Original Research Article

Studies on Bioefficacy of Aqueous Plant Extracts against *Pectobacterium carotovorum* causing Black Leg and Soft Rot of Potato

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Abstract

Studies were conducted in the Department of Plant Pathology, Odisha University of Agriculture and Technology, Bhubaneswar following inhibition zone technique to test the bio-efficacy of plant extracts in vitro against *Pectobacterium carotovorum* causing pre-emergence and post-emergence rotting, black leg in field soft rot in potato. The seeds of locally grown trees, spices and weeds are used in the tests. These were *Terminalia chebula* (Chebulic myrobalan), *T. belerica* (Beleric myrobalan), *Emblcia officinalis* (Indian goose berry), (Azadirachata indica) (Neem), (Greater cardamom), *Rauvolfia serpentina* (Snake root), *Coriandrum sativum* (Dhania), *Cuminum cyminum* (Cumin), *Nigella sativa* (Black cumin), *Foeniculum vulgare* (Fennel), *Piper nigrum* (Black pepper), *Cassia fistula* (Indian laburn), *Cassia tora* (Senna tora). It was observed that *R.serpentina* exhibited maximum size of inhibition zone 13.53 followed by *A.subulatum* (11.33) against the test bacterial species. The inhibition zone was found to be 10.0mm in seed extracts of *T.chebula* and dried flower extracts of *Syzygium caryophyllus* while 9.30mm in *F.vulgare*, 8.68 in *C.cuminus* and 8.03mm in *N.sativa*. There was no statistically significant difference in inhibiting activity between *A.aromaticum* (7.97mm) and *A.indica* (7.92mm). The inhibition was same (7.01mm) in both *T.bellirica* and *C.viscosa*. In *P.nigrum*, *C.fistula* and *C.tora* also expressed same inhibiting activity (6.95mm). Minimum inhibition zone was observed in *E.officinalis* (6.68mm).

Keywords

Bioefficacy, Aqueous plant extracts, Antibacterial activity, Black leg and Soft rot

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Introduction

Potato (*Solanum tuberosum*) is considered as the most potential and nutritionally superior crop for fighting against hunger in both developing and under developed countries. Globally potato diseases mainly revealed the presence of 30 fungal, 7 bacterial and 36 viral diseases causing loss individually or collectively to the crop. The blackleg and soft rot cased by *Pectobacterium carotovorum* is an important disease of potato of the country, damaging the crop during pre and post emergence stage, growing period, harvest and post harvest operations like storage, transport and also at consumer level. The disease was found to be severe in immaturely harvested and peeled tubers while transported under poorly ventilated condition and under normal package of practices, i.e. 4% in north western
plains and 10% in eastern and peninsular India and 4-8% in hills (Somani and Shekhawat, 1990). In different locations of Odisha the black leg incidence varied from 1.35 to 4.36% in growing period and 4.19 to 6.47% of soft rot of tubers during harvest (Biswa and Dhal, 2013). The use of plant products have remarkable effects in plant disease management (Cowan, 1990 and Newman et al., 2000). The use organic farming is now gaining popularity. Different parts of many plants have antimicrobial properties. In this context the seeds of some commonly grown trees, weeds, medicinal plants and spices were tested against *P. carotovorum* causing black leg and soft rot of potato.

The seeds used in the studies were *Terminalia chhebula* (Chhebulic myrobalan), *T. belerica* (Beleric myrobalan), *Emblica officinalis* (Indian goose berry), *Azadirachata indica* (Neem), (Greater cardamom), *Rauwolfia sepentina* (Snake root), *Coriandrum sativum* (Dhania), *Cuminum cyminum* (Cumin), *Nigella sativa* (Black cumin), *Foeniculum vulgare* (Fennel), *Piper nigrum* (Black pepper), *Cassia fistula* (Indian laburn), *Cassia tora* (Senna tora) as well as dry flower buds of *Syzygium aromaticum* (clove) were collected (Table 1). These were washed several times in sterilized water and air dried. Fifty grams from selected seeds and dried flower buds along with 50ml of double distilled water were taken grinded with the help of pestal and mortar to a fine pulp. The pulp was filtered through two layers of muslin cloth and gently pressed to get maximum filtrate.

The filtrate from each plant part was collected and kept separately in different sterile specimen tubes and centrifused at 1500 rpm for 15 minutes. The supernatant liquid was drawn carefully into a 5ml syringe and then passed through membrane filter of 0.45nm size to sterilize the extract. The filter sterilized extract of each part collected in sterilized specimen tube with screw cap and stored in deep freeze maintained at -20°C. The extracts were evaluated in vitro following the inhibition zone technique.

In this technique, two drops of bacterial suspension of each test bacterium were transferred on to the petriplate containing NSA medium and spreaded over the surface of the medium with the help of a sterilized glass spreader. Three sets of Hi-media discs (5mm), soaked for one minute in each plant extracts were placed on the media surface of each petriplate at the equidistance from the centre. In each set four numbers of discs were used to hold sufficient quantity of the plant extract. Two sets of petridishes were used for testing each plant extract petriplates were incubated at 27±1°C for 24 hours in a BOD incubator. After the incubation period, the

Materials and Methods

The clean and healthy seeds of fifteen plants, i.e. *Terminalia chhebula* (Chhebulic myrobalan), *T. belerica* (Beleric myrobalan), *Emblica officinalis* (Indian goose berry), (Azadirachata indica (Neem), (Greater cardamom), *Rauwolfia sepentina* (Snake root), *Coriandrum sativum* (Dhania), *Cuminum cyminum* (Cumin), *Nigella sativa* (Black cumin), *Foeniculum vulgare* (Fennel), *Piper nigrum* (Black pepper), *Cassia fistula* (Indian laburn), *Cassia tora* (Senna tora) as well as dry flower buds of *Syzygium aromaticum* (clove) were collected (Table 1). These were washed several times in sterilized water and air dried. Fifty grams from selected seeds and dried flower buds along with 50ml of double distilled water were taken grinded with the help of pestal and mortar to a fine pulp. The pulp was filtered through two layers of muslin cloth and gently pressed to get maximum filtrate.

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petriplates were examined for development of inhibition zone around the discs. The diameters of each zone of inhibition was measured and recorded and analysed statistically to assess the antimicrobial properties of plant extracts against each test bacterium (Gomez and Gomez, 1984). In control the paper discs were soaked in sterilized water.

**Results and Discussion**

All the selected aqueous plant extracts exhibited various levels of antibacterial activity against *P. carotovorum*, the test bacterial species (Table 2). The aqueous seed extracts of *R. serpentina* exhibited highest length of inhibition zone 13.53 (Fig.1) followed by *A. subulatum* (11.33mm) against the test bacterial species. The inhibition zone was found to be 10.0mm in seed extracts of *T. chebula* while 9.30mm in *F. vulgare*, 8.68 in *C. cyminum* and 8.03mm in *N. sativa*.

There was no significant difference in antibacterial activity between *A. aromaticum* (7.97mm) and *A. indica* (7.92mm). The antibacterial activity was as par as in *T. bellirica* (7.00mm) and *C. viscosa* (7.01mm) while in *C. sativum* it was 7.28mm. The seed extracts of *P. nigrum*, *C. fistula* and *C. tora* expressed same activity (6.95 mm). Minimum inhibition zone (Fig.2) was observed in *E. officinalis* (6.68mm). No zone of inhibition of bacterial growth was observed in control. The inhibition zone in aqueous seed extracts ranged from 6.68mm to 13.53mm (Fig.3).

Several workers reported on the antimicrobial properties of above mentioned seed extracts and also the different parts of respective plants. Negi et al (2014) recorded the antibacterial activities of *R serpentine*. In *A. subulatum* bio-chemical and biological activities had been studied by Bisht et al., (2011).

Antibacterial activity of black myrobalan (*Terminalia chebula*) against *Helicobacter pylori* had been studied and reported (Malekzadeh et al., 2001). Rathre and Qureshi (2016) compiled the traditional uses and pharmacological behaviour of *F. vulgare*. Lacobellis et al., 2005 reported the antibacterial activity of *C. cyminum*. Parihar et al (2012) detected the antioxidant immunomodulatory and antimicrobial activity of *Amomum aromaticum* against *Klebsiella pneumonia*. Dharmarate et al (2018) reported antibacterial properties of *T. bellirica* against selected multi drug resistant bacteria.

Datta and Kundabala, (2013) studied the antimicrobial efficacy of endodontic irrigants from *Azadirachta indica*. Saeed and Tariq (2007) reported the antimicrobial activities of *Emblica officinalis* and *Coriandrum sativum* against gram positive bacteria and *Candida albicans*. Kalane et al., (2011) studied the antimicrobial activity of *Cassia tora*. Zou et al., (2015) worked on antibacterial mechanism and activities of black pepper chloform extract.

The antibacterial activity of black myrobalan (*Terminalia chebula*) against *Helicobacter pylori* had been reported (Malekzadeh et al, 2001). Mnif and Aifa (2015) compiled the beneficial effect of cumin (*Cuminum cyminum L.*) from traditional uses to potential biomedical applications. Raja Ratna Reddy et al., (2016) recorded the antimicrobial activity of *Azadirachta indica* (neem) leaf, bark and seed extracts. And Nunez and Aquino (2012) recorded antimicrobial property of *S. aromaticum*.

The inhibition zone in aqueous seed extracts ranged from 6.68mm to 13.53mm (Fig.3). It was indicated all the seeds used in the test had antibacterial properties against *P. carotovorum*. 

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| Sl.no | category       | Scientific Name       | Common name (English) | Family   | Traditional uses                                                                                                                                 |
|-------|----------------|-----------------------|-----------------------|----------|--------------------------------------------------------------------------------------------------------------------------------------------------|
| 1     | Medicinal tree | Terminalia chebula    | Chebulic myrobalan    | Combretaceae | It is used in treatment of constipations, colic pain, kidney dysfunction, eye diseases and sore throat. (https://vikaspedia.in/agriculture,Basa et al.,2017) |
| 2     | Medicinal tree | T.belerica            | Beleric myrobalan     | Combretaceae | It is used in treatment of constipations, colic pain, kidney dysfunction, eye diseases and sore throat. Seeds are edible(Kumar et al.,2018)          |
| 3     | Medicinal tree | Emblica officinals    | Aonla                 | Euphorbiaceae | Used in treatment of constipations, colic pain, kidney dysfunction, eye diseases and sore throat.(Sharma et al.,2003)                             |
| 4     | Spices         | Amomum subulatum      | Greater cardamom      | Zingiberaceae | It is antimicrobial cardiac stimulant, carminative, diuretic stomachi (Bisht et al.,2011)                                                          |
| 5     | Medicinal plant| Rauvolvia serpentina  | Snake root            | Apocynaceae | Used for various ailments such as snakebites, insomnia, hypertension and insanity (Singh et al., 2017,Negi et al.,2014)                             |
| 6     | Weed plant     | Cleome viscosa        | Wild mustard          | Cleomaceae | Used against fever, diarrhea, cardiac stimulant and carminative (Perumal Samy et al.,1999)                                                          |
| 7     | Spices         | Coriandrum sativum    | Coriander             | Apiaceae | Seeds are antidiabetic, anti-inflammatory and lowers cholesterol. It is used as diuretic, carminative, stimulant, nagelstein, antihelminetic, hypoglycaemic (Waheed et al.,2006) |
| 8     | Spices         | Cuminum cuminum       | Cumin                 | Apiaceae | Seeds used as food additive, popular spice, flavouring agent in many cuisines. It is used against hypolipidemica, cancer and diabetes(Mnif and Aifa ,2015) |
| 9     | Tree           | Azadirachata indica   | Neem                   | Meliaceae | Anti oxidant, antimalarial, antimutagenic, anticarcinogenic, anti inflammatory, antihyperglycaemic, antiulcer and anti diabetic purposes (Venugopalan and Visweswaran, 2013) |
| 10    | Shade tree     | Cassia tora           | Senna tora            | Fabaceae | Used against leprosy, bronchitis and cardiac disorders (Maity et al.,1998)                                                                         |
| 11    | Spices         | Nigella sativa        | Black cumin           | Ranunrulaceae | Commonly used for culinary and medicinal purposes as a remedy of hypertension and diabetes and as hypoglycemic, anti-inflammatory, antiulcer and broncho dilator (Bereksi et al., 2018) |
| 12    | Spices         | Foeniculum vulgare    | Fennel                | Apiaceae | Fennel seeds helps in digestion, prevents acne, mouth fresher, beats bad breath(Al-Timimi ,2019)                                                   |
| 13    | Spices         | Piper nigrum          | Black pepper          | Piperaceae | It is used as spice. It exhibit sedating, detoxification, hypotensive and anticancer activities. (Butt et al., 2012)                              |
| 14    | Spices         | Amomum aromaticum     | Aromatic cardamom     | Zingiberaceae | Seeds are used to make a gangle or mouth wash to treat toothache, gingivitis and parodontosis. Seeds are antibacterial and use against stomachic, alleviate dyspepsia, fatulance, colic, vomiting, diarrhoea, cough (Basak et al., 2017) |
| 15    | Shade tree     | Cassia fistula        | Indian laburnum       | Fabaceae | The bark is used in treatment of inflammatory swellings and as a cleaning agent for ulcers and wounds. It is believed to decrease purulent discharge and act as local antiseptic. The seeds are are antibilious, asperitif, carminative and laxative (Ajaya Kumar, et al., 2017) |
| 16    | Spices         | Syzygium aromaticum   | Cloves                | Myrtaceae | The dried flower buds contain high antioxidants, regulate blood sugar, reduce stomach ulcers, may promote bone health (Chaieb et al.,2007)            |
Table.2 Inhibition of growth of bacterial species by aqueous seed extract under *in vitro* condition

| Sl.No | Scientific Name         | Common name (English) | Local name   | Diameter of inhibition zone in mm |
|-------|-------------------------|-----------------------|--------------|-----------------------------------|
| 1     | *Terminalia chebula*    | Chebulic myrobalan     | Harida       | 10.00(3.24)                       |
| 2     | *T.myrobalan*           | Beleric myrobalan     | Bahada       | 7.00(2.74)                        |
| 3     | *Emblica officinals*    | Aonla                 | Aonla        | 6.68(2.68)                        |
| 4     | *Amomum subulatum*      | Greater cardamom      | Bada alaicha | 11.33(3.44)                       |
| 5     | *Rauvolfia serpentine*  | Snake root            | Patal garuda | 13.63(3.72)                       |
| 6     | *Cleome viscosa*        | Wild mustard          | Banasorisha  | 7.01(2.74)                        |
| 7     | *Coriandrum satium*     | Coriander             | Dhania       | 7.28(2.79)                        |
| 8     | *Cuminum cyminum*       | Cumin                 | Jeera        | 8.68 (3.03)                       |
| 9     | *Azadirachata indica*   | Neem                  | Nimba        | 7.92(2.86)                        |
| 10    | *Cassia tora*           | Senna tora            | Chhota chakunda | 6.95(2.73)                       |
| 11    | *Nigella sativa*        | Black cumin           | Kala jeera   | 8.03(2.92)                        |
| 12    | *Foeniculum vulgare*    | Fennel                | Panamadhuri  | 9.30(3.13)                        |
| 13    | *Piper nigrum*          | Black pepper          | Golamarcha   | 6.95(2.73)                        |
| 14    | *Amomum aromaticum*     | Aromatic cardamom     | Alaicha      | 7.97(2.91)                        |
| 15    | *Cassia fistula*        | Indian laburnum       | Sunari       | 6.95(2.73)                        |
| 16    | *Syzygium caryophyllus* | Clove                 | Labanga      | 10.00(0.71)                       |
| 17    | Sterilized Water        |                       |              | 0.00(0.71)                        |

Figures in parentheses are in \(\sqrt{x+0.5}\) transformed values
Fig.1 Inhibition zone observed in *R. serpentina*, Fig.2-Inhibition zone observed in *E. officinallis*

Fig.3 Histogram showing zone of inhibition (in mm) in different aqueous extracts against the test pathogen

The antibacterial property of *T. chhebula* (Chhebulic myrobalan), *T. belerica* (Beleric myrobalan), *E. officinalis* (Indian goose berry), *A. subulatum*, (Greater cardamom), *R. serpentina* (Snake root), *C. sativum* (Dhania), *C. cymimum* (Cumin), *N. sativa* (Black cumin), *Foeniculum vulgare* (Fennel), *Piper nigrum* (Black pepper), *A. aromaticum* (aromatic cardamom) against *P. carotovorum* are reported to be new in India. Use of such extracts as tuber treatment at planting time, basal drenching in field and also tuber treatment in storage after harvest are to be tested.

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