Available online at www.ijpab.com

DOI: http://dx.doi.org/10.18782/2582-2845.8934

ISSN: 2582 – 2845

Ind. J. Pure App. Biosci. (2022) *10*(4), 1-8

Indian Journal of Pure & Applied Biosciences

Peer-Reviewed, Refereed, Open Access Journal

Research Article

Incidence and Histopathology of Bacterial Leaf Spot Pathogen *Xanthomonas axonopodis* pv. *vesicatoria* (Doidge) Dye in Brinjal Seeds (*Solanum melongena* L.) Grown in Jaipur Area of Rajasthan

Nandini Sharma[1] and Dilip Kumar Sharma[2*]

[1]Botany Research Lab, Agrawal P.G. College, Jaipur, Rajasthan, India

[2]Director, Vardhman Mahaveer Open University (VMOU), Kota, Rajasthan-324010, India

*Corresponding Author E-mail: drdilipsharma12@gmail.com

Received: 22.04.2022 | Revised: 26.06.2022 | Accepted: 10.07.2022

ABSTRACT

A survey was conducted to examine seed-borne microorganisms' prevalence in the Jaipur region. On Tween-80 agar medium (semi-selective medium), 110 seed samples of brinjal (Solanum melongena L.) were shown to have a 09-100 per cent incidence of bacterium Xanthomonas axonopodis pv. vesicatoria (Xav). On the basis of discolorations and signs on the seed surface, these obtained seed samples were divided into three categories. Asymptomatic (06.25-97.50 per cent), moderately (shrivelled) discoloured (02.50-83.25 per cent), and highly discoloured (04.25-100 per cent) seeds were found in dry seed inspection. For histopathology examinations, two seed samples naturally infected (lab ac nos. SM001; SM011) with a 100% incidence of Xav were chosen. Necrosis was seen in the embryo and endosperm after manually bisecting severely affected seeds in third category. In non-symptomatic seeds, the pathogen was detected only in the layers of the seed coat (funiculus); however, in moderately discoloured and heavily discoloured seeds, Xav was found in the seed coat and endosperm, including the embryo. The bacterium generated necrosis, lytic cavities, and clusters of bacterium in the embryo and endosperm. Aggregation was observed in the seed decrease in cell contents and bacterial cells. The pathogen was discovered both extra- and intra-embryonic.

Keywords: *Incidence, bacterial leaf spot, seed discolourations, Xanthomonas axonopodis pv. vesicatoria, seed-borne disease, histopathology.*

INTRODUCTION

Brinjal (*Solanum melongena* L.) is a widespread solanaceous (nightshade family) vegetable crop produced for human consumption across the world. It's a nutrient-dense crop with a lot of anthocyanin, phenols, glycoalkaloids (solasodine), amide proteins, and dry matter.

Cite this article: Sharma, N., & Sharma, D. K. (2022). Incidence and Histopathology of Bacterial Leaf Spot Pathogen *Xanthomonas axonopodis* pv. *vesicatoria* (Doidge) Dye in Brinjal Seeds (*Solanum melongena* L.) Grown in Jaipur Area of Rajasthan, *Ind. J. Pure App. Biosci. 10*(4), 1-8. doi: http://dx.doi.org/10.18782/2582-2845.8934

Protein (1.4 g), fat (0.3 g), minerals (0.3 g), fibre (1.3 g), moisture (92.7 g), carbohydrates (4.0 g), vitamin A (124LU), Vitamin C (12.0 mg), Thiamine (0.04 mg), Potassium (2.0 mg), Sodium (3.0 mg), Sulphur (44.0 mg), Calcium (18 mg), Chlorine (52.0 mg), Copper (0.17 mg), Magnesium (16 mg), Oxalic acid (18 m (0.9 mg) occurs in per 100g of edible portion. In Brazil, powdered brinjal infusion is taken to lower blood cholesterol levels. West Bengal, Bihar, Gujarat, Orissa, Karnataka, Maharashtra, Andhra Pradesh, and Uttar Pradesh are the main producing states in India. Jaipur, Kota, Alwar, Bharatpur and Sriganganagar are the primary producing districts in Rajasthan, covering 6.08 hectares and generating 25.79 MT in 2015-16 (Anonymous, 1985 & Kumar et al., 2017).

Bacterial leaf spot disease is caused by *Xanthomonas axonopodis* pv. *vesicatoria* (syn: *X.c.* pv. *vesicatoria*), that is a significant disease of tropical and subtropical regions that first emerged on brinjal plants (Neergaard, 1977, Bradbury, 1986, & Richardson, 1990). The bacterium is Gram-negative rod-shaped and invades the plant's aerial portion and transmits via infected seeds. The bacterial disease changed the biochemical contents of the seeds, lowering their quality and increasing yield loss. On semi-selective medium, Tween-B, Xav was obtained from symptomatic leaves and other areas of the plant (McGuire, & Jones & Sasser, 1986). The occurrence of disease and the localization of bacteria are determined by host resistance, infection mechanism, and current environmental conditions. Seeds contaminated with seed-borne bacteria have the potential to spread the infection from infected to healthy plants. The identification of the pathogen in seed components may aid in the development of efficient disease control techniques. As a result, a study was carried out to determine the pathogen's position within the brinjal seed tissues.

MATERIALS AND METHODS

To investigate the bacterial location in seeds, two naturally infected brinjal seed samples (lab ac nos. SM001; SM011) with a high incidence of *Xanthomonas axonopodis* pv. *vesicatoria* on agar media (100 percent on Tween-80 medium) were chosen.

(1) Incidence and Dry Seed Examination

Brinjal seed samples were obtained from the market, farmer's fields, and storage homes in 2014-15 and examined dry. A total of 110 seed samples were gathered from the key areas of Rajasthan's Jaipur districts (which is divided into East, West, North, and South Jaipur). 400 brinjal seeds were randomly chosen from each sample and examined with the naked eye first, then using a stereoscopic binocular microscope (10-40) (Anonymous, 1985). Asymptomatic, shrivelled, and heavily discoloured seeds were classified based on numerous indications and symptoms such as water-soaked patches, oozing, discolouration, and shrivelling, brown or black spots on seed surfaces (Sharma, 2007, & Sharma & Agrawal, 2010). The degree of discolouration, shape, size and various outgrowths were examined on the seed surface. To determine the per cent incidence of Xav in brinjal seed samples, all seed samples were cultured on a nutrient agar medium.

(2) Microtome studies

The classified seeds (asymptomatic and symptomatic discoloured) from selected seed samples were soaked in distilled (sterilized) water for 30 minutes in a hot air oven at 60°C to the microtomes examination. These softened seeds were employed in successive microtome and hand-cut sections for histopathological examinations (Verma & Agrawal, 2018). These softened seeds that were fixed in 70% alcohol were placed in glass vials for 48 hours, then dehydrated via a tertiary-butyl alcohol series; infiltered and embedded in paraffin wax. The embedded seeds and plant material were sectioned into blocks of 8-10 micron thickness, deparaffinized, dyed with saffranine, and lastly mounted in DPX (Johanson, 1940). A compound microscope was used to examine microtome slices (X-20-100). For the study, the softened seeds are sliced with a sterilizing blade.

RESULTS AND DISCUSSION

All of the gathered seeds samples were divided into three major categories based on xenomorphic characters: symptomatic [moderately discoloured (02.50-83.25 percent) and heavily discoloured seeds (04.25-100 percent)] and asymptomatic (06.25-97.50 percent) seeds (Table-2). Seeds that were asymptomatic were dull ochre in colour, often C-shaped or angular in shape, flattened, somewhat reticulate, smooth, and had thick walls but no obvious symptoms. Water-soaked translucent shimmering spots on the surface of shrivelled discoloured seeds varied from light brown to black. Seeds that were heavily discoloured and had dark brown to black discolouration and blotches on the surface shrivelled and were decreased in size (Fig.1 A). Incubation of such discoloured symptomatic seeds produced the pathogen. Infection with Xav has a negative impact on seed quality, causing discolouration, shrivelling, and water-soaked symptoms in brinjal seeds. When such discoloured seeds were bisected, dark brown coloured embryos were discovered, as contrast to asymptomatic seeds. On agar media, such seeds grew *Xanthomonas axonopodis* pv. *vesicatoria* after incubation. Seed-borne bacterial infections cause seed discolouration and reduce yields (Verma & Agrawal, 2018). Microtome investigations were performed on both selected seed samples from each category to determine the bacterium's location. *Pseudomonas syringae* in sunflower (Godika, & Agrawal & Singh, 2000) and pigeonpea seeds (Sharma et al., 2001), *X.a.* pv. *vesicatoria* in chilli (Sharma, 2007), *Ralstonia solanacearum* in tomato (Sharma, 2007) and brinjal seeds (Sharma & Sharma, 2014), and *X.c.* pv. *campestris* in rape & mustard (Sharma, & Agrawal & Singh, 1992) were previously reported to cause discoloured seeds with water-soaked symptoms (translucent areas). Bacteria's capacity to colonise in germinating seeds is an important step in disease transmission. In this study, on the surface of brinjal seeds hair like appendages were found shed in heavily infected seeds, like pseudo-hairs in tomato (Sharma & Agrawal, 2010).

The bacterial cells appear in the coccus or rod shaped in 3 and 4 seeds out of 10 seeds in outer and inner tests in each sample ac nos SM001 and SM011 in xenomorphically described first category (asymptomatic seeds). The bacterium was also found in the seeds' hilum. The bacterium was not found in the endosperm, and space in between the seed coat and the endosperm, as well as between the endosperm and the embryo in symptomatic seeds (Fig. 2). The infection was discovered to have harmed the quality of brinjal seed batches.

The seeds in the second category (moderately discoloured seeds) were found discoloured in the centre or had water-soaked spots (Fig.1 A). In acc numbers SM001 and SM011, the pathogen invaded the hilum, in 3 and 4 seeds out of 10 seeds, respectively; outer layer (4 and 5 seeds) and inner layer of the seed coat (5 and 7 seeds). In 3 and 5 seeds, cells of bacterial were occurs in between the inner layer of the seed coat and the endosperm, and in endosperm 4 and 6 seeds in both samples, respectively (Fig.1C-E). Out of 10 seeds, the pathogen was found in the embryonal axis in 3 and 2 seeds, respectively (Fig. 2).

The seed size was decreased, shrivelled, and flat in the third category (heavily discoloured), revealing considerable discolorations due to heavily infection of Xav (Fig. 2). In seed samples acc nos SM001 and SM011, the bacteria was found around the hilum (5, 6 seeds), exterior layer (7, 9 seeds) and inner seed coat layer (8, 9 seeds), and endosperm (9, 8 seeds). In both seed samples, clusters of bacterial cells were seen in the embryonal axis (7, 6 seeds) and cotyledons (8, 7 seeds) (Fig.1G, H) (Table-1). This category included bacterial cell aggregation or clumping, as well as the depletion of cell contents in various components. The lysis or disintegration of endosperm cell walls was also observed (Fig.1F) (Table-1).

Cells of Xav were found to be restricted to the area between the seed coat,

endosperm, radicle, and hilum region in moderately and extensively discoloured seeds in the current investigation. There are also reported aggregates of bacterial cells in the micropylar area. Symptomatic seeds with brown to black discolouration and water-soaked translucent regions on the seed surface are thought to be results of infection of bacteria. Bacteria on the seed surface can cause systemic or vascular infection, and they are often detected in the other seed tissues and seed coat layers (Skoric, 1927, Neergaard, 1977, & Singh & Mathur, 2004). *Xanthomonas axonopodis* pv. *vesicatoria* is thought to penetrate brinjal seeds through the funiculus and micropyle opening. Bacterial infections have been shown to penetrates through micropyle (Skoric, 1927), wounds (Khristov, 1968), stomata (Tabei, 1967, & Fukuda et al., 1990), funiculus (Naumann, 1963) or mechanical damage in previous research (Tabei et al., 1989, & Agrios, 2005).

Due to infection, the infected seeds displayed cell necrosis, lysis, disintegration, or a decrease in cell contents. Both in asymptomatic and symptomatic seeds had pathogen colonies at the hilum or funiculus area. Similarly, in pea, the raphe of seeds with vascular elements provides a good entry point for *Pseudomonas syringae* (Verma & Agarwal, 2018). The bacteria is present in soft parenchymatous tissue's inter- and intracellular spaces and induces cell lysis. The bacterial cavities might be little or enormous, and they are all filled with slime. The micropylar infection begins through the funiculus by bacterial mass adhering to the faniculus cavity. Under field circumstances, *Pseudomonas syringae* pv. *pisi* invades the parenchyma and may enter the vasculature. Internally, the bacterium *P. s.* pv. *syringae* was discovered in certified wheat seeds collected from Bangladesh wheat fields (Rashid, 1995). *Pseudomonas syringae* pv. *lachrymans* infection of cucumber seeds begins intracellularly and intercellularly in the funiculus (Wiles & Walker, 1951, & Kritzman & Zutra, 1983). In artificially infected plants, the *X. c.* pv. *campestris* penetrates in cabbage seeds via funiculus (Cook, & Larson & Walker, 1952). Infestation of *X. a.* pv. *phaseoli* in bean seed occurs in the micropyle and/or funiculus (Zaumeyer, 1930). According to Cook et al. (1952), the development of cells or clusters of bacterial cells around the hilum area indicated pathogen penetration through the funiculus. In cabbage (Bandyopadhyay & Chattopadhyay, 1985), cotton (Tennyson, 1930, & Brinkerhoff & Hunter, 1963), rape and mustard, and pigeonpea, the seed bacteria are found underneath the seed coat.

Xanthomonas axonopodis pv. *vesicatoria* was identified extra and intra embryonal in brinjal seeds in this investigation, colonising the outer layers of seed coat in asymptomatic seeds (healthy appearing seeds) and up to the embryo in symptomatic seeds. *Xanthomonas campestris* in cow pea (Sharma et al., 2001), *X.c.* pv. *campestris* in rape and mustard (Sharma, & Agrawal & Singh, 1992), *Ralstonia solanacearum* and *X.a.* pv. *vesicatoria* (Sharma, 2007) and in pegion pea (Gaikwad & Kore, 1981, & Sharma et al., 2001). Internal infection was reported in chalazal halves and micropyle of the seed coats, as well as in embryo rarely (Tennyson, 1936; & Brinkerhoff & Hunter, 1963, 1964). Externally and internally up to endosperm, *X. c.* pv. *glycinus* and *X. oryzae* pv. *oryzae* were detected (Fang et al., 1956, Srivastava & Rao, 1964, Groth, 1983, & Mukerjee & Singh, 1983).

In the present investigation, it was investigated that the bacterium was found associate with floral bud and persistent calyx in transmission studies. Similar study was reported in seed of *Capsicum annuum* by isolating the *Xanthomonas vesicatoria* in cortical and vascular tissues of pedicel, ovary (Crossan & Morehart, 1964, & Sharma, 2007); *Acidovorax citrulli* (causes bacterial fruit blotch of cucurbits) was observed in the cotyledons of pistil-inoculated seeds and perisperm-endosperm layers (Dutta, Avci, & Hahn & Walcott, 2012), *X.a.* pv. *vesicatoria* in chilli and tomato seeds (Sharma, 2007), *X.c* pv. *campestris* in mustard and rape seeds (Sharma et al., 1992), and pigeon pea (Sharma et al., 2001). The bacterium *P. s.* pv.

lachrymans found in the embryo and confined to the outermost layers of the radicle (Nauman, 1963).

Table 1: Location study of *Xanthomonas axonopodis* pv. *vesicatoria* in seeds of brinjal in various categories

Seed categories	Components of seed						
	Hilum	Seed Coat		Space in between endosperm and seed coat	Endosperm	Embryo	
		Outer layer of testa	Inner layer of testa			Embryonal axis	Cotyledons
Sample Ac. No. SM001							
1. Asymptomatic seeds	0	2	1	0	0	0	0
2. Moderately discoloured seeds	3	4	5	3	4	3	2
3. Heavily discoloured seeds	5	7	8	8	9	7	8
Sample Ac. No. SM011							
1. Asymptomatic seeds	1	1	2	0	0	0	0
2. Moderately discoloured seeds	4	5	7	5	6	2	2
3. Heavily discoloured seeds	6	9	9	9	8	6	7

Table 2: Incidence of bacterium *Xanthomonas axonopodis* pv. *vesicatoria* in seeds of brinjal in various categories grown in Jaipur area of Rajasthan

S. No.	Area	No. of sample studied	Asymptomatic	Moderately discolored (shriveled)	Heavily discolored	White crusted seeds (Actinomycetes)
1.	North Jaipur	31	06.25-88.25	07.75-60.50	07.75-100	05(02.75-27.25)
2.	South Jaipur	32	07.50-84.75	11.25-83.25	04.25-35.25	09 (05.25-27.25)
3.	East Jaipur	28	15.50-90.50	13.25-78.75	06.25-38.25	07 (04.25-12.75)
4.	West Jaipur	19	10.75-97.50	02.50-62.75	06.25-50.75	06 (06.50-15.25)
	Total	110	06.25-97.50	02.50-83.25	04.25-100	02.75-27.25

Fig. 1: Histopathology of brinjal seeds naturally infected with *Xanthomonas axonopodis* pv. *vesicatoria*

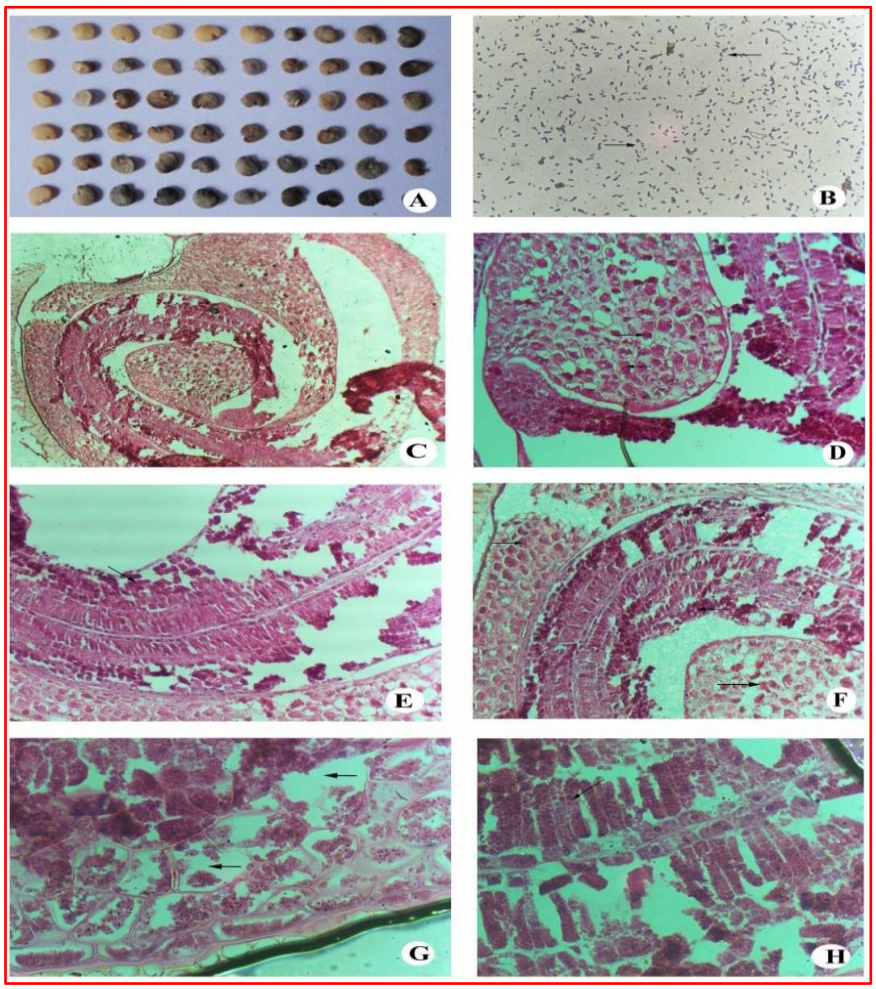
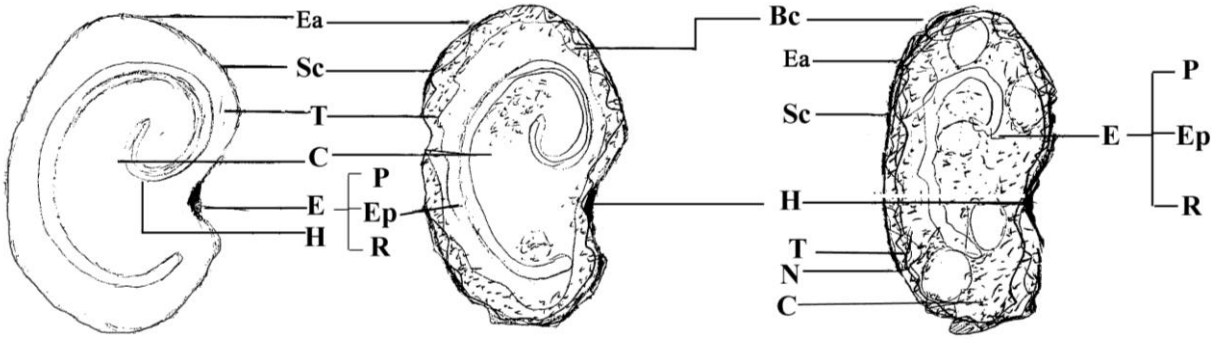


Fig. 1A-Seed rows in dry seed examination as asymptomatic, moderately discoloured and heavily discoloured (from left to right and upper to lower) seeds respectively, **B**-Cells of Xav in Gram's staining x1000 (note the coccoid, rod shaped cells), **C**-whole seed with bacterial cells showing depletion, lysis and necrosis of seed x250, **D**-Part of moderately discoloured seed showing heavy colonization on Xav in endosperm, cotyledons tissue, and seed coat (outer and inner layer) x250, **E**- embryo showing lysis of host cells and depletion of cell contents x250, **F**- bacterial cell in seed coat of seed and endosperm x250, **G**-

heavily discoloured seeds showing the presence of pathogen at endosperm and embryo x1000, **H-** heavily discoloured seed showing the cells of the pathogen in endosperm cotyledons and radicle x1000.

Fig. 2: Semi diagrammatic presentation of *Xanthomonas axonopodis* pv. *vesicatoria* in naturally infected seeds of brinjal



I. Asymptomatic seed **II. Moderately discoloured seed seed** **III. Heavily discoloured**

Bc = Bacterial cells H = Hilum (funiculus) R = Redicle
C = Cotyledons N = Necrosis Sc = Seed coat
E = Embryo P = Plumule T = Testa
Ep = Epicotyl Ea = Epidermal appendages

CONCLUSIONS

The bacterium, Xav was reported to be aggregated in space between seed coats, hilum region, endosperm and radicle. The presence of bacterial cells in abundance at funiculus area may suggest that the possible mode of invasion in seed is via this part leading to systemic infection. Bacterial masses occur in between the cells of parenchymatous tissue and counter palisade. The colonization of bacteria may cause the host cell necrosis, reduction in cell contents and formation of lytic cavities.

Acknowledgement

The authors are grateful to Prof. Kailash Agrawal, Head, Department of Botany, University of Rajasthan, Jaipur, faculty members of P.G. Department of Botany for valuable support and academic guidance. The authors are also thankful to all the scientists whose work is cited and could not acknowledge unknowingly and persons that directly or indirectly engaged in writing in this paper and during practical work.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

Conflict of Interest

The author declares no conflict of interest.

Author Contribution

Both authors contributed equally to establishing the topic of the research and design experiment.

REFERENCES

Agrios, G. N. (2005). Plant pathology 5 the edition. *Elsevier, Academic Press*, pp-952.

Anonymous, (1985). International seed rules for seed testing International Seed Testing Association (ISTA). *Seed Science & Technology 4*(3-49), 50-177.

Anonymous, (2015). Indian horticulture database, pp-302. http://nhb.gov.in/area-pro/NHB_Database_2015.pdf.

Bandyopadhyay, S., & Chattopadhyay, S. B. (1985). Incidence of black rot of cabbage and cauliflower under different conditions of infection.

Indian Journal Agriculture Science, 55, 350-354.

Bradbury, J. F. (1986). Guide to Plant Pathogenic Bacteria. *CAB International Mycological Institute (CMI)*, UK pp-332.

Brinkerhoff, L. A., & Hunter, R. E. (1963). Internally infected seeds as a source of inoculum for the primary cycle of bacterial blight of cotton. *Phytopathology 54*, 1397-1401.

Cook, A. A., Larson, R. H., & Walker, J. C. (1952). Relation of the black rot pathogen to cabbage seed. *Phytopathology 42*, 316-320.

Crossan, D. F., & Morehart, A. L. (1964). Isolation of *Xanthomonas vasicatoria* from tissues of *Capsicum annuum*. *Phytopathology 54*, 356–359.

Fang, C. R., Lin, C. F., & Chu, C. L. (1956). A preliminary study on the disease cycle of the bacterial leaf blight of rice. *Acta Phytotaxonomica Sinica 2*:, 173-185.

Fukuda, T., Azegami, K., & Tabei, H. (1990). Histological studies on bacterial black node of barley and wheat caused by *Pseudomonas syringae* pv. *japonica*. *Annals of Phytopathological Society of Japan 56*(2), 252-256.

Gaikwad, B. M., & Kore, S. S. (1981). Bacterial leaf spot and stem canker of pigeon pea caused by *Xanthomonas cajani*. *Indian Journal Mycology of Plant Pathology 11*, 50-56.

Godika S., Agrawal K., & Singh T. (2000). Histopathological and biochemical changes in seeds of sunflower infected with *Pseudomonas syringae*. In: Proceeding of Indian Phytopathological Society-Golden Jubilee: International conference. *Integrated plant Disease Management of Sustainable Agriculture. Vol. II*. New Delhi: IARI; 2000. p. 1131-32.

Groth, D. (1983). Seed transmission of the bacterial pustules pathogen in soybeans. *Iowa Seed Science* 5(2), 1-10.

Johanson, D. A. (1940). Plant Microtechniques. *Tata McGraw Hill Book Company, New York 11*, 523.

Kristov, A. (1968). Bacterial wilt, a dangerous disease of tomato and other plants in Bulgaria, *Gradinarstvo, 10*(5), 27-28.

Kritzman, G., & Zutra, D. (1983). Systemic movement of *Pseudomonas syringae* pv. *lachrymans* in the stem, leaves, fruits and seeds in cucumber. *Can. J. Plant Pathol. 5*, 273–278.

Kumar, P., Shaunak, I., Thakur, A. K., & Srivastava, D. K. (2017). Health promising medicinal molecules in vegetable crops. *J Genetics Genomes 1*(102), 1-4.

Mukerjee, P., & Singh, R. A. (1983). Histopathological studies on infected rice seed with *Xanthomonas campestris* pv. *oryzae* and mode of its passage from seed to seedling. *Seed Res*. *11*, 32–41.

Sharma, N., & Sharma, D. K. (2014). Incidence and seed transmission of *Ralstonia solanacearum* (Smith) in brinjal (*Solanum melongena* L.) seeds. *International Journal of Plant Pathology, 5*, 63-69.

Naumann, K. (1963). Uber das Auftreten von Bakterien in Gurkensamensaus Fruchten, die durch *Pseudomonas lachrymans* infiziert Warem. *Phytopathology Z. 48*, 258-271.

Neergaard, P. (1977). Seed Pathology. *The MacMillan Press Ltd., London*: 1187.

Richardson, M. J. (1990). An annotated list of seed-borne diseases (4th edn). Proceedings International Seed Testing Association Zurich, Switzerland.

Sharma, D. K. (2007). Seed-borne and post-harvest bacterial diseases of chilli (*Capsicum* spp.) and tomato (*Lycopersicon esculentum* Mill.) crops and their management, Ph.D. Thesis, Univ. of Rajasthan, Jaipur.

Sharma, D. K., & Agrawal, K. (2010). Incidence and colonization of *Ralstonia solanacearum* in tomato

seeds. *Journal of Mycology and Plant Pathology 40*(1), 115-119.

Sharma, J., Agarwal, K., & Singh, D. (1992). Detection of *Xanthomonas campestris* pv. *campestris* (Pammel) Dowson infection in rape and mustard seeds. *Seed Research 20*, 128-133.

Sharma, M., Kumar, D., Agarwal, K., Singh, T., & Singh, D. (2001). Colonization of pigeon pea seed by *Xanthomonas campestris* pv. *cajani*. *Journal of Mycology and Plant Pathology 31*(2), 216-219.

Singh, D., & Mathur, S. B. (2004). Histopathology of Seed-Borne Infections. CRC Press LLC, 2000 N.W. Corporate Blvd., Boca Raton, Florida, pp- 282.

Skoric, V. (1927). Bacterial blight of pea: overwintering, dissemination and pathological histology. *Phytopathology 17*, 611–627.

Srivastava, D. N., & Rao, Y. P. (1964). Seed transmission and epidemiology of bacterial blight disease of rice in North India. *Indian Phytopathology 17*, 77-78.

Tabei, H. (1967). Anatomical studies of rice plants affected with bacterial leaf blight with special reference to stomatal infection at the coleoptile and the foliage leaf sheath of rice seedling. *Ann. Phytopathol. Soc. Japan 33*, 12–16.

Tabei, H., Azegami, K., Fukuda, T., & Goto, T. (1989). Stomatal infection of rice grain with *Pseudomonas glumae*. The causal agents of the bacterial grain rot of rice. Nippon shokubutsu Byori Gakkaiho. *Annals of Phytopathological Society Japan 55*(2), 224-225.

Tennyson, G. (1936). Invasion of cotton seed by *Bacterium malvacearum*. *Phytopathology 26*, 1083–1085.

Verma, A. K., & Kailash, A. (2018). Location and histopathology of seed-borne bacterial pathogen *Pseudomonas syringae* pv. *pisi* carried by pea seeds. *Journal of Applied Biology & Biotechnology 6*(1), 20-22.

Wiles, A. B., & Walker, J. C. (1951). The relation of *Pseudomonas lachymans* to cucumber fruits and seeds. *Phytopathology 41*, 1059–1064.

Zaumeyer, W. J. (1930). The bacterial blight of beans caused by *Bacterium phaseoli*. *U.S. Dept. Agric. Bull. 180*, 1–36.

McGuir, R. G., Jones, J. B., & Sasser, M. (1986). Tween media for the semi-selective isolation of *Xanthomonas campestris* pv. *vesicatoria* from soil and plant material. *Plant Dis. 70*, 887-891.

Rashid, A. Q. (1995). Detection of seed-borne *Pseudomonas syringae* pv. *syringae* in wheat. *Plant Varieties Seeds 8*, 47-54.