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IN VITRO ACTIVITY OF RHIZOME AND BULB EXTRACTS ON *XANTHOMONAS AXONOPODIS* PV. *VESICATORIA* (DOIDGE) DYE IN CHILLI SEEDS GROWN IN RAJASTHAN

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The rhizome and bulb extracts of few medicinal plants were used to study their antimicrobial efficacy against *Xanthomonas axonopodis* pv. *vesicatoria* (Doidge) Dye, causal agent of bacterial leaf spot disease in chilli. For the seed treatment onion (*Allium cepa*), garlic (*A. sativum*) and ginger (*Zingiber officinale*) extracts were tested in filter paper disc assay, seeded agar method and seed treatment methods at pure and diluted concentrations. The extract of garlic followed by ginger found most effective improves seed germination and control of pathogen at 100% concentration as compared to check. The study indicates the effectively controlled of the pathogen and reduced the disease incidence significantly in non-hazardous manner and environment friendly.

Keywords: Antibacterial control; bacterial incidence; rhizome and bulb extracts; chilli; *Xanthomonas axonopodis* pv. *vesicatoria*.

Introduction

Bacterial leaf spot (BLS) disease of sweet pepper (*Capsicum annuum*) and tomato (*Lycopersicon esculentum*) caused by *Xanthomonas axonopodis* pv. *vesicatora* is recorded from several countries of eastern and southern Africa, USA, Ethiopia, Kenya, Malawi, Mozambique and South Africa[1,2]. In India, bacterial leaf spot disease was first reported from Pune, Maharastra in 1948 by Patel *et al.* in chilli. In Rajasthan, the disease is responsible for 7.5 to 16.6 per cent loss at 22-34°C temperature with high humidity[3,4].

Xanthomonas axonopodis pv. *vesicatora* (Doidge) Dye (syn: *Xanthomonas campestris* pv. *vesicatoria*) (XAV) a gram-negative, rod-shaped bacterium attack on host plant[5,6]. The isolates were gram's negative, KOH solubility test positive, levan negative, lipase activity positive, oxidase negative, starch hydrolyzing, gelatin hydrolyzing, arginine variable, produced as circular, raised, yellow, mucoid colonies on Tween-80 agar medium, did not reduce nitrate and no rotting of potato tissue, induced positive hypersensitivity reaction on tobacco leaves by local necrosis and desiccation. XAV suspected from symptoms fruit was confirmed by isolation on semi-selective media including Tween B[7,8].

XAV was found seed-borne (10-15%) also subsists on infected plant debris, weeds and volunteer tomato plants[9]. The losses in marketable fruits may be more than

50%[10,11]. The incidence was less than 5% persisted from one season to next in crop debris or on weed hosts[12]. The bacterial identification studied by available detailed description of bacterial species described by various scientists[13-19].

Materials and methods

Seed treatment bulb and rhizome extracts in SBM- Fresh and health looking (without any symptoms) samples as garlic (*Allium sativum*), onion (*A. cepa*) and ginger (*Zingiber officinale*) were bought from the market and preserve in aseptic conditions for the test against *Xanthomonas campestris* pv. *vesicatoria.*

In the experiment, 10 g fresh bulb/rhizome were washed thoroughly and crushed in double sterile distilled water at the rate of 1g tissues in 1 ml of water (1: 1 w/v) using pestle and mortar and filtered through double layered cheesecloth. The filtrate was treated as stock solution.

Two seed samples of chilli, Ca-1227 and Ca-1234 naturally infected with XAV were treated individually with the aqueous extracts of plant material for 4 hrs in two different concentrations, pure (100%, w/v) and diluted (30% v/v) in triplicate (100 seeds/ sample). Seeds soaked in sterile distilled water were treated as check. All the treated and untreated seeds were incubated on moistened blotter papers and per cent seed germination, seedling symptom, incidence of the bacteria and inhibition of the pathogen observed on 8th days in standard blotter method[20]. The percent control of pathogens was calculated by the following formula-

[Percent control = Incidence in check (C) - incidence in treatment (T) /Incidence in check (C) x 100]

Filter paper disc assay and seeded agar method- Two another methods namely seeded agar method and filter paper disc method (disc diffusion method) were also carried out to find out the antibacterial activity of plants parts against XAV. Bacterial suspension (10 ml) of test bacterium was spread by sterile L-rods on nutrient agar medium. Filter paper discs of 8 mm diameter impregnated with extracts were placed in the inoculated plates similarly the filter paper discs soaked in double sterile distilled water placed in the middle of the plate and incubated at 30±2° C for 48 hrs. In seeded agar method the wells (8 mm diameter) on nutrient agar medium (already seeded) using sterilized cork borer were yielded. The (1 ml v/v) crude form of suspension of bulb/rhizome extracts was place in wells by using sterile syringe. The diameter or zone of inhibition was recorded upto 6 days in intervals of 24 hrs at 25 ± 2°C for each test agent.

The inhibition zones were measured in diameter (mm) around of the discs and calculated to compare the antibacterial activity of the test plant extracts. The inhibition annulus was calculated by following formula[21-24].

[Activity index (AI) = Inhibition zone of sample/ Inhibition zone of standard]

Results and Discussion

Aqueous garlic extract is made up of antioxidant ingredient used as a broad spectrum antibiotic, killing bacteria, fungus and viruses. The antimicrobial activity of aqueous garlic extract against selected microorganisms namely *Salmonella entercolitis, Escherichia coli, Enterococcus faecium, Pseudomonas aeruginosa, Staphylococcus aureus, Acetobacter aceti, Xanthomonas campestris, Lactobacillus plantarum, Streptococcus pyogenes, Leuconostoc gasicomitatum, Mucor* spp., *Saccharomyces cerevisae, Penicillium notatum, Aspergillus flavus* isolated from spoilt tomato fruit was determined using

Table 1. *In vitro* effects of seed treatment of rhizome/bulb extracts on seed germination and incidence of *Xanthomonas axonopodis* pv. *vesicatoria* in chilli

Rhizome/ bulb extracts	Treatment (Conc.) (%) (w/v)	SEED SAMPLES					
		Ca-1227			Ca-1234		
		Seed germination (%)	Incidence of pathogen (%)	Control of pathogen (%)	Seed germination (%)	Incidence of pathogen (%)	Control of pathogen (%)
Check	-	66.7 (54.76)	63.3 (52.71)	0(0)	72.5 (58.37)	60.0 (50.77)	0 (0)
Zinger	**100**	73.3** (58.89)	36.7** (37.29)	42.1** (40.46)	82.5** 65.27)	42.5 (40.69)	29.2 (32.71)
	30	73.3 (58.89)	46.7 (43.11)	26.2 (30.79)	80.0 (63.44)	47.5 (43.57)	20.3 (26.78)
Onion	**100**	63.3 (52.71)	47.5 (43.5)	24.96 (29.76)	77.5 (61.68)	50.0 (45.0)	16.7 (24.12)
	30	60.0 (50.77)	60.0 (50.77)	5.6 (13.69)	77.5 (61.68)	52.5 (46.43)	12.7 (20.88)
Garlic	**100**	86.7** (68.61)	23.3** (28.86)	63.5** (52.83)	90.0**(71.56)	30.0** (33.21)	50.0** (45.0)
	30	80.0** (63.44)	33.3** (35.24)	47.6** (43.62)	87.5** (69.30)	35.0** (36.27)	41.7** (40.22)
CD at 5%	-	6.20	5.64	11.91	4.52	5.05	12.80
CD at 1%		8.60	7.82	16.59	6.15	6.87	17.42

Values are the mean of 3 replicates. Values in parentheses are angular transformed values.

Table 2. *In vitro* evaluation of antibacterial activity of some plant parts extracts against seed-borne bacteria in chilli and tomato

S. No.	Extracts	Parts used	Concentration wt/v	IZ (mm)	AI
1.	Garlic (*Allium sativum*)	**Bulb**	**100**	**25.33**	**3.17**
			50	**19.67**	**2.46**
			25	**18.00**	**2.25**
2.	Ginger (*Zingiber officinale*)	**Rhizome**	**100**	**16.33**	**2.04**
			50	**14.00**	**1.75**
			25	**12.67**	**1.58**
3.	Onion(*Allium cepa*)	**Bulb**	**100**	**12.67**	**1.58**
			50	**11.33**	**1.38**
			25	**10.33**	**1.21**

* Diameter of filter paper (well) disc (8 mm) included inhibition zone in check.

diffusion method from Nigeria. The aqueous extract of garlic between 10 to 40 mg/ml in concentration possesses antimicrobial properties on these organisms except *A. flavus*[25]. Pathogenic intestinal bacteria which are diarrheagenic in humans and animals have been reported to be easily inhibited by garlic[26]. The minimum inhibitory concentrations (MIC); 40 mg/ml of aqueous garlic extract on *Staphylococcus aureus* was determined [27]. Garlic and onion has been in use since ancient times in India and China for a valuable effect on various diseases to inhibit the food borne pathogens like *Salmonella, Shigella* and *S. aureus*and to cure several critical diseases[28-31]. The aqueous garlic extract has been attributed to the presence of thiosulfinates (e.g, *allicin*) which found effective against microorganism[32].

The improvement in the seed germination in infected seeds was 86.7 and 90% after treating the seeds with extract of bulb of garlic at 100% concentration

followed by extract of rhizome of ginger (73.3 and 82.5%) at same concentration in ac. nos. Ca-1227 and Ca-1234 as compared to check (66.7 and 72.5%) respectively. The reduction in incidence of the pathogen at 100% concentration was (23.3 and 30%) in extract of bulb of garlic followed by extract of rhizome of ginger (36.7 and 42.5%) in both the samples as compared to check (63.3 and 60%) respectively. The per cent control of the pathogen was 63.5 and 50% at 100% concentrations due to extract of bulb of garlic followed by extracts of rhizome of ginger at same concentrations (42.1 and 29.2%) in both the samples respectively (Table 1).

In filter paper disc assay and seeded agar method the rhizome/bulb extracts were used at 100, 50 and 25% concentration to evaluate inhibition of bacterial pathogens. Out of 3 extracts, the highest inhibition zone and activity index were shown by extract of garlic (25.33 mm and 3.17) followed by ginger (16.33 and 2.04) extract of onion (12.67 mm and 1.58) at 100% concentration against *X. c.* pv. *vesicatoria* (Table 2).

The reduction in incidence and improvement in seed germination by pathogens *Pseudomonas syringae* pv. *syringae* and *Ralstonia solanacearum* was reported extracts of ginger and garlic at 100% concentration in chilli[33]. Different combination of bioagents for the biocontrol of *Ralstonia solanacearum* were tried[34-36]. The maximum reduction in primary inoculum was found in those treatments in which plant extracts were applied *i.e.* garlic extract (-0.0821 unit/day) followed by *Trichoderma viride* (-0.0386 unit/day) *Azotobacter chrookocum* + phosphobacterin (-0.0365 unit/day) and onion extract (-0.0106 unit/day). In 60 days recording, maximum reduction was found in *Glomus mosseae* treated plot followed by onion extract (-0.0225 unit/day) and hing + tunner power (-0.038 unit/day), whereas at 90 days treatment with bleaching powder + lime (-0.088 unit/day) caused maximum reduction of inoculum.

A biformulation (Plb. 3 isolate of *Bacilluis* spp.) + inert carrier (talc) at different ratio + carboxy methyl cellulose at 10% of total weight (spore power and talc as sticker) was found to be effective in producing inhibition zone of varying sized in spot test against different pathovars like *X. axonopodis* pv. *campestris* (13 mm), *X. a.* pv. *citri* (20 mm), *X. a.* pv. *malvacearum* (10 mm), *X. a.* pv. *Mangiferaeindicae* (16 mm) and *X. oryzae* pv. *oryzae* (18 mm)[37].

Bhaudharia and kumar (2004)[24] used *Lawsania innermis, Solanum dalcamara* and *Allium sativum* against pathogenic bacteria *Escherichia coli, Eubacter aerogens, Proteus mirabilis* and *Staphylococcus aureus* by filter paper disc method. *Lawsania innermis* found effective as compared to antibiotics (Check).

Plants and their derivatives are good alternative source to managing microbial diseases due to presence of various secondary metabolites or bioactive compounds used as natural pesticides[38-40]. The plant extract are environmentally safe and alternatives chemicals in integrated disease management programs [41] because chemicals having several hazardous effect and accumulate in animal tissues. So the medicinal plant tried against pathogen due to bioactive and biochemical compounds that are non-phytotoxic and easily biodegradable [42-47].

Various leaf extracts at 25, 50 and 100% concentration tried against *Xanthomonas axanopodis* pv. *vesicatoria* and *Parthenium hysterophorus* followed by *Lantana camara* in filter paper method and

seeded agar method found effective at 100% concentration[33,47].

Six medicinal plant extracts was tested *in vitro* using filter paper disc assay and seed treatment method against *Pseudomonas syringae* pv. *pisi* in pea for their antibacterial effectiveness and *A. sativum* followed by *T. chebula* found effective. Seed treatment with aqueous extract of *A. sativum* improved seed germination (94.6%) and control the incidence of the pathogen in seeds (85.5%) [48].

Seed treatment with *T. bellirica* and *A. sativum* showed maximum seed germination and control of *Xanthomonas pisi*[49]**.** The antibacterial activity of 30 leaf extracts against 11 strains of *X. campestris* pv. *mangiferaeindicae* tried and out of thirty 12 leaf extracts showed antibacterial activity. It screened 2300 plant species so as to know their antibacterial activity against the bacteria like *Escherichia coli* and *Staphylococcus aureus*[50]. Extract of garlic in hot water showed significant inhibitory effect on *Xanthomonas citri* and *Erwinia carotovora* and human pathogens[51].

In the present study, XAV was controlled by extract of garlic followed by extract of ginger and onion. These extracts showed improvement in seed germination and reduction in incidence of *X. c.* pv. *vesicatoria* as compared to check. The relative percent control of the pathogen was as follows-

Allium cepa* > *Zingiber officinale*> *A. sativum

The methanolic leaf extracts of *Acacia nilotica, Sida cordifolia, Tinospora cordifolia, Withania somnifera* and *Ziziphus mauritiana* against *Bacillus subtilis, Escherichia coli, Pseudomonas fluorescens, Staphylococcus aureus* and *Xanthomonas axonopodis* pv. *malvacearum* has been reported[52]. The aqueous extracts of garlic, clove and onion were found effective against *X. axonopodis* pv. *vignaradiatae* during foliar application[53]. The plant extracts of *Allium cepa, Azadirachta indica, Tamarix aphylla, Vernonia anthelmentica, Plumbago zelanicum* and *Tegetis erecta* showed significant antibacterial activity at 50% concentration against *X. campestris* pv. *campestris in vitro* and showed improved seed germination as compare to streptomycin[54].

In the present study, the activity index by aqueous extracts of *Allium sativum* (25.33), *Zingiber officinale* (16.33) and *A. cepa* (12.67) individually were found most effective against the pathogen at 100% concentration in filter paper and seeded agar method as compared to check. The minimum activity index was shown by garlic (3.17, 2.46 and 2.25), followed by ginger (2.04, 1.75 and 1.58) and onion (1.58, 1.38 and 1.21) at three concentrations 100, 50 and 25% respectively.

Conclusion

The present study revealed that various plant extracts (aqueous) were used for the antibacterial activity of onion (*Allium cepa*), garlic (*A. sativum*) and ginger (*Zingiber officinale*) tested in filter paper disc assay, seeded agar method and seed treatment methods. The extract of garlic bulb followed by rhizome extract of ginger and extract of onion bulb showed improved seed germination and reduction in incidence of *X. c.* pv. *vesicatoria*. The use of these plant extracts found effective to control the diseases in eco-friendly and non hazardous manner.

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