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A PERSPECTIVE ON COMMON SCAB (*STREPTOMYCES SCABIEI*) DISEASE MANAGEMENT STRATEGIES IN POTATO CROP

^aAbdul Rehman, ^aJahanzaib Sandhu, ^bMuhammad W. Alam, ^cSaira Mehboob^aDepartment of Plant pathology, University of Agriculture Faisalabad, Pakistan.^bDepartment of Plant Pathology, University of Okara, Pakistan.^cInstitute of Plant Pathology, Ayub Agriculture Research Institute Faisalabad, Pakistan.

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ABSTRACT

Potato common scab (CS), caused by *Streptomyces scabiei*, and is a major potato disease that causes deep pitted lesions on the surface of the tuber. The market value and quality of tubers with CS are reduced, resulting in significant economic losses to potato growers. Traditional control strategies such as irrigation and reduced soil pH help to reduce CS but are not efficient methods. Celest® 100 FS (Fludioxanil), Emesto® 24% FS (Penflufen) and Bio magic® (*Bacillus subtilis*) were tested on CS in this study. A disc diffusion method was used to evaluate the efficacy of these products under in vitro conditions at three different concentrations: 100 ppm, 200 ppm, and 300 ppm. After 7 days, results showed that Bio Magic was the most effective at reducing the growth of *Streptomyces scabiei*, followed by Emesto 24 FS and Celest 100 FS at 300 ppm. The maximum inhibition percentage by Bio Magic was 45.05%, followed by Celest 100FS at 38.38% and Emesto 24FS at 31.47%. In greenhouse experiments, Celest 100FS treated potato tubers produced the highest yield of 2250g per pot (10" x 10" inch), followed by Bio Magic at 2000g, Emesto 24FS at 1850g, and the non-treated control at 1300g. The data indicated that Celest 100FS provided the best control of CS in greenhouse trials.

Corresponding Author: Abdul Rehman

Email: arb041@hotmail.com

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INTRODUCTION

Potato scab is one of the major concerns for farmers after late blight and bacterial soft rot. It damages the tubers' quality, such that it is unacceptable for consumption and seed potato production (Loria *et al.*, 1997; Wanner and Kirk, 2015). Potato (*Solanum tuberosum* L.) is one of the world's most widely cultivated crops, following maize, rice, and wheat. During 2018-19, the total potato production area in Pakistan was approximately 196.2 thousand hectares, with an approximate production of 4578.9 thousand tonnes (Economic Survey of Pakistan, 2018). Diseases are a major yield-limiting factor for potatoes (Majeed *et al.*, 2017). Besides biotic factors, soil alkalinity is considered the main reason for CS

development during the season (Bouchek-Mechiche *et al.*, 2000). Four different species of *Streptomyces* exist, viz., *Streptomyces scabiei*, *S. ipomea*, *S. acidiscabies*, and *S. turgidiscabies* (Healy *et al.*, 2000). *Streptomyces scabiei* is one of the most important pathogens (Keinath and Loria, 1991).

Streptomyces scabiei enters the plant tissues through lenticels and wounds, causing CS by producing Thaxtomin (Lawrence *et al.*, 1990). The pathogen is a soil inhabitant, saprophytic, and can survive in the soil on diseased tubers for more than a decade. Once it is established in the soil, it is very difficult to control (Kritzman and Grinstein, 1991). Presently, there are no registered chemicals for CS in Pakistan except for highly

toxic and expensive fumigants. Due to lack of resistance germplasm, various approaches to minimize losses are crop rotation and use of fungicides. Recently, biocontrol approaches have gained importance because they are less hazardous.

Therefore, the objective of this study was to evaluate and compare the efficacy of two different commercial fungicides (Celest 100 FS and Emesto 24FS) and the bio-pesticide (*Bacillus subtilis*) on the growth of *Streptomyces scabiei* and CS disease using infested soil from a production field with a known history of CS.

MATERIALS AND METHODS

Soil sample collection and isolation of *Streptomyces scabiei* from infected potato tubers

Infested soil was collected from production fields in the potato-growing regions of Pakistan for pathogen isolation and for greenhouse pot experiments. The soil was classified into five different categories, such as 1% or less, 1-10%, 11-20%, 21-50%, and 50% or above CS prevalence (Driscoll *et al.*, 2009) from the previous season. Potato tubers showing typical symptoms of CS were collected and brought to the laboratory at the University of Agriculture, Faisalabad, Pakistan. Samples were stored at 4 °C prior to use.

Tubers were washed with distilled water and scab portions from infected tubers were cut into small pieces using a sterile scalpel using 70% ethanol. Pieces were surface-disinfested with 1.5% sodium hypochlorite for 2 minutes and then rinsed with distilled water. These pieces were grinded to form a homogenised paste by mixing 1 ml of Tris-HCL into 2 ml of Eppendorf tubes. Tubes have been incubated at 55 °C for 2 hours to

remove unwanted microorganisms. A ten-fold dilution was made before pouring onto Nutrient Agar (NA) media plates at 1 mL/plate. An aliquot of 100 l from the suspension was used to spread on NA plates for bacterial growth. The plates were incubated at 28 °C for 1-2 days. Sarwar *et al.* (2019). *Streptomyces scabiei* was identified based on typical growth characteristics, diffusible pigments, and spore colors compared with already identified *Streptomyces* spp.

Evaluation of fungicides and biopesticide on *in vitro* growth of *S. scabiei*

A disc diffusion method was used to determine the efficacy of three products (Celest, Emesto, and Bio Magic) against CS (Bonev *et al.*, 2008). To make the stock solutions, 1 gram of pure active ingredient was mixed into 100 µl of distilled water. Different concentrations were prepared by using stock solutions (Table 1). The pure culture at the rate of 100 l was spread with the help of a sterile swab on the nutrient agar media plates. Uniform bacterial growth was obtained by streaking the plate with the swab in one direction and then rotating the plate 90 degree and streaking again in the same direction. This rotation was repeated three times. Filter paper discs were dipped into the solutions of each product and were placed separately in the center of each Petri dish. The control treatment consisted of filter paper dipped in distilled water. Three replications of each treatment were made. The plates were incubated at 28 °C for 7 days, and the effect of each treatment was determined by measuring growth rate (mm) using a colony counter.

Table 1. Sources of fungicides, biopesticide and their tested concentrations.

Common Name	Active Ingredient	Concentrations	Manufacturers
Celest 100 FS	Fludioxanil	100 ppm	Syngenta
		200 ppm	
		300 ppm	
Emesto 24FS	Penflufen	100 ppm	Bayer
		200 ppm	
		300 ppm	
Bio magic	<i>Bacillus subtilis</i>	100 ppm	Roshan
		200 ppm	
		300 ppm	

Effect of fungicides and biopesticide on disease reduction under greenhouse pot assay

A greenhouse pot experiment was carried out in the

research area of the Department of Plant Pathology at University of Agriculture Faisalabad, Pakistan. The pots were filled with classified soil separately and placed in the

green house. Concentrations of three products were prepared according to table 2. Potato tubers were dipped in the solutions for 10 minutes and allowed to air dry. Then, tubers were sown into the pots. Three replications of each treatment were made. Potato tubers treated with only water served as the non-treated control. A solution of 50 mL of *Streptomyces scabiei* (1×10^8 CFU/mL) was added to all the treatments to ensure the disease development on potato tubers. Potatoes were allowed to grow under regular watch and care. After 13 weeks, tubers were harvested and CS levels were determined using a disease rating scale (Table 3) (Driscoll *et al.*, 2009). The weight (yield) of each tuber was also determined (g).

Table 2. The concentrations of fungicides and biopesticide used for pot assay.

Common names	Application rate
Celest 100 FS	40 mL/100 kg of seed
Emesto 24FS	10 mL/100 kg of seed
Bio magic	750 g/acre

Table 3. Rating scale used to classify the severity of common scab infection on potato tubers (Driscoll *et al.*, 2009).

Rating	Scab lesion percent surface area	Pitted lesion percent surface area
0	0	0
1	1-10	0
2	11-20	0
3	21-50	1-5
4	51-80	6-25
5	81-100	>25

Statistical analysis

The whole experiment was conducted under a completely randomized design. Analysis of variance was carried out using Minitab 16. The least significant difference (LSD) at p 0.05 was applied to find differences among treatments (Gomez and Gomez, 1984).

Table 4. Mean effect of various concentrations of fungicides and bio pesticide against the *Streptomyces scabiei* growth inhibition zone after 7 days of interval.

Treatments	Concentrations			Overall Mean
	100 ppm	200 ppm	300 ppm	
Celest 100 FS	25.33 c	33 b	45.33 c	34.55
Emesto 24FS	21.67 d	28.33 c	35 d	28.33
Bio magic	30.67 b	37.33 b	53.67 b	40.55
Control	90 a	90 a	90 a	90

RESULTS

Effect of fungicides and biopesticide against the *in vitro* growth of *S. scabiei*

The Bio Magic treatment was found most effective in reducing the growth of *Streptomyces scabiei* (53.67%) as compared to control (90%) at a concentration of 300 ppm after three days, followed by the Celest 100 FS (45.33%) as compared to control (90%) at a concentration of 300 ppm. As a result, Emesto was found to be the least effective, with a 35% growth reduction compared to the control (90%) after three days of incubation (Table 4).

Evaluation of products on common scab in green house pot assays

Potatoes were collected from the pots after 13 weeks of sowing. Each tuber was evaluated for every treatment individually. Each tuber was observed and placed in one of the six classes described above (table 5). Soil with a disease incidence of 51% or above showed the highest disease severity, while the minimum disease severity was recorded in soil with a 1% or less disease incidence. The maximum disease severity was recorded in the non-treated control, while the best result was shown by Celest 100 FS, followed by Bio Magic. Emesto 24 FS was found to be the least effective as compared to other treatments, but it was significantly different than the non-treated control.

The effect of various treatments (Celest 100 FS, Emesto 24 % FS, and Bio Magic) on yield of the potato crop against the potato scab The analysis of variance showed that all the treatments significantly contributed to the increase in potato yield. The maximum yield of 2250 g was obtained with Celest 100 FS treated potatoes, followed by Bio magic (*B. subtilis*) with a value of 2000 g, while Emesto 24% FS was found to be the least effective with a value of 1850 g yield. The minimum yield was obtained in the control with a value of 1350 g (Figure 1).

Table 5. Effect of various treatments on disease reduction in green house pot assay.

Treatments	Different categories of soil				
	1% or less	1-10%	11-20%	21-50%	51% or above
Celest 100 FS	0.2	1.2	1.5	2.1	3
Emesto 24FS	0.4	1.4	1.7	2.3	3.3
Bio magic	0.6	1.7	2	3.3	3.8
Control	1.8	2.5	2.6	4	4.3

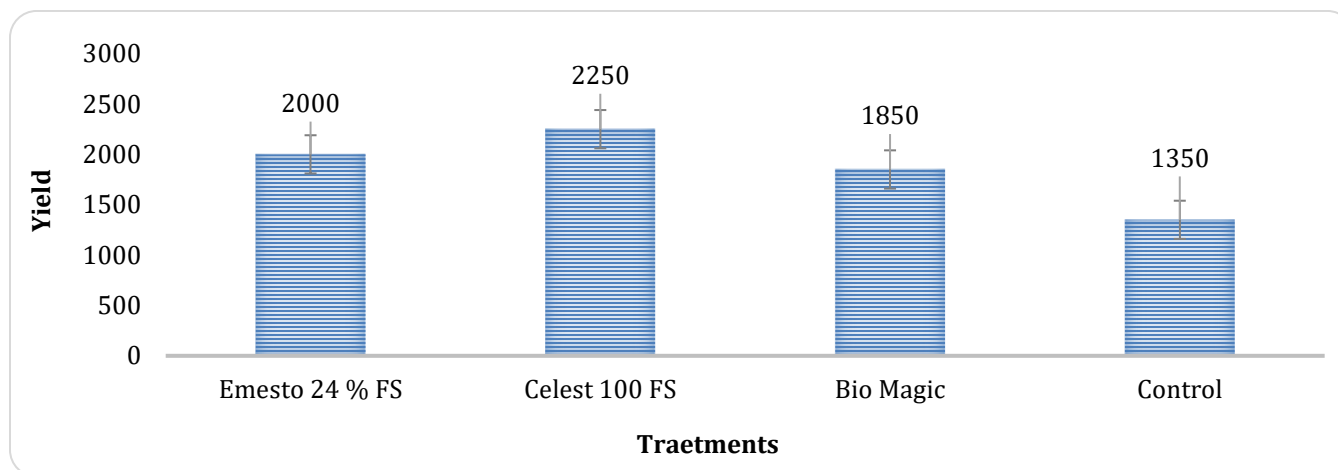


Figure 1. Effect of various treatments on the yield (grams) of potato crop against the potato scab.

DISCUSSION

There are limited options available to control potato scab in Pakistan and elsewhere. The disease incidence has been increasing due to inadequate control strategies. Different strategies such as chemical treatment, irrigation, soil acidification, crop rotation, and using tolerant varieties have been used for the management of the disease (Davis *et al.*, 1976; Adams and Lapwood, 1978; Waterer, 2002; Li *et al.*, 1999; Bouček-Mechiche *et al.*, 2000; Agbessi *et al.*, 2003). Indiscriminate use of chemicals is not only responsible for environmental pollution but also for ecological disturbance. To overcome these problems, biocontrol agents were used as alternative strategies because they were antagonists and left no harmful residual effects (Raaijmakers *et al.*, 2002; Hyder *et al.*, 2020; Bashir *et al.*, 2020). Traditionally, common scab disease has been controlled by using fungicides like Ridomil and Rhizolex under in vitro and in vivo conditions (Hosny *et al.*, 2014). Efforts have also been made to control this disease by seed treatment with chemical fungicides like fluazinam, mancozeb, and benzothiazole (Santos-Cervantes *et al.*, 2017).

The present study was conducted to investigate the effect of certain fungicides such as Celest 100 FS, Emesto FS, and biopesticide *Bacillus subtilis* on *Streptomyces*

scabiei under in vitro and green house pot assays. Stein (2005) noted that *B. subtilis* reduced the growth of *S. scabiei* by producing antibiotics (Stein, 2005), but two main antibiotics played a key role in antagonism. These are Macrolactin A and Iturin A and are stable at high temperatures and a wide range of pH (Han *et al.*, 2005). The use of different antagonistic microorganisms has now become a viable approach as compared to traditional management against plant pathogens (Lugtenberg and Kamilova, 2009). Various microorganisms, including *Pseudomonas* spp. (Arseneault *et al.*, 2013; Singhai *et al.*, 2011), antagonistic *Streptomyces* spp. (Sarwar *et al.*, 2019), *Brevibacillus laterosporus* (Chen *et al.*, 2017), *Bacillus amyloliquefaciens* (Lin *et al.*, 2018), The genus *Bacillus* contains many beneficial plant bacteria that help the plant grow by various means, such as antimicrobial compound secretions, increasing the availability of plant nutrients, competing with other pathogenic bacteria, and inducing the plant's defense (Klopper *et al.*, 2004; Ongena and Jacques, 2008; Spaepen *et al.*, 2009; Fan *et al.*, 2011; Bibi *et al.*, 2017; Ali *et al.*, 2014).

The disease severity of common scab was significantly decreased, and yield was increased when potatoes were treated with fludioxonil, *E. cloacae*, and mustard meal

(Al-Mughrabi, 2010). Until now, there has been no report of using a *Bacillus* species as a suppressive strain to control common scab disease; here we report the use of *Bacillus subtilis* as a biocontrol agent. In this study, penflufen, fludioxanil, and *Bacillus subtilis* were tested *in-vitro* against *S. scabiei*, and these treatments had antagonistic effects in petri plates. In general, the best results were shown by *B. subtilis* in all tested concentrations of treatments. These results were similar to those reported by Barakat (1970). In the pot assay, the minimum disease severity was observed on potatoes planted in soil having 1% or less disease severity, while the maximum disease severity was observed on those potatoes planted in soil having 50% or above disease severity. The minimum disease severity was observed on potato tubers treated with Celest 100 FS and Bio magic (*Bacillus subtilis*), but Emesto 24% FS also showed good results as compared to control. Our results also closely agree with those reported by Barakat (1970), Lapwood *et al.* (1973), and Errampalli and Johnston (2001). Farmers' incomes will be equally affected by the above approaches, like fungicides, biocontrol agents, and application of certain chemicals as seed treatments, which will be equally beneficial for farmers to increase their incomes by minimizing crop losses.

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CONFLICT OF INTEREST

The authors have not declared any conflict of interests.

AUTHORS CONTRIBUTIONS

Jahanzaib Sandhu and Abdul Rehman conducted the practical experiments, collected the data, and analyzed the data, while, Abdul Rehman, Muhammad W. Alam, and Saira Mehboob worked as advisors and wrote the paper.

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