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Evaluation of different fungitoxicants against seed discolouration of paddy *in vitro*

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Abstract
Seed discolouration is an important constraint in paddy. It reduces germination, vigour as well as marketability of the produce. There are several pathogens responsible for the seed discolouration. During investigation it was found that, *Fusarium moniliforme* is responsible for tip discolouration and *Curvularia lunata* is responsible for base discolouration of seed. For management, eight chemicals were screened against these pathogens *in vitro* and revealed all the chemicals were significantly inhibit the growth of pathogen. Thiophanate methyl at 0.1% concentration recorded maximum mycellial growth inhibition of *Curvularia lunata* (92%) followed by tricyclazole at 0.5% (91.80%) where as Copper oxide at 0.2% concentration recorded maximum mycellial growth inhibition of *Fusarium moniliforme* (90.91%) followed by Carbendazim at 0.1% (90.51%).

Keywords: Seed discolouration, *Fusarium moniliforme*, *Curvularia lunata*, Paddy, Fungicide

Introduction
Rice is a widely distributed crop having many ecotypes and adapted to various environmental conditions. It is the staple food of Indian sub continent. Being the second largest grower of paddy, India approximately produces 166.5 million metric tons in 2017 with highest productivity in Punjab (4019 kg/ha). There are many constraints which reduces the productivity of paddy. Out of which, biotic factors like disease are important. Rice crop is attacked by more than 50 diseases which may appear at any growth stage of crop growth (Arshad *et al.*, 2009) [2]. Apart from commonly found disease such as blast, bacterial leaf blight, sheath blight, sheath rot, brown spot and bakane, there are many more disease of paddy which causes huge loss to the crop. It is also known to be attacked by many seed-borne diseases of major and minor importance. These pathogens are known to cause damage at different stages like storage, seed germination, seedling establishment, vegetative growth and reproductive phase. The infected seeds may fail to germinate or may transmit disease from seed to seedling and from seedling to growing plants (Fakir *et al.* 2002) [6]. Seeds from infected panicles become discoloured and sterile, thereby reducing yield and quality (Mew and Gonzales 2002) [8]. It may cause discolouration or distinct lesions on grain or whole panicle, panicle blight, brown/black spots on grain, discolouration of florets (Groth *et al*., 1991) [7]. The disease should be treated in early stage for better management. Use of healthy seeds for sowing is the key for better crop stand. Seed treatment is the initial step of crop protection for a disease free crop. Uses of fungicides are very convenient and easy for the farmers for managing diseases. Hence, present investigation was carried out in order to evaluate the efficacy of different fungicides against the pathogen responsible for seed discolouration *in vitro*.

Material and Methods
Collection of seed samples
For collection of seed sample, Paddy fields of Central farm, OUAT, Bhubaneswar was surveyed. Paddy seed sample of medium duration variety Mandakini was collected from random rice plants during the month of September and brought to the laboratory in separate polythene bags. The seed samples collected from field were examined using magnifying glass and stereo binocular microscope. It was segregated in to different groups basing on the types of symptom they exhibit.

Isolation of pathogen from disease sample

The seed samples of each category were placed in moist chamber separately after surface sterilization in order to know the pathogen responsible for different symptoms. After five days of incubation mycelia growth was observed from the surface of seed. It was identified as *Curvularia lunata* and *Fusarium moniliformi* responsible for base discolouration and tip discolouration respectively by microscopic observations.

Bio assay of different fungicides against test pathogens

Different fungicides were tested against the test pathogens in order to know their efficacy to inhibit their mycelia growth *in vitro* at specified concentrations adopting poisoned food technique (Nene and Thapliyal, 1973) [9]. The required amount of different chemicals were weighed and incorporated into sterilized, cooled but liquefied form of potato dextrose agar medium to get a desired concentration of poisoned media after proper mixing. Twenty ml of poisoned medium of each fungicide was poured into 90 mm sterilized Petri dishes. Three replications were maintained for each treatment. All the plates were inoculated with actively growing 7 mm mycelial disc of test fungi after proper solidification. These plates were incubated at 25±1 °C for seven days and then colony diameter was recorded. Percent inhibition of mycelial growth over control was calculated by using Vincent formulae (1947) [11].

$$I = \frac{(C - T)}{C} \times 100$$

Where

I = Per cent inhibition of mycelium
C = Growth of mycelium in control
T = Growth of mycelium in treatment

Statistical analysis

The experiment was laid in completely randomized block design with nine treatments and three replications. The differences between treatments were evaluated by analysis of variance (ANOVA) using OPSTAT statistical software (CCS HAU, Hisar).

Results and Discussion

Bioassay of fungicides on growth inhibition of *Curvularia lunata*

All the fungicides tested were significantly reduced the fungal colony of *Curvularia lunata*. The fungicide thiophanate methyl at 0.1% concentration recorded maximum mycellial growth inhibition (92%) followed by tricyclazole at 0.5% (91.80%) which was at par. The next best treatments were thiram at 0.2% (90.30%), carbendazim at 0.1% (90.00%) and Copper oxychloride at 0.2% concentration (89.20%) which was at par with each other. Copper hydroxide recorded more than 80% of mycelial inhibition i.e. 85.20%. The least mycelia growth inhibition of 68.80% was recorded in mancozeb at 0.1% concentration. In total, all the fungicides registered a significant mycelia inhibition of more than 60% of *Curvularia lunata* causing base disolouration of paddy seed (Table 1 and Figure 1).

Bio efficacy of fungicides on mycelia growth inhibition of *Fusarium moniliforme*

All the fungicides inhibited the radial growth of *Fusarium moniliforme* to an appreciable level. The fungicide Copper hydroxide at 0.2% concentration recorded maximum mycelial growth inhibition (90.91%) followed by Carbendazim at 0.1% (90.51) which were at par with each other. The next best treatments were Tricyclazole at 0.05%, Thiophenate Methyl at 0.1%, Copper Oxychloride at 0.2% and Thiram at 0.2% recording 82.41%, 81%, 79.70% and 79.30% inhibition over control respectively. The least inhibition was recorded in Mancozeb (63.91%) followed by Captan (68.90%) at 0.1% and 0.2% concentration which were significantly different from each other. All the treatments recorded more than 60% of mycelial growth inhibition over control.

The effectiveness of Thiophanate Methyl and Mancozeb against *Curvularia* and *Fusarium* was reported by Butt *et al.* (2011) [4] who explained Thiophanate Methyl and Mancozeb suppressed growth of *Curvularia* by 50% and has a insignificant effect on *Fusarium*. Bhuiyan *et al.* (2013) [3] observed elimination of seed borne fungi and increase the seed germination of 25.70% over control when seed was treated with Carboxin+ Thiram at 0.3% of seed weight. Seed treatment with synthetic fungicides and foliar application of synthetic fungicide are most effective to control rice grain discoloration disease (Agarwal *et al.*, 1989; Arshad *et al*., 2009) [2]. Arshad *et al* (2009) [2] found Carbendazim followed by Thiophenate methyl, Dithane M-45 and Ridomil works well against grain discoloration of paddy and showed maximum germination and least seedling mortality. The most effective disease control of grain discoloration of rice was recorded by fungicide such as mancozeb or propiconazole at the boot leaf stage followed by salt 20 days after 50% flowering (Deka *et al*, 1996) [5]. These findings of earlier workers are in confirmation with present result.

Table 1: Bio assay of different chemicals against *Curvularia lunata* and *Fusarium moniliforme*

Treatments	Chemical name	Dose (%)	% inhibition of *Curvularia lunata* over control	% inhibition of *Fusarium moniliforme* over control
T_1	Tricyclazole	0.05	91.80	82.41
T_2	Mancozeb	0.1	68.80	63.91
T_3	Captan	0.2	74.10	68.90
T_4	Copper Oxychloride	0.2	89.20	79.70
T_5	Thiram	0.2	90.30	79.30
T_6	Thiophanate Methyl	0.1	92.00	81.00
T_7	Carbendazim	0.1	90.00	90.5
T_8	Copper hydroxide	0.2	85.20	90.91
T_9	Control		0.00	0.00
	SE(m)±		0.433	0.511
	CD at 1%		1.298	1.531

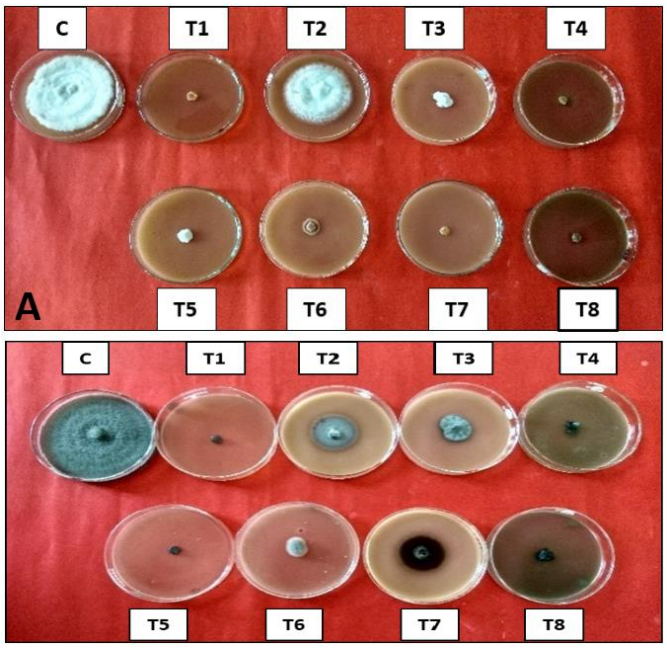

Fig 1: Bio-assay of fungicides against (A) *Fusarium* (B)*Curvularia*, C: Control, T_1: Tricyclazole, T_2: Mancozeb, T_3: Captan, T_4: copper oxychloride, T_5: Thiram, T_6: Thiophanate methyl, T_7: Carbendazim, T_8: Copper Oxide

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