Fundamental and Applied Agriculture
Vol. 6(1), pp. 8–18: 2021
doi: 10.5455/faa.15278

PLANT PROTECTION | ORIGINAL ARTICLE

In vitro evaluation of bacterial antagonists in controlling seed borne fungi associated with four oilseeds in Bangladesh

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ARTICLE INFORMATION

Article History
Submitted: 18 Oct 2020
Accepted: 17 Nov 2020
First online: 27 Mar 2021

Academic Editor
Md Abdullahil Baki Bhuiyan
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ABSTRACT

Effect of bacterial antagonists was assessed using blotter incubation method of seed health testing. Four oilseeds in Bangladesh *viz.*, mustard var. Binasorisha-4, soybean var. Binasoybean-3, sesame var. Binatil-3 and peanut var. Binachinabadam-4 and three bacterial bioagents, *Bacillus subtilis*, *Bacillus thuringiensis* and *Pseudomonas fluorescens* were used in this study. Besides these antagonists, Vitavax 200 was used as a positive check treatment along with an untreated control. Among the tested antagonists, in comparison to control treatment, *B. subtilis* increased the germination of Soybean (91.67%), Sesame (94.0%) and Peanut (83.33%). In case of mustard, highest germination (96.0%) was observed in seed treated with *P. fluorescens*. Nine fungal mycoflora were recorded from the four non-treated control oilseed samples *viz.*, *Fusarium oxysporum*, *Aspergillus flavus*, *A. niger*, *Colletotrichum truncatum*, *Macrophomina phaseolina*, *Cercospora personata*, *Curvularia lunata*, *Penicillium* sp., and *Rhizopus* sp. The highest number of seed borne fungi was detected in untreated control. According to the observation, *B. subtilis*, *B. thuringiensis* and *P. fluorescens* significantly inhibited seed borne fungi of tested seeds over control treatment. However, Vitavax 200 was found highly effective to inhibit the seed borne fungi. Among the bioagents, *B. subtilis* suppressed highest number of seed borne fungi such as *A. niger* (mustard) and *Aspergillus flavus* (sesame), whereas *B. thuringiensis* suppressed the highest number of seed borne fungi such as *F. oxysporum* (Peanut), *Aspergillus flavus* (Peanut), *C. lunata* (Sesame) and *Penicillium* sp.(Peanut). *P. fluorescens* was found highly effective to inhibit different seed borne fungi such as *F. oxysporum* (Mustard), *Aspergillus flavus* (Soybean and Mustard), *Penicillium* sp. (Mustard and Sesame), *C. truncatum* (Soybean), *C. personata* (Soybean) and *M. phaseolina* (Soybean). In most cases, inhibition of seed borne fungi by *P. fluorescens* was statistically similar to Vitavax 200. Thus, all of the three tested bioagents were effective to control seed borne fungi associated with oil seed crops. Specifically, *P. fluorescens* was found to be the most effective bioagent in controlling seed-borne fungi.

Keywords: Bacterial antagonist, *Bacillus subtilis*, *Pseudomonas fluorescens*, oilseed, seed borne fungi

Cite this article: Saha TK, Naznin S, Monjil MS. 2021. *In vitro* evaluation of bacterial antagonists in controlling seed borne fungi associated with four oilseeds in Bangladesh. Fundamental and Applied Agriculture 6(1): 8–18. doi: 10.5455/faa.15278

1 Introduction

Quality seeds play an important role in the production of healthy crops. But seeds are found to be responsible for disease transmission because they carry several pathogens which get associated either in the field or in the post-harvest storage condition (Agarwal and Sinclair, 1997). The growth of toxigenic fungi can adversely affect grain quality (Zhai et al., 2014). Pathogen free healthy seed is needed for desired plant populations and good harvest. Many plant pathogens

are known to seed-borne, which presence or absence on seed surface determine the quality of seed (Rennie, 1998). Seed borne diseases have been found to affect the growth and productivity of crop plants (Islam et al., 2009). In Bangladesh, out of 16% annual crop losses due to plant diseases, at least 10% loss is incurred due to seed-borne disease (Fakir, 1983). It has also been demonstrated that seed-borne fungi are responsible for poor health of seeds in many crops (Neergaard, 1973). As many as 490 seed-borne diseases are known to attack 759 different crop plants in Bangladesh of which at least 200 are of major concern (Fakir, 1980).

Oilseed crops (Brassicaceae) have been grown all over the world and are considered important crops due to their economic value. In Bangladesh four important oilseeds are mustard, soybean, groundnut and sesame. Among oilseeds in Bangladesh, in fiscal year (FY) 2016-17, soybeans are the fourth ranked crop in terms of total planted area at 9.82% of total oilseed planted area; mustard dominates with 67%, followed by sesame (10.88%) and groundnuts (11.55%), respectively (USDA, 2018). The full potential of the oil-seed crops is so far from being exploited and the yield levels in Bangladesh are low due to several biotic and abiotic factors. Among the several biotic limiting factors for successful oilseed production, susceptibility to disease is one of the major constraints. Fungal infection during storage of seed is a significant constraint among several factors limiting the production of oilseeds (Klein et al., 2006). The most isolated genera of fungi from diseased soybean are *Aspergillus*, *Colletotrichum*, *Fusarium*, *Penicillium*, *Rhizopus*, *Macrophomina*, *Alternaria*, *Cercospora* and *Curvularia* (Shovan et al., 2008). The predominant species of the genera from Peanut are *Fusarium*, *Aspergillus*, *Penicillium*, *Rhizopus*, *Sclerotium*, *Rhizoctonia* etc. (Gachomo, 2004). Sesame is being affected by *Fusarium*, *Aspergillus*, *Penicillium* and *Rhizopus* (Suleiman et al., 2013). The predominant isolated fungal genera from diseased mustard are *Alternaria*, *Fusarium*, *Rhizoctonia*, *Penicillium*, *Aspergillus*, *Curvularia* and *Rhizopus* (Rai et al., 2015).

The need of high quality seed is becoming very essential to achieve optimum plant stand but the maintenance of viability and checking rapid deterioration of seed is posing a serious problem in the seed industry. It is obvious that the seed loses its viability and vigor during storage similar to other biological materials. The loss of seed viability due to seed deterioration is inevitable which is mainly dependent on physical, physiological and chemical composition of seed (Malik, 2013). But the rate of deterioration could be slow down to a greater extent during storage by imposing certain biological seed treatments before storage (Debeaujon et al., 2000). Adaption of these techniques for a particular crop require standardization work as the response of seed to the pre-sowing treatment vary with chemicals, their concentration, duration of treatment, type of seed lot etc. (Islam et al., 2003). Several fungicides are available in Bangladesh to control seed-borne pathogens. But, the indiscriminate use of chemicals and their residual toxicity adversely affect the non-target animals including human beings besides affecting the seed quality (Aktar et al., 2009). Hence, the alternate and safe approach is the treatment of seeds with biological agents which is eco-friendly, economical and available (Sharma et al., 2015).

In view of the recurrent occurrence of fungal diseases in oilseed crops, present investigations were carried to evaluate three different bacterial antagonists *viz*, *Bacillus subtilis*, *B. thuringiensis* and *Pseudomonas fluorescens* against seed borne infection of oilseed crops.

2 Materials and Methods

2.1 Laboratory experiment

The laboratory experiment was conducted in the Mycology Section, Professor Golam Ali Fakir Seed Pathology Centre (PGAFSPC), and Microbiology and Bio-control Laboratory, Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh during January, 2017 to April, 2018. Adequate amount of seeds (250 g for each variety) were collected from Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh. These were Mustard (*Brassica juncea*) var. Binasorisha-4, Soybean (*Glycine max*), var. Binasoybean-3, Sesame (Sesamum indicum) var. Binatil-3 and Peanut (*Arachis hypogaea*) var. Binachinabadam-4. Three bacterial antagonists were used to treat the seeds such as *Bacillus subtilis*, *Bacillus thuringiensis* and *Pseudomonas fluorescens*. These bioagents were collected from Microbiology & Bio-control Laboratory, Department of Plant Pathology, Bangladesh Agricultural University. Previously, Microbiology & Bio-control Laboratory isolated several bacterial bioagents from native sources and screened their bio-control activities.

2.2 Experimental treatments

There were five treatments used in the experiment. These were (i) T1 = Seed treatment with *Bacillus subtilis*, (ii) T2 = Seed treatment with *Bacillus thuringiensis*, (iii) T3 = Seed treatment with *Pseudomonas fluorescens*, (iv) T4 = Seed treatment with Vitavax 200 @ 0.2% of seed weight, and (v) T5 = Control (Untreated). Seed treatment was done following the method described by Islam and Monjil (2016). Briefly, required amount of seeds (400 seeds per treatment) were soaked in a bacterial suspension at least 107 CFU per mL for 10 minutes. After treatment, seeds were placed in open air (25 $\pm$ 1 °C) for drying (4-5 h). Chemical fungicide,

Vitavax 200 (@ 0.2% of seed weight) was tested as a seed treating chemical. Five grams of seeds from each sample and 0.01 g of Vitavax 200 were taken in 250 mL of Erlenmeyer flask and were shaken for 10 min for proper coating (Islam et al., 2013). The experiment was conducted following Completely Randomized Design (CRD).

2.3 Blotter incubation method

To detect the seed borne fungi associated with the seeds in seed samples, the blotter incubating method was used following International Rules for Seed Testing (ISTA, 2009). In this method, three layers of blotting paper (Whatman filter No. 1) soaked in sterilized water were placed at the bottom of 9 cm diameter plastic petridish. Thereafter, treated and untreated seeds (Control) were placed in wet blotter paper with 25 seeds for Mustard and Sesame and 10 seeds for Soybean and Peanut in each plate.

Total 400 seeds were tested for each crop for each treatment following the rules of ISTA (2009). Seeds in petridises were kept for incubation under the temperature of 22 $\pm$ 2 °C for 7-9 days. To keep the filter paper moist, adequate sterilized water was supplied. Each individual seed was observed under stereomicroscope (ZEISS, Stemi 508) to identify the association of seed borne fungi. Most of the fungi were detected by observing their growth characters on the incubated seeds following the key outlined by Mathur and Kongsdal (2003) and Habib et al. (2011). The temporary as well as permanent slides were also prepared and observed under compound microscope for proper identification. Photographs of the identified pathogens were taken using Compound Microscope (ZEISS, Primo Star) at 10× and 40× magnifications (Bio-safety laboratory, Department of Plant Pathology, Bangladesh Agricultural University). The fungi were identified to species level, wherever possible, following the keys of Mathur and Kongsdal (2003).

2.4 Data analysis

The collected data were statistically analyzed using STAR (Statistical Tool for Agricultural Research) statistical package to find out the variation resulting from experimental treatments. The significance of difference between the pair of means was compared by LSD test at 5% level of probability (Gomez and Gomez, 1984). Mean differences were judged by Duncan's Multiple Range Test (DMRT).

3 Results

3.1 Seed borne fungi identified

Nine fungal mycoflora were recorded from the four untreated control oilseed samples *viz.*, *Fusarium oxysporum*, *Aspergillus flavus*, *Aspergillus niger*, *Colletotrichum truncatum*, *Macrophomina phaseolina*, *Cercospora personata*, *Curvularia lunata*, *Penicillium* sp., and *Rhizopus* sp. (Fig. 1). Four fungal species (*F. oxysporum*, *A. flavus*, *A. niger* and *Penicillium* sp.) were indentified in Mustard var. Binasorisha-4. Total five fungi mycoflora were identified in Soybean var. Binasoybean 3 *viz.*, *F. oxysporum*, *C. truncatum*, *A. flavus*, *M. phaseolina* and *C. personata*. In Sesame var. Binatil 3, identified four fungal species were *A. flavus*, *A. niger*, *Penicillium* sp. and *C. lunata*. Four fungi were identified in peanut seeds. These were *F. oxysporum*, *A. flavus*, *Penicillium* sp. and *Rhizopus* sp. During identification following characteristics of isolated fungi were detected.

3.1.1 *Fusarium oxysporum*

Fungus produces sparse to abundant growth, covering part or whole seed. Mycelium can be white to cream color. Slimy masses of conidia which were seen running along hyphae. Micro conidia were generally produced in abundance, they were of various shapes, oval, elliptical or reniform, usually non-septate but 1-septate conidia can be seen. Macroconidia were hyaline, thin walled, 3-5 septate, falcate to almost straight, pointed at both ends.

3.1.2 *Aspergillus flavus*

Growth of the fungus on seed was characterized by immature, white heads and mature heads in shades ranging from yellowish cream to green. Conidiophores bearing the heads were clearly seen when the growth is light. They were long hyaline terminating in bulbous heads. Conidia globose to subglobose, usually rough, yellowish – green.

3.1.3 *Aspergillus niger*

Brown to black globose conidial heads on long, erect, hyaline conidiophores were characteristics of this fungus. The mycelia contained single conidiophores or small groups of conidiophores and at times they cover parts of seed or whole seed. Conidia more or less globose. Dark brown often rough or echinulate.

3.1.4 *Colletotrichum truncatum*

Growth of the fungus on seed consists of acervuli which were generally single, rarely in groups and black sclerotia with setae. Setae in acervuli was bigger than conidial masses which were usually dull orange to pinkish bright orange. Acervuli could sometimes be seen on the top of sclerotia. Conidia cylindrical, straight ends obtuse, hyaline, aseptate.

3.1.5 *Macrophomina phaseolina*

Light to heavy growth on seed, consisting of large pycnidia overgrown with greyish hyphae especially surrounding the neck of pycnidia. Pycnidiospores of *M. phaseolina* were often seen oozing from ostiol of pycnidia in the form of cirrhus which was white and wet in the beginning, becomes drier with age. They were one-celled, hyaline, ellipsiod to obovoid, thin walled.

3.1.6 *Cercospora personata*

Growth of the fungus consisted of long, stiff, dark gray conidiospores, each bearing a needle like, white to silvery white conidium. Conidia hyaline, acicular, mostly straight, truncate base, tapering apex, 0-22 septate, thickened hilum.

3.1.7 *Curvularia lunata*

A few isolated, dark conidiophores, short to long, bearing clusters of black, shiny conidia were present at the tip. The growth was extensive covering part of seed or whole seed. The arrangement of conidia on conidiophores was acropleurogenous. Conidia smooth walled, 3 septate, mostly curved but some straight, third cell from the base the largest and darkest, end cells subhyaline or pale, tip cell rounded, basal cell usually with a scar.

3.1.8 *Penicillium* sp.

The thallus (mycelium) typically consists of a highly branched network of multinucleate, septate, usually colorless hyphae. Many-branched conidiophores sprout on the mycelia, bearing individually constricted conidiospores. The conidiospores were the main dispersal route of the fungi, and often were green in color.

3.1.9 *Rhizopus* sp.

Often growth of fungus covered the whole seed and extends to blotters. Even from one infected seed the dense mycelium can cover the whole petridish. Sporangia spherical, black, contain numerous spores. Sporangiospores are one-celled, spore shape may vary from globose to oval, ellipsoid, polygonal or angular, even striate.

3.2 Seed germination and seed borne infection of oil crops

3.2.1 Mustard var. Binasorisha-4

Seed germination of mustard was significantly influenced by antagonists which ranged from 86.67% to 96.00% . Maximum seed germination (96.00%) was recorded in *P. fluorescens* treated seed followed by Vitavax 200 (94.67%). However, the lowest and minimum seed germination (86.67%) was recorded in control treatment (Table 1). Seed borne infection by *F. oxysporum*, *A. flavus*, *A. niger* and *Penicillium* sp. was significantly influenced by the antagonists (Table 1 and Fig. 2). In case of *F. oxysporum*, infection ranged from 0.00% to 8.00%, where the highest seed borne infection (8%) was recorded in control followed by *B. subtilis* treated seed (4.67%). The lowest seed borne infection (0.00%) was recorded in Vitavax 200. Seed borne infection by *A. flavus* was ranged from 0.67% to 6.00%, where the highest seed borne infection (%) was recorded in control (6.00%) followed by *B. subtilis* (4.47%). The lowest seed borne infection (0.67%) was recorded in Vitavax200 followed by *P. fluorescens* (2.00%). Infection of seeds by *A. niger* was ranged from 0.00% to 6.00%, where the highest seed borne infection (%) was recorded in control (6.00%) followed by *B. thuringiensis* (2.67%). The lowest seed borne infection (0.00%) was recorded in Vitavax 200 followed by *P. fluorescens* (1.33%). Infection range of *Penicillium* sp. was from 0.00% to 6.00%, where the highest seed borne infection (%) was recorded in control (6.00%) followed by *B. subtilis* (3.03%). The lowest seed borne infection (0.00%) was recorded in Vitavax 200.

3.2.2 Soybean var. Binasoybean-3

Seed germination of soyabean was significantly influenced by these antagonists and ranged from 75.00% to 91.67%. Maximum germination (%) was recorded in *B. subtilis* treated seed (91.67%) followed by *P. fluorescens* (83.33%) and minimum germination (75.00%) was recorded in untreated control treatment (Table 2). Seed borne infection of five identified fungi, *F. oxysporum*, *C. truncatum*, *A. flavus*, *M. phaseolina* and *C. personata* was significantly influenced by the three antagonists (Table 2 and Fig. 2). Seed borne infection by *C. truncatum* was ranged from 0.00% to 13.33%, where the highest seed borne infection (%) was recorded in control (13.33%) followed by *B. thuringiensis* (6.67%). Seed borne infection was not observed (0.00%) in Vitavax 200 and *P. fluorescens*. Infection range of *F. oxysporum* in soybean seeds was 0.00% to 10.00%, where the highest seed borne infection (%) was recorded in control (10.00%) followed by *B. thuringiensis* treatment (3.63%). The lowest seed borne infection (0.00%) was recorded in Vitavax 200. Second lowest *F. oxysporum* was observed in *P. fluorescens* treated seeds (2.33%). In case of *A. flavus*, infection ranged from 0.00% to 12.83%, where the highest seed borne infection (%) was recorded in *B. thuringiensis* (12.83%) followed by *B. subtilis* (11.67%). Zero seed borne infection was recorded in Vitavax 200 and *P. fluorescens*. Infection of soybean seeds by *M. phaseolina* ranged from 0.00% to 6.67%, where the highest seed borne infection (%) was recorded in *B. thuringiensis* (6.67%)

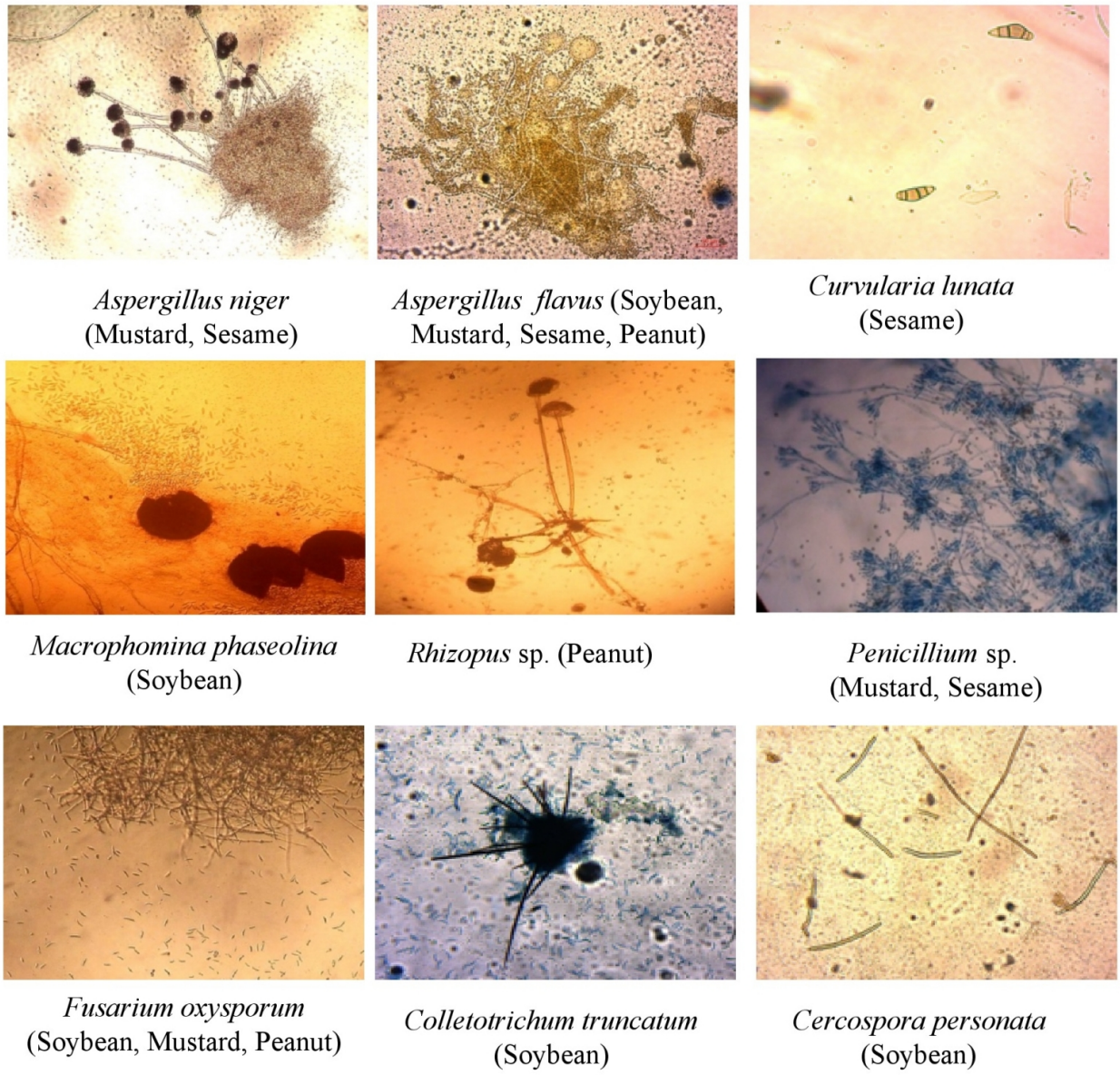

Figure 1. Detection of fungi associated to four soilseed crops (mustard, soybean, sesame and peanut) using blotter incubation method. Fungal detection was done by preparing semi-permanent slides under compound microscope (ZEISS, Primo Star)

Table 1. Efficacy of different antagonists as seed treatment on seed germination (%) and seed borne infection (%) of Mustard var. Binasorisha-4

Treatment	Germination (%)	% Seed borne infection			
		F. oxysporum	*A. flavus*	*A. niger*	*Penicillium* sp.
T1	92.67 ab	4.67 ab	4.47 ab	1.33 c	3.03 ab
T2	88.67 bc	4.00 abc	4.00 ab	2.67 b	2.67 ab
T3	96.00 a	2.00 bc	2.00 bc	1.33 c	2.00 b
T4	94.67 a	0.00 c	0.67 c	0.00 d	0.00 b
T5	86.67 c	8.00 a	6.00 a	6.00 a	6.00 a
Sig. level	*	**	*	**	*

Each value represents the mean of four replications. In a column, figures with same letter do not differ significantly where figures with dissimilar letter differ significantly; '*' and '**' indicate 5% and 1% level of significance, respectively; T1 = *B. subtilis*, T2 = *B. thuringiensis*, T3 = *P. fluorescens*, T4 = Vitavax 200, and T5 = Control (Untreated).

Table 2. Efficacy of differents antagonist as seed treatment on seed germination (%) and seed borne infection (%) of Soybean var. Binasoybean-3

Treatment	Germination (%)	% Seed borne infection				
		C. truncatum	*F. oxysporum*	*A. flavus*	*M. phaseolina*	*C. personata*
T1	91.67 a	5.00 b	3.33 bc	11.67 a	1.67 b	3.33 abc
T2	78.33 b	6.67 ab	3.63 bc	12.83 a	6.67 a	6.67 ab
T3	83.33 ab	0.00 b	2.33 a	0.00 b	0.00 b	1.67 bc
T4	81.67 b	0.00 b	0.00 c	0.00 b	0.00 b	0.00 c
T5	75.00 b	13.33 a	10.00 b	10.00 ab	3.33 ab	8.33 a
Sig. level	*	**	**	*	*	*

Each value represents the mean of four replications. In a column, figures with same letter do not differ significantly where figures with dissimilar letter differ significantly; '*' and '**' indicate 5% and 1% level of significance, respectively; T1 = *B. subtilis*, T2 = *B. thuringiensis*, T3 = *P. fluorescens*, T4 = Vitavax 200, and T5 = Control (Untreated).

followed by control (3.33%). Seed borne infection was not observed in Vitavax 200 and *P. fluorescens*. In case of highest seed borne infection of *C. personata* was observed in control (8.33%) and lowest was found in Vitavax 200 (0.00%) followed by *P. fluorescens* (1.67%)

3.2.3 Sesame var. Binatil-3

Seed germination of sesame was significantly influenced by different antagonist and ranged from 87.33% to 96.00%. Maximum seed germination (%) was recorded in Vitavax 200 treated seed (96.00%) followed by *B. subtilis* (94.00%) and minimum seed germination (87.33%) was recorded in control (Table 3). Seed borne infection of the four identified fungi (*A. flavus*, *A. niger*, *Penicillium* sp. and *C. lunata*) was significantly influenced by the three different antagonists (Table 3 and Fig. 2). In case of *A. flavus*, infection ranged from 0.00% to 8.00%, where the highest seed borne infection (%) was recorded in control (8.00%) followed by *P. fluorescens* (4.33%). The lowest seed borne infection (0.00%) was recorded in Vitavax 200.

In case of *A. niger*, infection ranged from 0.67% to 5.33%, where the highest seed borne infection (%) was recorded in control (5.33%) followed by *B. thuringiensis* (1.33%). The lowest seed borne infection (0.00%) was recorded in Vitavax 200 and *B. subtilis*. Seed borne infection of *Penicillium* sp., were ranged from 0.00% to 9.33%, where the highest seed borne infection (%) was recorded in control (9.33%) followed by *B. subtilis* (4.00%). The lowest seed borne infection (0.00%) was recorded in Vitavax 200 followed by *P. fluorescens* (0.67%) In case of *C. lunata*, infection ranged from 0.00% to 5.33%, where the highest seed borne infection (%) was recorded in control (5.33%) followed by *P. fluorescens* (3.33%). The lowest seed borne infection (0.00%) was recorded in Vitavax 200.

3.2.4 Peanut var. Binachinabadam-4

Seed germination of peanut was significantly influenced by antagonist and ranged from 63.33% to 95.00%. Maximum germination (%) was recorded in Vitavax 200 treated seed (95.00%) followed by

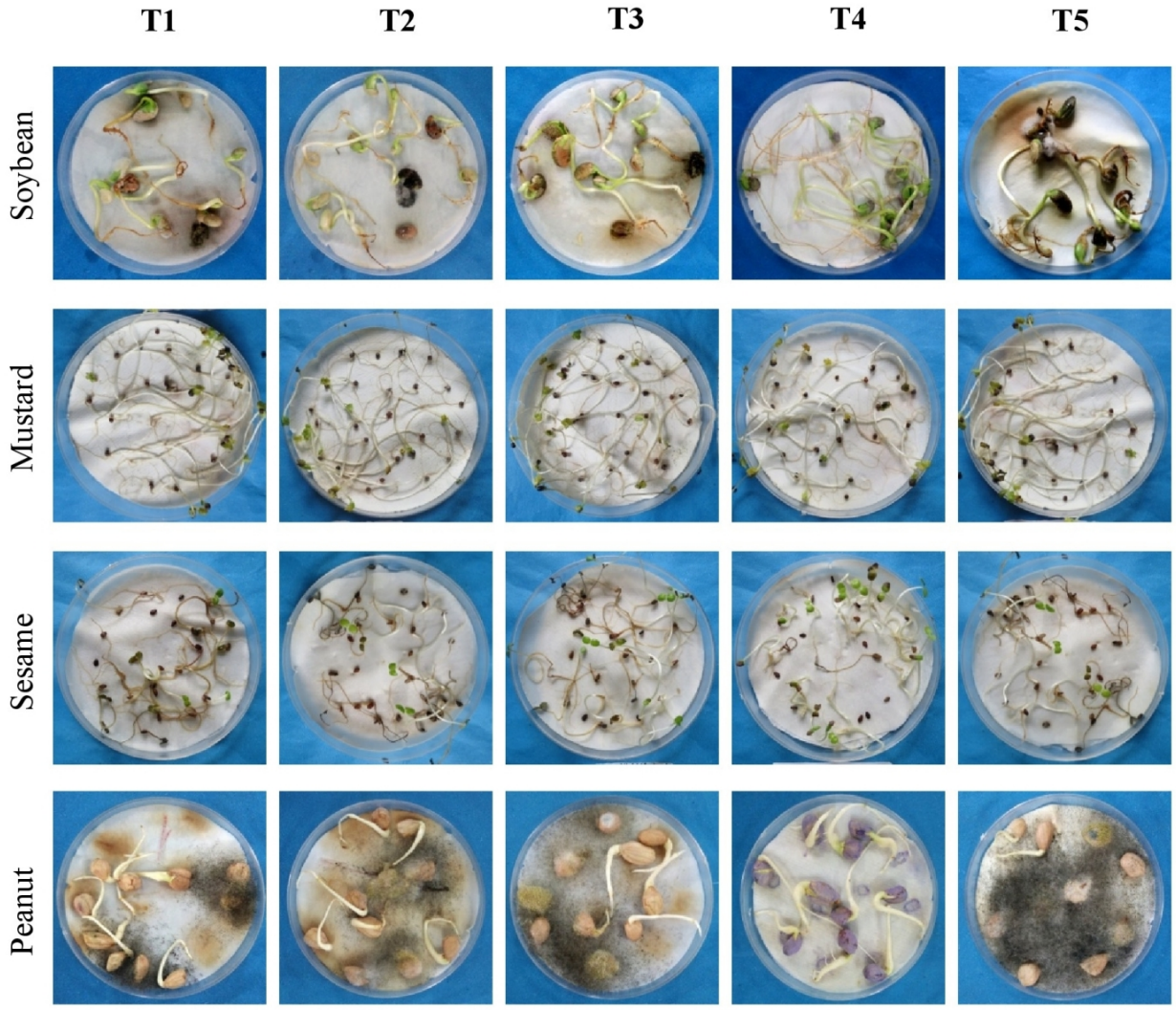

Figure 2. Effect of treatments on seed germination and fungal infection on seeds of mustard, soybean, sesame and peanut. Observation of seed germination and fungal growth using blotter incubation method. Treatments were (i) T1 = Seed treatment with *Bacillus subtilis*, (ii) T2 = Seed treatment with *Bacillus thuringiensis*, (iii) T3 = Seed treatment with *Pseudomonas fluorescens*, (iv) T4 = Seed treatment with Vitavax 200 @ 0.2% of seed weight, and (v) T5 = Control (Untreated).

Table 3. Efficacy of different antagonists as seed treatment on seed germination (%) and seed borne infection (%) of Sesame var. Binatil-3

Treatment	Germination (%)	% Seed borne infection			
		A. flavus	*A. niger*	*Penicillium* sp.	*C. lunata*
T1	94.00 ab	2.67 bc	0.00 b	4.00 b	2.67 abc
T2	90.00 bc	3.33 bc	1.33 b	2.00 c	2.00 bc
T3	91.33 abc	4.33 ab	0.67 b	0.67 c	3.33 ab
T4	96.00 a	0.00 c	0.00 b	0.00 c	0.00 c
T5	87.33 c	8.00 a	5.33 a	9.33 a	5.33 a
Sig. level	*	**	**	**	*

Each value represents the mean of four replications. In a column, figures with same letter do not differ significantly where figures with dissimilar letter differ significantly; '*' and '**' indicate 5% and 1% level of significance, respectively; T1 = *B. subtilis*, T2 = *B. thuringiensis*, T3 = *P. fluorescens*, T4 = Vitavax 200, and T5 = Control (Untreated).

Table 4. Efficacy of different antagonists as seed treatment on seed germination (%) and seed borne infection (%) of stored seed of Peanut var. Binachinabadam-4

Treatment	Germination (%)	% Seed borne infection			
		F. oxysporum	*A. flavus*	*Penicillium* sp.	*Rhizopus* sp.
T1	83.33 b	21.67 b	11.67 a	11.67 a	11.67 b
T2	78.33 bc	20.00 b	3.33 bc	6.67 ab	8.33 bcd
T3	71.67 cd	22.33 b	10.00 ab	8.33 a	10.00 bc
T4	95.00 a	6.67 c	1.67 c	0.00 b	5.00d
T5	63.33 d	33.33 a	16.67 a	13.33 a	16.67a
Sig. level	**	**	**	**	*

Each value represents the mean of four replications. In a column, figures with same letter do not differ significantly where figures with dissimilar letter differ significantly; '*' and '**' indicate 5% and 1% level of significance, respectively; T1 = *B. subtilis*, T2 = *B. thuringiensis*, T3 = *P. fluorescens*, T4 = Vitavax 200, and T5 = Control (Untreated).

B. subtilis (83.33%) and minimum seed germination (63.33%) was recorded in control (Table 4). Seed borne infection of *F. oxysporum*, *A. flavus*, *Penicillium* sp. and *Rhizopus* sp. was significantly influenced by the bacterial antagonists (Table 4 and Fig. 2). In case of *F. oxysporum*, infection ranged from 6.67% to 33.33%, where the highest seed borne infection (%) was recorded in control (33.33%) followed by *P. fluorescens* (22.33%). The lowest seed borne infection (%) was recorded in Vitavax 200 (6.67%). In case of *A. flavus*, infection ranged from 1.67 % to 16.67 %, where the highest seed borne infection (%) was recorded in control (16.67%) followed by *B. subtilis* (11.67%). The lowest seed borne infection (%) was recorded in Vitavax 200 (1.67%). In case of *Penicillium* sp., infection ranged from 0.00% to 13.33%, where the highest seed borne infection (%) was recorded in control (13.33%) followed by *B. subtilis* (11.67%). The lowest seed borne infection (0.00%) was recorded in Vitavax 200. In case of *Rhizopus* sp., infection ranged from 5.00% to 16.67%, where the highest seed borne infection (%) was recorded in control (16.67%) followed by *B. subtilis* (11.67%). The lowest seed borne infection (%) was recorded in Vitavax 200 (5.00%).

4 Discussion

The antagonists (*B. subtilis*, *B. thuringiensis* and *P. fluorescens*) were found to be effective in controlling seed borne diseases and improving seed germination of oil seed crops. All the antagonists have positive effect on germination of four oil seeds. In addition, these antagonists reduced the seed-borne fungi which in some cases was statistically similar with the results of Vitavax-200 treated seeds. As the species of Bacillus synthesize various types of lipopeptides which have antimicrobial properties therefore, they could inhibit the growth of plant pathogens (Shafi et al., 2017). Gong et al. (2014) found the inhibitory substances, Bacillomycin D against *A. flavus* synthesized by *B. subtilis*. Bacillomycin D was found to be effective against target fungus after separation and purification of metabolic liquid produced by the bacteria.

Bacillomycin D caused severe injury to both cell wall and cell membrane of fungal spores and hypha (Gong et al., 2014). Cao et al. (2012) investigated the effects of *B. subtilis* (SQR 9) on Fusarium wilt of cucumber, where application of *B. subtilis* (SQR 9) significantly decreased the Fusarium wilt disease incidence and promoted plant biomass. *B. subtilis* was found to be antagonistic against *A. brassicae*, *A. brassicicola*, *A. dauci*, *A. linicola*, *C. lini*, *F. moniliforme*, *M. phaseolina*, and *Rhizopus* sp. (Umechuruba, 2004).

B. thuringiensis isolates produce chitinase and cellulase which antifungal activity coupled with auxin production and phosphate solubilisation are desirable for a potential plant growth promoting agent (Kumar et al., 2015). Kassogué et al. (2016) stated that under *in vitro* conditions, seed treatment with *B. thuringiensis* strains improved seed germination over the control. We found *P. fluorescens* increased the germination of mustard (96.00%) which were significantly higher than the control (untreated seeds). *P. fluorescens* also inhibited the seed borne fungi such as *F. oxysporum* (mustard), *A. flavus* (soybean and mustard), *Penicillium* sp. (mustard and sesame), *C. truncatum* (soybean), *C. personata* (soybean) and *M. phaseolina* (soybean). Many scientists have discussed the effective use of *P. fluorescens* in crop seeds. *P. fluorescens* strain (Pf1), effectively inhibited the mycelial growth of *M. phaseolina* (Jayashree et al., 2000). Application of *P. fluorescens* strain (558) significantly reduced anthracnose in mango caused by *C. gloeosporioides* when the fruits were inoculated by the antagonist (Koomen and Jeffries, 1993) in the UK. Shanmugam et al. (2002) conducted a study to test the effect of *P. fluorescens* (Pf1) on co-inoculation in peanut to control root rot, a severe soil-borne disease caused by *M. phaseolina*. Strain (Pf1) resulted in significantly inhibition of *M. phaseolina* mycelial growth under *in vitro* conditions. *P. fluorescens* strain (3JW1) had multiple effects including reduction of *A. flavus* infection and aflatoxin contamination (Yang et al., 2017). Etebarian et al. (2005) evaluated *P. fluorescens* isolate (1100-6) as a potential biological control agent for apple blue mold caused by *P. expansum* or *P. solitum* and found that *P. fluorescens* isolate (1100-6) could be an important new biological control for *P. expansum*. In the plant rhizosphere, *P. fluorescens* produces a wide spectrum of bioactive metabolites, that is, antibiotics, siderophores, volatiles, and growth-promoting substances; competes aggressively with other microorganisms; and adapts to environmental stresses. In addition, *Pseudomonads* are responsible for the natural suppressiveness of some seed and soil borne pathogens. It suppresses the growth of pathogenic microorganisms by various mechanisms, namely, production of antibiotics, bacteriocins, siderophores, hydrolytic enzymes such as β-1,3-glucanase and chitinases, and other metabolites such as phytoalexins and induction of systemic resistance (David et al., 2018).

5 Conclusion

Based on the findings of the present study, it can be concluded that bacterial antagonists have potential to improve seed germination and inhibit the seed borne fungal infection associated to oil seed crops. Inhibition of fungal flora by bacterial bioagents is comparable to seed treating chemical, Vitavax 200, particularly in case of *P. fluorescens*.

Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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The Official Journal of the **Farm to Fork Foundation**
ISSN: 2518–2021 (print)
ISSN: 2415–4474 (electronic)
http://www.f2ffoundation.org/faa