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RESEARCH ARTICLE

Microbicidal effects of magnetic field and irradiation on plant pathogenic *Thielaviopsis paradoxa* and *Pectobacterium cartovorum*

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Abstract

Abiotic controls such as Ultraviolet irradiation (UV) and magnetic fields (MF) have a microbicidal effect on the growth survival rate of plant pathogens. Date palm black rot disease is caused by the phytopathogenic fungus *Thielaviopsis paradoxa (Th. Paradoxa*), while soft onion rot is caused by the phytopathogenic bacteria *Pectobacterium cartovorum* (*Pe. cartovorum*). The 100-280 nm peak UV wavelength was employed for (0, 5,10, 15, 20, 25 and 30 minutes). The effect of UV wavelength was used on the above fungi and bacteria on solid nutrient agar (NA) medium and measured the growth number colony forming unit (CFU)/ml) on the Petri dish. The MF Single field strength of 10 gausses was used on a liquid nutrient broth (NB) medium and measured the growth by the optical density (O.D) and CFU for 30 minutes at 28 °C. The results of reduction show best effect for MF at 5 cm was 99%, with a growth rate of 1.1 x 10^2 compared with *Pe. cartovourum*. The O.D results of *Pe.cartovorum* at UV ray was 0.09 and colony forming unit 1.5 × 10^7 CFU/ml). At the end of the exposure time of optical density, the (O.D) and CFU were 1.2 and 1.1x10^2 CFU/ml, respectively. When a direct magnetic field is used to induce the fungus colony, it uses a method that is very different from colony formation in a petri dishes. The abiotic elements in this study have an impact on plant pathogen control and are seen as alternatives with an effect on the environment and economy.

Keywords: Abiotic control; magnetic field; *Pe. Cartovorum*; *Th. Paradoxa*; UV rays.

1. Introduction

Some important economic plants are infected with pathogenic fungal and bacterial diseases, including what has been studied as a fungal disease that affects date palms and a bacterial disease that affects vegetable plants such as onions. The period that plant pathogenic microorganisms remain infectious in a natural setting outside their host is not known. Generally, abiotic factors control like as Ultraviolet radiation (UV) and magnetic fields (MF), is the primary microbicidal agent in the environment (Nicholson et al., 2005). The sun's solar radiation contains a significant amount of UV radiation. Only UV wavelengths longer than 100-280 nm reach the Earth's surface after being filtered by the ozone (O_3) layer (Coohill *et al.*, 2008). Significant effects have demonstrated that extremely low-frequency magnetic fields (ELF 300 Hz) can influence biological systems. Thus, the microbicidal environmental effects of sunlight on bacteria and bacterial spores are very important in biodefense (Dudley *et al.*, 2002; Whitney *et al.*, 2003).

Furthermore, it has been suggested that the inhibition growth of *Pe. cartovorum* and *Th. paradoxa* were dependent on field strength, frequency, and microorganism type. The inhibition and decline of microbial growth rate were previously reported (Piatti *et al.*, 2002;

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Zhang *et al.,* 2003). Bacterial disease control is complicated, and effective control should be dependent on cultural practices and could benefit from various tools that allow for rapid and specific detection of the bacterial pathogens at low levels of infection (Abu-Obeid *et al.*, 2018).
Thielaviopsis paradoxa can exist in a mycelium or spores form, *Pe cartovorum* can exist in vegetative cells, and magnetic fields shift development towards the mycelium form. Suppress spore formation of vegetative cells of *Pe. cartovorum* is an effect paralleled with the diminished activity of alkaline phosphatase. Several studies have investigated the effects of low-frequency electromagnetic fields on DNA, enzyme activity, and cells. These studies have proved that MF influences a variety of cellular functions such as growth, cell membrane characteristics, gene expression, protein biosynthesis, enzyme activity, cell reproduction, and cellular metabolism. The biochemically multipurpose fungus *Th. paradoxa* produces many acids and degradative enzymes supporting its absorptive lifestyle. This metabolic diversity and its capability to use various carbon sources make *Th. paradoxa* a valuable cell factory for applications in many different industrial processes. This research describes the effect of a magnetic field and UV irradiation on the growth of pathogenic fungi *Th. paradoxa* and *Pe. Cartovorum*.

2. Materials and methods

2.1. Microorganism isolation and survival conditions

The microorganisms plant pathogen tested were *Th. paradoxa* and *Pe. Cartovorum* from date palm trees and onion, the isolated fungi were identified by using macro and microscopic techniques. The isolation and culturing of *Th. paradoxa* was conducted on potato dextrose agar (PDA) medium at 28 °C for 3 days. Spore of the isolated fungi were harvested from 7 day old culture by preparing a spore suspension of $2x10^7$ spores/ ml .one ml of the spore suspension was used for inoculating 100 ml of media. all flasks were then incubated at 28°C in shaking incubator at 150 rpm for 72h. Three replications were maintained, and the number of the fungal colony was measured by colony forming unit (CFU/ml).
On the other hand, *Pe. cartovorum* was cultured on nutrient broth (NB) and nutrient agar (NA) media at 28 °C. Bacterial suspension is altered by using a spectrophotometer to measure the optical density (O.D) of the suspension matter at 600nm. The bacterial number is $5x10^8$ CFU/ml before exposures to abiotic factors. In order to count the number of bacterial colonies using the spreading method for bacteria, 1 ml of the bacterial suspension was diluted using the solution in a series of dilutions. For *Pe.cartovorum* physiological and biochemical tests, including levan production from sucrose, presence of oxidase, catalase, and arginine dihydrolase, starch hydrolysis, and acids produced from D-sorbitol, D-glucose, lactose, inulin and D-xylose was carried out according to the methods described by Schaad *et al.* (2001).

2.2. Different irradiation and magnetic field

In a safety cabinet (laminar flow), a UV lamp is used with wavelength of 100-280 nm and the distance from UV tip to microorganism were set to 20 cm, and the current UV was set to 0.35 A to irradiate LED light (Liu *et al.*, 2019). The magnetic field strength of 10 Gauss was measured by Gauss meter, and the experimental cultured groups were placed with the magnetic field beside them (Liu *et al.*, 2022). The bar magnets' effect on the growth rate on liquid medium (test tube) and Petri dish was comparable to the control samples, but was different effect on the other samples (image 1).

Image 1. The bar magnets affect the growth rate of liquid and solid media.

2.3. Microbicidal effect by UV light on different media using colony forming assay (CFA)

Pe. Carotovorum cell suspensions was adjusted to 0.5 McFarland turbidity standards, while *Th.paradoxa* inoculum was spore suspension (10^5 conidia ml-1).the inoculum of each tested microorganism was cultured on NA medium and spread on PDA plates with cotton swabs. After that, 20cm above the agar medium surface, UV light was irradiated. The time series were validated using irradiation times of 0, 10,15,20,25 and 30 minutes. The biological effects were evaluated by performing a colony-forming assay (CFA). The cell suspensions of each tested microorganism were adjusted to 1×10^6 CFU/ml and 10^5 conidia ml^{-1} for *Pe. Carotovorum* and *Th.paradoxa*, respectively. After that, 4 petri dishes with NA or PDA media were inoculated with 10 µl of 10 fold serial dilutions of the cell suspension. The infected plates were then incubated at 28 °C for 24 hours for bacteria and 28° C for 72 hours for *Th.paradoxa*. Then the number of growing colonies was counted, and the survival rate was also expressed as a percentage of the non-irradiated control treatment. The experiment was repeated three times, and the average percentage of surviving microorganisms or growth reduction was also evaluated.

2.4. Statistical analysis

The data obtained were statistically analyzed by variance analysis (ANOVA) using SAS statistical software (SAS ver. 9.2). The means were compared using Duncan's multiple tests (Steel and Torrie, 1980).

3. Results and discussions

3.1. Microbial isolates and culture conditions

The current study was undertaken to determine the influence of magnetic field and UV irradiation on growth. The pathogenic fungus was isolated from date palm and identified as *Th. Paradoxa* by morphological and microscopically observations. It is known that the fungus Thielaviopsis (family Ceratocystidaceae) includes six fungal species, namely *Th. cerberus*, *Th. punctulata*, *Th. ethacetica*, *Th. musarum*, *Th. euricoi*, and *Th. paradoxa* (De Beer et al., 2014). The colony growth of fungal species was morphologically studied on the PDA medium at 28 °C. In this regard, the morphological features of *Th. paradoxa* were woolly, initially white, quickly becoming black colonies with conidial production. Fig. 1 (A and B) shows that the thick-walled aleuroconidia were light to dark brown, oval-shaped, and borne singly on the top

of short hyphae, while the phialoconidia were hyaline to pale brown, cylindrical-shaped, and formed lengthwise in chains (Alhudaib *et al.*, 2022). Moreover, *Th. paradoxa* colonies are usually fast-growing, pale, or brightly colored and may have a cottony aerial mycelium. These soil-borne pathogenic fungi cause severe infections and adversely affect the date quality and production (Abdelmonem *et al.*, 2007; Saeed *et al.*, 2016).

A **B** **C**

Figure 1. Symptoms of black rot disease caused by *Th. paradoxa* on date palm;A and B conidospores and *Th. Paradoxa* culture in PDA medium, respectively. C, Symptom on palm leaves and the whole plant

Also, it is known that the bacteria *Pe. carotovorum* causes soft rot disease in cabbage, potato, radish, and other crops, including onion, during cultivation, postharvest, transport, and storage, resulting in severe economic losses every year worldwide (Toth *et al.*, 2003; Gillis *et al.*, 2014; Opara and Asuquo, 2016; Rutolo *et al.*, 2018). Fig. 2 (A and B) shows the bacterial colonies of *Pe. cartovorum* isolated from onion plants are usually fast growing, in shades of grey, sometimes white, mainly consisting of a dense felt of conidiophores. *Pe. cartovorum* quickly spreads on the agar surface and fills a Petri dish with a typically candy-like cotton colony, initially white, which turns grey to yellow-brown, and the reverse is white to pale.

A **B**

Figure 2. soft rot disease: A, symptoms on onion bulb and B, *Pe. Cartovorum* bacterial growth on NA media

Table (1) shows some biochemical characteristics of the *Pe. cartovorum* isolated from the onion. Results indicate that *Pe. cartovourm* could positively produce acid from lactose, D-glucose, D-xylose, and inulin, whereas it produces no acid from D-sorbitol. Other biochemical characteristics, such as acid and levan production from sucrose, the presence of oxidase, catalase, nitrate reduction, and starch hydrolysis, allow *Pe. cartovroum* to be easily distinguished (Perombelon and van der wolf, 2002). However, the biochemical tests did not entirely differentiate *Pe. carotovorum*. They are also very slow and cannot be used to detect infected plants by the bacteria directly. Furthermore, the biochemical tests risk misidentifying closely related subspecies (Czajkowski *et al.*, 2015).

Table 1. The biochemical characteristics of *Pe. cartovorum* bacteria.

Bacteria	Levan	Oxidase	Catalase	Starch Hydrolysis	Nitrate reduction
	-	-	+	-	+
	Acid production from				
Pe. cartovorum	D-glucose	lactose	Inulin	D-xylose	D-Sorbitol
	+	+	+	+	-

3.2. The effect of irradiation on the growth rate of isolated microorganisms

The results in Fig. 3 demonstrate that the abiotic factor UV rays affect the growth rate of *Th. paradoxa* and *Pe. cartovorum*. The bacterial cells decreased after the initial experimental test from 3.5 x 10^7 CFU/ml to 1.5 x 10^1 CFU/ml for 30 min. There is an obvious decrease in the total account number of bacterial and fungi colonies following the increase in UV exposure. These findings were obtained using a UV filter, which is not present in sunlight (and thus is not environmentally relevant), but in the region (especially 280 nm radiation). The UV damage in bacterial spores is repaired during germination (Moeller, 2007). The growth inhibition of pathogenic bacteria *Pe. cartovourum* was more than the growth inhibition of the fungus *Th. paradoxa*.

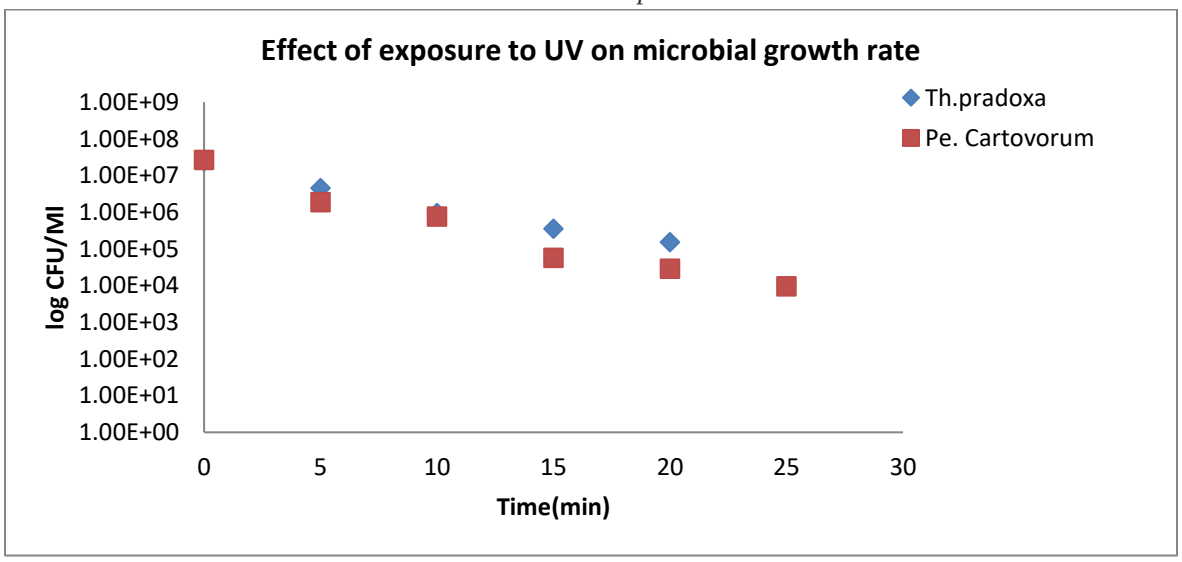

Figure 3. Effect of exposure to UV rays on the growth rate of *Th. paradoxa* and *Pe. cartovorum*.

3.3. The effect of magnetic field on the growth rate of isolated microorganisms

The exposure to magnetic fields slows the growth of isolated bacteria. The outcomes of the microbial development shown in fig (4) .it can be determined from the relationship between the developments of *Th. Paradoxa* on solid medium and how it was influenced by the magnetic field that the growth diameter was reduced on both liquid and solid media. High-intensity magnetic fields may be can influence cell membrane fluidity and other properties (Iwasakaa *et al.*, 2006)

Figure (4) shows magnetic fields' effect on the bacterial growth of *Pe.cartovorum* in both nutrient broth (liquid medium) and nutrient agar (solid medium) at comparable nutrient concentrations. Generally, the magnetic field-exposed samples grow at a slower rate. The magnetic field has a more significant impact on the growth rate, as minimal growth is observed in the bacteria treated with the magnetic field.

The effect of extremely low frequency (< 300 Hz) on the growth rate of Gram-negative bacteria and determining any morphological changes might have been caused by extremely low frequency - EMF (Inhan-Garip *et al.*, 2011). The growth patterns are not significantly affected, as each set's general trend of a bacterial growth curve is followed.

The difference from the control, on the other hand, grows with time, indicating that the inhibiting effect of a magnetic field persists across several generations of bacteria.

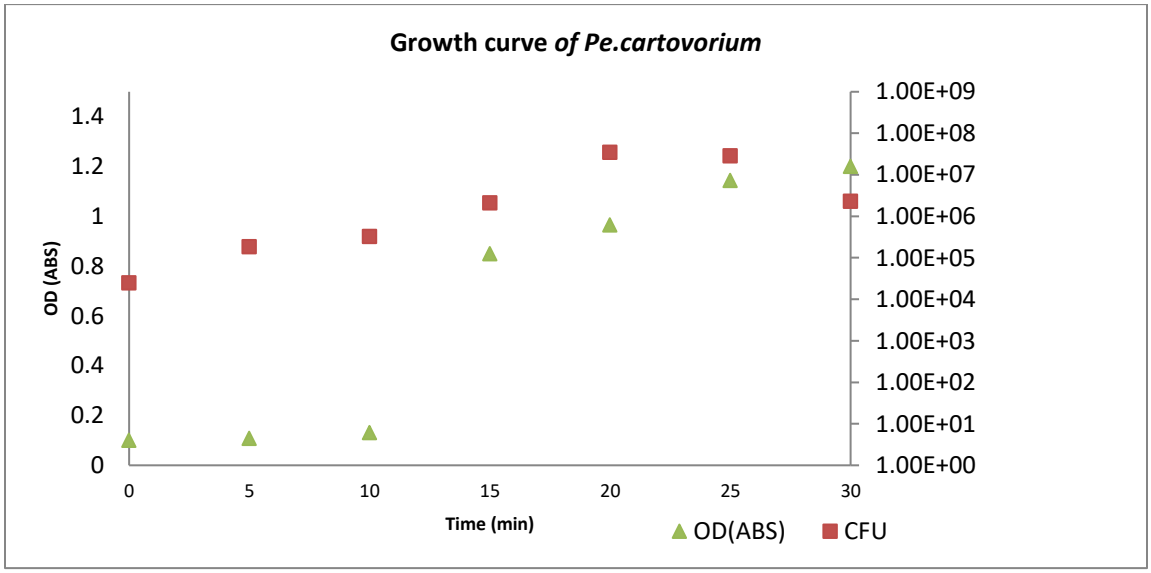

Figure 4. Growth curve of Pe. cartovorum on liquid and solid media.

Table (2) shows that *Th. paradoxa* growth number at magnetic field significant difference; there was stimulation in fungal growth compared to the bacterial growth. The bacterial growth number of the magnetic field of 6 x 10^5 CFU/ml was more than the fungal growth number of 5 x 10^3 CFU/ml. the microbial growth rate at highly low-frequency electromagnetic fields have been the reduction percentage in colony forming unit (Fojt *et al.*, 2004). Low-intensity electromagnetic fields have been shown to reduce the growth and sporulation of phytopathogenic microscopic fungi (Nagy and Fischl, 2004). Likewise, magnetic fields were linked to a decrease in microorganism growth (Piatti *et al.*, 2002; Zhang *et al.*, 2003), but it was concluded that cell viability varies with exposure time and magnetic intensity. The G^+

and G^- bacteria showed growth decrease and morphological changes as biological effects of exposure to the magnetic field compared to gram-positive and gram-negative bacterial strains (Inhan-Garip *et al.*, 2011). It has also been reported that an electromagnetic field can inhibit bacterial growth due to cell membrane damage (Bajpai *et al.*, 2012).

Table 2. Effect of magnetic field on the growth rate of *Th. paradoxa* and *Pe. cartovorum*.

Microorganisms	*Th. paradoxa*		*Pe. cartovorum*	
Exposure time/min	Log CFU/ml	Reduction%	Optical Density (O.D)	Log CFU/ml
0	6×10^5 a	00	0.09	3.5×10^7a
5	1×10^5 b	70	0.10	1.7×10^6 b
10	2.2×10^4 c	89	0.13	2×10^5c
15	2.1×10^4d	92	0.84	1.3×10^3d
20	5×10^3 e	95	0.96	1.2×10^3 d
25	4×10^2 e	99	1.11	9.3×10^2 d
30	1.1×10^2e	99	1.20	1.5×10^1 e

*The mean difference is significant at the 0.05 level.

4. Conclusion

The effects of the applied magnetic field (MF) and Ultraviolet irradiation (UV) seem to be beneficial for abiotic control of plant pathogens and have great potential as an alternative tool for microbial management. Abiotic control (MF and UV) inhibit the growth of plant pathogenic microorganisms *Th. paradoxa* and *Pe. cartovorum*. Magnetic field and Ultraviolet positively affected the nucleic acid and enzyme activity compared with plant pathogenic bacteria and fungi in the same conditions at a significant difference.

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Data presented in this study are available at fair request from the respective author.
Ethics Approval and Consent to Participate
This work carried out at the Botany department (microbiology and phytopathology) and followed all the department instructions.
Consent for Publication
Not applicable.
Conflicts of Interest
The authors declare no conflict of interest.

5. References

Alhudaib, K.A., El-Ganainy, S.M., Almaghasla, M.I., Sattar, M.N. (2022). 'Characterization and Control of *Thielaviopsis punctulata* on Date Palm in Saudi Arabia', *Plants (Basel)*, 18;11(3), pp. 250.

Abdelmonem, A., Rasmy, M. (2007). 'Major diseases of date palm and their control', *Commun. Inst. For. Bohem.*, 23, pp. 9–23.

Abu-Obeid, I., Khlaif, H., Salem, N. (2018). 'Detection and Identification of Potato Soft Rot *Pectobacterium carotovorum* Subspecies *carotovorum* by PCR Analysis of 16S rDNA in Jordan', *Agricultural Sciences*, 9, pp. 546-556.

Bajpai, I., Saha, N., Basu, B. (2012). 'Moderate intensity static magnetic field has bactericidal effect on E. coli and *S. epidermidis* on sintered hydroxyapatite', *J. Biomed. Mater. Res. B. Appl. Biomater*, 100B, pp. 1206– 1217.

Coohill, T.P., Sagripanti, J.L. (2008). 'Bacterial inactivation by germicidal ultraviolet radiation', *Photochem. Photobiol.* 84, pp. 1084– 1090.

Czajkowski, R., Jafra, S., Lojkowska, E., Potrykus, M., Van, D.W.J., Sledz, W. (2015). 'Detection, identification and differentiation of *Pectobacterium* and *Dickeya* species causing potato blackleg and tuber soft rot: A review', *Ann. Appl. Biol.*, 166, pp. 18-38.

Dudley, J.P., Woodford, M.H. (2002). 'Bioweapons, biodiversity, and ecocide: Potential effects of biological weapons on biological diversity', *Bioscience*, 52, pp. 583– 592.

De Beer, Z.W., Duong, T.A., Barnes, I., Wingfield, B.D., Wingfield, M.J. (2014). 'Redefining Ceratocystis and allied genera', *Stud. Mycol.*, 79, pp. 187–219.

Fojt, L, Strašák, L, Vetterl, V.R., Šmarda, J. (2004). 'Comparison of the low-frequency magnetic field effects on bacteria *Escherichia coli*, *Leclercia adecarboxylata* and *Staphylococcus aureus*', *Bioelectrochemistry*, 63(1), pp. 337–41.

Gillis, A., Rodriguez, M., Santana, A.M. (2014). '*Serratia marcescens* associated with bell papper (*capsicum annuum* L.) soft –rot disease under greenhouse conditions', *European Journal of Plant Pathology*, 138(1), pp. 1-8.

Inhan-Garip, A., Aksu, B., Akan, Z., Akakin, D., Ozaydin, A.N, San, T. (2011). 'Effect of extremely low frequency electromagnetic fields on growth rate and morphology of bacteria', *Int. J. Radiat. Biol.*, 87(12), 11, pp. 55–61.

Iwasakaa, M., Yaoita, M., Iwasawa, T., Ueno, S. (2006). 'combined effects of magnetic fields and temperature changes on 1-aminoaphthalene -8- sulfonic acid fluorensece in red blood cell ghost cell membrane', *journal of applied physics*, 99, 08S104.

Liu, C.Y., Tseng, C.H, Wang, H.C., Dai, C.F., Shih, Y.H. (2019). 'The Study of an Ultraviolet Radiation Technique for Removal of the Indoor Air Volatile Organic Compounds and Bioaerosol', *Int. J. Environ. Res. Public Health*, 17;16(14), pp. 25-57.

Liu, X., Pollner, B., Paulitsch-fuchs, H. A., Fuchs, C.E., Dyer, P.N., Loiskandl, W., Lass-Florl, C. (2022). 'Investigation of the effect of sustainable magnetic treatment on the microbiological communities in drinking water', *Environmental research*, 213, 113638.

Moeller, R., Douki, T., Cadet, J., Stackebrandt, E., Nicholson, L. W., Rettberg, P., Reitz, G., Horneck, G. (2007). 'UV-radiation-induced formation of DNA bipyrimidine photoproducts in *Bacillus subtilis* endospores and their repair during germination', *Int. Microbiol.*, 10, pp. 39– 46.

Nagy, P., Fischl, G. (2004). 'Effect of static magnetic field on growth and sporulation of some plant pathogenic fungi', *Bioelectro magnetics*, 25, pp. 316–318.

Nicholson, W.L., Schuerger, C. A., Setlow, P. (2005). 'the solar UV environment and bacterial spore UV resistance: considerations for Earth-to-Mars transport by natural processes and human spaceflight', *Mut. Res.*, 517, pp. 249– 264.

Opara, E.U., Asuquo, A.A. (2016). 'An overview of characterization and identification of soft rot bacterium Erwinia in some vegetable crops', *Greener J. Biol. Sci.*, 6(3), pp. 46–55.

Pérombelon, M.C.M., VanDerWolf, J.M. (2002). *'Methods for the detection and*

quantification of Erwinia carotovora subsp. atroseptica (Pectobacterium carotovorum subsp. atrosepticum) on potatoes: a laboratory manual', Scottish Crop Research Institute Annual Report 10. on potatoes: a laboratory manual. Scottish Crop Research Institute Annual Report 10.

Piatti, E., Albertini, M.C., Baffone, W., Fraternale, D., Citterio, B., Piacentini, M.P. (2002). 'Antibacterial effect of a magnetic field on Serratia marcescens and related virulence to Hordeum vulgare and Rubus fruticosus callus cells', *Comp Biochem Physiol B Biochem Mol. Biol.,* 132(2), pp. 359–365.

Rutolo, F.M., Clarkson, P.J., Covington, A.J. (2018). 'the use of an electronic nose to detect early signs of soft-rot infection in potatoes', *Biosystems Engineering*, 167, pp. 137-143.

Saeed, E.E., Sham, A., El-Tarabily, K., Abu Elsamen, F., Iratni, R., AbuQamar, S.F. (2016). 'Chemical control of black scorch disease on date palm caused by the fungal pathogen *Thielaviopsis punctulata* in United Arab Emirates', *Plant Dis.,* 100, pp. 2370–2376.

Steel, R.G.D., Torrie, J.H. (1980). *'Principles and procedures of statistics',* 2nd ed McGraw-Hill, New York.

Schaad, N.W., Jones, J.B., Chun, W. (2001). *'Laboratory Guide for Identification of Plant Pathogenic Bacteria',* 3rd Ed. APS Press, USA.

Toth, I.K., Bell, K.S., Holeva, M.C., Birch, P.R. **(**2003**). '**Soft rot Erwinia: from genes to genomes', *Mol. Plant Pathol.*, 4, pp. 17–30.

Whitney, E.A.S., Beatty, E.M., Taylor, H.T., Weyant, R.J., Sobel, J., Arduino, J. M., Ashford, D.A. (2003). 'Inactivation of *Bacillus anthracis* spores', *Emerg. Infect. Dis.* 9, pp. 623– 627.

Zhang, Q.M., Tokiwa, M., Doi, T., Nakahara, T., Chang, P.W., Nakamura, N., (2003). 'Strong static magnetic field and the induction of mutations through elevated production of reactive oxygen species in *Escherichia coli* soxR.', *Int. J. Radiat Biol.,* 79(4), pp. 281–286.