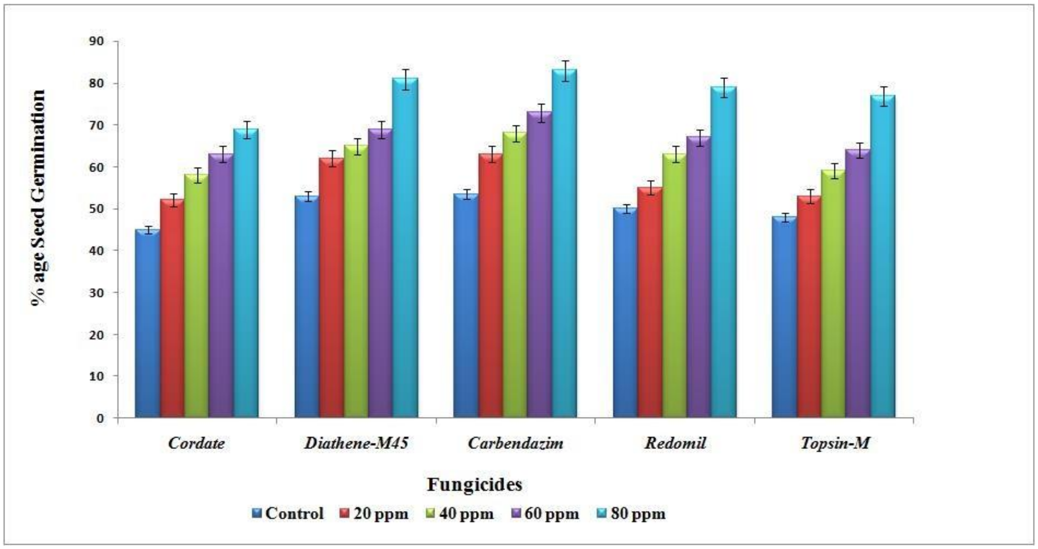

Figure 3: Efficacy of various fungicides on germination percentage of infested rice seeds by using standard blotter paper technique (means of two years data)