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

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A B S T R A C T

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 storage in potato. The seeds of locally grown trees, spices and weeds are used in the tests. These were *Terminalia chhebula* (Chhebulic myrobalan),*T. belerica* (Beleric myrobalan), *Emblica 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 be10.0mm in seed extracts of *T.chebula* and dried flower extracts of *Syzygium caryophyllus* while 9.30mm in *F.vulgare*, 8.68 in *C.cyminum* 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.officinallis* (6.68mm).

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 (Biswal 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), *Rauvolfia 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). Hence the present investigation comprising *in vitro* testing of abovementioned seed extracts was conducted in the Department of Plant Pathology, Odisha University of Agriculture and Technology, Bhubaneswar following inhibition zone technique (Valgas *et al.*, 2007).The study was undertaken keeping in view of use such extracts as tuber treatment at planting time, basal drenching in field and also tuber treatment in storage after harvest.

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), *Rauvolfia 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* (cloves) 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 filterate.

The filterate from each plant part was collected and kept separately in different sterile specimen tubes and centrifused at 1500 rpm for 15minutes.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 -20^0 C. The extracts were evaluated *in vitro* following the inhibition zone technique.

In this technique, two drops of bacterial suspension of each test bacterium was 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 ± 1^0C for 24 hours in a BOD incubator. After the incubation period, the

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 be10.0mm 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.officinallis* (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*. Dharmaratne etal (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 anti microbial 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.*

Table.1 Scientific name, common name, family and traditional uses of test plants

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*	Belericic 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*	*Rauvolfia serpentina*	Snake root	Apocynaceae	Used for various aliments 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,nagelsteic,antihelmtic,hypoglycaemic (Waheed *et al*,2006)
8	*Spices*	*Cuminum cyminum*	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 antidiabetic 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 freshner,,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 paradontosis. 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*	Belericic 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	Golamaricha	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)
	SE(m)±			0.07
	CD(P=0.05)			0.21

Figures in parentheses are in √x+0.5 transformed values

Fig.1 Inhibition zone observed in *R serpentina*, **Fig.2**-Inhibition zone observed in *E.officinallis*

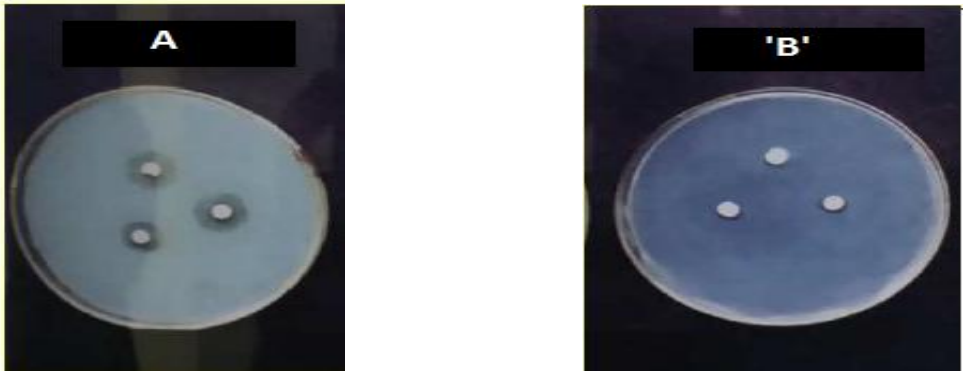
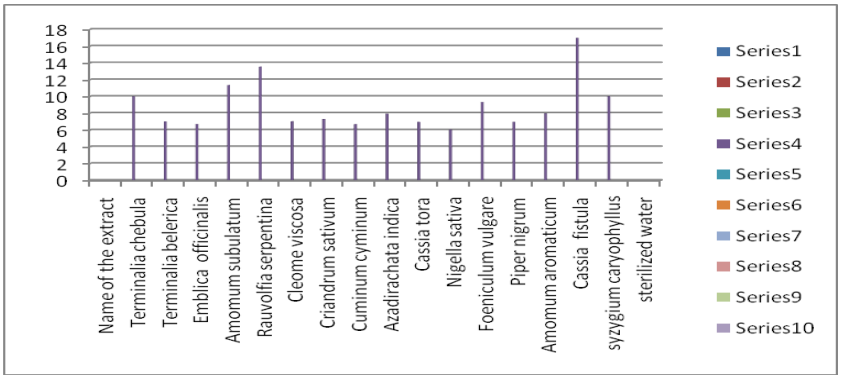


Fig.3 Histogram showing zone of inhibition (in mm) in different aqueus extracts against the test pathogen



The antibacterial property of *T. chhebula* (*Chhebulic myrobalan*), *T. belerica* (*Beleric myrobalan*), *E. officinalis* (Indian goose berry), (*A. subulatum)*, (Greater cardamom), *R. sepentina* (Snake root), *C. sativum* (Dhania), *C. cyminum* (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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