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Research Article

An Innovative *In Vitro* Agar Block Method for Screening of Effective Fungicides against *Fusarium Moniliforme* Causing Stalk Rot and Ear Rot of Maize

Tiru Zerald, Sarkar Monalisha, Chakraborty Arka Pratim, Pal Ayon and Mandal Parimal*

**Mycology and Plant Pathology Laboratory, Department of Botany, Raiganj University, Raiganj, Uttar Dinajpur.*

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ABSTRACT

Proper laboratory technique is one of the most important parts in research for screening of bioactive potential agent from diverse sources. There are same common laboratory techniques are used for screening of bioactive agent against pathogenic microorganisms such as disc diffusion method, agar-well diffusion method, poisoning food method. In the present study we designed an innovative agar block method for screening of effective fungicides and bioactive agents on growth of *Fusarium moniliformae* (ITC NO. 11,208.19) causing stalk rot and ear rot in maize at Utttar Dinajpur, West Bengal, India. Three potent fungicides- Taqat, SAAF and Policy showed highest percent of inhibition of radial growth (PIRG)- (88, 82.66 & 80) % with inhibition zone of (19.5, 12.5 & 16.5) mm against *F. moniliforme*. Lowest PIRG- (41.33 & 40) % with the range of zone of inhibition- (0.5) mm was observed when Dacotech and Dhanuka M-45 were used *in vitro* to control the growth of causal pathogen of stalk rot and ear rot disease of *Zea mays*. In a nutshell, this laboratory practice might be helpful for screening of effective bioactive agents against a large number of pathogenic microorganisms with a limited laboratory resource.

Keywords: *In vitro agar block method, Best effective fungicides (Taqat, SAAF & Policy), Fusarium moniliforme, Zea mays*

INTRODUCTION

The most effective and traditional farmer centric methods for disease control are the use of chemical fungicides. There are several fungicides which are being commercially and locally available, are being evaluated by several workers against different disease-causing pathogens. Some specific active ingredients like pyraclostrobin and fludioxonil were tested against *Fusarium* spp to check its growth by previous workers[1]. In another experiment conducted by Rahman et al. (2009)[2], foliar fungicides (strobilurin, premix of strobilurin and triazole, pyrazole-carboxamide) were used against major soil borne pathogens of soybean (*C. truncatum*, *F. virguliforme*, *M. phaseolina*, *P. irregulare*, *R. solani*, *S. sclerotiorum* and *S. glycines*). There was report by several researchers about the way of control of fungal pathogens that could be achieved by the use of different target site fungicides. That target site fungicides were the succinate dehydrogenase inhibitors (SDHIs), in addition to the well-known phenyl-pyrroles (PP fungicides) that affectecd the fungal osmotic signal transduction cascade and pathogen osmoregulation. Methyl benzimidazole carbamates (MBCs) showed the best inhibition activity for *Fusarium* species and *Aspergillus flavus in vitro*. *Fusarium* species and *A. flavus* exhibited different responses to fungicides and the use of fungicide mixture was supposed to be the best strategy to control toxigenic fungi on maize[3]. There are same common laboratory methods such as disc diffusion, agar-well diffusion, poisoning food method are being used by several workers for screening of effective fungicides, botanicals, synthetic chemical and other abiotic agents against different pathogens[4,5,6]. In recent years to screen antimicrobial activities, several bioassays such as disk-diffusion, well diffusion and broth or agar dilution are commonly used[7]. To study the antibacterial and antifungal activities of clove, oregano, thyme, cinnamon and cumin against food spoilage bacteria like *Bacillus subtilis* and *Pseudomonas fluorescens*, pathogens like *Staphylococcus aureus* and *Vibrio parahaemolyticus*, harmful fungi like *Aspergillus flavus*, agar well diffusion method was adopted by Liu et al. (2017)[8].

Although all these available techniques are used but they are supposed to be time consuming and need huge laboratory resources like glassware, growth medium and space. Keeping this in mind, in our present invention, we design an innovative *in vitro* agar block method for screening of effective bioactive agents from large number of available fungicides against *Fusarium moniliforme* causing ear rot and stalk rot diseases of maize. Objectives of our current study are not only to assess *in vitro* effects of fungicides on growth of pathogen but also to examine *in vitro* sensitivity of pathogen towards tested fungicides.

MATERIAL AND METHODS

**Author for Correspondence: drpmandalrgu@gmail.com*

Fungal isolate- causal pathogen of maize stalk rot and ear rot disease

The fungal isolate was isolated from maize diseased field located at Utttar Dinajpur, West Bengal, India. Fungal culture was identified from the Indian Type Culture Collection, New Delhi. The isolate was properly maintained on PDA slants at 4ºC and 7 days old fungal culture was used for further study.

Active ingredients of ten fungicides used for in vitro agar block technique

Ten different fungicides viz. SAAF (12%Carbendazim+63%Mancozeb), Magnet 8-64 (8% Metalaxyl+64%Mancozeb), Dhanuka M-45 (Dithiocarbamate), Antracol (Dithiocarbamate), Taqat (70% Captan + 5% Hexaconazole), Master (8% Metalaxyl +64% Mancozeb), Dacotech (Tetrachloroisophthalon), Bavistin (Benzimidazole), Kaguya (12% Carbendazim +63% Mancozeb33) and Policy (12% Carbendazim + 63% Mancozeb) were used for screening of their effectiveness against the causal pathogen causing stalk rot and ear rot of maize following the innovative method of agar block. Fungicides are commercially available with their trade names and within bracket indicates active ingredients and commercial formula of those fungicides.

Mode of actions of ten fungicides used for in vitro screening

(a) ***SAAF:*** Contact and systemic in action.

(b) ***Magnet 8-64:*** It is mixture of two fungicides – Mancozeb and Metalaxyl. Mancozeb acts by its contact action. Mancozeb is fungitoxic when exposed to air. It is converted to an isothiocyanate, which inactivates the sulphahydral (SH) groups in enzymes of fungi. Metalaxyl inhibits protein synthesis, growth and reproduction in fungi.

(c) ***Dhanuka M-45:*** Contact fungicide of dithiocarbamate group, which reduces the activity of enzymes in fungus which in turn reduces the energy production and finally results in death of the fungus.

(d) ***Antracol:*** Broad spectrum contact fungicide which affects several sites in the metabolism of fungal cells.

(e) ***Taqat:*** Contact and systemic in action.

(f) ***Master:*** The fungicide ensures durable protection during the period of active growth from inside due to the systematic activity of metalaxyl and from outside due to contact action of mancozeb.

(g) ***Dacotech:*** Broad spectrum, non-systemic fungicide.

(h) ***Bavistin:*** Broad spectrum systemic fungicide that inhibits mycelia growth, development of germ tissues. It disturbs the fungal metabolism by interfering and disturbing fungal cell membrane, thus weakening the pathogen.

(i) ***Kaguya:*** Broad spectrum, systemic and contact combination fungicide.

(j) ***Policy:*** Contact and systemic in action.

An innovative protocol adopted for agar block assay

Antifungal bioactive screening of different locally available abiotic agents was carried out following innovative agar block method as described in our Laboratory- Mycology and Plant Pathology Laboratory, Department of Botany, Raiganj University, West Bengal, India. In this method, 10ml of 2% agar solution was prepared in 20ml culture tube, capped and then sterilized at 121ºC with 15 lb in^{-2} for 15 minutes. After sterilization, 1mg of fungicides in order to make the final concentration of 0.1mg/ml were added and poured on sterile petriplates aseptically, uniform layer was made and solidified. Then 5 mm of agar block was cut using sterilized cork borer and stored at 4ºC for further use. For different fungicides different agar blocks were prepared as per the above method. The agar blocks containing fungicides were placed in the periphery on a freshly prepared PDA plates. Fungus pathogen of 7 days old culture on PDA medium were cut as a fungal disc using sterilized cork borer and placed onto the centre of test plates. It was then incubated at 25ºC for 10 days. Bioactive potentials of different fungicides were evaluated by measuring Percent of Inhibition of Radial Growth (PIRG) against the test fungal pathogen causing stalk rot and ear rot diseases in maize. In control, sterilized water was used instead of fungicides on agar block. PIRG was calculated using the following formula: $PIRG = (R_1 – R_2)/R_1 x100$.

R_1 = diameter of the fungal colony (cm) in the control; R_2 = diameter of the fungal colony (cm) on fungicide treated growth medium.

RESULTS

Identification of Fungal isolate

The Fungal isolate was identified as *Fusarium moniliforme* from the Indian Type Culture Collection, New Delhi with the accession number-(ITC NO. 11,208.19). *F. moniliforme* was examined under bright field microscope to study its morphological and microscopic features which depicted that hyphae were septate and hyaline, with abundant oval to clavate shaped microconidia.

In vitro screening of effective fungicides against Fusarium moniliforme

Radial growth of *F. moniliforme* (ITC NO. 11,208.19) causing ear rot and stalk rot disease in maize was studied following innovative *in vitro* agar block method with ten different available fungicides. Among ten fungicides, three of them such as Taqat, SAAF and Policy showed highest percent of inhibition of radial growth (PIRG) in the range of 80-88% with zone of inhibition in the range of (10-20) mm. Taqat fungicide showed highest percent of inhibition of radial growth (PIRG)-88% (Table 1).

Four other fungicides such as Dhanuka M-45, Antracol, Master and Dacotech showed lowest PIRG in the range of 40-60% with zone of inhibition between (0.5-10) mm. Lowest PIRG-40% with zone of inhibition- (0.5)mm was found when Dhanuka M-45 fungicide was used *in vitro* to control the growth of *F. moniliforme* (Figure 1).

DISCUSSION

Our current study depicts the proper assessment of *in vitro* fungicidal effects on growth of fungal pathogen as well as clearly indicates how *Fusarium moniliforme* is sensitive towards tested fungicides in *in vitro* sensitivity test. Three potent fungicides- Taqat, SAAF and Policy showed highest percent of inhibition of radial growth (PIRG)- (88, 82.66 & 80) % . Dacotech and Dhanuka M-45 showed lowest PIRG- (41.33 & 40) % against *F. moniliforme*. In support of our research findings, several research workers previously used different concentrations of SAAF (carbendazim12% and mancozeb 63%) in high dose in reducing spore germination and mycelial growth of *Drechslera oryzae*. Comparative evaluation of SAAF with other fungicides (companion and carbendazim) revealed that SAAF was effective at various concentrations. The minimum disease incidence with 12.5% was recorded by foliar spray of SAAF @ 0.75% which was followed by carbendazim[9]. There was also report by Adinarayana et al. (2013)[10] about the field efficacy of Taqat 75 WP fungicide against foliar fungal diseases of Vigna *mungo*. Taqat 75 WP at both the concentrations of 500 g and 750 g/ha was highly effective in controlling the incidence of foliar fungal diseases such as rust and powdery mildew in blackgram. Efficacy of Bavistin fungicide in controlling *Botrytis* gray mold disease in chickpea was reported by Rashid et al. (2014)[11].

Table 1. *In vitro* screening of ten different fungicides (0.1mg/ml) showing inhibition of mycelia growth of *Fusarium moniliforme* (ITC NO. 11,208.19) using innovative agar block method

Test Fungicides (0.1mg/ml)	Tested Fungal isolate	Percent of Inhibition of Radial Growth (PIRG)*	Inhibition zone (mm)
SAAF	*Fusarium moniliforme* (ITC NO. 11,208.19)	82.66±2.66	12.5±0.16
Magnet 8-64		73.33±2.78	10.0±0.10
Dhanuka M-45		40.00±2.33	0.5±0.04
Antracol		62.66±2.25	07.5±0.09
Taqat		88.00±2.69	19.5±0.21
Master		60.00±2.27	09.0±0.12
Dacotech		41.33±2.34	0.5±0.04
Bavistin		72.00±2.73	13.5±0.17
Kaguya		77.33±2.65	13.5±0.16
Policy		80.00±2.61	16.5±0.20

***(R_1–R_2)/R_1x100**; **R_1** = Diameter of the fungal colony (cm) in the control, **R_2** = Diameter of the fungal colony (cm) on fungicide treated plates; **mm= mili meter**; **±=S.E.**; Average of three replicates.

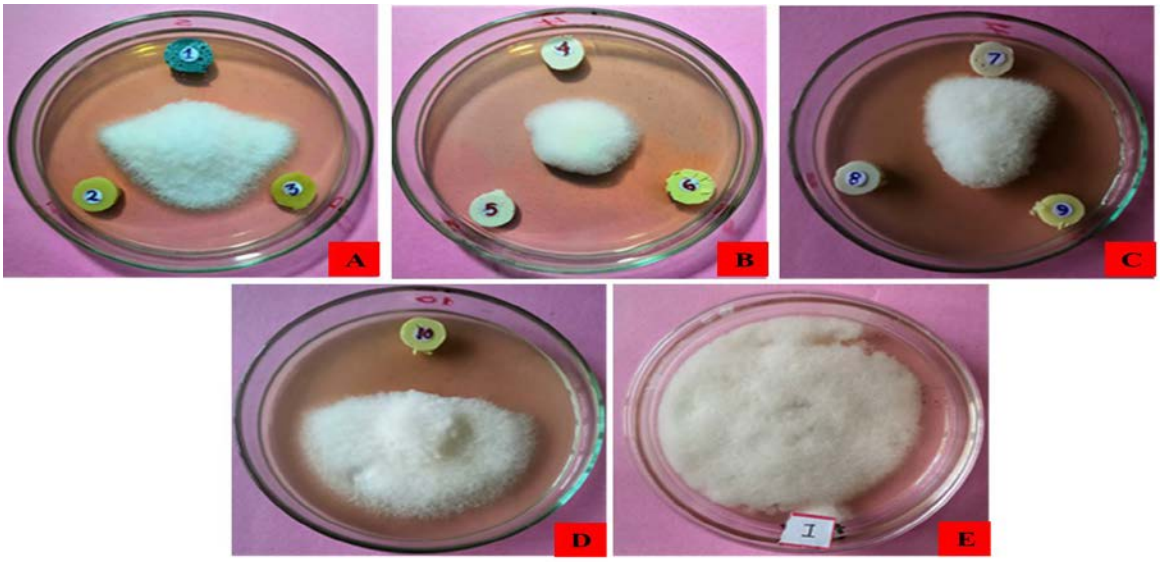

Figure 1

Figure 1. *In vitro* agar block method to screen ten different fungicides against *Fusarium moniliforme* (ITC NO. 11,208.19)

Agar block numbers with fungicides: (A): 1-SAAF, 2-Magnet 8-64 and 3- Dhanuka M-4; (B): 4-Antracol, 5-Taqat and 6-Master; (C): 7- Dacotech, 8- Bavistin and 9- Kaguya; (D): 10- Policy and (E): I- Control plate (*F. moniliforme*).

CONCLUSION

The overall findings of the present work clearly reveals that Taqat and SAAF fungicides can be successfully used to control *Fusarium moniliforme*- causal pathogen of stalk rot and ear rot diseases of maize. Cost effective and time-consuming innovative method of *in vitro* agar block can be adopted as new technique for screening of effective bioactive agents/botanicals against a large number of pathogenic microorganisms in research laboratories.

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CONFLICT OF INTEREST

No potential conflict of interest was reported by the authors.

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