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EXPLORATION OF BOTANICALS EXTRACTS AGAINST BLACK ROT OF CABBAGE CAUSED BY *XANTHOMONAS CAMPESTRIS* PV. *CAMPESTRIS*

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ABSTRACT
An investigation was carried out with an objective to extract and evaluate plant extracts against Black rot of cabbage caused by *Xanthomonas campestris* pv. *campestris* (*Xcc*). Out of 40 plants screened for the antibacterial activities, 9 plants viz., *Bixa orellana*, *Jatropha curcas*, *Lantana camara*, *Polyalthia longifolia*, *Polygonum hydropiper*, *Terminalia arjuna*, *Terminalia chebula, Zingiber officinale* and *Saraca asoca,* were found to have antibacterial property.The methanol extracts of *Polyalthia longifolia* and *Terminalia chebula*and chloroform extract of *Zingiber officinale* were found to be most effective against the pathogen in *in vitro* condition. The growth of the bacterium was inhibited significantly with increasing concentration of three plants extracts. However, the minimum inhibitory .concentrations of all the formulated plant extracts were observed at 0.2%. *P. longifolia* followed by *T. chebula* methanol extract at 0.2% was found to be effective in controlling the black rot of cabbage caused by *X. campestris* pv. *campestris*. The *in-vivo* study shows that the plant extract *Polyalthia longifolia* MeOH (20.91) showed higher disease control than *Terminalia chebula* MeOH (18.32) as compared to the untreated control. However, the streptomycin sulphate (24.66) has higher disease control the plant extracts.

INTRODUCTION

India is the second largest producer of cabbages and other brassica crops which accounts for 7.742 m. MT of the total production of cabbage from an area of 0.372 m. ha. (FAO, 2015). In which West Bengal is the highest cabbage producing state in the country and accounts for 27% of the total production of cabbage in the country. The state produces about 2.18 m. MT of cabbage from an area of 0.08 m. ha. with productivity of 28.0 t/ha. 0.34 lakh MT cabbage(Indian Horticulture Database, 2014). Among the bacterial diseases, Black rot of cabbage caused by *Xanthomonas campestris* pv. *campestris* caused huge losses, which can significantly reduce (> 50%) the yield of crops all over the world under favourable environmental conditions (William, 1980, Gupta, 1991). Further the bacterium is reported to have developed resistance to antibiotics like kanamycin, ampicillin, penicillin , streptomycin and also against the copper fungicide (Alshia *et al.,*2013) and therefore poses a challenge in their control. However, many researchers are exploring naturally occurring compounds constrain the pathogen attack as an alternative management systems to reduce pesticide dependent which is an increasing public concern on environmental issues (Bhardwaj and Sahu 2014, Cuthbertson and Murchie 2005; Singh, 2003; Singh *et al.,* 2014). As plant based bactericide has not yet been developed, the need to work on these has of immense importance. Several plants may contain antibacterial compounds against plant pathogenic bacteria which needs their evaluation. Present investigation was carried out to study the antibacterial activity of some of the potential plant extract formulations *in vitro* as well as *in vivo* condition.

MATERIALS AND METHODS

Isolation of the bacteria

The bacteria infected leave samples were collected from Jaguli Instructional farm, Mohanpur of Bidhan Chandra Krishi Viswavidyalaya, and adjoining farmer's field of the University. The isolation was done following standard techniques. The inoculated plates were incubated at 29 ± 1ºC in BOD incubator and were observed for development of bacterial colonies up to three days. For obtaining pure culture of Xanthomonads, bacterial colonies having translucent, yellow, smooth raised growth and which developed after 72 hrs of incubation were further purified by dilution plate method. Single isolated colonies of above characteristics were transferred to the slants of Potato Sucrose Peptone Agar (PSPA) medium. The bacterial culture thus obtained was preserved at 10ºC in the refrigeration and was used for further studies.

Collection and identification of the plants samples for extraction

Various widely growing plants parts were collected from University campus at Mohanpur, Nadia. The plant samples, duly identified by the department expertise, are dried at 50 ºC.Well dried powdered leaves or aerial part and well ground

seed were packed and used for extraction(Salie *et al.,* 1996).

Methods of extraction

The powdered plants are subjected to sequential extraction using the soxhlet apparatus for 6-8 hrs. Solvents viz., hexane, chloroform and methanol (MeOH) were used for extraction. The crude extract was collected, concentrated in a Buchi Rotary evaporator at 45 p C, transferred in a pre-weighed conical flask and evaporated to dryness (Wang *et al.,* 2004).

Preparation of primary emulsifiable concentrates of the extracts

Dried jelly like substances or oily extracts of known weight, were then made into emulsifiable concentrates by using light solvent naptha (LSN), for testing their efficacy against the test pathogens. Tween 80 was used as a surfactant.

Testing of Bio efficacy of the plant extract

Disk diffusion technique using a paper disc was used for the testing of Bio efficacy of the plant extract. A bacterial lawn was prepared using spread plate technique, 1 ml of the bacterial suspension which is of 0.1 OD was spread upon the PSPA agar plates and allow to dry for 15minutes aseptically under the laminar airflow.A filter paper disc impregnated with plant extracts is placed on bacterial lawn and were incubated at 29 ± 1ºC in BOD incubator for 24 hrs. Zone of Inhibition, i.e., the area of no growth around the disc was then measured and taken as observation. The relative percentage inhibition of the test extract with respect to Streptomycin Sulfate 100 ppm was calculated by using the formula as described earlier (Ajay *et al*., 2003; Gaurav *et al*., 2010).

The relative percentage inhibition by the test extract was calculated by using the following formula (Ajay *et al*., 2003; Gaurav *et al*., 2010).

$$\text{Relative percentage inhibition by the test extract} \quad \frac{(x - y)}{(z - y)} \quad 100$$

Where,

X: total area of inhibition of the test plant extract

Y: total area of inhibition of the solvent

Z: total area of inhibition of the antibiotic

Dose response studies

Dose response studies for the effective extracts against the pathogens were studied in vitro following paper disc method at four different concentrations *i.e.*, 0, 0.1, 0.2, 0.3 and 0.5% and the Minimum inhibitory concentration was determined (Carson and Riley 1995; Canillac and Mourey, 2001).

Evaluation of the selected plant extracts on the black rot of cabbage (*Xanthomonas campestris* pv. *campestris*)

A field experiment was conducted at Nagarukhra, Nadia during 2012-13, in order to test the efficacy of the plant extracts on black rot of cabbage caused by Xcc. The plot sizes were 5m x 5m. All the package of agronomic practices was followed except spraying of any fungicide and antibiotics other than the treatments. The plants spacing was maintained at 45cm x 45cm. The seeds was sown in the seed bed during the month of August and transplanted after one month. The first spraying was done after the appearance of the disease symptoms at the field. The second and third spraying were done 10 and 20 days after spraying and the observations were recorded after each spraying. Last observation was recorded 30 days after first spraying. The disease intensity (PDI) was calculated as percentage of infected leaves of cabbage. The per cent disease intensity (PDI) was computed as (Kashyap and Dhiman, 2010):

$$\text{PDI for severity} \quad \frac{\text{Sum of all disease ratings}}{\text{Total number of leaves} \quad \text{Maximum rating value}} \quad 100$$

For computing the PDI, 0-5 scale was used as follows:

0 = No disease

1 = Up to 20 per cent leaf area under symptoms

2 = between 21-40 per cent leaf area under symptoms

3 = between 41-60 per cent leaf area under symptoms

4 = between 61-80 per cent leaf area under symptoms

5 = > 80 per cent leaf area under symptoms

RESULTS AND DISCUSSION

A total of 40 plants were collected in and around the campus of B.C.K.V, Mohanpur, dried, extracted and used for testing the antibacterial activities against the plant pathogenic bacteria *X. campestris* pv. *campestris*, causal organisms of black rot disease of cabbage and were tested with solvent extracts *viz*, methanol, chloroform and hexane extract using standard technique of paper disc diffusion. The results were as shown in Table 1. Five methanol extracts, *viz*., *Bixa orellana, Polyalthia longifolia,Terminalia arjuna,Terminalia chebula* and *Saraca asoca* were able to show inhibition against the bacterium. Highest zone of inhibition (ZOI) was produced by *Terminalia chebula* with 542.81% which was at par with *Polyalthia longifolia* (51.38%), *Saraca asoca* (47.88%) and *Bixa orellana* (45.71%) while lowest ZOI was produced by *Terminalia arjuna* (42.21%). Chloroform extracts of nine plants *viz*., *Bixa orellana, Jatropha curcas, Lantana camara, Polyalthia longifolia,Polygonum hydropiper, Terminalia arjuna, Terminalia chebula, Zingiber officinale and Saraca asoca* were able to show inhibition against the bacterium. Highest ZOI was produced by *Polyalthia longifolia* with 51.52% of inhibition which was statistically at par with *Terminalia chebula*(48.27%), *Zingiber officinale* (45.23%) and *Saraca asoca* (44.22%). A minimum ZOI was produced by *Jatropha curcas* with 25.66% of inhibition and it was statistically at par with *Lantana camara* (29.50%). Two hexane extracts *viz*., *Lantana camara* and *Polyalthia longifolia* with relative percentage of 25.30% and 29.52% respectively were able to produce ZOI against the bacterium. Over all *Terminalia chebula* methanol (54.81%) gives the highest ZOI among the extracts followed by *Polyalthia longifolia* chloroform (51.52%) against the test bacterium. also reported the antibacterial activity of leaf and pericarp extracts of *Polyalthia longifolia*. Similarly, report of antibacterial property of these plant extracts has been reported by several researchers (Yumlembam and Borkar, 2014; Manasa *et al.,* 2014; Ambarish *et al*., 2011; Pinki and Sinha, 2013). Summarizing the results obtained from the preliminary screening of the plant extracts against the test pathogens it was observed that extracts obtained using hexane as the extracting solvent showed comparatively low bioactivity as compared to their counterparts obtained using chloroform

Table 1: Relative ZOI produced by 0.2% of the formulated plant extracts against *Xcc*by using paper disc diffusion method

Sl. No.	Scientific name	% of relative zone of inhibition		
		Methanol	Chloroform	Hexane
1.	*Bixa orellana* L.	45.71^{ab}	40.61^{bc}	0.00^{c}
2.	*Jatropha curcas* L.	ND	25.66^{e}	0.00^{c}
3.	*Lantana camara* L.	ND	29.50^{de}	25.30^{b}
4.	*Polyalthia longifolia* Sonn.	51.38^{ab}	51.52^{a}	29.52^{a}
5.	*Polygonum hydropiper* L.	ND	36.39^{cd}	0.00^{c}
6.	*Terminalia arjuna* Roxb.	42.21^{b}	37.21^{cd}	0.00^{c}
7.	*Terminalia chebula*	54.81^{a}	48.27^{ab}	0.00^{c}
8.	*Zingiber officinale* Roscoe	ND	45.23^{abc}	0.00^{c}
9.	*Saraca asoca*	47.88^{ab}	44.22^{abc}	0.00^{c}
10.	Solvent (LSN @ 0.2%)	0.00^{c}	0.00^{f}	0.00^{c}
11.	Control (Water)	0.00^{c}	0.00^{f}	0.00^{c}
	S Em ±	3.45	2.92	1.42
	CD(0.05)	10.48	8.52	4.14

ND = Not done (Figures followed by the same letter are not significant at P < 0.05)

Table 2: Percent disease inhibition after spray of plant extract on cabbage field infected with black rot.

Sl. No.	Treatment	Conc^{n}	0 DAS			10 DAS			20 DAS			30 DAS			Disease control(%)
			2012	2013	pool	2012	2013	pool	2012	2013	pool	2012	2013	pool	
1.	*Terminalia chebula* MeOH	0.2%	13.07^{b}	14.38^{a}	13.73^{a}	20.31^{b}	21.23^{c}	20.77^{c}	26.47^{c}	28.29^{c}	27.38^{c}	31.62^{b}	34.92^{b}	33.27^{b}	18.32
2.	*Polyalthia longifolia* MeOH	0.2%	14.01^{ab}	14.85^{a}	14.42^{ab}	18.87^{b}	21.18^{c}	20.03^{c}	25.27^{c}	25.89^{cd}	25.58^{cd}	29.42^{bc}	31.93^{b}	30.68^{c}	20.91
3.	Streptomycin Sulfate	0.01%	14.03^{ab}	14.63^{a}	14.33^{ab}	18.93^{b}	18.53^{d}	18.73^{c}	23.54^{c}	23.34^{d}	23.44^{d}	26.93^{c}	26.93^{c}	26.93^{d}	24.66
4.	Solvent	0.2%	14.65^{ab}	13.87^{a}	14.26^{ab}	28.08^{a}	25.80^{b}	26.94^{c}	40.54^{b}	40.74^{b}	40.64^{b}	48.74^{a}	49.74^{a}	49.24^{a}	2.35
5.	Control (Water)	-	15.67^{a}	15.39^{a}	15.52^{b}	29.95^{a}	28.04^{a}	29.00^{d}	44.12^{a}	44.84^{a}	44.48^{a}	50.70^{a}	52.48^{a}	51.59^{a}	-
	SEm	0.56	0.57	0.44	0.677	0.54	0.35	1.13	1.095	0.95	1.00	1.02	0.833		
	CD 5%	1.69	1.71	1.32	2.03	1.60	1.037	3.38	3.275	2.85	2.99	3.05	2.50		

Figures followed by the same letter are not significant at p < 0.05

and methanol. The two solvents have different polar abilities, with methanol having higher polarity and thus they tend to dissolve different compounds from the plant materials dipped in them. The difference observed in antibacterial activity of the extracts is likely to be due to the solubility of the active compound(s) in ethyl acetate and methanol or the presence of inhibitors to the antibacterial principle. Polar extracts such as aqueous, ethanol and methanolic extracts were more active towards the bacteria than the non-polar extracts such as Chloroform and Petroleum Ether (Packialakshmi and Naziya, 2014; Siddhuraju and Becker, 2003; Okigbo and Ogbonna, 2006). This suggests that the probable bioactive compounds present in the crude extracts were mostly mid to high polar which could be extracted using more polar solvents. This confirms previous reports that methanol is a better solvent for more consistent extraction of antimicrobial substances from medicinal plants as compared to other solvents, such as water, ethanol, and hexane (Ahmad *et al*., 1998;Bhardwaj and Sahu 2014, Eloff *et al*., 1998; Lin *et al*., 1999; Thomas, 2014).

With the availability of the plant source three plants extracts *viz*., methanol extracts of *Polyalthia longifolia* and *Terminalia chebula* and chloroform extract of *Zingiber officinale*, were chosen for further test. The different doses *viz*., 0.1%, 0.2%, 0.3% and 0.5%, of the three plant extracts were tested against the four plant pathogenic bacteria using paper disc method. However, the minimum inhibitory concentration of all the formulated plant extracts were observed at 0.2% against the *Xcc*.

Evaluation of selected plant extracts against plant disease under field condition against black rot of cabbage caused by

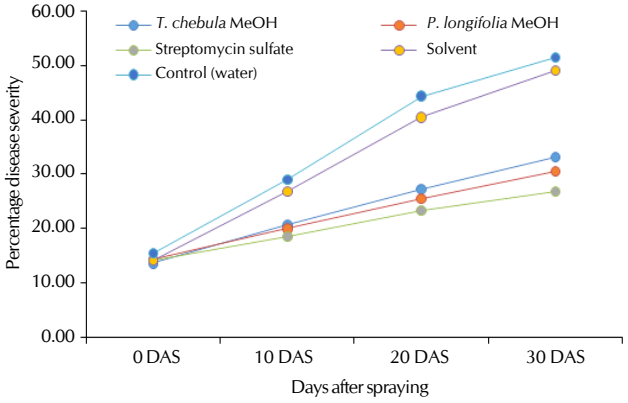

Figure 1: Graphical representation of disease severity on cabbage field affected by *X. campestris* pv. *campestris*

Xanthomonas campestris* pv. *campestris

The selected plant extract of *Polyalthia longifolia* and *Terminalia chebula*in MeOH was screened for their efficacy in the field against the black rot of cabbage caused by *X. campestris* pv. *campestris*. The experiment was carried out during the pre- Kharif season of 2012-13 on a farmer's cabbage field, situated at Nagarukhra, Nadia, West Bengal, which was severely infected with black rot of cabbage. The first symptoms were appeared at 30 DAT (Days after transplanting). The spraying was done after appearance of first visible symptoms. Data was recorded at 0 DAS (Days after spraying), 10 DAS, 20 DAS and 30 DAS. The disease intensity (PDI) was calculated as percentage of infected leaves of cabbage. The per cent

disease intensity (PDI) was computed and presented in Table 2, Fig 1.The results indicate that, the plants extract reduced black rot of cabbage when sprayed at a concentration of 0.2% of formulation (20 EC) under field conditions. The record of the two year pool data, before the spraying showed the PDI of 13.73, 14.42, 14.33, 14.26 and 15.52 on *Terminalia chebula* (MeOH), *Polyalthia longifolia* (MeOH), Streptomycin Sulfate, Solvent and Control (Water) respectively which were at par. 10 DAS the second reading showed that the PDI of Streptomycin Sulfate (18.73), *Polyalthia longifolia* MeOH (20.03) and *Terminalia chebula* MeOH (20.77) were statistically at par which was lower as compare to the Solvent (26.94) and Control (29.00). The same pattern was observed on 20 DAS (10 days after 2nd spraying). After 3rd spraying final observation was recorded on 30 DAS. The PDI result shows that Streptomycin Sulfate (26.93) had significantly better rest of the treatments. The plant extract *Polyalthia longifolia* MeOH (30.68) showed lower PDI than *Terminalia chebula* MeOH (33.27). The Solvent and Control showed maximum PDI of 49.24 % and 51.59% respectively. Sateesh *et al.,* (2004) reported that the foliar application of leaf extracts effectively reduced the incidence of bacterial blight diseases of rice under greenhouse condition. Vigo-Schultz (2006) reported an inhibition of dark rot in cauliflower, caused by *X. campestris* pv. *campestris*, *in vivo* occurred only in the leaves treated with 100 and 500 mg/litre of alcoholic extract of *Mikania glomerata* when applied concomitantly with the bacteria. This result was similar to the bordeaux mixture, indicating a control by direct antimicrobial activity. Bajpai (2010) reported that the oil extracts of *Metasequoia glyptostro boides* displayed remarkable *in vivo* antibacterial effect up to 65 to 100% disease suppression efficacy against the tested strains of *Xanthomonas* spp. on greenhouse-grown oriental melon plants (*Cucumis melo* L. var. *makuwa*). In the present finding the control of disease was recorded highest with Streptomycin Sulfate (24.66%), followed by *Polyalthia longifolia* (MeOH) (20.91%) and *Terminalia chebula* (MeOH) (18.32%). The disease progress rate was higher in the Solvent and control (water) treatments as compared to Streptomycin Sulfate, The Solvent also showed a small disease suppressive effect 2.35% which was almost negligible and at par to the untreated control. There were no phytotoxicity symptoms seen on the spray of the treatments on the cabbage plant.

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