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Incidence and Histopathological Study of *Xanthomonas Axonopodis* Pv. *Vesicatoria* in Tomato (*Lycopersicon Esculentum* Mill.) Seeds

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Abstract Dry seed examination of 75 seed samples of tomato (*Lycopersicon esculentum* Mill.) belonging to 13 districts of Rajasthan revealed 10-100% incidence of *Xanthomonas axonopodis* pv. *vesicatoria* (XAV) on Tween-80 medium. Two naturally infected seed samples of tomato carrying 100% incidence of XAV were selected and categorised into asymptomatic, moderately discoloured and heavily discoloured seeds. The heavily infected seeds were shrivelled with or without pseudo-hairs, with water-soaked symptoms and on bisecting such seeds the embryo and endosperm showed necrosis and browning. The pathogen was found confined to the outer seed coat layer particularly at ramnent of funiculus in the asymptomatic seeds. In moderately discoloured seeds pathogen was found in seed coat, space in between seed coat and endosperm. It colonised all the seed components including embryo and endosperm in heavily discoloured seeds. The pathogen caused necrosis, formation of lytic cavities, reduction in cell contents and aggregation of the bacterial cells. The pathogen was found extra-as well as intra embryonal.

Keywords: Tomato, *Xanthomonas axonopodis* pv. *vesicatoria*, seed-borne, Incidence, histopathology.

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Introduction

Bacterial specks and leaf spots diseases of tomato caused by *Xanthomonas axonopodis* pv. *vesicatoria* is one of the most important and widespread diseases in tropical, subtropical, warm and temperate regions of the world. The pathogen also attacked on pepper spreaded very fast in warm and humid environment (listed in A2 quarantine list), showed spot on seedlings in European countries, defoliation in young plants and spots on leaf, stem and

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fruits in older plants Black, Seal, Zakia, Nono-Womdium and Swai (2001) reported from Tanzania that in rainy season bacterial spot was found in most of the mainland vegetable regions of northern and southern high lands. In fields surveys of tomato disease incidence (varied greatly between years and fields) from <5% to >90% was recorded.

Karaca and Demir (1998) from Turkey isolated *Pseudomonas syringae* pv. *tomato, Corynebacterium michiganensis* pv. *michiganensis* and *Xanthomanas campestris* pv. *vesicatoria* from tomato that causes leaf spot disease. The pathogen has been reported to be seed-borne in tomato (Neergaard, 1977, Bradbury, 1986, Richardson, 1990). In the present study, incidence of pathogen in seed samples of tomato from Rajasthan state and histopathology have been studied.

Materials and methods

Seventy five seed samples of tomato collected from 13 districts of Rajasthan were subjected to dry seed examination, standard blotter method (Anonymous, 1985) and Tween-80 agar medium plate method (Lelliot and Stead, 1987, Saettler *et al.*, 1989). In dry seed examination, seed samples were categorised into asymptomatic, moderately discoloured and heavily discoloured seeds. The degree of discolouration, sign, shape, size, outgrowths on seed surface was studied. All the seed samples were incubated on Tween-80 medium to record the per cent incidence of the pathogen in the seed samples. The isolates of the bacterium were subjected to confirmative tests of identification (Schaad, 1988).

Seventy five seed samples of tomato collected from 13 districts of Rajasthan were studied by dry seed examination, incubation on moistened blotters (Anonymous, 1985) and Tween-80 agar (Lelliot and Stead, 1987, Saettler *et al.*, 1989) plate method to find the incidence of *Xanthomonas axonopodis* pv. *vesicatoria* in tomato seed samples. The culture were maintained on nutrient agar (NA) and pure colonies after 72 h of incubation at 30^{o}C typical bacterial colonies from seeds raised on YDC (Schaad and Kendrick, 1975) agar plate were re-transfer to YDC agar medium plates to obtain pure cultures which were subjected to various tests namely gram's staining, KOH solubility test, levan formation, oxidase test (Kovac's, 1956; Hildbrand and Scroth, 1972), potato soft rot test, nitrate reductase test (Fahy and Persley, 1983), arginine dihydrolysis, gelatin hydrolysis test, hypersensitivity test in tobacco and pathogenecity tests (Lelliot and Stead, 1987) for the identification of the bacterial species. For all the tests 24-48 h old cultures (Lelliot and Stead, 1987) and bacterial suspensions (Kiraley *et al.*, 1970) were used. The bacterial isolates identified by various methods as

described above were subjected to pathogenecity tests (Schaad, 1980) on the host plant and other plant species.

Two seed samples (ac. nos. Ly-1412 and Ly-1413) of tomato naturally infected with XAV and carrying 100% incidence as revealed on Tween-80 medium were selected for histopathological studies. Serial microtomed and hand cut sections were used and the sections stained with saffranin and fast green combinations (Johanson, 1940) were used.

Results

In dry seed examination, seed samples of tomato were categorised into asymptomatic, moderately discoloured and heavily discoloured seeds (Fig. 1A). The discoloured seeds showed shrivelling, water-soaked, translucent areas and bacterial ooze forming crust like growth on the seed surface. The seed surface of asymptomatic seeds had sufficient pseudo-hairs but in discoloured seeds these were mostly shed (Fig. 1A). The heavily discoloured seeds on bisecting found the discoloured embryo and endosperm with necrosis and browning.

The bacterial colonies isolated from various seed samples were produced convex to domed, circular, entire, yellow, mucoid, shiny and raised colonies on YDC agar medium and identified to be of *Xanthomonas axonopodis pv. vesicatoria*. The incidence was studied on Tween-80 and YDC agar medium. On Tween-80 medium Xcv appears as circular, raised, yellow colonies surrounded by zone of white crystals of calcium salt of fatty acids released from tween by lipolytic enzymes. The isolates were gram's negative, KOH solubility test positive, levan negative, lipase activity positive, Kovac's oxidase negative or weak, nitrates were not denitrified or reduced but catalase positive, starch hydrolyzing, gelatin hydrolyzing, arginine variable, no rotting of potato tissue occurred. The pathogen induced positive hypersensitivity reaction on tobacco leaves after infiltration. The colonies were smooth, circular and butyrous or viscid usually yellow (xanthomonadins) in colour, but a few non-pigmented strains also occurred on nutrient agar medium.

Incidence of the pathogen

The pathogen was recorded in 68 and 63 seed samples in untreated (10-82%) and pretreated (8-66%) seeds in standard blotter method. The incidence of pathogen on Tween-80 medium was 10-100% in 68 seed samples belonging to 13 districts of Rajasthan. The seed samples of Jaipur (20-100%), Bikaner (30-100%), Sawai Madhopur (40-100%), Karoli (30-90%), Sikar (80-100%) and Alwar (30-70%) districts revealed relatively high incidence of the pathogen (Table 1).

Histopathological studies

Two seed samples (ac. nos. Ly-1412 and Ly-1413) of tomato carrying naturally infection (100%) of pathogen were selected for histopathological studies. In sections of asymptomatic seeds the aggregation of the bacterial cells was confined to ramenent of funiculus (hilum), outer and inner layers of seed coat in both the samples studied (Table 2; Figs.1, 2). The pathogen also colonized in between inner layer of seed coat.

Moderately discoloured seeds, revealed the pathogen colonisation of most of the seed components. The bacterial cells and their clumps were observed at ramenents of funiculus (hilum), outer layer of seed coat, inner layer of seed coat (Fig.1C) and endosperm (Table 2; Fig. 1D). In endosperm, seeds revealed the bacterial colonies and lytic cavities. The pathogen was localized in all seed components including embryonal axis and in between endosperm and embryonal axis in a few seeds in both samples.

In heavily discoloured seeds, aggregation of bacterial cells and their clumps were observed at ramenent of funiculus, outer and inner layers of seed coat, endosperm and embryo (Table 2; Fig.1E). In some seeds the endosperm cuticle was not intact, depletion of cell contents, formation of lysogenous cavities, aggregation of bacterial cells and necrosis were observed (Figs. 1 F & G). The pathogen was also found aggregated in between seed coat layers and endosperm and in the cotyledons (Fig. 1 H & I, Fig. 2). On sectioning some seeds showed deformed endosperm and brown to black embryo. In heavily infected seeds, lytic cavities formed due to disruption of cells were quite frequent in the cotyledonary tissue was observed. The cotyledons also showed necrosis.

Discussions

Infection of XAV in seeds of tomato affected seed quality adversely causing discolourations, shriveling, shedding of pseudo-hairs and water- soaked symptoms. Such symptoms caused by *Xanthomonas campestris* in cow pea (Kishun, 1989), by *X. c.* pv. *campestris* in rape and mustard (Sharma *et al.*, 1992), pegion pea (Gaikwad and Kore, 1981, Sharma *et al.*, 2001, by *Ralstonia solanacearum* in tomato (Sharma and Agrawal, 2010) have also been reported. Neergaard (1977) mentioned that *X. campestris* occurred on seed coat surface but those causing vascular or systemic infection are frequently found in the seed coat and other tissues of seed. The bacteria have been reported to be present below the seed coat in cabbage (Bandyopadhyay and Chattopadhyay, 1985), rape and mustard (Sharma *et al.*, 1992) and pegion pea (Sharma *et al.*, 2001).

In the present, seeds with discolourations were found associated with pathogen. Similar observations have been reported in other crops like cowpea, mustard, sunflower, pigeon pea etc. In cowpea, shrivelled seeds showed brown discolorations of seeds in bean halo disease caused by *Pseudomonas phaseolicola* (Neergaard 1977). Discoloured seeds with water-soaked translucent areas on seed surface due to *P. syringae* have been reported in sunflower (Godika *et al*., 2000). Brown, pinkish discolourations by *Xanthomonas campestris* pv. *campestris* in mustard (Sharma *et al*., 1992) and *X. cajani* pv. *cajani* in pigeon pea (Sharma *et al*. 2001) have been reported.

In this study, the bacterium was found associated with the pseudo-hairs and also at hilar region. Verma (1990) reported that this may be due to gas exchange, water transport that is through the funiculus during the development of seed. Formation of cells or clumps of bacterial cells near hilar region suggested the penetration of pathogen through funiculus as also suggested (Cook *et al.*, 1952). *Xanthomonas campestris* pv. *phaseoli* caused common and fuscous blight in *Phaselous* spp. and *Dolichos lablab* confined to be harboured both within the seed and on the seed coat (Mortensen, 1994a).

X. c. pv. *malvacearum* located internally and externally on the seed. Internally in seed, it was located in chalaza, micropylar end of the seed coat and in the embryo (Brinkerhoff and Hunter, 1963, Hunter and Brinkerhoff, 1964). *X. c.* pv. *glycines* and *X. oryzae* pv. *oryzae* were found located externally and internally up to endosperm (Fang *et al.*, 1956, Srivastava and Rao, 1964, Groth, 1983). *Pseudomonas syringae* pv. *phseolicola* in severly infected seeds of bean (*Phaseolus* sp.) are found associated in the hilum region of seed, surface of cotyledons and embryo (Taylor *et al.*, 1979).

In the present study, large number of cells or clumps was observed at funiculus (hilum) and it suggested being the site of penetration of bacteria in the seed. Earlier studies have been demonstrated that the bacterial pathogens may penetrate through micropyle, funiculus (Naumann, 1963), through wounds (Khristov, 1968) and through stomata (Tabei *et al.*, 1989, Fukuda *et al.*, 1990).

Thus, the pathogen was found to be extra- as well as intra embryonal in seeds of tomato. It was confined to outer layer of seed coat and funiculus in asymptomatic seeds and seed coat, in inner layer of testa, endosperm and embryo in moderately and heavily discoloured seeds. It was very interesting to observe that the pseudo-hairs present on seed surface get readily shed due to bacterial infection. The bacterial cells were found in abundance at the ramanents of funiculus suggesting the possible mode of invasion and infection in seed through this area leading to systemic infection as earlier reported.

Table 1. District wise occurrence and incidence of various bacterial species in tomato in SBM and on various semi-selective medium

Districts	*Xanthomonas axonopodis* pv. *vesicatoria*		
	UT	PT	TWEEN-80
Ajmer	04(32-40)	04(24-30)	04(40-60)
Alwar	07(24-64)	06(16-58)	07(30-70)
Bharatpur	05(12-34)	05(8-22)	05(10-50)
Bikaner	04(24-48)	04(16-48)	04(30-100)
Churu	01(58)	01(56)	01(60)
Dausa	05(40-50)	05(34-44)	05(50-80)
Jaipur	20(10-50)	17(8-46)	20(20-100)
Jodhpur	03(24-46)	03(22-44)	03(30-80)
Karoli	07(12-82)	07(8-66)	07(30-90)
Kota	02(26,38)	02(24,28)	02(35,40)
Sikar	04(40-70)	04(38-64)	04(80-100)
Sawai Madhopur	05(20-42)	05(12-42)	05(40-100)
Tonk	01(00-00)	01(00-00)	01(00-00)
Total	68(10-82)	63(8-66)	68 (10-100)

Table 2. Location of *Xanthomonas axonopodis* pv. *vesicatoria* in different parts of seeds of tomato in various categories of seeds in microtome sections (10 seeds/category/sample)

Seed categories	Seed Components						
	Remnant of funiculus (hilum)	Spermoderm Outer seed coat layer	Inner seed coat layer	Space in between endosperm and seed coat	Endosperm	Embryo Embryonal axis	Cotyledons
Sample Ac. No. Ly-1412							
Asymptomatic seeds	1	2	1	0	0	0	0
Moderately discoloured seeds	2	6	5	7	6	2	0
Heavily discoloured seeds	4	9	9	8	8	7	7
Sample Ac. No. Ly-1413							
Asymptomatic seeds	1	1	1	0	0	0	0
Moderately discoloured seeds	3	7	7	5	7	3	0
Heavily iscoloured seeds	4	10	9	9	6	8	6

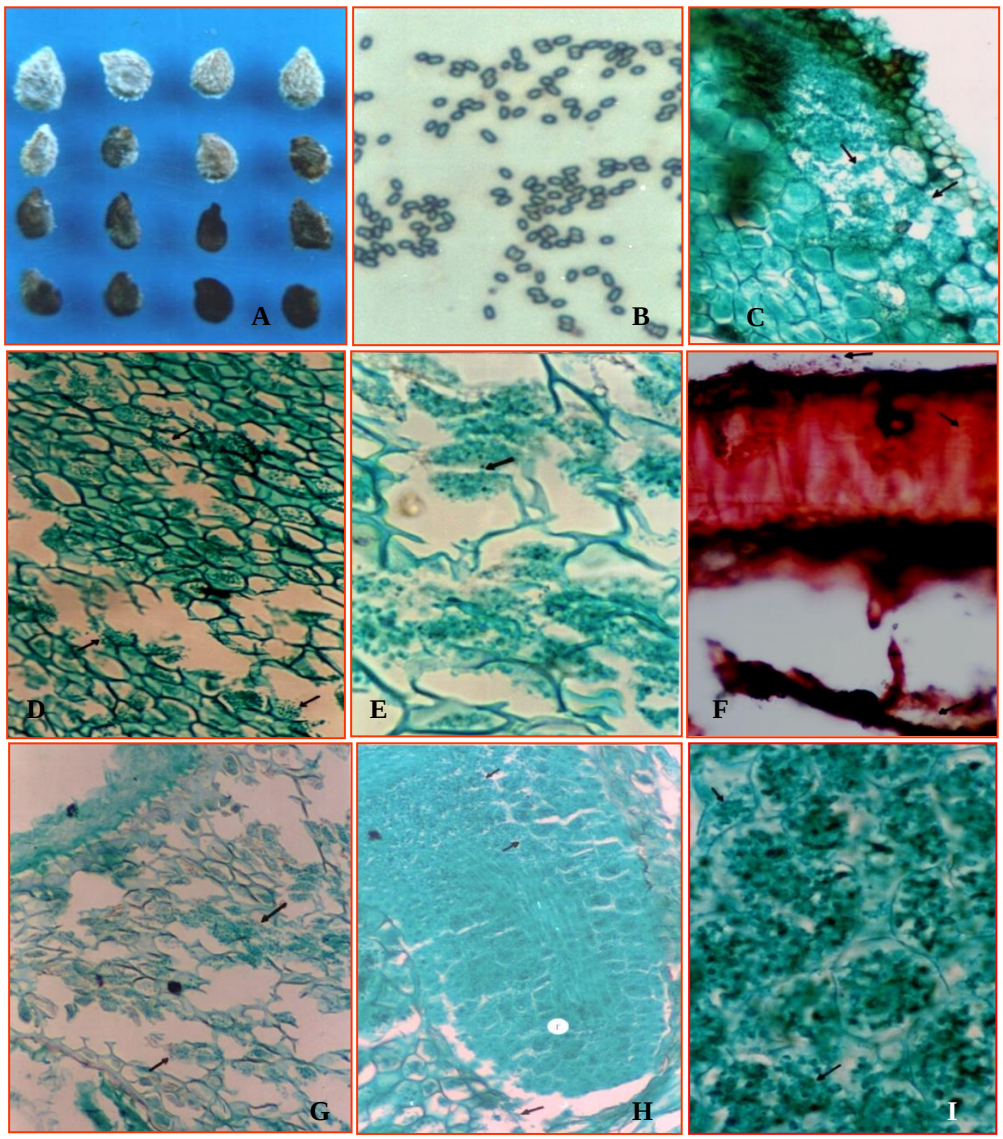

Fig. 1. Location of *Xanthomonas axonopodis* pv. *vesicatoria* in naturally infected seeds of tomato
A = Dry seed examination: asymptomatic (upper most layer), moderately discoloured (2nd row) and heavily discoloured seeds (lower last two rows), B = the cells of the pathogen on Gram's staining 1000 X, C = parts of LS of moderately discoloured seed showing lysis of host cells and presence of bacterial cells (→) in endosperm X 250, D &E = a part of LS of moderately discoloured seeds showing the bacterial cells (→) in endosperm cells, Note the lysis of cells and loss of content X 250 and X 1000, F = Parts of L.S. of moderately discoloured seed showing bacterial ooze at the outer layer of testa and in between the testa layers, Note the disintegration of inner layer of testa X 100, G = Parts of L.S. of moderately discoloured seed showing the bacterial cells (→) in endosperm and remnant of funiculus, Note the lysis of of inner layer of testa and endosperm X100, H & I = Parts of L.S. of moderately discoloured seed showing lysis of cells and presence of pathogen (→) in redical and endosperm X 250 and X 1000.

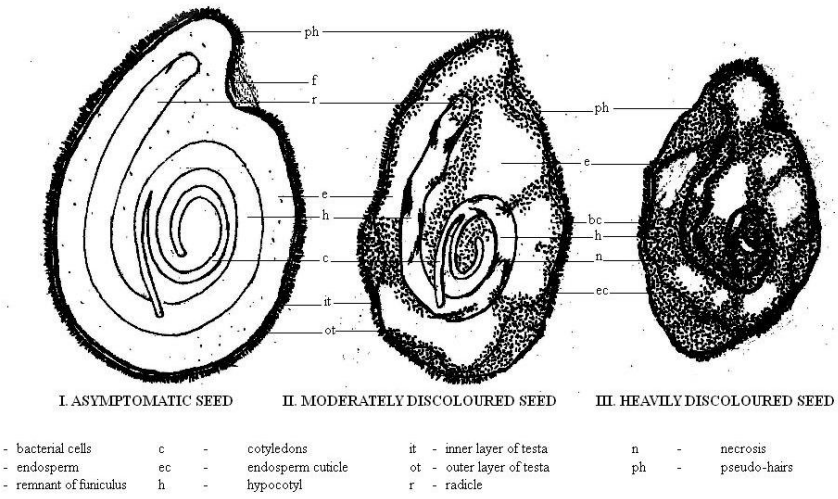

Fig. 2. Semi-diagrammatic representation and location of *Xanthomonas axonopodis* pv. *vesicatoria* in naturally infected seeds of tomato (bc =bacterial cells; c = cotyledons; e = endosperm; ec = endosperm cuticle; h = hypocotyls; it = inner layer of testa; n = necrosis; ot = outer layer of testa; ph = pseudo-hair; f = remnant of funiculus; r = radical)

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References

Anonymous (1985). International seed rules for seed testing International Seed Testing Association (ISTA). Seed Science and Technology 4:50-177.

Bandyopadhyay, S. and Chattopadhyay, S. B. (1985). Incidence of black rot of cabbage and cauliflower under different conditions of infection. Indian Journal Agriculture Science 55:350-354.

Black, R., Seal S., Zakia, A., Nono-Womdium, R. and Swai, I. (2001). Bacterial spot (*Xanthomonas campestris* pv. *vesicatoria*) of tomato and sweet pepper in Tanzania. Plant Pathology 50:810-810.

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

Brinkerhoff, L. A. and 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. and Walker, J. C. (1952). Relation of the black rot pathogen to cabbage seed. Phytopathology 42:316-320.

Fahy, P. C. and Persley, G. J. (1983). Plant bacterial diseases. A diagnostic guide. Academic Press, London, New York, Sydney. 393 pp.

Fang, C. R., Lin, C. F. and 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. and 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 56:252-256.

Gaikwad, B. M. and 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., Agarwal, K. and Singh, T. (2000). Histopathological and biochemical changes in *Pseudomonas syringae.* Indian Phytopathol 1131-1132.

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

Hunter, R. E. and Brinkerhoff, L. A. (1964). Longevity of *Xanthomonas malvacearum* on and in cotton seed. Phytopathology 54:617-617.

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

Karaca, I and Demir, G. (1998). Investigations on seed-borne bacterial pathogen in some plants. Doga Tiirk Tarim ve Ormancilik Dergisi 12:120-131.

Kiraley, Z., Klement, Z., Solymosy, F. and Vörös, J. (1970). Methods in Plant Pathology. Akademiai Kiadó, Budapest. Journal of Agricultural Technology 7:197-205.

Kishun, R. (1987). Loss in yield of tomato due to bacterial wilt caused by *Pseudomonas solanacearum.* Indian Phytopathology 40:152-155.

Kovacs, N. (1956). Identification of *Pseudomonas pyocyanea* by the oxidase reaction. Nature London 178:703.

Lelliot, R. A. and Stead, D. E. (1987). Methods for the diagnosis of bacterial diseases of plants. In: Methods in Plant Pathology. Vol. 2 (Ed. Preece, T.F.), Blackwell Scientific Publication, Oxford, London. 216 pp.

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 pp.

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

Saettler, A. W., Schaad, N. W. and Roth, D. A. (1989). Detection of bacteria in seed (edt.). APS Press St. Paul, Minnesota. 122 pp.

Schaad, N. W. (1980). Laboratory guide for identification of plant pathogenic bacteria (Edt.). For Bacteriology Committee of American Phytopathological Society, St. Paul, Minnesota. 72 pp.

Schaad, N. W. (1988). Laboratory guide for identification of plant pathogenic bacteria (2^{nd} edn). APS Press (The American Phytopathological Society) St Paul, Minnesota. pp. 164.

Schaad, N. W. and Kendrick, R. (1975). A qualitative method for detecting *Xanthomonas campestris* in crucifer seed. Phytopathology 65:1034-1036.

Sharma, D. K. and Agrawal, K. (2010). Incidence and colonization of *Ralstonia solanacearum* in tomato seeds. Journal of Mycology and Plant Pathology 40:115-119.

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

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

Sharma, M., Agrawal, K. and Singh, T. (2002). Incidence and seed transmission of *Xanthomonas campestris* pv. *cajani* in pigeon pea. Journal of Mycology and Plant Pathology 32:1-5.

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

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

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

Taylor, J. D., Dudley, C. L. and Presly, L. (1979). Studies of halo-blight infection and disease transmission in dwarf beans. Annals of Applied Biology 93:267-277.

Verma, R. (1990). Studies on seed-borne mycoflora and disease of moth bean and cowpea grown in Rajasthan. (Doctor of Philosophy's Thesis). Rajasthan University. Jaipur, India.

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