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Research Article

IN-VITRO EVALUATION OF PLANT EXTRACTS FOR THE MANAGEMENT OF BLACK LEG OF POTATO

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Abstract

Potato crop is facing serious threat due to blackleg disease caused by *Pectobacterium atrosepticum*. For pathogen isolation and identification, diseased samples were collected, processed and confirmed through Koch's postulates, gram staining, KOH test and Potato tuber assay. Nine plants extracts *P. guajava* (Guava), *P. granatum* (Pomegranate), *S. pinnata* (Plum), *S. cumini* (Kalajam), *M. indica* (Mango), *T. indica*, *L. chinensis* (Litchi), *V. amurensis* (Grapes) and *C. verum* (Cinnamon) were evaluated against Pa by using inhibition zone technique under Completely Randomized Design (CRD). Maximum inhibition zone was measured by *S. cumini* (7.640 mm) followed by *C. verum* (6.566), *C. vinifera* (4.159), *T. indica* (3.437), *P. granatum* (2.918), *p.guajava* (2.844), *S. pinata* (2.751), *M. indica* (2.344), *L. chinensis* (2.251) as compared to control, respectively. Interaction between treatments and concentrations indicated that C1 concentration of *S. cumini* (6.955) expressed maximum inhibition zone as compared to *C. verunn* (5.177), *C. vinifera* (3.177), *T. indica* (3.288), *P. granatum* (1.844), *p.guajava* (2.066), *S. piñata* (2.288), *M. indica* (1.066), *L. chinensis* (1.733) mm while C2 concentration of S. cumini (7.455) also expressed maximum inhibition zone followed by *C. verum* (7.177), *C. vinifera* (4.122), *T. indica* (3.344), *P. granatum* (3.344), *p. guajava* (2.677), *S. piñata* (3.344), *M. indica* (2.733), *L. chinensis* (2.344) mm and at C3 concentration exhibited 8.511,7.344, 5.177, 3.677, 3.566, 3.788, 3.400, 3.566, 2.677 mm inhibition zone of treatments as compared to control respectively. While interaction between treatments and hours showed that *S. cumini* (6.844, 7.622, 8.455) exhibited maximum inhibition zone and *L. chinensis* (1.566, 2.233, 2.955) expressed minimum inhibition zone at H1, H2 and H3 as compared to control respectively.

Keywords: Potato, *Pectobacterium atrocepticum*, management, plant extracts

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1. INTRODUCTION

Potato is a member of family Solanaceae with genus Solanum and species tuberosum. It is perennial crop but cultivated as annual crop. It is major field crop of temperate regions (Czajkowski et al. 2011). It is tuber bearing crop cultivated all over the world. It is 4th most important crop after maize (*Zea mays*), wheat (*Triticum aestivum*) and rice (*Oryza sativa*) because of its yield and high nutritive value. Potato contains 20.6% carbohydrates, 2.1 % proteins and 0.3% fat. In developing countries, potato is main cash crop for farmers with easy cultivation. In many countries, potato is used as staple food. It is rich source of starch, vitamins and some essential amino acids like leucine, tryptophan and isoleucine and antioxidants (Vanaei et al. 2008). Potato crop is damaged by many biotic factors like bacteria, virus and nematodes etc. Among bacterial diseases, black leg of potato caused by *Pectobacterium atrosepticum* is major threat to potato crop (Bhutta et al. 2005). In Pakistan, it was firstly reported in 1984 from swat valley (Rashid et al. 2012). Symptoms of black leg includes soft rottening of tubers and rapid wilting of healthy stem and leaves. Stems turns into inky black color. Maceration of tubers take place due to pectinolytic and cell wall degrading enzymes released by bacteria. Rotted mother tubers considered primary source of inoculum which transfer the disease to healthy tubers (Farran et al. 2006). To deal with blackleg disease, use of resistant potato varieties is ideal method. To identify resistance source against black leg, screening of potato germplasm is done. Many methods to screen potato germplasm against black leg disease but field experiment is authentic method. It is time taking method so other management strategies must be considered for disease control which will be eco-friendly and economical.

Different plant extracts evaluated against black leg disease for its management i.e. neem aqueous extract reduce the disease incidence and disease severity in field as well as in storage. Ironweed and Siamese cassia aqueous leaf extracts also effective against disease and show antibacterial activity (Bdliya and Dahiru, 2006). El Astal (2004) used aqueous garlic extract which have high antibacterial actively and reduce the disease. Neem (*Azadirachta*), onion (*Allium sepa*) and garlic extract proved effective to reduce the disease, their results recorded by measuring inhibition zone technique. These results are related to the Jahan et al, (2007), they used neem oil against bacteria and observed inhibition zone against bacteria. Plant extracts have minimum health hazard effects, cost effective and environment friendly as compare to chemicals. They are easily biodegradable. Different plant extracts proved effective against black leg of potato (Rashid et al., 2012). So, in this study, potential of different plant extracts was evaluated for the management of black leg disease of potato.

2. MATERIALS AND METHODS

2.1 Isolation of pathogen from infected sample and its confirmation

Pathogen was isolated form infected potato plants having typical symptoms of black leg by using standard isolation protocols (Riker and Riker, 1936). Diseased portions of plants were transferred on filter paper for drying purpose after cutting and disinfection with 0.1% mercuric chloride solution and then placed on petri plates (9mm) containing nutrient agar (NA) media. These petri plates after wrapping were placed in incubators at 27oC±2oC. Bacteria isolated on the petri plates showed the characteristics and morphological characters (colony color and cell shape) of Pa further identified and confirmed by Koch's postulates.

2.2 *In-vitro* antibacterial potential of Plant extracts against Pa

Extracts of nine plants *P.guajava* (Guava), P. granatum (Pomegranate), *S. pinnata* (Plum), *S. cumini* (Kalajam), *M. indica* (Mango), *T. indica*, *L. chinensis* (Litchi), *V.amurensis* (Grapes) and *C. verum* (Cinnamon) were evaluated against *Pectobacterium atrosepticum* under lab conditions through inhibition zone technique (Barry et al., 1979). Fresh leaves of the test plants were collected from Horticultural nursery, UAF. All plant leaves were surface sterilized with 70% ethanol to remove all debris. 75 g leaves of each plant were brought to lab and their fine powder was prepared by electric blender (AG014, MAKUTE). Then ethanolic extract was prepared by mixing 20 g of leaves powder of each plant in 100 ml ethanol. To remove all debris and leaves parts, ethanolic extract was filtered with four layers of muslin cloth and re-filtered by using Whatman filter paper number 41. The extract considered as standard and stored at 4°C. Bacterial suspension (108 CFU/ml) by using spectrophotometer (Hitachi U-2001, model 121-003) was prepared. Petri plates (9cm) having nutrient agar media were fully streaked by sterile spreader. 1 cm (dia.) small piece of blotter paper was cut and dipped into each concentration (10, 20 and 40%) of tested extract and put it in the center of petri plate. To avoid any impurity, plates were kept at 4°C for 24 h and then incubated (RTI-250, Robus) at 28 ± 2°C. Trial was designed under Complete Randomized design and inhibition zone were measured after 12, 24 and 36 hours with digital Vernier caliper (500-196, Mitutoyo). In control treatment distilled water was used. Data of inhibition zones were measured and analyzed with Fisher's Least Significant Difference Test (LSD) (Steel and Torrie , 1997).

3. RESULTS

3.1 Isolation, purification and Identification of bacterial pathogen

Bacteria isolated on the petri plates showed the characteristics and morphological characters (colony colour and cell shape) of Pa. further identified and confirmed by Koch's postulates, KOH test, gram staining and tuber assay. In result of pathogenicity test, inoculated plants showed typical black symptoms after seven days.

3.2 *In-vitro* evaluation of different Plant extracts against Pa

ANOVA for inhibition zone (mm) indicated that all the treatments (T), concentrations (C), hours (H) and their interactions (T×C) and (T×H) expressed significant while (C×H) and

(T×C×H) expressed non-significant results. (Table. 20) While treatments showed that inhibition zone was maximum by *S. cumini* (7.640) followed by *C. verunn* (6.566), *C. vinifera* (4.159), *T. indica* (3.437), *P. granatum* (2.918), *p.guajava* (2.844), *S. pinata* (2.751), *M. indica* (2.344), *L. chinensis* (2.251) as compared to control respectively. (Fig.1)

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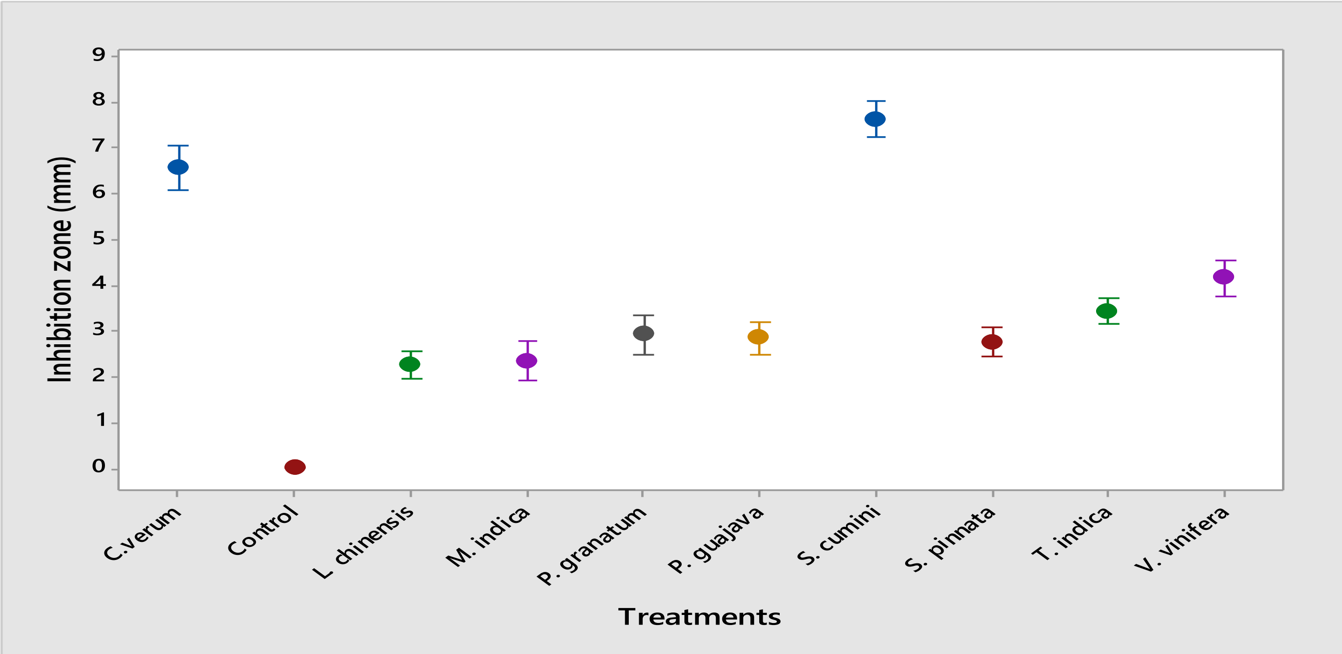

Fig. 1 Evaluation of plant extracts against *Pectobacterium atrosepticum* under lab. conditions

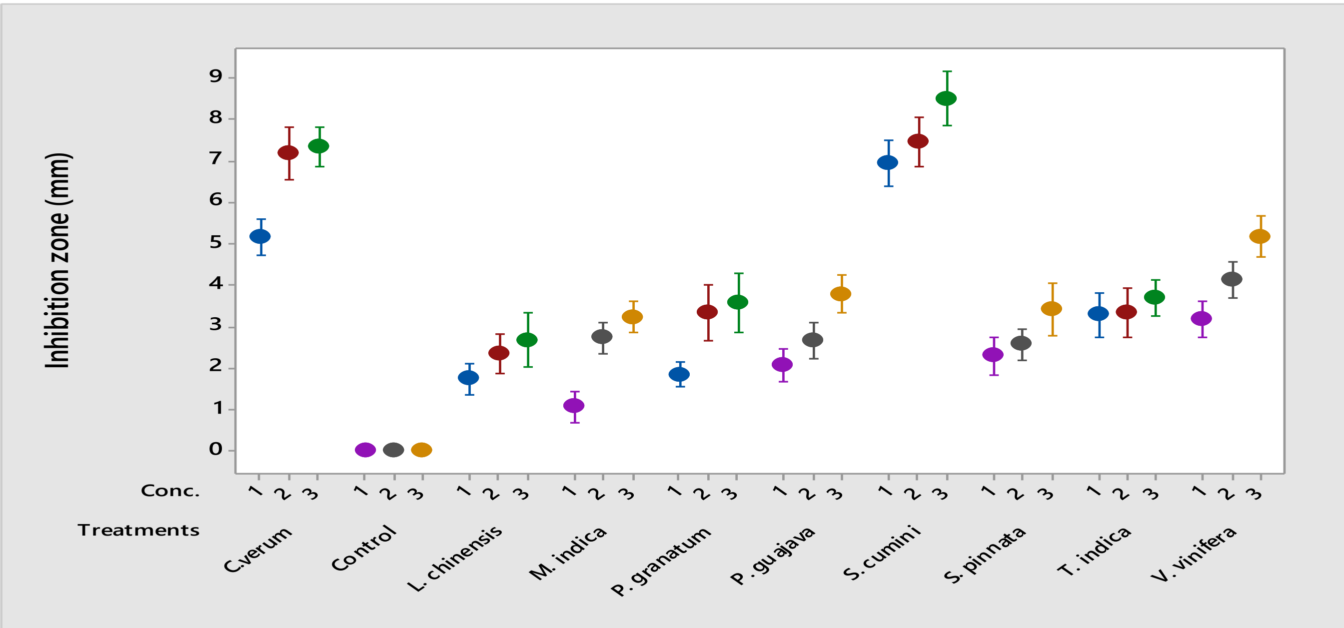

Fig. 2 Evaluation of interaction b/w treatments and concentrations against Pectobacterium atrosepticum under lab. Conditions

Interaction between treatments and concentrations indicated that C1 concentration of *S. cumini* (6.955) expressed maximum inhibition zone as compared to *C. verunn*

(3.344), *M. indica* (2.733), *L. chinensis* (2.344) mm and at C3 concentration exhibited 8.511,7.344, 5.177, 3.677, 3.566, 3.788, 3.400, 3.566, 2.677mm inhibition zone of treatments as compared to control respectively (Fig. 2).

While interaction between treatments and hours showed that *S. cumini* (6.844, 7.622, 8.455) exhibited maximum inhibition zone and *L. chinensis* (1.566, 2.233, 2.955) expressed minimum inhibition zone at H1, H2 and H3 as compared to control respectively. (Fig. 3).

chemicals leads to environmental along with health hazards (Russell, 2005). These are persistent in nature and left their residues in the surroundings for many years. Several plant species have been reported to possess antifungal potential (Singh et al., 2004). These naturally occurring ingredients in plants with adequate concentrations are less phytotoxic, safe, ecofriendly, easily biodegradable and

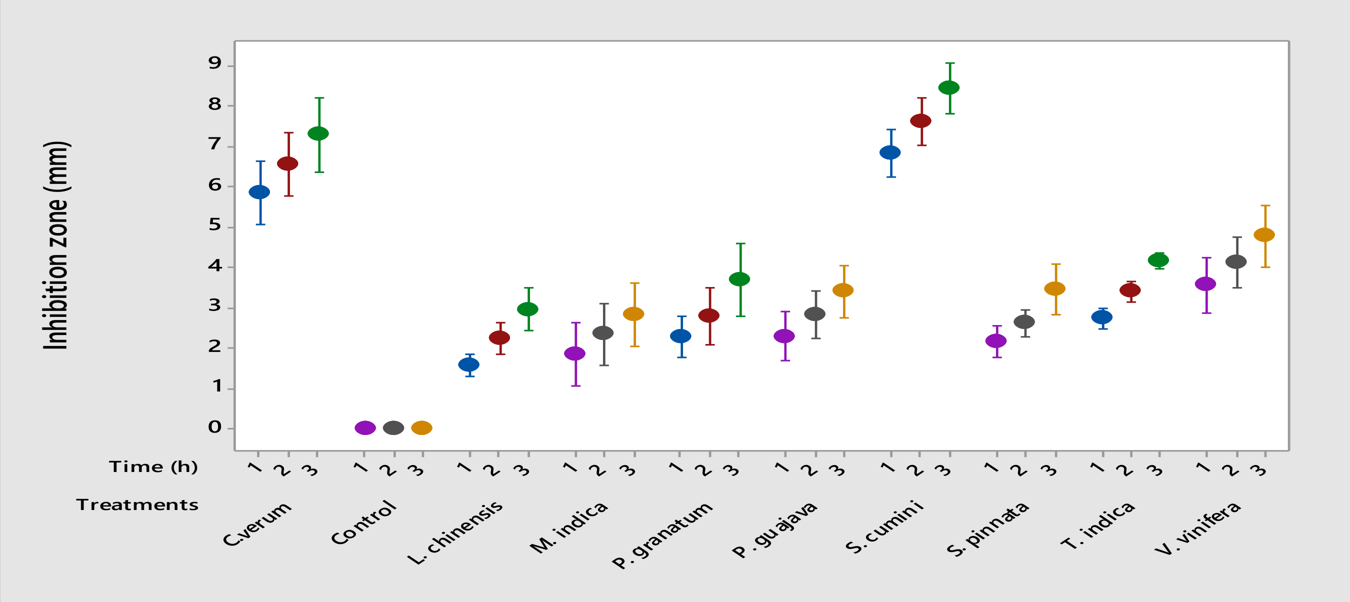

Fig. 3 Evaluation of interaction b/w treatments and time against Pectobacterium atrosepticum under lab. conditions

4. DISCUSSION

In potato and other many crops, the disease and maceration of plant tissue cause by most important pectolytic bacteria (*E. chrysanthemi* and *Erwinia carotovora*) (Duarte et al. 2004). The soft rot of stem and inky black symptoms progresses the severe blackleg disease in the field. During storage, the diseased potato tuber showed black soft rot and die the plants which affected by pathogen. By plant debris and potato tubers the pathogen is carried but if environmental conditions are not favourable the pathogen does not develop the disease symptoms and pathogen present in inactive form. The bacteria transmitted from diseased to healthy tubers and cause rotting, in the potato crop cause great loss not only in stored potatoes but also in the field. It causes blackleg under high temperature but mainly causes stem rot. The major source of infection in potato that produce the disease is infected or rotting tuber and infested crop residues. (Ali et al. 2014). No doubt chemicals provide rapid and easy control towards disease, but continuous use of these

translocated (Sitara et al., 2008). These botanical pesticides are thought to be an alternative of chemicals which are much detrimental to plants, animals and human beings (Asthana, et al., 2001). Plants extracts are used in numerous formulations like essential oils, cakes, spray, dipping solution, fumigants and water solutions (Siripornvisal and Ngamchawee, 2011) So, plant extracts provide a safe and eco-friendly way to manage blackleg disease of potato (Cuthbertson and Murchie, 2005). So, in contemporary study nine plant extracts were evaluated and it was found that reduction in bacterial growth in lab. condition and minimum incidence of blackleg was expressed by *S. cumini*. Results of the current study are supported by the work of Wang et al., (2004) evaluated 88 plant extracts against *Pectobacterium* and concluded that among 88, 19 plant extracts showed significant results in reducing bacterial growth. Outcomes of the present study are also supported by the work of Alabi and Olorunju, (2004) who concluded that plant extracts have a variety of antimicrobial compounds which not only control plant diseases but also have a potential to increase yield of potato. Method of application and suitable formulation of plant products is very much important for disease management. Commercialization of ecofriendly plant products especially *S. cumini* products, can be further improved by the addition of synthetic additives and improved application methods.

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