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Management of chilli anthracnose by botanicals fungicides caused by *Colletotrichum capsici*

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Abstract
Chloroform extract of Ginger (*Zingiber officinale* Roscoe.) rhizome, Clerodendrum (*Clerodendrum infortunatum* L.) mature leaf and methanol extract of Polyalthia (*Polyalthia longifolia*) mature leaf were tested against *C. capsici* radial growth, biomass production and spore germination on following poisoned food technique at 20, 100, 200 and 400 µg/ml and carbendazim at 1, 5, 10, 20µg/ml was taken as standard fungicide control. The effective concentration of plant extracts and fungicide carbendazim were also tested *in vivo* and field condition following artificial inoculation by pin-prick method of fully matured fruits about to ripe harvested chilli fruits. The extracts formulated with solvent and surfactant (20EC) were sprayed on harvested fruits both before inoulation and after inoculation and incubated in moist chanmer at 28±1 °C. Under field condition the botanical formulations were sprayed on chilli plnts bearing mature fruits both naturally and artificial inoculated conditions at 400µg/ml.All the plant extract formulations showed inhibition of radial growth, biomass production and spore germination with increased dose relationship. However, the fungicide carbendazim showed highest activity than the botanical formulations at lower doses. Under *In vitro* highest radial growth inhibition (57.78%) and spore germination inhibition (62.70%) at 400µg/ml was observed in polyalthia-methanol and highest inhibition of biomass production was observed in ginger-chloroform (32.78%). Under *in vivo* condition, all the treatments showed reduced lesion diameter but Clerodendrum-chloroform showed less lesion diameter compared to other treatments while in field condition both in natural infection and artificial infection, clerodendrum-chloroform showed less percent infected fruits in clerodendrum-chloroform followed by ginger-chloroform and polyalthia-methanol. Based on the results, the plant extract of *Clerodendrum infortunatum* could be developed and used as an effective alternative to synthetyic chemicals for post-harvest anthracnose of chilli both under field and post-harvest condition.

Keywords: Plant extracts, Antifungal activity, Chilli, Anthracnose, *Colletotrichum capsici,* Post-harvest

1. Introduction
Chilli (*Capsicum annum*), an important spice crop in the world and India is one of the leading producers and exporters of chilli in the world. Anthracnose of chilli or fruit rot disease causes the major share of crop loss (Saxena, 2016) [2] in the field as well as in the storage and transport. The causal fungus, *Colletotrichum capsici* (Syd. & P.Syd.) is hemibiotrophic in nature. The pathogen may initiate the infection in the green fruit but remain quiescent till ripening of fruits. During ripening the lesion starts to develop very fast and may cause total ripe fruit decay in the field and during post-harvest stages of storage and transport. High moisture content, nutrient composition and pH of the ripe fruits support the growth of the fungus.
Synthetic fungicides are generally recommended and used to control the disease in the field. However, the use synthetic fungicides at ripening stage or on harvested fruits has a greater likelihood of direct environment food chain contamination; Unnikrishnan and Nath, 2002) [27]; Zahida and Masud, 2002 [30]; off-odour effects and development of resistance (Fogliata *et al*., 2001). These ill-effects of synthetic fungicides prompted to develop safe alternative strategies for reducing losses due to ripe and postharvest decay (Nashwa 2012) [23].
Therefore an attempt to modify this condition, an integrated management practice and use of natural products leading to organic production of chilli may guarantee as effective, economic and environment friendly (Kashyap et.al. 2010; Mondal and Mondal 2012; Kabir et.al. 2014) [15, 18, 14]. In our laboratory Bhutia *et.al*. (2015) [32] reported the use of plant extracts for effective management of anthracnose of Banana caused by *Colletotrichum musae* while Rupert *et.al*. (2016) [33] reported the use of plant extracts for management of black rot of Cabbage caused by *Xanthomonas campestris* pv *campestris*.
The objective of the present research work is to study the effect of some common and widely

available plant extracts on management of anthracnose disease of chilli caused by *C. capsici* under field condition and post-harvest management with an aim to develop a safe and effective product against chilli anthracnose.

2. Materials and method

2.1 Pathogen Culture

Colletotrichum capsici was cultured for 1-2 weeks on Potato Dextrose Agar (PDA) at 25 °C. The isolate was obtained from infected chilli fruit. Spores were harvested by adding 3-4ml of sterile distilled water to the petri dish. The spores were than rubbed with a sterile glass rod to free them the PDA medium and the suspension was passed through two layers of cloth. The suspension was diluted with water to obtain spore concentration of 10^6 spores/ml was determined with Haemocytometer.

2.2 Preparation of Plant Extracts

The extraction of the powdered plants for hot extraction was done following extraction with organic solvents using Soxhlet apparatus (capacity 250 ml) for 6-8 hrs. Well dried powdered plant materials (50 g) were packed and used for extraction (Sultana et.al 2009). Chloroform and methanol were used for extraction @ 150ml each. The crude extract was collected, concentrated in a Buchi Rotavapor at 45 ^{0}C, transferred in a pre-weighed conical flask and evaporated to dryness.

2.3 Preparation of botanical pesticide formulation 20% EC (w/w)

Extracts (2 g) of each plant were taken in a beaker (250 ml). Surfactant mixture of (A) N-Alkaline Sulfonate and (B) K-Alkaline Sulfonate were added @ 4% of the total formulation (20 EC) along with 76 % of light solvent naptha (LSN). The entire formulation procedure was developed and standardized at Bio-formulation Laboratory, Dept. of Ag. Chemicals, BCKV, Mohanpur (Majumder, 2014) [31].

2.4 *In vitro* applicability of the plant extracts against radial growth, biomass production and spore germination of *Colletotrichum capsic*

Antifungal tests of the crude extracts of different plant extracts against *C. capsici* was done following *In vitro* methods *i.e* poisoned food technique and broth dilution assay described by (Grover and Moore,1962 and Pitarokili et.al. 2003) and spore germination assay in groove slides (Rana *et al.,* 1997) at four different concentration 20µL, 100µL, 200µL and 400 µL. The test fungus was inoculated in different medium mixed with plant extracts as well as carbendazim (1µL, 5µL, 10µL and 20µL), used as check and incubated at temperature of 28 ±1 °C and was calculated from four replicates of fungi after every 24hrs untill full growth of fungus in control sets, calculated by formula (Bhutia *et.al.,* 2015) [32]

$$\% \text{ Growth inhibition (GI)} = \frac{\text{Growth in control (GC)} - \text{Growth in treatment (GT)}}{\text{Growth in control (GC)}} \times 100$$

2.5 Efficacy of plant extracts on artificially inoculated anthracnose development in chilli under in vivo condition

The chilli fruits (cv.Beldanga) about to ripen were harvested from Jaguli field, BCKV, Mohanpur, Nadia, West Bengal, India, and brought to the laboratory where they were washed under running tap-water, air-dried and surface sterilized. Set of 20 fruits were used per replication. One set of fruits were first treated with plant extracts, pin pricked and then inoculated with spore (DBI) and another sets of fruits were first pin pricked, inoculated with spore suspension and dipped in plant extracts (DAI). A standard fungicide Bavistin 50WP (carbendazim) 1g/lt and a control was used to compare with the treatments. The fruits were stored in plastic trays to maintain a high relative humidity (approx. 80%) in a BOD at 28±1 °C. lesion length of disease fruits were taken.

2.6 Efficacy of plant extracts on naturally and artificially infected fruits under field condition

Under field condition the plant extracts were sprayed under naturally infected fruits and artificially infected fruits (spraying with spore suspension of 10^6 spores/ml) in Jaguli Farm, BCKV, Mohanpur, Nadia, West Bengal, India. Under artificial condition the plant extracts were sprayed in two ways i.e., spraying of inoculum before spraying with plant extract (SAI) and spraying of inoculum after spraying with plant extracts (SBI) and disease was calculated on the basis of percent fruit infected.

2.7 Data analysis: The *In vitro* and in vivo experiments were arranged completely randomized design and field experiments in randomized block design and the in vivo and field experiments were analysed as di factorial analysis by using softwear MSTATC.

3. Results

3.1 *In vitro* screening of the plant extracts against radial growth, biomass production and spore germination of *C. capsici* at different concentration.

The results obtained from the table 1, 2 and 3 indicate that with increase in concentration of plant extracts and fungicides, inhibition of radial growth, biomass production and spore germination of *Colletotrichum capsici* increases but the dose required by the standard fungicide is less compared to plant extracts. Highest radial growth inhibition (57.78%) and spore germination inhibition (62.70%) at 400 µg/ml was observed in polyalthia-methanol and highest inhibition of biomass production was observed in ginger-chloroform (32.78%) whereas lowest radial growth inhibition (57.78%) and spore germination inhibition (15.40%) at 400 µg/ml was observed in clerodendrum-chloroform and lowest inhibition of biomass production was observed in polyalthia-methanol (64.60%)

Table 1: Radial growth inhibition of *C. capsici* by plant extracts and synthetic fungicide at different concentrations

Sl no	Treatments	*Per cent* inhibition of radial growth			
		20µg/ml	100µg/ml	200 µg/ml	400µg/ml
1	Ginger chloroform	12.2	24.4	37.8	53.3
2	Clerodendrum chloroform	6.11	8.33	28.89	32.78
3	Polyalthia chloroform	15	25	51.67	57.78
		1 µg/ml	5 µg/ml	10 µg/ml	20 µg/ml
4	Carbendazim	0	0	2.32	3.35

Table 2: Inhibition of biomass production of *C. capsici* by plant extracts and synthetic fungicide at different concentrations

S.l no	Treatments	*Per cent* inhibition of biomass production			
		20µg/ml	100µg/ml	200 µg/ml	400µg/ml
1	Gingher chloroform	2.4	23.6	48.7	73.8
2	Clerodendrum chloroform	5.7	55.7	57	70.3
3	Polyalthia methanol	13.1	37.8	56.6	64.6
		1 µg/ml	5 µg/ml	10 µg/ml	20 µg/ml
4	Carbendazim	5.23	9.71	15.19	21.63

Table 3: Spore germination Inhibition of *C. capsici* by plant extracts and synthetic fungicide at different concentrations

Sl. no	Treatments	*Per cent* inhibition of spore germination			
		20µg/ml	100µg/ml	200 µg/ml	400µg/ml
1	Gingher chloroform	0	3.3	19	42.3
2	Clerodendrum chloroform	0	1.3	12	15.4
3	Polyalthia methanol	3.7	10.6	34.7	62.7
		1 µg/ml	5 µg/ml	10 µg/ml	20 µg/ml
4	Carbendazi	0	0.27	0.91	5.8

The values are the mean of four replications

3.2 Percent of anthracnose (*Colletotrichum capsici*) infected fruit of Chilli under artificial inoculum spray under field condition following plant extract formulation spraying during May, 2014

Percent anthracnose infected fruits were recorded after 10 days of inoculation after 3rdspraying at 30 days where spraying of plant extract and carbendazim. is done in 2 ways i.e., spraying of plant extracts after inoculum spray (SAI) and spraying of plant extracts before inoculum spray (SBI) is present in the table.4. And Fig.1.Similarly a solvent system (used for preparation of formulation) was used for spraying to check the phytotoxic effects. Lowest percent anthracnose infected fruits was observed in the fruits sprayed with clerodendrum extract (23.84%) and highest in fruits sprayed with solvent (58.68%) irrespective of time of inoculation. Among the two types of inoculum spraying, SBI shows lowest percent anthracnose infected fruit than SAI irrespective of treatments.

In SAI and SBI lowest percent anthracnose infected fruit was observed in plants sprayed with clerodendrum (23.49% and 24.18%) and highest in solvent (66.82% and 57.08% respectively). In all the cases control treatment has the highest infected fruits comparing to all the treatments.

Table 4: Percent of anthracnose (*Colletotrichum capsici*) infected fruit of Chilli under artificial inoculum spray following plant extract formulation spraying during May, 2014

Sl. No	Treatment	Percentage of infected fruit		
		SAI	SBI	Mean of infected fruit irrespective of time of inoculation
1	Ginger chloroform	51.40(45.81)	27.22(31.45)	39.31
2	Clerodendrum chloroform	23.49(28.99)	24.18(29.46)	23.84
3	Polyalthia methanol	46.78(43.15)	50.66(45.38)	48.72
5	Solvent (A+B+LSN)	66.82(54.83	57.08(49.07)	58.68
4	Carbendazim (1g/lit water)	60.29(50.94)	46.12(42.78)	56.47
6	Control	73.18(58.81)	85.69(67.77)	79.43
	Mean of infected fruit irrespective of treatments	53.12	49.03	
		treatment	Time of inoculation	Time of inoculation X treatments
	SEm±	0.25	0.10	0.36
	CD at 5%	0.72	0.29	1.04

A=N-Alkaline Sulfonate, B= K-Alkaline Sulfonate, LSN= light solvent naptha.
SAI= Spraying with plant extract after inoculation with pathogen
SBI= Spraying with plant extract before inoculation with pathogen
Values are the mean of 4 replications.
**Figure within parenthesis indicates arc-sine transformed value

Fig 1: Effect of *Z. officinale* extract (A), *C. infortunatum* extract (B) and Polyalthia longifolia extract (C) and untreated control (D) in artificially inoculated on chilli (cv.Beldanga) by *C. capsici* at ambient condition (80-82 percent R.H., 27 ± 1 °C),after 10 days of storage.

3.3 Percent anthracnose infected fruits of chilli under field condition (natural incidence) following plant extract formulation spraying during September-October, 2014.

Percent of anthracnose infected fruits were recorded at 10 days after1st spraying, 10 days after 2nd spraying, 10 days after 3rd spraying with plant extracts at are presented in table 5. Similarly a solvent system (used for preparation of formulation) was used for spraying to check the phytotoxic effects. The disease observed at different days showed reduced percent anthracnose infected fruits in all the treatments except in control it increases. The less percent anthracnose infected fruit was observed in plants spraying with clerodendrum-chloroform extract (4.49%) followed by ginger-chloroform extract (6.45%) and polyalthia-methanol (7.38%).

Table 5: Percent anthracnose infected fruits of chilli under field condition (natural incidence) following plant extract formulation spraying during September-October, 2014.

SL. No.	Treatments	Percent anthracnose infected fruits at difference days of observation		
		10 days after 1st spray	10 days after 2nd spray	10 days after 3rd spray
1	Ginger chloroform	22.75(28.49)	21.10(27.35)	6.45(14.72)
2	Clerodendrum chloroform	15.60(23.27)	14.01(21.98)	4.49(12.24)
3	Polyalthia methanol	30.65(33.62)	21.22(27.43)	7.38(15.76)
	Solvent (A+B+LSN)	21.71(27.77)	42.78(40.85)	45.51(40.72)
5	Carbendazim (1g/L of water)	42.78(40.85)	22.45(28.28)	12.07(20.33)
6	Control	23.30(28.86)	49.51(44.72)	54.73(47.71)
S.Em. (±)		0.26	0.25	0.55
CD 5%		1.12	1.06	1.90

A=N-Alkaline Sulfonate, B= K-Alkaline Sulfonate, LSN= light solvent naptha.
Values are the mean of 4 replications
**Figure within parenthesis indicates arc-sine transformed value

3.4 Anthracnose development and lesion diameter on artificially inoculated Chilli (cv. Beldanga) following dipping in plant extracts

Application of all the three plant extracts significantly reduce the infection in chilli fruits evident from the results shown in the table.6. After storage at 28±1 °C for 10 days, among the different treatments clerodendrum-chloroform (3.95mm) showed comparatively less lesion length in chilli compared to carbendazim and solvent irrespective of time of inoculation and among the two types of inoculation less lesion diameter was observed in case of dipping of fruits in plant extracts before inoculation irrespective of time of treatments (4.44mm). In all the cases lesion diameter was highest in control treatments.

Table 6: Lesion diameter of anthracnose infected fruits of chilli caused by *C.capsici* following treatment with plant extracts.

Sl. No	Treatment	Lesion diameter (mm) at 10 days after inoculation		
		DAI	DBI	Mean lesion diameter irrespective of time of inoculation
1	G.C	4.23±1.97	5.85±0.77	5.04
2	C.C	4.33±2.28	3.58±2.19	3.95
3	P.M	5.93±1.88	2.95±1.15	4.44
4	Solvent (A+B+LSN)	13.75±4.66	6.53±3.40	10.14
	Carbendazim (1g/lit water)	6.53±3.40	2.18±1.93	4.35
5	Control	13.78±4.36	7.23±1.42	10.51
	Mean lesion diameter irrespective of treatments	6.96	4.44	
Lesion diameter at 10 days after inoculation		Treatment	Time of inoculation	Time of inoculation xTreatments
	S.Em. (±)	0.97	0.61	1.37
	CD 5%	2.83	1.78	3.99

A=N-Alkaline Sulfonate, B= K-Alkaline Sulfonate, LSN= light solvent naptha.
DAI= Dipping in plant extract after inoculation with pathogen spore
DBI= Dipping in plant extracts before inoculation with pathogen spore

3.5 Phytotoxicity observation under field condition

The phytotoxic effect of the solvent used for preparation of plant extracts were checked under field condition at 400µg/ml and 600µg/ml concentrations are presented in a table.7 where no phytotoxic effects like leaf specking, necrosis, chlorosis, leaf cupping and plant stunting were observed at 400µg/ml but at 600µg/ml concentration phytotoxic effects was observed.

Table 7: Phytotoxic studies under field condition with solvents in chilli at 400µg/ml and 600µg/ml

doses	leaf speckling	necrosis	chlorosis	Leaf cupping	plant stunting
400µg/ml	nill	nill	nill	nill	nill
600µg/ml	present	present	present	present	present

4. Discussion

The plant extracts are reported to have antifungal properties (Fawzi 2009 and Boughalleb et.al, 2005) [9, 4] and are safe for living beings and environment. Exploitation of chemicals which are naturally available in plants which retards the growth of various undesirable microorganisms have prominent role in development of commercial pesticides for crop protection strategies in future, especially for the

management of different diseases of plants (Varma and Dubey,1999, Gottlieb 2002) [28, 12].

The *In vitro* tests of plant extracts is an initial step for selection of plant with antifungal properties against pathogens followed by in vivo tests to check the positive results of *In vitro* tests (Gorris and Smid,1995; Tegegne *et.al* 2008) [11, 24]. In particular, with increase in concentration the polyalthia-methanol extract exhibited inhibitory effect on spore germination and radial growth while Ginger-chloroform extract exhibits a strong inhibitory effects on fungal biomass production followed by clerodendrum-chloroform and polyalthia-methanol. With increase in concentration of these plant extracts implied an increase in the active ingredients of the formulation which act on the test pathogens thereby affecting its physiological processes, lowering the growth of the pathogens (Tijjani 2014) [1]. The inhibitory activity of plant extracts may be due to direct toxic effect on the pathogen (Vijayan1989)The antifungal activities of the plant extracts is mainly due to the presence of secondary plant metabolites like terpinoides, phenols, flavonoides, alkaloids has been reported earlier (Mohamed and EI-Hadidy,2008) [17].

In vivo study showed that ginger-chloroform, clerodendrum-chloroform and polyalthia-methanol extract as well as Carbendazim was effective extract to controlling the anthracnose of chilli fruits under particular storage condition and in particular, the clerodendrum-chloroform extract was found highly effective over Carbendazim. A similar report was established by Ekhuemelo 2016 [7], reduction in the severity of anthracnose disease by the application of the plant extracts, comparable with mancozeb. The effect of extract on plant pathogens were reported in various literatures (Shukla 2012) [3]. These are consistent with earlier reports indicating the fungicidal properties of natural plant products and their potential to control plant diseases (Enikuomehin, 2005; Opara and Obani, 2009; Tunwari and Nahunnaro, 2014) [8, 20, 26]. The fungicidal activity of these plants may be attributed to variations in the chemical constituents of the various plants and their variable effects on plant pathogens (Nduagu *et al*., 2008) [19] and treated food commodities are non-injurious thus increasing shelf-life of the commodities (Tripathi, 2008) [25].

Regarding the time of application of plant extract, there were no such huge difference between extracts applied before inoculum treatment and extracts applied after inoculum treatment in both *in vivo* and field conditions, may be due to systemic action of the plants extracts against *C.capsici* which does not allow the pathogen to cause much infection upto a certain level. Under field condition reduced percent infected fruit can be seen when the plant extracts were applied thrice but under natural incidence, infection was less comparing to artificial inoculation may be due to increased inoculum comparing to natural infection. Chowdhury 2007, while working on anthracnose of Mango reported that thrice application of plant extracts inhibited fruit infection leds to highest fruit retention. Therefore the use of plant extracts as an antimicrobial agents can be an interesting field of investigation for research works.

Regarding phytotoxicity of the plant extracts, the solvents sprayed under field condition were used to check the phytotoxicity of the solvents used for plant extract preparation where there were no phytotoxic effects was observed at 400µg/ml but the plant showed phytotoxic effects at 600µg/ml. Bhutia 2013, reported under field condition the plant extracts did not show any phytotoxic effects when applied at 0.2% concentration but at 0.3% phytotoxicity have been observed. From this observation we can say that plant extract is used for disease control and not the solvent used for preparation of plant extract.

Regarding the dose response, the dose required by plant extract is more comparing to standard fungicides which may be due to formulation techniques. So improved formulation procedures should be applied for better efficacy the plant extracts.

In conclusion, the results reported here show that though clerodendrum-chloroform was less effective under *In vitro* condition but the extract was most effective comparing to other extracts in controlling disease of chilli in storage as well as field caused by *C.capsici*. Besides more works have to be done on mechanism of action of plant extracts in molecular level. Further shelf life studies on efficacy of plant extracts are carried in laboratory so that the product should be commercially formulated, explored and confirmed in large-scale experiments in farmer's field as well as storage condition to control post-harvest anthracnose of chilli.

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