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EFFECTS OF *PSEUDOMONAS SYRINGAE* AND *XANTHOMONAS CUCURBITAE* ON SEED GERMINATION OF CUCURBITS

Md. Estiak Khan Chowdhury[1,2], Md. Enamul Haque[3], Fahmida Begum Mina[3], Sumon Karmakar[3], Mutasim Billah[3], Meherun Nesa[4], Biswanath Sikdar[3] and Md. Faruk Hasan[4,*]

1. Institute of Veterinary Pathology, Justus Liebig University, FB 10-Veterinary Medicine Frankfurter Str. 96, 35392, Giessen, Germany.
2. Department of Genetic Engineering and Biotechnology, University of Rajshahi. Rajshahi, P.O. Box 6205, Bangladesh.
3. Department of Biotechnology and Genetic Engineering, Bangabandhu Sheikh Mujibur Rahman Science and Technology University, Gopalganj, P.O. Box 8100, Dhaka, Bangladesh.
4. Department of Zoology, University of Rajshahi, Rajshahi, P.O. Box 6205, Bangladesh.
5. Department of Microbiology, University of Rajshahi. Rajshahi-6205, Bangladesh.
***Corresponding author e-mail: faruk_geb@ru.ac.bd**

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ABSTRACT
Pseudomonas syringae and *Xanthomonas cucurbitae* affect the leaves, stems, and fruits of cucurbit plants contributing to a widespread bacterial disease of cucurbits. The present study was conducted to evaluate the germination rate of the seeds of five species of cucurbit, *viz.* bitter gourd, ridge gourd, bottle gourd, cucumber, and pumpkin, by inoculating bacteria *P. syringae* and *X. cucurbitae* at the seedling stage of development. The germination test was carried out by using the 'Top-of-paper' method. Bitter gourd seeds showed an 80% germination rate in control, but seeds inoculated with *P. syringae* and *X. cucurbitae* showed a 20% germination rate after 90 hours of incubation. A similar delay resulted for ridge gourd seeds inoculated with *P. syringae* and *X. cucurbitae* showed highest 25% and 41.67% germination, respectively. Bottle gourd seeds inoculated with *P. syringae* and *X. cucurbitae* showed a maximum of 63.64% and 72.73% germination respectively, after 120 hours. After 60 hours of incubation, cucumber seeds inoculated with *P. syringae* and *X. cucurbitae* showed 50% and 70% germination, respectively. However, after 90 hours of incubation, cucumber seeds inoculated with isolate *P. syringae* and *X. cucurbitae* showed the highest of 100% germination. Similarly, pumpkin seeds inoculated with *P. syringae* and *X. cucurbitae* showed a maximum of 70% and 80% germination after 50 hours of incubation. The experiment revealed that inoculation of *Pseudomonas syringae* and *Xanthomonas cucurbitae* at the seedling stage cucurbits have impaired seed germination significant delay in seed vigor over control.

1. Introduction

Cucurbits are members of the Cucurbitaceae family and are home to some of the most popular garden crops in the world (Enroth 2020; Rahman 2016). Cucurbits are well-known and popular vegetable because of its rich nutrient profile and versatile uses in culinary, therapeutic and cosmetic purposes (Wang 2021). The incidence of phytopathogenic agents, such as bacterial disease, and the use of inferior quality seeds play an important role among the numerous factors resulting in a lower yield of these crops (Gouda et al. 2018). At least 10 percent of the total of 16 percent annual crop losses attributed to plant diseases are owing to seed-borne diseases (Khaskheli et al. 2019). One of the primary concerns for cucurbit cultivation is the infestation of seed-borne bacterial, leaf spot disease and fungal diseases, which inhibits growth, germination, the establishment of yield, and crop consistency (Chowdhury et al. 2024; Rahman 2016). Bacterial spot, angular leaf spot, bacterial wilt, cucurbit yellow vine, and bacterial root rot are the most common bacterial diseases in Bangladesh (Tumpa et al. 2018). Two of the most prolific bacteria are *Pseudomonas syringae* and *Xanthomonas cucurbitae* that can contribute to widespread bacterial disease in cucurbits worldwide. These bacteria can invade the leaves, stalks, and fruit of cucurbit plants (Sharma 2018). *P. syringae* is a non-spore-forming rod, motile, and aerobic bacterium (Liu et al. 2016). It causes angular leaf spot (Liu et al. 2016) and light-tanning (Sharma et al. 2016). *X. cucurbitae* is an anaerobic, non-spore-forming rod bacterium. It infects leaves and fruit of cucurbit crops and causes the bacterial leaf spot disease of cucurbits (Sharma et al. 2016). A germination test is carried out to determine the accession of germinates under optimal conditions. It generates typical seedlings (Kildisheva et al. 2020). Laboratory experiments seek to seed priming technology as a key technique to increase crop production more frequently, rapidly, and ultimately (Garcia et al. 2022). The germination of a seed in the laboratory test is interpreted as the emergence and growth of the seedling to a point where its vital structures' feature shows whether a useful plant can be further grown under favorable field conditions (Ruttanaruangboworn et al. 2017). The germination rate of a seed lot is a significant predictor of the seed's efficiency on the field. If the germination rate was expressed as the 90% germination rate, it indicates that 90 out of 100 seeds would potentially germinate under ideal conditions (Poorter et al. 2016). This knowledge is critical in estimating the optimum seed rate and deciding whether a particular seed lot will yield a successful crop. The germination and advancement of seeds is the first stage of inefficient plant production. The capability of seeds to germinate and emerge in the soil is the path for plant development (Finch-Savage and Bassel 2016). Seed testing and effect of bioagents on cucumber seed mycoflora, seed germination, and seedling viability and vigor were evaluated, previously (Chowdhury et al. 2020; Sharma et al. 2023; Finch-Savage and Bassel 2016). *Pseudomonas syringae* and *Xanthomonas cucurbitae* can affect the cucurbit plants. It is necessary to examine the effect of these bacterial strains at the seedling stage of development. Moreover, to evaluate bacterial pathogenicity in the seedling stage, plant producers will make a range of management choices, including seed commitment, determining seeding rates, and weed planting avoidance.

To study the interactions between cucurbit plants and the bacterial pathogens- *Pseudomonas syringae* and *Xanthomonas cucurbitae* strains, whether being virulent or avirulent seedling stage of development and carry out molecular genetic analysis to find resistance genes for higher yield in further studies. The inoculation of seeds with microorganisms persists as a feasible approach to facilitate the successful growth of seedlings. The effect of inoculation of bacteria is more vulnerable for most plants, especially in seed germination and seedling growth stages. It is a crucial stage in producing crops and

necessary for optimum yield and sustainable plants' populations (Souza et al. 2015). Germination of bacterial inoculated seeds may be induced, prevented, or stayed regulated at levels of control depending on various factors that contaminate pathogens and others (Ndeddy Aka and Babalola 2016). The present experiment aims to evaluate the ability of either inhibiting or stimulating the germination of cucurbits seeds by inoculating bacteria *Pseudomonas syringae* and *Xanthomonas cucurbitae* in the seedling stage of development.

2. Materials and methods

2.1 Experimental design

The present experiment was conducted at Professor Joarder DNA and Chromosome Research Laboratory, Department of Genetic Engineering and Biotechnology, University of Rajshahi, Rajshahi-6205, Bangladesh, from July 2018 -July 2019.

2.2 Seed samples collection and storage

Five species of gourds seeds viz. *Momordica charantia*, *Luffa acutangular*, *Lagenarea siceraria*, *Cucumis sativus*, and *Cucurbita pepo* were collected from the commercial nursery Rajshahi district, Bangladesh. The seed samples were collected in sterile, airtight plastic bags separately to prevent extraneous contamination. The seeds of different cucurbits species were stored at 5ºC to keep the seeds dry and cool for further studies.

2.3 Bacterial inoculum collection and preparation

Bacteria used as inoculums in the germination experiment were *Pseudomonas syringae* and *Xanthomonas cucurbitae*. The symptoms were identified based on diseased symptoms by the Bangladesh Council of Science and Industrial Research (BCSIR), Binodpur, Rajshahi. Bacterial isolates were obtained from the culture collection of the Professor Joarder DNA and Chromosome Research Laboratory, Department of Genetic Engineering and Biotechnology, University of Rajshahi, Rajshahi-6205, Bangladesh.

The process for disinfection and isolation was carried out according to (Costa et al. 2012) with slight modifications. The sample was placed in 100 ml of LB liquid medium and incubated at 37°C overnight. A sterile loop was used to streak bacteria onto a fresh LB agar plate after incubation in LB liquid medium and again incubated at 37°C for 16 hours. Afterward, a single colony was gathered by loop and streaked for pure culture on another new media plate. The pure cultures were preserved on LB slant at 4°C for a short duration.

2.4 Preparation for germination test

A random sample of seeds from each accession was taken by spreading the seed lot on a clean surface and mixing thoroughly. Approximately 400 seeds were used for the germination test, which was divided into three replicates. Most seeds can germinate at a temperature between 20 to 30 °C, sufficient water in the substrate, and a proper amount of light given to the seeds (Bicksler 2011).

2.5 Procedure of germination test

'Top-of-paper method' was used for the germination test in this present study. This method is one of the most efficient methods for species with a seed diameter of less than 2 mm, such as seedlings, forage

grasses, and different seed varieties (Powell 2009). The absorbent paper was used as a substrate for germination in this method.

2.6 Humidification of dry seeds in the sample

Before germination testing, miniaturization of the seeds found extremely dry with moisture content below 8% elevated to 15–17%. Three very moist paper towels were placed flat inside a large polythene box. The seeds were placed on top of the moist paper, and the box was covered with a secure lid. Then, depending on the primary moisture content, the box was placed in an incubator at 20°C for 24 hours or more. The high-quality paper must be used as a substrate (Gómez-Favela et al. 2017). The paper had a neutral pH of 6–7, which was maintained during the germination test.

2.7 Seed dressing and surface sterilization

The seeds were soaked in a 1% solution of sodium hypochlorite for ten minutes. The seeds were rinsed twice in sterile distilled water and then treated with the 3 g/kg dose of the fungicide (3 g thiram, 30 % wettable powder Kg^{-}1seeds). The seeds were mixed thoroughly with the fungicide for 3-5 min, placed on sterile paper towels in the open air to dry, and then kept sterile plastic containers for further use.

2.8 Germination of seeds through top-of-paper method

Selected seeds have inoculated the strains and incubated in a shaker for 24 hours. 50µl liquid bacterial culture was used for all the seeds except control after a regular interval. All seeds, including control and treatment seeds, were treated with an equal volume of distilled water. 50µl liquid bacterial culture was used for all the seeds except control after a regular interval. Then the containers were covered and ensured no airlock resulting from excess moisture on the covers. It was placed in an incubator maintained at 20°C for germination of the seeds. The number of seeds was counted that have germinated. Finally, the seed germination rate was calculated in percentage by using the following formula:

Germination rate (%) = (Number of germinated seeds / Total number of seeds) ×100

2.9 Data analysis

The experiments were conducted in a randomized complete block design (RCBD), with each treatment replicated three times. Each replicate consisted of 10-15 seeds. The collected data were calculated in percentage (%) and generated graph displayed in the results by Microsoft excel 2013.

3. Results

3.1 Effect of bacteria on germination of bitter gourd

A germination test was carried out where ten seeds of bitter gourd were used as control. In the control medium, a total of eight seeds germinated after 90 hours of incubation, and the germination rate was 80%. After 100 hours, seeds inoculated with *P. syringae* and *X. cucurbitae* showed a maximum of 30% and 30% germination, respectively. Which were shown in Figure 1A-C and 2.

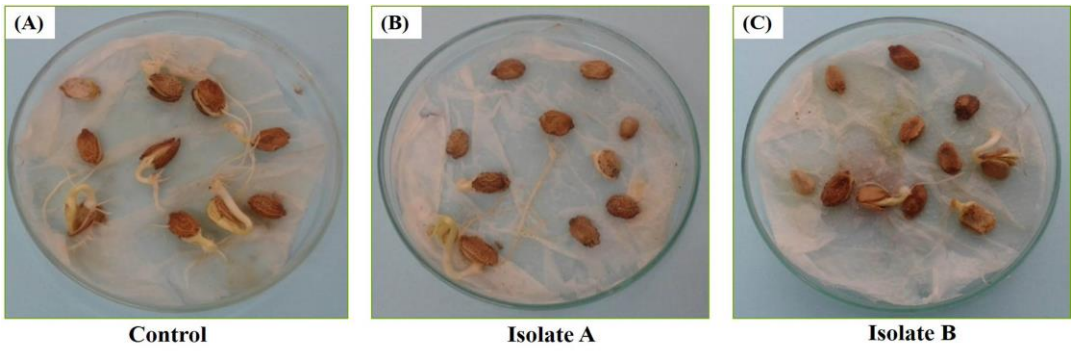

Figure 1: Germination of bitter gourd seeds, (A) Control medium, (B) *P. syringae* and (C) *X. cucurbitae*.

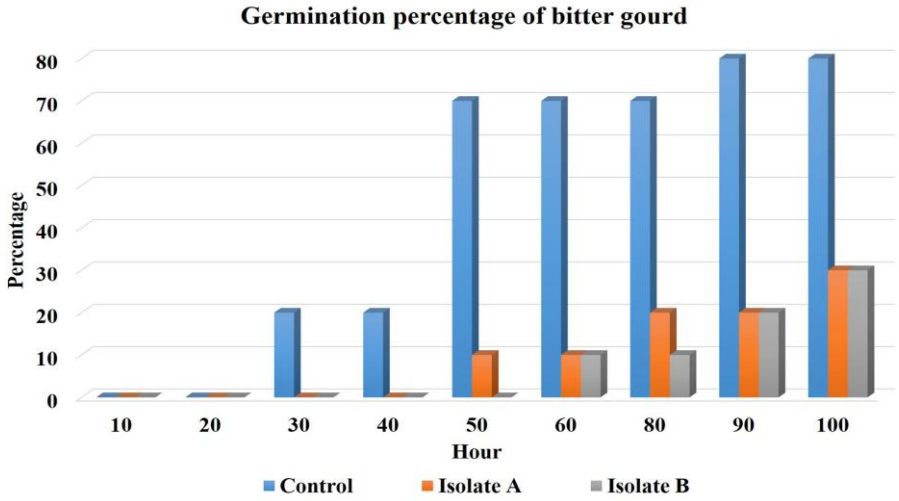

Figure 2: Germination percentage of bitter gourd seeds inoculated with *P. syringae* and *X. cucurbitae*

3.2 Effect of bacteria on germination of ridge gourd

Germination test carried out where 12 seeds of ridge gourd were used as control. In the control medium, a total of eight seeds germinated after 70 hours of incubation, and the germination rate was 66.67%. After 90 hours, seeds inoculated with *P. syringae* and *X. cucurbitae* showed a maximum of 25% and 41.67% germination, respectively, shown in Figure 3A-C and 4.

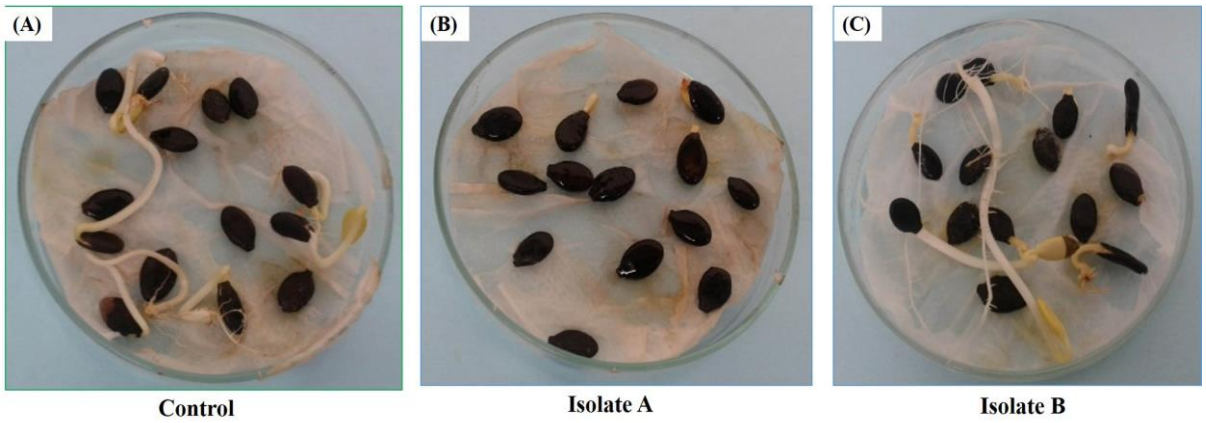

Figure 3: The germination of ridge gourd seeds; (A) Control medium, (B) *P. syringae* and (C) *X. cucurbitae* after 90 hours.

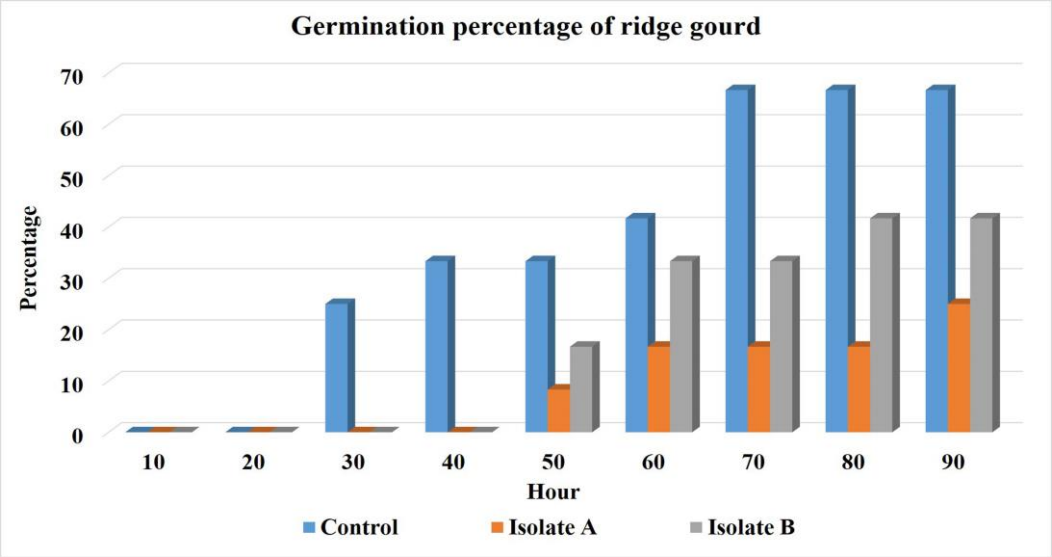

Figure 4: Germination percentage of ridge gourd seeds inoculated with *P. syringae* and *X. cucurbitae*

3.3 Effect of bacteria on germination of bottle gourd

Germination test carried out where 11 seeds of bottle gourd were used as control. In the control medium, a total of nine seeds germinated after 60 hours of incubation, and the germination rate was 81.81%. Seeds inoculated with *P. syringae* showed 54.55% germination after 70 hours, and *X. cucurbitae* showed 54.55% germination after 40 hours. After 120 hours, seeds inoculated with *P. syringae* and *X. cucurbitae* showed a maximum of 63.64% and 72.73% germination, respectively, which were shown in Figure 5A-C and 6.

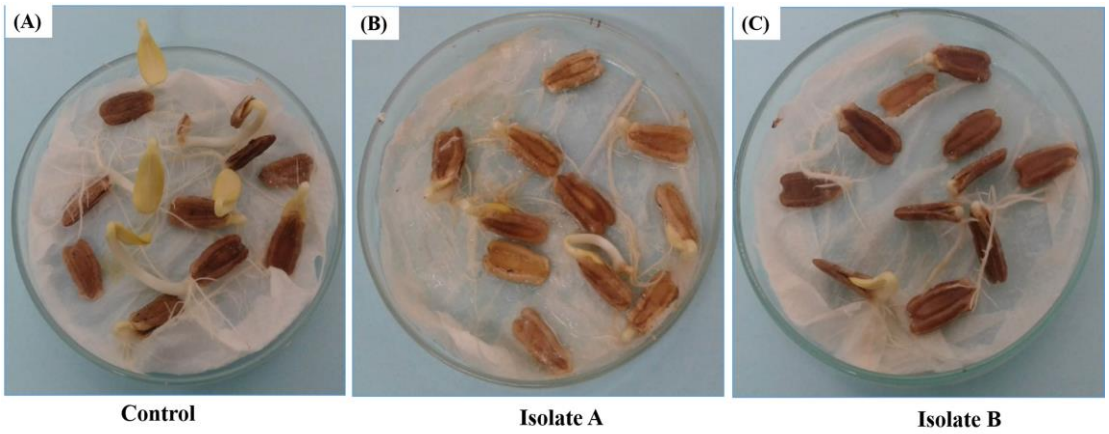

Figure 5: Germination of bottle gourd seeds; (A) Control medium, (B) isolate A and (C) isolate B after 120 hours of inoculation

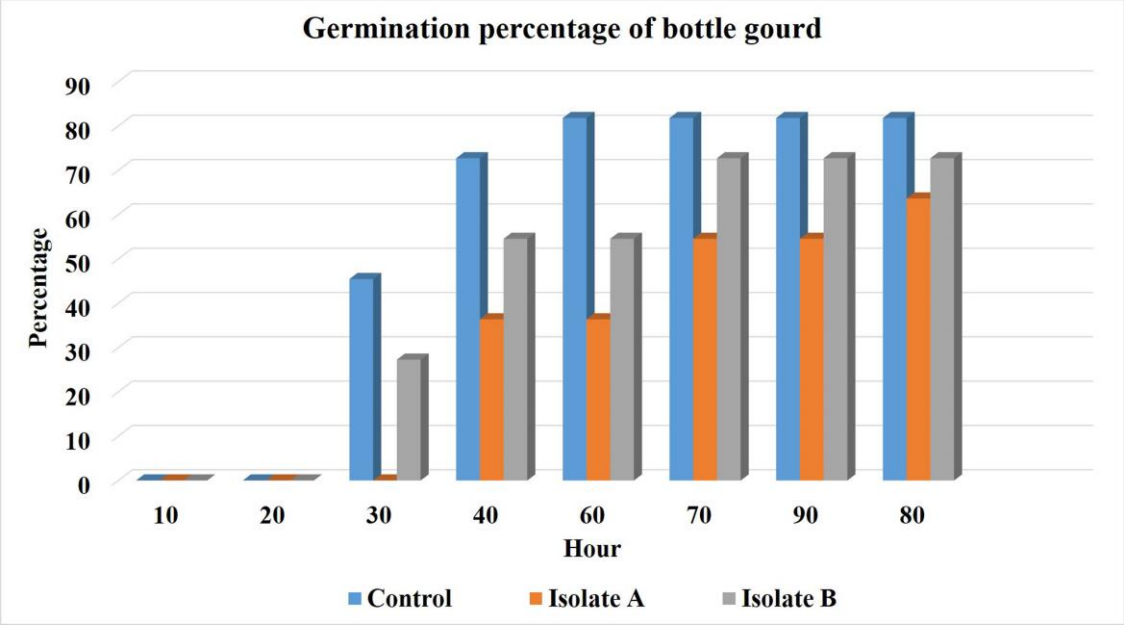

Figure 6: Germination percentage of bottle gourd seeds inoculated with *P. syringae* and *X. cucurbitae*

3.4 Effect of bacteria on germination of cucumber

A germination test was carried out where ten seeds of cucumber were used as control. In the control medium, a total of ten seeds germinated after 60 hours of incubation, and the germination rate was 100%. Seeds inoculated with *P. syringae* showed 50% germination after 50 hours, and *X. cucurbitae* showed 70% germination after 50 hours. After 90 hours, seeds inoculated with *P. syringae* and *X. cucurbitae* showed a maximum of 100% germination was displayed in Figure 7A-C and 8.

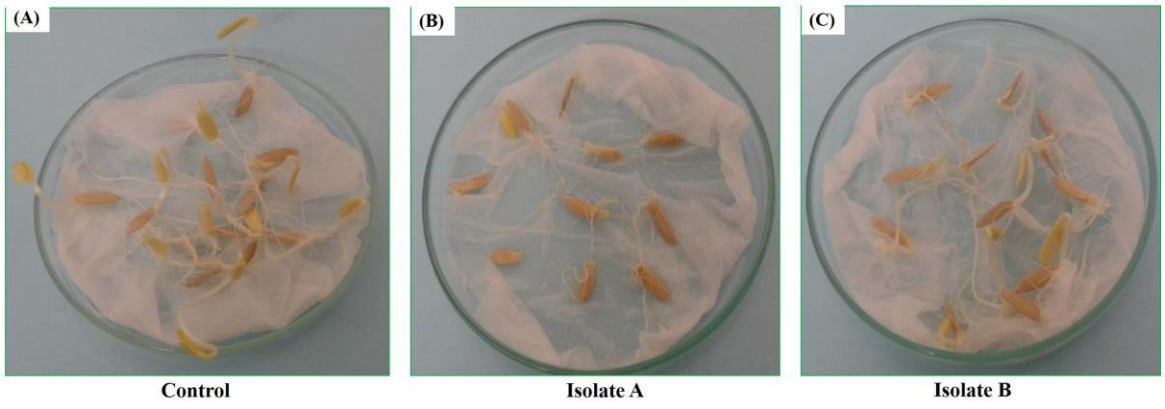

Figure 7: Germination cucumber seeds; (A) Control medium (B) *P. syringae* and (C) *X. cucurbitae* after 90 hours of inoculation

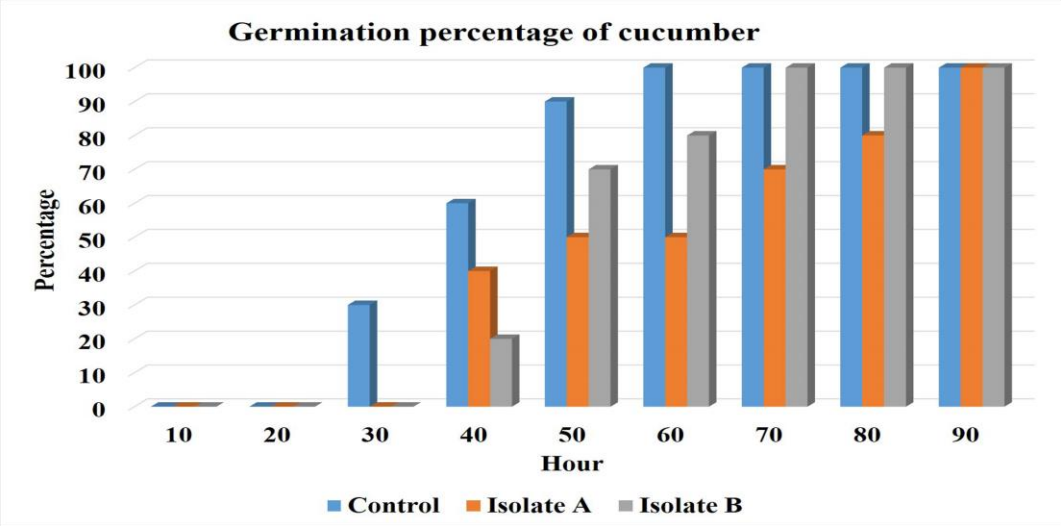

Figure 8: Germination percentage of cucumber seeds inoculated with *P. syringae* and *X. cucurbitae*

3.5 Effect of bacteria on germination of pumpkin

Germination test carried out where 10 seeds of pumpkin were used as control. In control medium, total 7 seeds germinated after 30 hours of incubation and the germination rate was 70%. Seeds inoculated with *P. syringae* showed 50% germination after 30 hours and seeds inoculated with *X. cucurbitae* Showed 60% germination after 30 hours. After 50 hours, seeds inoculated with *P. syringae* and *X. cucurbitae* showed maximum 70% and 80% germination, respectively. Which were shown in Figure 9A-C and 10.

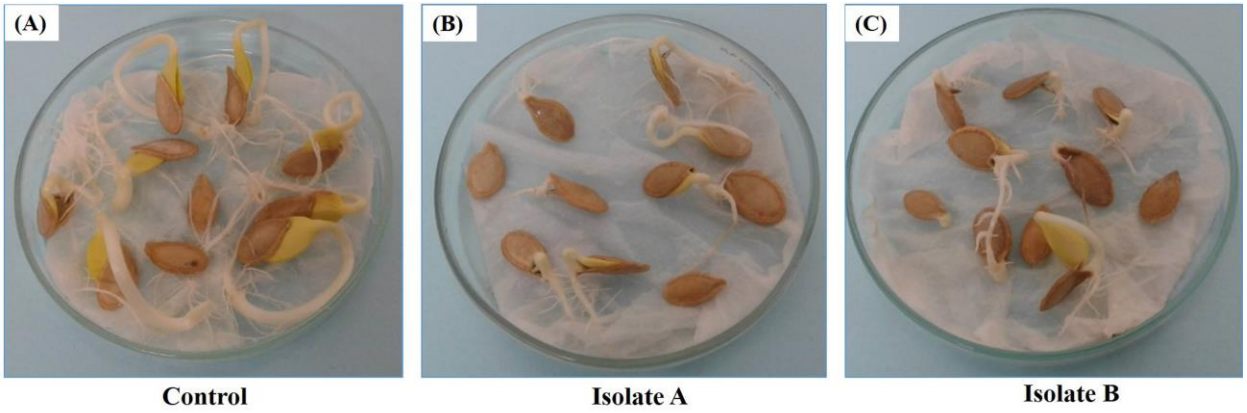

Figure 9: Germination of pumpkin seeds; (A) Control medium, (B) *P. syringae* and (C) *X. cucurbitae* after 50 hours of inoculation.

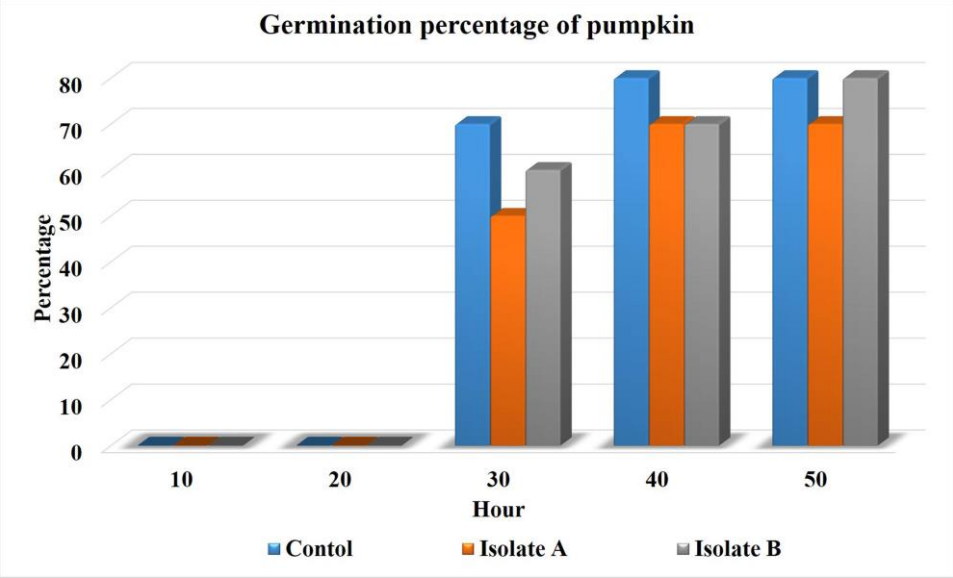

Figure 10: Germination percentage of pumpkin seeds inoculated with *P. syringae* and *X. cucurbitae*

4. Discussion

In this present study, evaluation of the ability of either inhibiting or stimulating the germination of seeds of five cucurbits viz. *Momordica charantia*, *Cucumis sativus*, *Lagenarea siceraria*, *Luffa acutangular* and *Cucurbita pepo* were conducted by inoculating two bacterial strains *Pseudomonas syringae* and *Xanthomonas cucurbitae*. These two bacterial strains affect crops during different stages of development causing various bacterial diseases of cucurbit plants (D. K. Sharma 2018). *Pseudomonas syringae* attacks the leaves, stems, and fruit of cucurbit plants (Liu et al. 2016). In leaves, the bacterium develops tiny, angular patches, which gradually become brownish. The infected leaf tissue often dries out and drops down, leaving holes with an irregular shape and strongly infected leaves with yellow effect. Angular leaf spot, caused by *Pseudomonas syringae* is a widespread bacterial disease of cucurbits worldwide. Lesions can also appear on the petals, stem and fruit that may also be visible with white crusty exudates (A. Sharma et al. 2016). *Xanthomonas cucurbitae* infects leaves and fruit of cucurbit crops. Symptoms on the leaves are small (2-4 mm), chlorotic to beige spots (Ravanlou and Babadoost 2015). On fruits, the spots may be colonized by secondary fungi and bacteria, which grows in thin, slightly sunken circular areas, 1-3 mm in diameter, and in the beige center and dark brown halo (Liu et al. 2016). The present experiment is conducted with these two bacterial strains by inoculating them into five species of cucurbit seeds in the seedling stage of development to check whether these two isolates can result positively by stimulating germination rate or negatively by inhibiting the rate of germination, as there is no clear mention in previous literature about the pathogenicity of these bacteria towards seedlings of cucurbits. There has been similar previous study (Kumar et al. 2017) on evaluating bacterial pathogenicity of *Ralstonia solanacearum* towards 6-7 days old tomato seedlings inoculated by leaf clipping method. *R. solanacearum* is a phytopathogenic bacterium that colonizes the xylem vessels of host plants leading to a lethal wilt disease. It has been observed that *R. solanacearum* triggered disease symptoms in the seedlings at the inoculation site and eventually spread down to the root region (Kumar et al. 2017).

From our experiment, it was observed that, in each case, the germination rate over time had been delayed and inhibited through bacteria inoculation in comparison with control. In the experiment, germination test carried out where 10 seeds of bitter gourd were used as control and after 90 hours of incubation, the germination rate was 80%, while seeds inoculated with *P. syringae* and *X. cucurbitae* showed 20% germination rate after 90 hours, resulting a significant delay in seed vigor over control. Similar delayed result was observed in the germination test of ridge gourd seeds in which, the germination rate was 66.67% after 90 hours of incubation in control treatment, while seeds inoculated with isolate A and isolate B showed maximum 25% and 41.67% germination, respectively. These observations showed that when seeds were inoculated with bacteria, bacterial colonization impairs seed germination resulting significant delay in seed vigor over control. These observations are consistent with the result from earlier study (Harper and Lynch 1979) as the effect of the bacterium *Azotobacter chroococcum* inoculation on barley seeds showed delay in germination. Similar effects observed with wheat as they were attributed to damage by seed borne species of *aspergillus* and *penicillium* (Griffin 1966).

In the present study, all seeds, including control and treatment seeds were treated with the equal volume of distilled water. 50µl liquid bacterial culture was used for all the seeds except control after a regular interval. The intermediate level of 50µl liquid bacterial culture as inoculum appeared as the typically efficient level of growth augmentation (Leifert and Cassells 2001; Thomas et al. 2007). The delay in seed germination following inoculation with isolate A and B may not be attributed in part to the level of inoculum used for seed inoculation in our experiment. The inhibition of seed germination by some microorganisms tends to be correlated with oxygen microbial affinity. In addition to colonization of seed in the ear or during storage, microorganisms can grow during seed germination and when oxygen supply is reduced, they can compete for oxygen and impair germination (Gaber and Roberts 1969). Similar experiment (Harper and Lynch 1981) in the germination of seed inoculated with a saprophytic fungus *Gliocladium roseum* resulted delayed germination as oxygen uptake by inoculated seed did not increase and germination was completely suppressed. In our experiment, the seeds which were found very dry with moisture content below 8%, were necessarily raised the moisture content by humidification to 15–17% before testing for germination and containers were covered ensuring that there is no air lock resulting from excess moisture on the covers to prevent the substrate from drying and allow diffusion of oxygen. The interaction between five cucurbit plant species and the bacterial pathogen *Pseudomonas syringae* and *Xanthomonas cucurbitae* strains were observed as virulent in the seedling stage of development and further studies need to be carried out on molecular genetic analysis to find resistance genes for developing disease resistant varieties.

In the present study, germination test of the seeds of bottle gourd in control medium resulted in 81.81% of germination rate after 60 hours of incubation, while seeds inoculated with isolate A showed 36.37% germination after 40 hours resulting delay in germination rate initially, but increased germination rate was observed after 70 hours at 54.55% germination of the seeds inoculated with Isolate A. Bottle gourd seeds inoculated with Isolate B Showed 54.55% germination after 40 hours, which is higher than the germination rate of seeds inoculated with Isolate A, but lower than the control treatment of seeds. After 120 hours, seeds inoculated with isolate A and isolate B showed maximum 63.64% and 72.73% germination, respectively. Germination test of the seeds of cucumber resulted 100% germination in control medium after 60 hours of incubation whereas seeds inoculated with isolate A and B showed 50% and 70% of germination, respectively after 60 hours. But, after 90 hours seeds inoculated with isolate A and isolate B showed maximum 100% germination which is equal to the germination rate of

control seeds. Similar results were observed for pumpkin seeds as after 30 hours of incubation, germination rate was 70% for control treatment, while seeds inoculated with isolate A showed 50% and Isolate B showed 60%, resulting a slight reduced percentage in seed germination. After 50 hours, seeds inoculated with isolate A showed maximum 70% germination and isolate B showed maximum 80% germination which is equal to the germination rate of the control seeds. Similar result was observed (Thomas et al. 2007) in the inoculation of papaya seeds with *Microbacterium sp., Pantoea sp., Cedecea sp., Brevundimonas sp., Bacillus sp., Sphingomonas sp., Mrthylobacterium sp. and Agrobacterium sp*. inoculates, which led to delayed germination or slow seedling growth initially, during the first 2-3 weeks after sowing but thereafter, some of the inoculates showed gradual improvement in germination except *Brevundimonas* and *Agrobacterium* inoculates. They caused a significant reduction in seedling vigor over control (Thomas et al. 2007).

Dressing seeds with fungicides, such as- Thiram or Benlate, or sterilizing surfaces with sodium hypochlorite, minimizes fungal attacks during germination tests (Hussain et al. 2017). However, it may impact the outcomes of the germination experiments and may pose a health threat to seed analysts (Zaller et al. 2016). In our study, the seeds were soaked for ten minutes in a 1% solution of sodium hypochlorite for sterilization and, 3g Kg^{-1} dose of the fungicide (3 g thiram, 30 % wettable powder Kg^{-1} seeds) was used for seed dressing. Based on previous results (Vejsadova et al. 2002), two sterilization substances: 7.2% Ca(OCl)2, and 0.5% NaOCl, were applied alone or in combination with 70% ethanol. In contrast, Botlagunta and Babu (2024) stated the growth enhancement and changes in bacterial microbiome of cucumber plants exhibited by biopriming with some native bacteria. The findings revealed that sodium hypochlorite significantly inhibited the germination rate, that embryos were impaired, and that germination was significantly lower, as much as 50% lower in *D. incarnata subsp. serotina*. Another study (Sui et al. 2012) found that some sampling swabs were positive for *S. aureus* and other bacteria following initial disinfection with 0.5% sodium hypochlorite on ventilator faceplates and pasteurization of Y-pieces and water traps of breathing circuits, indicating that 0.5% sodium hypochlorite and pasteurization did not kill all *S. aureus* and other bacteria. The delay in seed germination may partly be due to the use of fungicides or sodium hypochlorite in our experiment, but further experiments are needed to validate this.

While the above-mentioned studies on the effect of microbial inoculation on germination of various vegetable species were conducted under different conditions, our study was employed under optimum laboratory conditions and unveiled new data on the delay in seed germination of the five cucurbit seeds when they were inoculated at the seedling stage of development with the isolate A and B. This is an apparent disadvantage of crop production, as our experiment confirms that these two bacterial isolates have the potential to attack cucurbits at all stages of development.

5. Conclusion

The germination of *Momordica charantia, Cucumis sativus, Lagenarea siceraria, Luffa acutangular* and *Cucurbita pepo* have significantly delayed by *Pseudomonas syringae* and *Xanthomonas cucurbitae*. These two bacterial strains can attack cucurbits at all stages of development including seedling stage and this plant-pathogen interaction resulted virulent. More studies using other seed species of cucurbits may appear necessary in order to assess the pathogenicity of these bacterial inoculums under laboratory conditions, as well as control measures for these cucurbit species will be addressed in future studies.

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Author contributions

MEKC, MEH and MFH conceived the investigation plane and prepared the first draft of manuscript. FBM, MB and SK carried out data analysis and assist to improve manuscript. MFH and BS reviewed and finalized the manuscript.

Conflict of interest

The authors declared no competing interests.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

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