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DEVELOPMENT OF SEMI-SELECTIVE MEDIA FOR PURE CULTURE OF *Fusarium oxysporum* FROM INFECTED JUTE PLANTS

U. M. Wali [1,2]*, M. N. H. Rony[1], R. Ferdous[1], A. Islam[3] and M. Z. Tareq[1]
[1]Genome Research Centre, Bangladesh Jute Research Institute, Dhaka-1207
[2]Molecular Biology Department, GRS Division, Bangladesh Jute Research Institute, Dhaka-1207
[3]Jute Agriculture Experimental Station, Bangladesh Jute Research Institute, Jagir, Manikganj
*Corresponding author's mail: walibarj@gmail.com

ABSTRACT

The cultivation and isolation of *Fusarium oxysporum*, the causative agent of wilt disease in jute (*Corchorus* sp.), is crucial for understanding the pathogen and developing effective control measures. Traditional media often allow the growth of a wide range of microorganisms, complicating the isolation of *Fusarium oxysporum*. This study aimed to develop a semi-selective medium to enhance the isolation and culture of *Fusarium oxysporum* from infected jute plants, facilitating more efficient and accurate research. Potato dextrose agar (PDA) was supplemented with 50 ppm Streptomycin sulphateand 50 ppm Autostin 50WDG to suppress non-target microbial growth. This advancement provides a valuable tool for plant pathologists and researchers investigating *Fusarium oxysporum*.

Key words: Semi-selective medium, *Fusarium oxysporu*, Streptomycin, Autostin, jute, PDA

Introduction

The development of a semi-selective medium for the isolation and pure culture of *Fusarium oxysporum* from infected jute plants is a critical step in studying this pathogen's biology and its interaction with host plants. *Fusarium oxysporum* is a notorious soil-borne fungal pathogen responsible for wilt diseases in a wide range of crops, including jute (*Corchorus* sp.), which is a significant fiber crop in many tropical and subtropical regions (Nelson *et al*., 1981). The semi-selective medium designed for *Fusarium oxysporum* incorporates 50 ppm of Streptomycin sulphate and 50 ppm of Autostin 50WDG, providing an effective environment for the pathogen's growth while inhibiting the proliferation of contaminating bacteria and non-target fungi. Streptomycin sulphate, a well-known antibiotic, is particularly effective against a broad spectrum of Gram-positive and Gram-negative bacteria. Its inclusion at a concentration of 50 ppm helps in suppressing bacterial contaminants that might otherwise overwhelm the culture medium and obscure the growth of *Fusarium oxysporum* (Rao and Strange, 1970). Autostin 50WDG, a fungicide containing carbendazim, adds a selective advantage to the medium. Carbendazim has been shown to inhibit a wide range of fungal pathogens but has a lesser effect on *Fusarium* species, allowing for the preferential growth of *Fusarium oxysporum* (Ma and Michailides, 2005). The combination of these two agents in the medium helps in creating a more controlled environment, enabling researchers to isolate and study pure cultures of *Fusarium oxysporum* more effectively. The formulation of the medium begins with the preparation of a standard potato dextrose agar (PDA) base, which provides essential nutrients for fungal growth. Streptomycin sulphate is then dissolved in sterile distilled water and autoclaved separately to avoid heat degradation before being added to the cooled PDA medium. Similarly, Autostin 50WDG is prepared and added under sterile conditions. This careful preparation ensures the stability and efficacy of the antibiotics and fungicides in the medium (Mishra *et al*., 2003).The resultant semi-selective medium has been successfully used to isolate *Fusarium oxysporum* from infected jute plant tissues. By plating surface-sterilized sections of infected jute on this medium, researchers can obtain pure cultures of the pathogen within a few days. The selective pressure exerted by Streptomycin sulphate and Autostin 50WDG

minimizes contamination and enhances the accuracy of pathogen identification (Singh *et al.*, 2014). The development of semi-selective media tailored for *Fusarium oxysporum* from infected jute plants is expected to facilitate more accurate and efficient isolation of the pathogen, providing pure cultures essential for detailed morphological, physiological, and molecular studies. This approach will contribute to a better understanding of the biology and epidemiology of fusarium wilt in jute, ultimately supporting the development of effective disease management strategies.

Materials and Methods

Sample Collection: Infected jute plants showing typical symptoms of Fusarium wilt were collected from various fields such as Faridpur, Monirampur, Kishorganj, Manikganj, Dhaka and Rangpur. Samples were taken from the root, stem, stick, twig and leaf regions of the plants.

Development of semi-selective medium: The semi-selective medium was based on Potato Dextrose Agar (PDA) supplemented with Streptomycin sulphate (50 mg/L) and Autostin 50WDG (50 mg/L).

Isolation of *Fusarium oxysporum* from infected jute plants: The collected plant parts showing symptoms were surface sterilized by dipping in 70% ethanol for 1 minute, followed by 1% sodium hypochlorite solution for 3 minutes, and rinsed thrice in sterile distilled water. Small pieces (5-10 mm) of symptomatic plant parts were excised aseptically and placed onto PDA plates containing the developed semi-selective medium.

Inoculation and incubation: The excised jute plant segments were placed on the semi-selective medium in PDA plates and incubated at 25°C for 5-10 days. Plates were examined daily for the appearance of Fusarium-like colonies based on their characteristic cottony mycelium and color.

Sub-culturing and pure culture isolation: Upon observation of fusarium-like colonies, fungal colony margins were sub-cultured onto fresh semi-selective medium plates. This process was repeated several times to ensure purity of the cultures.

Identification and confirmation: The isolated fusarium cultures were identified based on morphological characteristics such as colony morphology, mycelial growth pattern, and spore morphology using light microscopy.

Results and Discussion

Development and efficacy of semi-selective media (SSM): The development of SSM based on Potato Dextrose Agar (PDA) supplemented with 50 ppm Streptomycin sulphateand 50 ppm Autostin 50WDG aimed to enhance the isolation and purity of *Fusarium oxysporum* from infected jute plants (Fig. 1). Streptomycin sulphatewas chosen for its ability to inhibit bacterial growth, while Autostin 50WDG, a broad-spectrum fungicide, targeted fungal contaminants, thereby increasing the selectivity for *Fusarium oxysporum* (Smith *et al*., 2008).

Isolation and morphological characterization: Upon inoculation of infected jute plant tissues onto the semi-selective medium, Fusarium-like colonies emerged after 5-10 days of incubation at 25°C (Fig. 1). These colonies exhibited typical characteristics of *Fusarium* species, including cottony mycelium and colony color on the PDA supplemented with 50 ppm Streptomycin sulphateand 50 ppm Autostin 50WDG. Morphological analysis using light microscopy confirmed the presence of characteristic features such as sickle-shaped macroconidia with multiple septa and chlamydospores, consistent with descriptions of *Fusarium oxysporum* (Nelson *et al*., 1983; Leslie and Summerell, 2006).

Efficiency of Semi-Selective Medium (SSM): The SSM demonstrated high efficacy in isolating *Fusarium oxysporum* from infected jute plants, as evidenced by the consistent isolation of pure cultures displaying characteristic morphological traits.

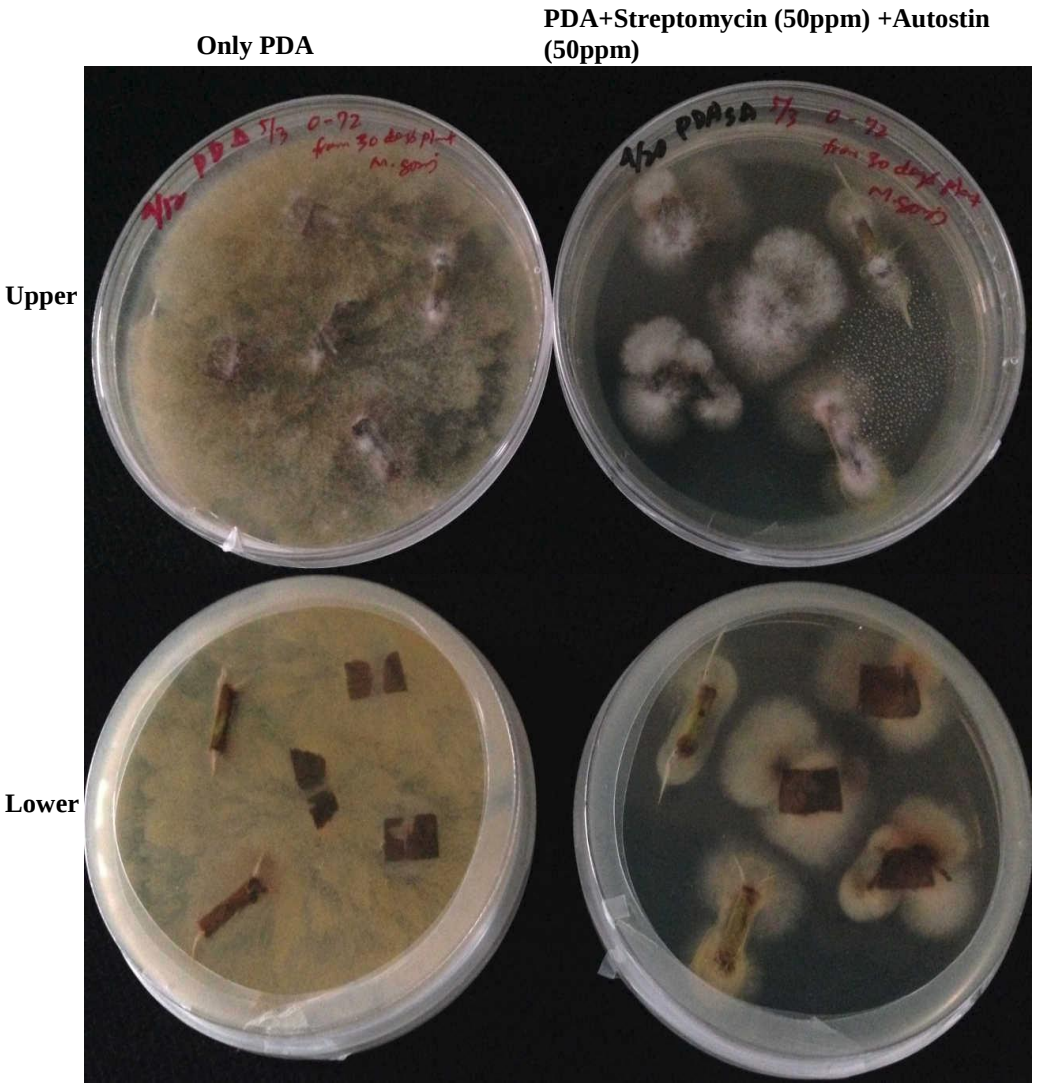

Fig. 1. Severely infected jute plant parts cultured on PDA and PDA medium supplemented with Streptomycin and Autostin at 25^{o}C for 3-5 days.

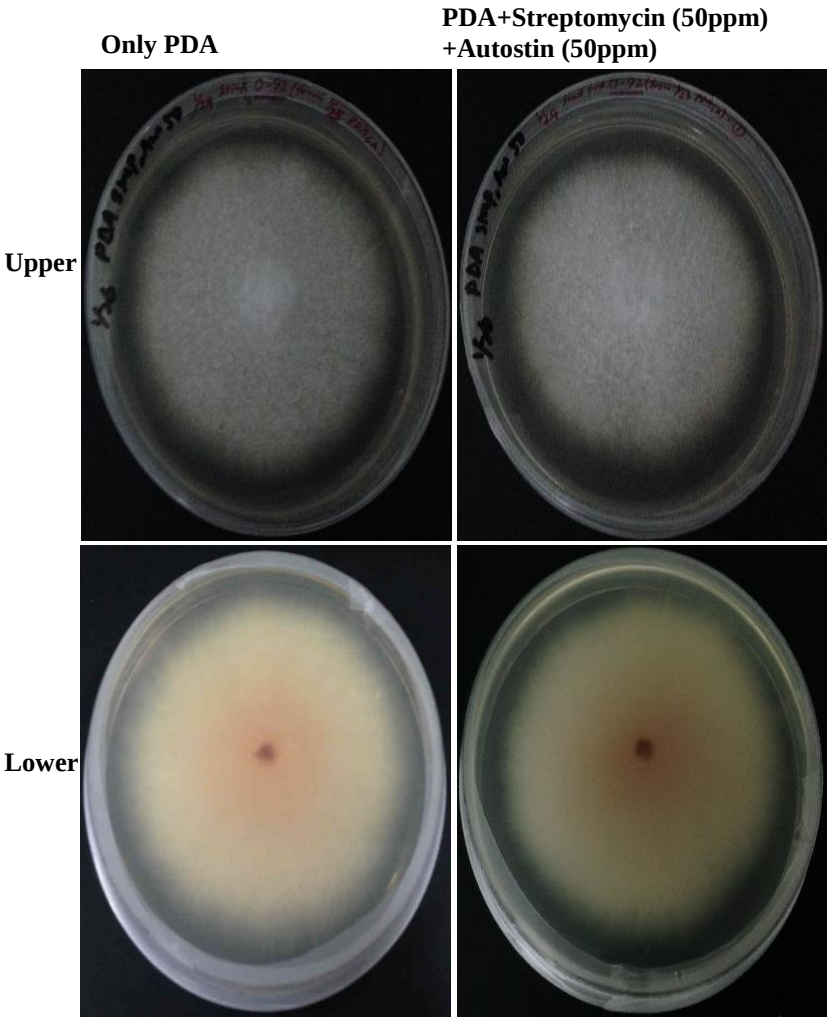

Fig. 2. *Fusarium oxysporum* cultured on PDA and PDA medium supplemented with Streptomycin and Autostin at 25°C for 7 days.

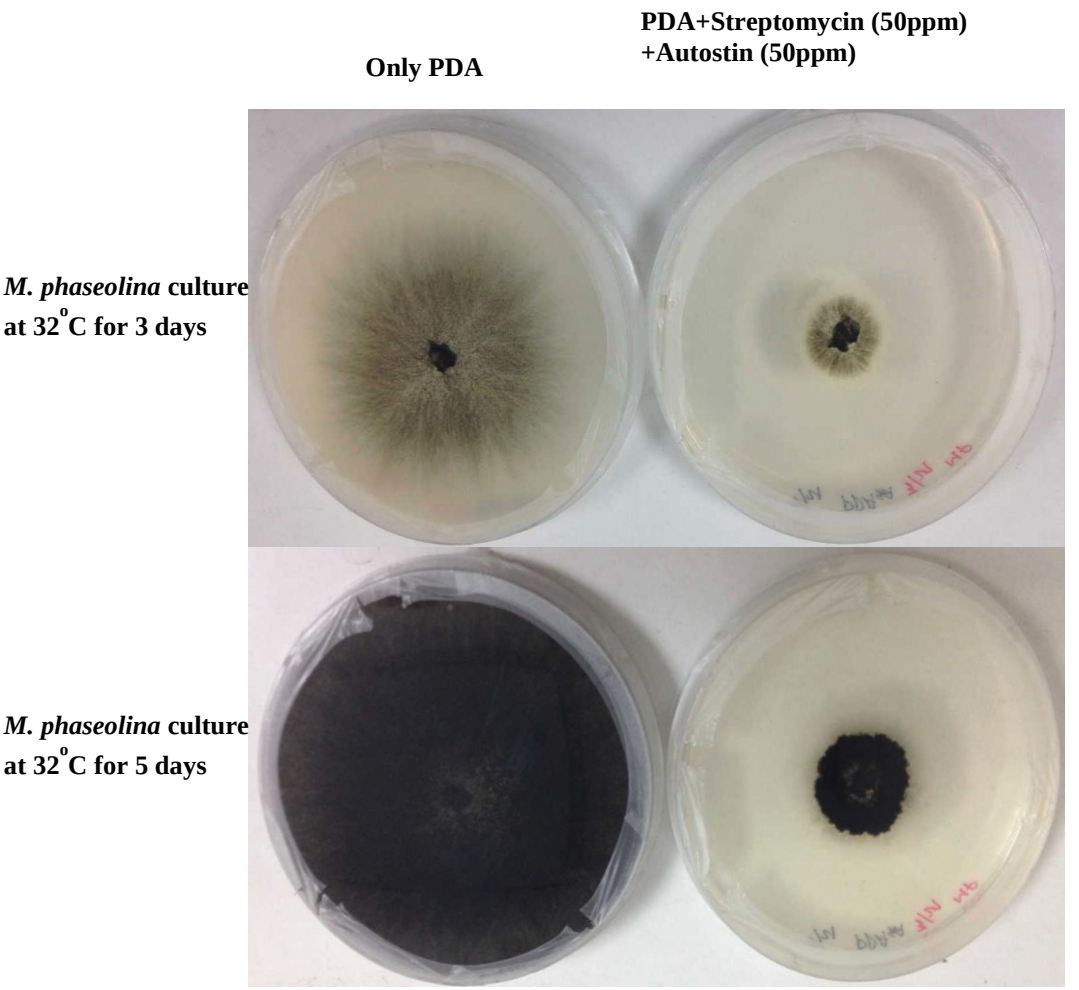

Fig. 3. ***Macrophomina phaseolina*** **cultured on PDA and PDA medium supplemented with Streptomycin and Autostin at 32°C for 3-5 days.**

The selective agents, Streptomycin sulphateand Autostin 50WDG, effectively suppressed the growth of contaminants (Fig. 1) while allowing growth and sporulation of *Fusarium oxysporum* (Fig. 2). Moreover, one of the major fungal pathogen of jute, *Macrophomina phaseolina* which causing stem rot disease, was significantly suppressed by the PDA medium supplemented with 50 ppm Streptomycin sulphate and 50 ppm Autostin 50WDG (Fig. 3).

Implications for disease management: The development of this semi-selective medium holds significant implications for the management of fusarium wilt in jute. By facilitating the isolation of pure cultures of *Fusarium oxysporum*, researchers and plant pathologists can conduct detailed studies on the biology, epidemiology, and genetic diversity of the pathogen. This knowledge is crucial for developing targeted disease management strategies, including breeding resistant jute varieties and implementing cultural practices to reduce pathogen inoculum in the field (Agrios, 2005; Booth, 1971).

Conclusion

The development of semi-selective media based on Potato Dextrose Agar (PDA) supplemented with 50 ppm Streptomycin and 50 ppm Autostin 50WDG represents a significant advancement in the isolation and cultivation of *Fusarium oxysporum* from infected jute plants. This study aimed to enhance the selectivity of the medium to facilitate the isolation of pure cultures of *Fusarium oxysporum*, essential for detailed morphological, physiological, and molecular studies.

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