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ISOLATION AND MOLECULAR CHARACTERIZATION OF *Xanthomonas campestris* FROM TOMATOES PLANT (SEED) IN WESTERN DELTA REGION OF DELTA STATE

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ABSTRACT

Tomatoes plant and seeds have been affected by bacterial contamination and early infection, during its developmtal stage resulting plant disease cases in affected farm-lands of Delta State. Our study investigates isolation and molecular characterisation of Xanthomonas campestris from tomatoes plant in Oghara. Four locations in the study area were surveyed while freshly harvested tomato and suspected bacterial leaf spot infected tomato plant samples were collected and analyzed. Standard microbiological techniques for the isolation of bacteria were employed. Morphological and biochemical characteristics, viz., Gram's reaction, colonial characteristics, pigment production, KOH test, catalase test, starch hydrolysis, acid production, and gelatin liquefication tests, were carried out. Further confirmation tests employing molecular characterization using the bacterial universal primer fdl rp2 which targets the 16S rRNA region of Xanthomonas campestris pv vesicatoria as a specific primer pair, and Xanthomonas race specific primers were used. Our results revealed 75 presumptive strains that showed positive results to the various phenotypic tests performed. It was observed that 13 bacterial strains were confirmed from all four locations as X. campestries, with occurrences as follows: leaves 2 (20%), bacterial leaf spot infected tomato seed or bulb 4 (25%), freshly harvested tomato seed 1 (25%), root and surrounding soil 4 (13.3%), and stem 2 (13.3%). A high level of bacterial contamination was observed on the bacterial leaf spot affected tomato samples compared to the freshly and aseptically collected and analyzed sample. The exposure of harvested tomato bulbs to the environment while negating appropriate interpersonal hygiene may have spawned contamination, infection of tomato bulbs, and the distribution of the potential pathogen in the study area. The need for appropriate implementation of inter-personal hygienic practice among farmers while handling the tomato bulb harvest cannot be overemphasized.

KEYWORDS: *Xanthomonas campestris, Xanthomonas campestris pv vesicatoria, Tomatoes, Primer fdl rp2, 16SrRNA gene sequencing*

INRODUCTION

Fruits and vegetables are vital sources of nutrients, vitamin and energy for humans, and are required for proper growth and development. Tomatoes are botanically defined as fruits because they are formed from a flower and contain seeds. Still, they're most often utilized like a vegetable in cooking. In fact, the US Supreme Court ruled in 1893 that the tomato should be classified as a vegetable on the basis of its culinary applications.

Fruits and vegetables are edible part of a mature ovary of a flowering plant which are usually eaten raw either fleshy or dry. Such fleshy fruits are classified as berry (orange, tomato, pineapple, pawpaw and banana); drupes (plume, coconut, almond, cherry) and pomes such as apple and pear. The dry fruits, unlike the fleshy fruits which have unlayered pericarp, are classified into dehiscent (pod, follicle and capsule) and indehiscent fruits like achene, samara, cashew etc. (Jay, 2000).

Tomatoes are (for example) a major vegetable crop that has grown in popularity over the last century. It is grown in almost every country around the world in greenhouses, net houses, and open fields (Garrido and Luque-Romero, 2014). The plant is adaptable, and the crop is divided into two types: fresh market tomatoes and processing tomatoes. Tomato fruits are not only delicious, but they are also high in vitamins A and C. Vitamin A is necessary for bone growth, cell division and differentiation, immune system regulation, and the maintenance of the surface linings of the eyes, respiratory, urinary, and intestinal tracts. Vitamin C aids in the formation of collagen, a protein that provides structure to bones, cartilage, muscle, and blood vessels. It also aids in the absorption of iron and helps to maintain capillaries, bones, and teeth (Maria *et al*., 2014). Lycopene, a powerful antioxidant that can help prevent the development of many types of cancer, is another important nutritional benefit gotten from tomatoes. Cooked tomatoes and tomato products are the best sources of lycopene because it is released from the tomato when it is cooked. Raw tomatoes contain about 20% of the lycopene found in cooked tomatoes. However, raw or cooked tomatoes are thought to be the best source of this antioxidant (Dewanto *et al*., 2002; US Department of Agriculture, release 28)

However, a number of challenges have threatened their survival, including changes in climatic condition, pests and microbial contamination (Igere *et al*., 2020; 2021). The use of agrochemicals, particularly in pest and disease management, is the most difficult challenge in tomato farming. Pesticide overuse has resulted in a high accumulation of chemical residues, posing a threat to the production of safe tomatoes in a world where people are concerned about diet, the environment, and worker welfare (Karungi *et al*., 2011). In addition, tomatoes seed surface contamination by microbes has also been one of the concerns as it had been linked with the ubiquitous nature of microorganisms.

Over the years, there has been an increase in the need to isolate and identify microorganisms associated with food (tomatoes) contamination and spoilage with a view to controlling as well as increasing the market value of such food types (Akinyele and

Akinkunmi, 2012). One potential pathogen which has been associated with the contamination of tomatoes is the *Xanthomonas* species.

Xanthomonas species are potential plant associated pathogen, which has outlived its relevance in recent times, causing diseases especially among tomato a fleshy fruit or berry. It is a Gram-negative, aerobic, short rod-shaped bacterium belonging to the family *Pseudomonadaceae*. This genus includes several pathovars, which are mainly plant pathogens that produce extracellular proteases and Type II secretion systems (2TSS) chiefly, for bacteria colonization of host plant. It produces a characteristic yellow pigment, xanthomonadin and/or xanthan gum which often is used chemotaxonomically and may be applied in commercialised diagnostics. Most strains are non-pathogenic with those that infects and affects tomatoes being *Xanthomonas campestris. Campestris*. Other members include *X. campestris pv. Vesicatoria* which is the causal pathogen for bacteria spots disease of tomato (*Lycopersicon esculentum mill*) in Nigeria especially in the humid South Eastern Nigeria (Bashan *et al.*, 1982). It is also asssociated with tomato leaf blight which is considered the most important and most destructive disease in tomatoes as it infect all cultivated varieties of tomatoes and pepper (Alvarez, 2000).

The classification and implication of *Xanthomonas* spp in bacterial leaf spot (BLS) was previously unknown, until few years ago when bacterial leaf spot causing Xanthomonads was divided into four groups (A, B, C, and D) based on its pathogenicity and physiological characteristics(Alvarez, 2000). In 2004, a new classification system was proposed which change the name of *X. campestris pv vesicatoria* to *X. euvesicatoria* (previously group A), and recognized the species *X. vesicatoria* (group B), *X. perforans* (group C), and *X. gardneri* (group D). (Sigillo *et al.,* 2012). The principal hosts of BLS causing *Xanthomonads* are tomatoes and peppers, though other incidental hosts have been recorded, mainly among weeds. The Group A members contains most of the pepper infecting strains, though some strains from groups B and D have also been reported to cause infectious symptoms among pepper (Ibrahim *et al.,* 2012). Strains from all four groups have been isolated from infected tomato plants only and both pepper and tomato plants, while some may only infect one of these plants (Mirik *et al.,* 2008). When symptoms on the fruit do occur, they start as pale-green, water-soaked areas and eventually become raised, brown, and rough (Kong *et al*., 2019). The bacterial colonization of the Leaves and stems of intracellular spaces induces the macroscopically visible symptoms including water-soaked lesions on the leaves that later become necrotic. (Frank *et al*, 2005; Daniella *et al*, 2003). Though these spots start out at about 24 inches in diameter, they increase in size and number, eventually causing the leaves to drop off (Frank et al., 2005). Tomato plants may drop 50–100% of their foliage due to infection from Xantomonads. Bacterial Leaf Spot (BLS) may also affect the stems of plants, leading to elongated, raised, light-brown cankers which are less than 25 inches long. (Daniella *et al.*, 2003). It is important to note that defoliation may

occur among pepper than in tomatoes plants, hence tomato plants with BLS often have a scorched appearance due to their diseased leaves. *X. campestris pv. vesicatoria* may also survive on tomato and pepper plants, seeds, and debris from infected plants as it cannot live in the soil for more than a few weeks (Daniella *et al.*, 2003). In cold climates, *X. campestris pv. vesicatoria* infection mostly arise from contaminated seed materials. When it survives on seeds, infection spread towards the cotyledons of the growing plant as it emerges from the seed coat while in some cases the internal structure of seeds are infected producing diseased plants from the point of germination. Systemic symptoms such as wilting, yellowing, and dwarfing are not typical of germination infected plants (Daniella *et al.*, 2003). During warm temperatures and high humidity, *X. campestris* pv. *vesicatoria* infection results greenhouses and nurseries concerns as the favourable conditions encourages bacteria growth while wet soils and overcrowding aids easy transmission of diseases among plants (Rosenthal *et al.,* 2018; Isakeit *et al.,* 2012)

Outbreaks or spoilage of tomatoes on its leaves and stem occur almost every year, which occasionally results loss of relevant resources of the economy and the populace in an environment. Such sustained losses later reach serious proportion were it affects many farmers, marketers, and packers who take no action to correct faulty situation that may contribute to spoilage. Marketing of spoilt tomato and tomato products is characterized by peculiar off-taste and odour known as "flat-sour" which can have serious effects on human health. (Singh and Sharma, 2007).

Going by considerations of health consequences associated with spoilt tomato, the isolation and identification of *X. campestris* becomes a necessity. By studying and characterising the potential pathogen present in spoilt tomato samples, preventative and/or control measures may be developed. Also, policy development for farmers and marketers implementation of proper hygiene during the cultivation and marketing may also be re-emphasized. The study involves the collection of infected and spoilt tomato samples from Oghara nexus for the isolation and molecular characterisation of *X. campestri* present in tomatoes.

MATERIALS AND METHOD

Study Area

This study was conducted within the Western Delta region (Oghara nexus) of the State. Samples were obtained from four community markets (Ajagbodudu, Ogharefe, Oghareki and Otefe) locatedin Western Delta, Delta State, Nigeria.

Sample Collection

A total of forty ripe tomato samples was collected from four major markets (10 from each market) within the study area (Oghara). In addition to the collected samples, soil samples of tomato farmland, tomato leaves, and stem were also collected to presumptively source-track the source of contamination of tomatoes. Each samples were collected into a sterile polythene bag labelled according to the respective sampling point in order to avoid mix up, before transport to the Laboratory for immediate analysis.

Sample Analysis

Samples homogenate were pre-prepared asceptically by brief pressure-

washing with sterile water, serial dilution to 10^3 and secured/preserved prior to inoculation on pre-prepared agar plate. These agar plates include Nutrient starch cycloheximide agar (NSCHA) and trioxocarbonated Yeast extract dextrose agar (TYDA) which were constituted as previously described by various investigators (Randhawa and Schaad, 1984; Wydra *et al.,* 2004; Kirimura *et al.,* 2022; Tebaldi *et al.,* 2022; Van der Wolf *et al*., 2022)) for the isolation of *Xanthomonas* species from the samples. Presumptive colonies of *X. campestris* were observed as raised milkish and round colonies with about 0.2–1.8 mm in diameter after three days incubation. Colonies were purified on nutrient agar, stored and nucleic acids were extracted from strains.

Other Presumptive Morphological and Biochemical Test

Pure isolates were subjected to a battery of *in vitro* culture-based presumptive test. The cultural morphology of isolates and biochemical characteristics applied included Gram's reaction, carotenoids plant pigment production on agar plates (yellow pigment; xanthomonadin), Potassium Hydroxide test, catalase test using 3% hydrogen peroxide, starch hydrolysis, acid production and gelatin liquefication test were carried out following the methods applied by various related investgators (Randhawa and Schaad, 1984Wydra *et al*., 2004; Van der Wolf *et al*., 2022)

Genomic DNA Extraction

Genomic DNA of the presumptive isolates was extracted using the crude DNA extraction method previously described by Igere and his colleagues (Igere *et al*., 2020). Briefly the 24 hr pure culture were harvested onto microfuge tube containing 500 ml of sterile DH_2O and votex or mix. The suspension was heated to boiling for 15 min and centrifuged. The supernatant was collected and used as DNA extract/template.

Agarose Electrophoresis

Agarose electrophoresis was conducted using a Sigma-based tris acetate-EDTA (TBE) of 50× (Sigma Aldrich, Dorset, UK), which was re-constituted to a 1X TBE running buffer. Gel was prepared by dissolving 1.5 g of agarose powder (Sigma Aldrich) in 100 mL of running buffer and heated to boiling. The prepared gelwas casted on a minigel tray (Anachem, Dorset, UK), allowed to polymerise, placed carefully in an electrophoresis tank filled with 1 × TBE Buffer and electrophoresed (electrophoresis machine CLS-AG100, Warwickshire, UK) at 100 V for 50 min. The gel was visualized on a Gel doc imaging system (Bio Rad Hercules, Califonia, USA).

Polymerase Chain Reaction PCR Analysis

PCR sequencing preparation cocktail consisted of 10 µl of 5x GoTaq colourless reaction, 3 µl of 25 mM MgCl2, 1 µl of 10 mM of dNTPs mix, 1 µl of 10 pmol each of the 16S rRNA gene according to Van der Wolf and his colleagues (2022) with forward primer (16SF GTGCCAGCAGCCGCGCTAA) and reverse primer (16SR: AGACCCGGGAACGTATTCAC) and 0.3 units of Taq DNA polymerase (Promega, USA) was used. The primers allowed amplification of the 16s RNA genes of the isolates. PCR was carried out in a GeneAmp 9700 PCR System Thermalcycler (Applied Biosystem Inc,

USA) with a PCR profile consisting of an initial denaturation at 94°C for 5 min; followed by a 30 cycles consisting of 94°C for 30secs, 30secs annealing of primer at 56°C and 72°C for 1 min 30 secs; and a final termination at 72°C for 10 mins and hold at 4°C.

RESULTS

The result of our study was presented in figures and Tables, as shown below. Table 1 shows the description of the sampling points, the time of sample collection during study and date of collection and Figure 1 shows the agarose electrophoresis photo of isolates, Table 1 shows the biochemical test results of isolates, it reveals the enzyme-based characterization techniques of the various isolates. This includes their major SAMPLE sources and the confirmed isolates using PCR.

Forty (40) samples of tomatoes plant in Oghara environs were collected randomly and analyzed microbiologically and the isolates were identified as *X. campestries* and yeast.

The most frequently isolated organism was *Xanthomonas campestries*. The observed result is an indication that the hygienic condition of the tomatoes plant has fallen below acceptable standard for human consumption.

Table 1: Shows the observed strains density in various sources of study sample

Plant and parts sources	**Total heterotrophic count**	**Total Presumptive isolates**	**Total confirmed isolates**
Suspected Bacterial leaf spot infected Tomatoes			
Bulb	5.30 to 7.3 log 10	16 (21.3%)	4 (25%)
Stem	5.23 to 7.8 log 10	15 (21.4%)	2 (13.3%)
Freshly harvested Tomatoes Bulb	2.30 to 3.3 log 10	4 (5.3%)	1 (25%)
Leaves	3.10 – 7.5log10	10 (14.29%)	2 (20%)
Soil	1.1 to 2.1 log 10	30 (42.86%)	4 (13.3%)
Total		75	13 (17.33%)

The above describes the isolates recovered from various sources

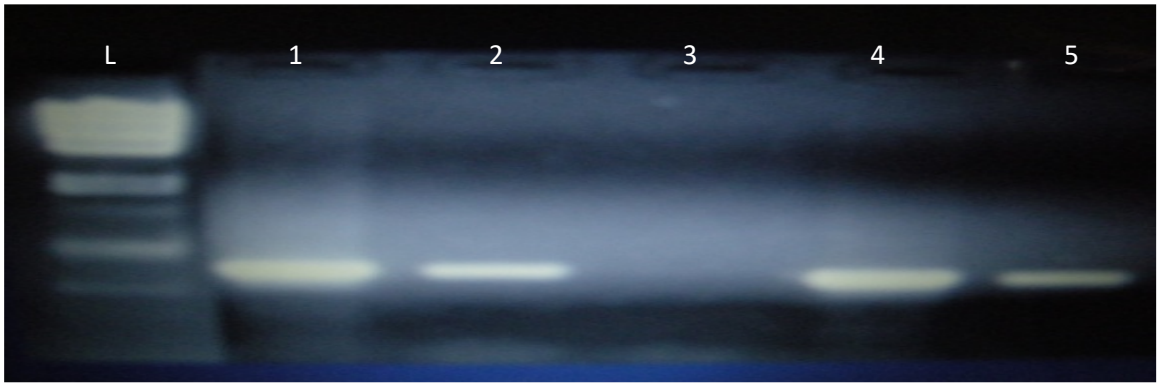

Fig. 1: Shows the gel photo of detected strains. It reveals the 16S rRNA gene (320 bp) in some isolates, L represents a molecular marker of 1.2kb, while numbers 1, 2, 4 & 5 are positive isolates while 3 is negative

Limitation of Study

The study is limited to the isolation and detection of *X. campestris* from tomatoes bulb using molecular biology techniques (Polymerase chain reaction). Other applied techniques were presumptive identification of the suspected isolates prior to the gen-based confirmation. We have not described the antimicrobial susceptibility testing in this perspective since we are currently studying potential antimicrobial agents which may be used for the elimination of the contaminating bacterial without affecting the market value of the tomatoes bulb.

DISCUSSON

Reports on the isolation of *Xantomonas campestris* from various sources of tomato plant parts and farmland environmental soil as well as BLS infected tomatoes bulb was conducted applying the standardised methods as previously described by various investigators. This was done to access and source-track the presence of the potential pathogen in tomatoes bulb and point of contamination and cause of tomatoes spoilage. We compared the presence of the potential pathogen in both spoilt and freshly harvested tomatoes while employing appropriate hygienic condition. The heteroptophic count revealed that high density of organism was present in spoilt tomatoes compared to the freshly harvested tomatoes employing appropriate hygienic practice. Such high density is an indication that there is possibility of early spoilage and deterioration of such tomato samples, if such tomato is taken to the market and it may result possible spoilage. Table 1 shows the number of isolates recovered from various sources indicating that such bacteria strains thrive and inhabit both the spoilt BLS infected tomato, the root/environmental soil, leaves and the apparently healthy freshly harvested tomato. Following the study of various authors, it is therefore evident that *X. campestris* is the major potential pathogen of tomatoes as reported by various investigators (Randhawa and Schaad, 1984; Wydra *et al*., 2004; Kirimura *et al*., 2022). In an earlier study by Bashan and his colleagues, it was reported that the strain that infects and affects tomatoes was *X. campestris pv. campestris* (Bashan *et al*.,

1982). *X.campestris pv. Vesicatoria* is the causal organism for bacteria spots of tomato (*Lycopersicon esculentum mill*), since it is the most important bacterial disease of tomatoes in Nigeria especially in the humid southeastern Nigeria (Bashan *et al.*, 1982). Leaf blight caused by the bacterium *X. campestris pv. Vesicatoria* is considered the most important and most destructive disease of tomatoes, infecting all cultivated varieties of tomatoes and pepper. (Alvarez, 2000).

It is important to also note that tomatoes are susceptible to microbial contamination and infection basically because it contains all the necessary nutrients required for microbial development, growth and metabolism (Kirimura *et al*., 2022). This is the encouraging factor for the proliferation of potential bacterial pathogen and *X campestris* survival/contamination of tomatoes. The need to ascertain quality of tomatoes plant and tomatoes products must remain sacrosanct to ensure safety from infection, retain its market value, consumption tendency of its products and promote economic advancement. Our laboratory analysis conducted on tomatoes plant within four (4) localities in Oghara nexus revealed some strains of bacteria species which were different from *X. campestries*. Seventy-five presumptive strains were recovered which indicate potential unhygienic handling condition or affront in the interpersonal hygienic practice while handling tomatoes in Oghara nexus. It is important to note that the observation of such organisms does not in all cases represent infection and/or spoilage of tomato since the strains were present in both presumed spoilt/infected tomato and apparently healthy tomato bulb. Other strains of organisms found during the study were yeast, however, our interest was mainly to confirm the presence of the milky colonies (*X. campestries*) present in the sampled tomato. In addition, the presence of yeast is an indication of cross contamination during the transport or carriage from farmland to road and marketplaces. Other potential source of microbial contamination arising from yeast presence may include spore formation/germination, pollination amongst reproducing plants, unhygienic disposal of waste etc., carriage of tomato in basket by traders, poor handlers education, washing of tomatoes with contaminated water etc. (Dada *et.al,* 1993). From the study, the critical points of contamination of tomatoes may include soil, and mono cropping which may be controlled by aseptic techniques.

The molecular detection further confirmed the presence of *X. campestris* in tomato plant parts as shown in Table 1. Fig 1 shows the gel photo of detected strains which was observed at a base pair size of 320. It was also observed that amongst 154 samples collected, 75 were presumptive isolates of *X. campestris* strains, while 13 (17.3%) were confirmed by PCR. Similar reports of related investigators have revealed the 16S rRNA gene (320 bp) in isolates confirming the presence of the strains in various part of the tomatoes as observed in this study (Ibrahim and Al-Saleh, 2012).

CONCLUSION

Although the ubiquitous nature of bacteria and other microorganisms remains a natural phenomenon necessary

for the survival of bacterial, it has become a hotspot for the contamination of various food sources when appropriate hygienic practice is negated. It is therefore evident that *X. campestris* is the major contaminant of tomato commercially available in the local market. The need for appropriate interpersonal hygiene is suggestive.

Research on the microbial quality of tomatoes is very important and adequate steps must be taken to prevent potential contamination and spoilage by microorganisms. The organisms isolated from the tomatoes plant indicates poor interpersonal hygiene and reveals the necessity for adroit management of farmland, preservation and control during tomatoes production.

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REFERENCES

Alvarez, M. E. (2000). Salicyclic acid in machinery of hypersensitive cell death and disease resistance. Programmed cell death in higher plants, 185-198.

Akinyele, B. J. and Akinkunmi, C. O. (2012). Fungi associated with the spoilage of berry and their reaction to electromagnetic field. *Journal of Yeast and Fungal Research*, 9(4): 49–57.

Dada, S., S., Tubosun, I. A., Lancelot, J.R. and Lar, A. U. (1993). Late archean for the reactivated basement of Northeastern Nigeria. *Journal of Africa Earth Science (and the Middle East),* 16(4)

Bashan, Y., Diab, S. and Okon, Y. (1982). Survival of *Xanthomonas campestris pv. vesictoria* in pepper seeds and roots in symptomsless and dry leaves in non-host plants and in soil. *Plant and Soil Journal,* 68(2), 161-171.

Daniella, B., Noel, L., Thieme, F. and Bonas, U. (2003). Genomic approaches in *Xanthomonas campetsris pv. vesicatoria* allow fishing for virulence genes. *Journal of Biotechnology*. 106: 203-214.

Dewanto, V., Wu, X., Adom, K. and Liu, H. (2002). Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. Journal of Agricultural and Food Chemistry 50, 3010-3014.

Frank, D. J., Hoppman, R. and Miller, K. G. (2005). Control of Brittle development by the acting regukators capping protein and the Arp 2/3 complex. *Annual Drosophilia Research Conference,* 46: 2033.

Garrido, J. and Luque-Romero, J. (2014). Integrated pest management in Mediterranean greenhouses. European Crop Protection.

Ibrahim, Y. and Al-Saleh, M. (2012). First report of bacterial spot caused by *Xanthomonas campestris* pv.

vesicatoria on sweet pepper (*Capsicum annuum* L.) in Saudi Arabia. *Plant Disease*, 96(11): 1690-1690.

Igere, B. E., Okoh, A. I. and Nwodo, U. U. (2020). Antibiotics susceptibility testing reports; A basis for environmental/Epidemiology surveillance and infection control amongst Environmental Vibrio cholerae. *International journal of Environmental resources of public Health,* 17: 5685.

Igere, B. E., Igolukumo, B. B., Eduamodu, L. E. and Odjadjare, E. O. (2021). Multi drug resistant *Aeromonas* species in Annelida; An evidence of pathogen harbouring leech in Recreation of water nexus of Oghara Nigeria. *Environmental Science of Afirca,* 20(2): 145-166,

Isakeit, T., Hassett, B. T. and Ong, K. L. (2012). First report of leaf spot of sesame caused by *Xanthomonas* sp. in the United States. *Plant disease*, 96(8): 1222-1222.

Jay, J. M. (2000). *Modern Food Microbiology*. Aspen publishers incorporated Gaithersburg, Maryland. A Wolters Kluwer Company, United States of America, 425-470.

Karungi, J., Kyamanywa, S., Adipala, E. and Erbaugh, M. (2011). Pesticide utilization, regulation and future prospects in small scale horticultural crop production systems in a developing country, pesticides in the modern world - pesticides use and management, Dr. Margarita Stoytcheva (ed.) 307-459.

Kirimura, K., Cao, W., Onda, Y., Yoshioka, I. and Ishii, Y. (2022). Selective and high-yield production of ethyl α-d-glucopyranoside by the α-glucosyl transfer enzyme of *Xanthomonas campestris* WU-9701 and glucose isomerase. *Journal of Bioscience and Bioengineering*, 134(3): 220-225.

Kong, C., Horta de Passo, V., Fang, Z., Yang, L., Zhuang, M., Zhang, Y. (2019). Complete Genome Sequence of Strain WHRI 3811 Race 1 of *Xanthomonas campestris* pv. campestris, the Causal Agent of Black Rot of Cruciferous Vegetables. *Molecular Plant-Microbe Interactions*, 32(12): 1571-1573.

Mirik, M., Selcuk, F., Aysan, Y. and Sahin, F. (2008). First outbreak of bacterial black rot on cabbage, broccoli, and brussels sprouts caused by *Xanthomonas campestris* pv. *campestris* in the Mediterranean Region of Turkey. *Plant Disease*, 92(1): 176-176.

Randhawa, P. S. and Schaad, N. W. (1984). Selective isolation of *Xanthomonas campestris* pv. *campestris* from crucifer seeds. *Phytopathology*, 74(3): 268-272.

Rosenthal, E. R., Ramos Sepulveda, L., Bull, C. T. and Koike, S. T. (2018). First report of black rot caused by *Xanthomonas campestris* on arugula in California. *Plant disease*, 102(5): 1025.

Sigillo, L., Senape, V., Serratore, G. and Bravi, R. (2012). *Fusarium oxysporum* f. spp. and *Xanthomonas campestris*

pathovars: two important pathogens of wild rocket fourth range productions in Southern Italy. In VI International Symposium on Brassicas and XVIII Crucifer Genetics Workshop 1005 (pp. 555-561).

Singh, D. and Sharma, R. (2007). *Postharvest disease of fruit and vegetables and their management*. In: Prasad, D edition sustainable pest management. Daya House, *New Delhi, India*. 51-54.

Tebaldi, N. D., Pereira, J. N. P., Sagata, E., Mota, L. C. B. M., da Hora Junior, B. T., Piazzoli, D. and Klosowski, A. C. (2022). Occurrence and detection of *Xanthomonas vasicola* pv. *vasculorum* in naturally contaminated maize seeds in Brazil. *Tropical Plant Pathology*, 1-5.

van der Wolf, J., Krijger, M., Mendes, O., Brankovics, B., Bonants, P., Didden, L. and Meekes, E. (2022). Molecular characterization of *Xanthomonas* species isolated from Araceae and the development of a triplex TaqMan assay for detection of *Xanthomonas phaseoli* pv. *dieffenbachiae*. *European Journal of Plant Pathology*, 163(1): 167-179.

Wydra, K., Khatri-Chhetri, G., Mavridis, A., Sikirou, R and Rudolph, K. (2004). A diagnostic medium for the semi-selective isolation and enumeration of *Xanthomonas axonopodis* pv. *vignicola*. *European journal of plant pathology*, 110(10): 991-1001.