JOURNAL OF CURRENT RESEARCH IN SCIENCE (ISSN 2322-5009)
CODEN (USA): JCRSDJ
2014, Vol. 2, No. 4, pp: 521-525
Available at www.jcrs010.com

ORIGINAL ARTICLE

ISOLATION AND IDENTIFICATION OF PHYTOPATHOGENS FROM DISEASED VEGETABLES AT DISTRICT KOHAT, KHYBER PAKHTUNKHWA PAKISTAN

Abdul Basit*, Iqbal Ahmad, Kashif Rahim, Mehwish Shafiq
Department of Microbiology, Kohat University of Science and Technology (KUST), Khyber PakhtunKhwa, 26000, Pakistan

ABSTRACT: Phytopathogenic bacteria and fungi utilize the vegetables and plant cells as privileged food source and cause devastation under appropriate environmental conditions. In the present study, investigation deals with the isolation and identification of the phytopathogens from diseased vegetables in the fields of District Kohat, Khyber PakhtunKhwa Pakistan. A total of 54 vegetable samples of *Allium cepa* (Onion), *Capsicum frutescens* (Green Chili), *Hibiscus esculenta* (Okra), *Luffa acutangula* (Ridge Gourd), *Lagenaria siceraria* (Bottle Gourd), *Momordica charantia* (Bitter Gourd), *Solanum melongena* (Eggplant) and *Spinacia oleracea* (Spinach) included various diseased leaves, fruits and onion bulbs were used for isolation of phytopathogenic bacteria and fungi. Many pathogenic bacteria species namely *Erwinia species, Ralstonia species, Xanthomonas specis* were identified on the basis of morphological, cultural characteristics and biochemical profile. Similarly fungal species of *Alternaria, Aspergillus* and *Fusarium* were also identified from diseased vegetables. These findings revealed that an average of 29.17% qualitative loss and 40.33% quantitative loss of vegetables occurs, which results a cumbrous loss in economy of Kohat.

KEYWORDS: Phytopathogens, identification, Diseased Vegetables, Kohat.

INTRODUCTION

Vegetables are the important food as they supply sufficient amounts of phenolics, flavonoids, ascorbic acid, riboflavin, folic acid, carotene, and minerals (Khanzadi, 2011). These vegetables are susceptible to phytopathgens along with a number of destructive pests, plant viruses and nematodes. The total loss of vegetable on this account has been estimated up to 20-30% but if the pathogens are allowed to develop, this loss may increase up to 80-90% (Iqbal *et al.*, 1996; Glazebrook, 2005).

Bacterial and fungal diseases cause heavy losses in vegetables. Black rot, caused by the vascular bacterium *Xanthomonas campestris* pv. *campestris*, is one of the most serious diseases of vegetable *Brassica* spp. (Cab International, 2006; Williams, 1980) while *Xanthomonas cucurbitae* causes leaf spots of cucurbits worldwide (Bradbury, 1986; Maringoni *et al.*, 1988). Blackleg, wilt diseases and soft rot is caused by gram negative, facultative anaerobic rod shaped bacterium *Erwinia carotovora* includes *Er. umylouura, Er. sulicis, Er. rubrifaciens, Er. tracheiphila, Er. nigrguens, Er. quercznu,* and *Er. Mallotiuoru* (Bradbury, 1986; Maringoni *et al.*, 1988). Among the range of production issues bacterial pathogen *Erwinia tracheiphila*, continue to rank high among limiting factors annually recurring in many areas (Russell *et al.*, 2010). Brown rot caused by *Ralstonia solanacearum* is a quarantine disease considered as one of the most important bacterial diseases of Vegetables (Leah *et al.*, 1999).

Fungi that cause diseases in vegetables include *Penicillium* spp, *Aspergillus* spp, *Alternaria* spp, *Botrytis cinerea*, *Monilinia laxa, Fusarium solani* and *Rhizopus stolonifer* (Ogawa *et al.*, 1995). *Aspergillus* is one of the major fungi species that produce aflatoxin (Palumbo *et al.*, 2008). Similarly *Alternaria* species infect the vegetable crops and reduce yield both qualitatively and quantitatively. It has been estimated that complex of three *Alternaria* species *A. brassicicola*, *A. brassicae* and *A. japonica* responsible for significant reductions in yield quantity and quality (Shresta *et al.*, 2000).

Many approaches required to reduce the impact of phytopathogens on the quality, quantity and economy of production. Conventional control measures involve the execution practices to prevent further infections, elimination of infected plant tissue and proper disposal to stop the transmission of the pathogen. Control of phytopathogens can also be achieved by the use of synthetic pesticides, antibiotics (e.g., tetracycline and streptomycin) (Kiran *et al.*, 2009).

Pakistan is primarily a farming country. Vegetable production is increasingly becoming an important component of Pakistan's agriculture (Government of Pakistan, 2004a).

Corresponding Author: Abdul Basit, Department of Microbiology, Kohat University of Science and Technology (KUST), Khyber PakhtunKhwa, 26000, Pakistan. **E-mail:** aabdul.9090@gmail.com

Vegetables are not even consumed domestically but are also exported regularly and play an important role in Pakistan economy (Government of Pakistan, 2004b). All over the Pakistan, more than 63 varieties of vegetables distributed in 44 genera, are grown on large scale (Athar and Bokhari, 2006).

District Kohat, Khyber PakhtunKhwa Pakistan is well-known land for the production of vegetables in Pakistan. District Kohat is a semi-arid region, with plenty of natural wealth of plant species. Local people of district Kohat are somehow depending on their cultivated vegetables or crops to fulfill their nutritional needs and economy (Hussain *et al.*, 2010). Phytopathogens and their gross loss per yield have a remarkable effect on the economy of their people.

Therefore the present study investigation deals with the isolation and identification of the phytopathogens from diseased vegetables in the fields of District Kohat.

MATERIALS AND METHODS

2.1. Samples Collection

In the present study, eight different diseased vegetables i.e. *Allium cepa* (Onion), *Capsicum frutescens* (Green Chili), *Hibiscus esculenta* (Okra), *Luffa acutangula* (Ridge Gourd), *Lagenaria siceraria* (Bottle Gourd), *Momordica charantia* (Bitter Gourd), *Solanum melongena* (Eggplant) and *Spinacia oleracea* (Spinach) were collected from six areas of District Kohat Khyber PakhtunKhwa, Pakistan. During a random selection of affected parts of vegetables, a total of 54 samples were collected specifically 16 samples from Bilitang, 06 samples from College Town and 08 each samples from, Dhoda, Jarma, Kharmatoo and Togh Bala respectively. Affected parts of each cultivar were cut with sterile surgical blade and stored in sterile bags. All the labeled samples were then transported to "Department of Microbiology, Kohat University of Science and Technology, Kohat" for further analysis.

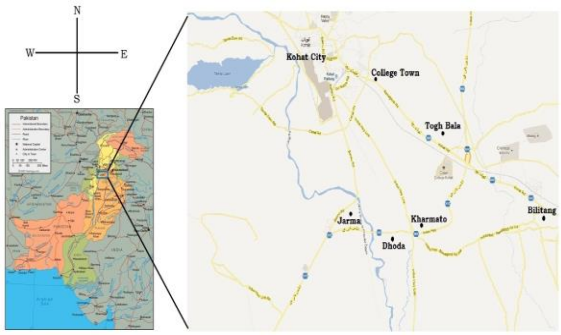

Figure 1: Map of district Kohat showing sampling area

2.2. Sample Inoculation

From the most affected portion of the plant, two small pieces each affected portion was cut aseptically. The infected portion bits were surface sterilized in 70 % alcohol or 1 % sodium hypochlorite and washed in three series of sterile water to remove traces of alcohol. Each of the two pieces from the infected portion were then inoculated aseptically at the center (point inoculation) of each part of Petri plate containing Nutrient agar (NA) and Sabouraud Dextrose Agar (SDA) respectively. The inoculated plates containing SDA were incubated at 25C° for 48 hours while the plates containing NA were incubated at 37C° for 48 hours. Observations were made for development of colonies.

2.3. Identification of Pathogens

Identification of bacteria was carried out based on their colony characteristics and Gram's staining and biochemical profile.

Fungal isolates were identified on the basis of morphological characteristics of colonies appeared on plates which were further identified by preparing the slides using Tape touch method as described by Harris with slight modification. Observations were made for the identification of fungal pathogens on the basis of the morphology and spore characteristics.

RESULTS AND DISCUSSION

3.1. Identification of bacterial isolates

Total 29 bacterial isolates were found from 54 samples. Bacterial species of *Erwinia, Ralstonia,* and *Xanthomonas* was identified was based on their morphological characteristics, gram staining biochemical profile and their symptoms on plants (Table 1 and 3).

Erwinia species were observed as half-white colored, round colonies which on Bitter Gourd and Spinach showed brown lesions on both fruits and leaves of the samples. *Ralstonia* species had white-colored, round and dense colonies resulting in moulds, soft watery rots and sudden death of fruits and leaves of Green Chili, Ridge Gourd and Eggplants.

Similarly *Xanthomonas* species exhibited a yellow-colored, round and dense colonies which had the effect of de-greening, stunting and brown rot spots on Onion, Okra and Bottle Gourd affecting Bulb of Onion, leaves and fruits of these vegetables.

Table 1: Gram reaction and cultural characteristics of bacterial isolates

Source	Isolated Organism	Gram Reaction	Culture Characteristics on Nutrient Ager
Onion (*Allium cepa*)	*Xanthomonas Spp*	Gram Negative	Yellow colored round colonies
Green Chili (*Capsicum frutescens*)	*Ralstonia Spp*		White-colored, round and dense colonies
Okra (*Hibiscus esculenta*)	*Xanthomonas Spp*	Gram Negative	Yellow colored round colonies
Ridge Gourd (*Luffa acutangula*)	*Ralstonia Spp*		White-colored, round and dense colonies
Bottle Gourd (*Lagenaria siceraria*)	*Xanthomonas Spp*	Gram Negative	Yellow colored round colonies
Bitter Gourd (*Momordica charantia*)	*Erwinia Spp*	Gram Negative	Half-white colored, round colonies
Eggplant (*Solanum melongena*)	*Ralstonia Spp*		White-colored, round and dense colonies
Spinach (*Spinacia oleracea*)	*Erwinia Spp*	Gram Negative	Half-white colored, round colonies

3.2. Identification of fungal isolates

Similarly 25 fungal isolates were found from 54 samples. Three fungal isolates namely *Alternaria species, Aspergillus species* and *Fusarium species* were identified on the bases of cultural and morphological characteristics (Table 2).

Alternaria species showed the black-colored growth on the plate and affecting the Spinach and Eggplant. Similarly *Aspergillus* species were identified on the basis of its relevant symptoms on Onion bulbs and leaves as well as its green-colored growth on SDA, *Fusarium* species were observed to be the most frequent among all the samples, characterized by its pink-colored growth and its effects on Green Chili, Okra, Ridge Gourd, Bottle Gourd and Bitter Gourd. Microscopic observation confirmed the structure of the spores and sporangia of respective fungi.

Table 2: Cultural characteristics of fungal isolates

Source	Isolated organism	Culture characteristics on SDA
Onion (*Allium cepa*)	*Aspergillus spp.*	Green-colored growth
Green Chili (*Capsicum frutescens*)	*Fusarium spp.*	Pink-colored growth
Okra (*Hibiscus esculenta*)	*Fusarium spp.*	Pink-colored growth
Ridge Gourd (*Luffa acutangula*)	*Fusarium spp.*	Pink-colored growth
Bottle Gourd (*Lagenaria siceraria*)	*Fusarium spp.*	Pink-colored growth
Bitter Gourd (*Momordica charantia*)	*Fusarium spp.*	Pink-colored growth
Eggplant (*Solanum melongena*)	*Alternaria spp.*	Black-colored growth
Spinach (*Spinacia oleracea*)	*Alternaria spp.*	Black-colored growth

Table 3: Biochemical characteristics of bacterial isolates

Bacterial isolates	Oxidase Test	Levan Test	KOH Solubility Test	Catalase	Indole Test	Sugar fermentation			
						Dextrose	Lactose	Sucrose	Mannitol
Erwinia spp	+			+	+			–	
Xanthomonas spp	–			+					
Ralstonia spp	+	+	+			+	+	+	+

Positive test = (+), Negative test = (-)

3.3. Identification based on symptoms

30 of the samples were leaves; 21 were fruit while a 3 were onion bulbs.

3.4. Disease Symptoms Observed in Onion

Stunting and reduction in bulb size and Black colored spots on the scales of the bulb while black discoloration at the neck of leaves was found in the fields. Due to these symptoms 30% loss impacts on yields was found.

3.5. Disease Symptoms Observed in Green Chili

Fuzzy, grey, mould growths on chili fruit and Irregular, brown to dark brown spots with concentric rings inside the spots on the fruit was observed while Wilting of the plant and crumbling of the leaves followed by yellow color was also observed. These symptoms cause 45% yield loses in green chili.

3.6. Disease Symptoms Observed in Okra

Small rough spots and yellow fruit color was observed. Also small yellowish-green to brown spots on the leaves and Yellowing and stunting of the plant was observed followed by wilting and rolling of the leaves in Okra. 50% impacts on yield's loss were investigated in Okra due to above diseased symptoms.

3.7. Disease Symptoms Observed in Ridge Gourd

Spotted, distorted, wrinkled and reduced in size of fruit. Stunted plant and the leaves give the appearance of yellow and wilted growth and the stem turns dark have been observed. 40 % yield loses were investigated.

3.8. Disease Symptoms Observed in Bottle Gourd

Skin of the fruit showed soft, dark green water soaked lesions which gradually developed into a watery soft rot and Chlorotic small and circular or elliptical in shape with black or brown color. While small, dark, and angular lesions on the leaves were observed. Due to these diseased

symptoms 35% impacts on yields were investigated.

3.9. Disease Symptoms Observed in Bitter Gourd

Tan-colored circular to oval lesions on the fruit surface were observed. Leaves become brown starting from the leaf edge showing a blighted appearance and Pale, brown, olivaceous brown, grayish or black were also observed. 30% yield lose were investigated in Bitter gourd fields.

3.10. Disease Symptoms Observed in Eggplant

Brown to dark brown soft watery rot covering the fruit completely was observed. While small, light-colored lesions on leaves were observed in Eggplant. 35% yield losses were recorded in Eggplant fields.

3.11. Disease Symptoms Observed in Spinach

Small circular or irregular black lesions spread over the leaves were observed in Spinach plants.40% losses in yield were recorded due to these symptoms.

Several diseases in vegetables are mostly caused by fungi and bacteria, including leaf spot, Wilt, leaf blight, black spot and Fruit rot. In present study same symptoms of diseases were found roughly in every field that was surveyed. These cause an average qualitative loss of 29.17% while the average quantitative loss was 40.33%, which greatly affects the economy. It was also observed that the control measures adopted by the farmers were not satisfactory furthermore farmers have poor knowledge regarding plant diseases controlling strategies.

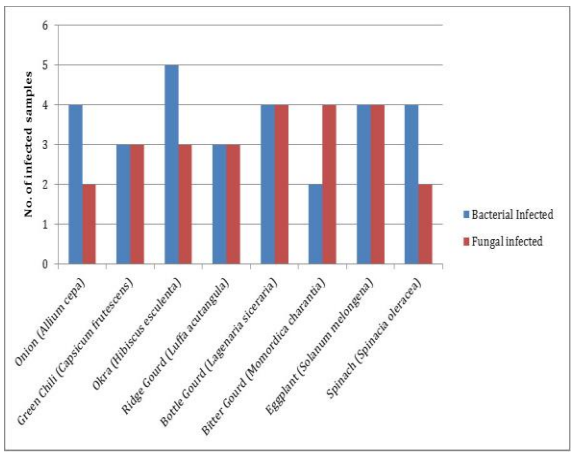

Figure 2: Showing the no of infected bacterial and fungal isolates obtained from diseased vegetables

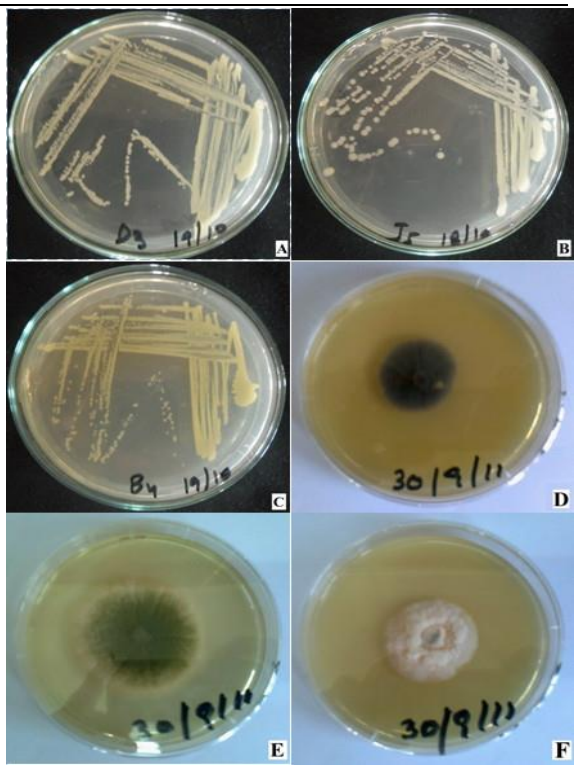

Figure 3: Shows the fungal and bacterial isolates observed after the sample inoculation, on SDA and NA respectively (A) *Erwinia spp.,* (B) *Ralstonia spp.,* (C) *Xanthomonas spp.,* (D) *Alternaria spp.,* (E) *Aspergillus spp.,* (F) *Fusarium spp.*

REFERENCES

Athar M, Bokhari TZ. Ethno botany and production constraints of traditional and commonly used vegetables of Pakistan. J Vegetable Sci 2006; 12: 27-38.

Bradbury JF. Guide to Plant Pathogenic Bacteria. Slough, UK: CAB International 1986; pp:309.

Cab International. Crop Protection Compendium. CAB International, Wallingford, UK 2006.

Glazebrook J. Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. Annual Review of Phytopathology 2005; 43: 205–227.

Government of Pakistan. Agricultural statistics of Pakistan 2003-2004. Ministry of Food, Agriculture and Livestock. Food, Agriculture and Livestock Division, Islamabad, Pakistan 2004a.

Government of Pakistan. Fruit, Vegetables and condiments statistics of Pakistan 2003-04. Food, Agriculture & Livestock Division, Islamabad, Pakistan 2004b.

Hussain J, Rehman NU, Khan AL, Hamayun M, Hussain SM, Shinwari ZK. Proximate and essential Nutrients evaluation of selected vegetable species from Kohat Region, Pakistan. Pak J Bot 2010; 42(4): 2847-2855.

Iqbal M, Verkerk RHJ, Furlong MJ, Ong PC, Syed AR and Wright DJ. Evidence for resistance to Bacillus thuringiensis (Bt) subsp. Kurstaki HD-1, Bt subsp. Aizawai and Abamectin in field populations of Plutella xylostella from Malaysia. Pestic Sci 1996; 48: 89–97.

Khanzadi FK. Nutrient Composition, Phenolic Content and Free Radical Scavenging Activity of Some Uncommon Vegetables of Pakistan. Pak J Pharm Sci 2011; 24(3): 277-283.

Kiran K, Linguraju S, Adiver S. Effect of plant extract on Sclerotium rolfsii, the incitant of stem rot of ground nut. J Mycol Pl Pathol 2009; 36: 77-79.

Leah T, Aharon M, Orly E. Survey of Bacterial and Fungal Seedborne Diseases in Imported and Domestic Potato Seed Tubers. Phytoparasitica 1999; 27(3): 1-12.

Maringoni AC, Leite RPJ, Komori N. Nova doença bacteriana do pepino (Cucumis sativus L.), causada por Xanthomonas campestris pv. cucurbitae (BRYAN) DYE, no Brasil. Summa Phytopathologica 1988; 142: 25-230.

Ogawa JM, Dehr EI, Bird GW, Ritchie DF, Kiyoto V, Uyemoto JK. Compendium of Stonefruit Diseases. APS Press, USA 1995.

Palumbo JD, Baker JL, Mahoney NE. Isolation of bacterial antagonists of Aspergillus flavus almonds Microbial Ecol 2008; 52(1): 45-52.

Russell L, Groves DL, Bill H. Vine Crop Pest Management and Pollinators. Wisconsin Crop Management Conference 2010; pp:49.

Shresta SK, Mathur SB, Munk L. Alternaria brassicae in seeds of rapseed and mustard, its location, transmission from seed to seedlings and control. Seed Science and Technology 2000; 28: 75-84.

Williams PH. Black rot: a continuing threat to world crucifers. Plant Dis 1980; 64: 736-742.