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MANAGEMENT OF *COLLETOTRICHUM CAPSICI* (SYD.) BUTLER AND BISBY CAUSING FRUIT ROT OF CHILLI USING FERMENTED LEAF EXTRACTS

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Abstract

Chilli (*Capsicum annum* L.) is one of the important commercial vegetable crop which is grown extensively almost throughout the world. It originated in the American tropics which propagated widely. Its crop suffers due to more than 40 fungal species, of which *Colletotrichum capsici* is one of the most destructive species causing seedling rot or damping off at seedling stage in a nursery, leaf spot or die back at different stages of crop growth and fruit rot or anthracnose at fruiting stage leading to reduced fruit yield and marketability. The fungus *Colletotrichum capsici* infects both unripe and ripe chilli fruits and survives on seed as acervuli and microsclerotia. Infection of *C. capsici* will be higher in mature stage than in the early stage of chilli plant. The use of chemical fungicide is a common practice for the control of anthracnose. However, continuous use of chemical fungicides lead to negative impacts on the environment, soil and human health. So, development of new alternative strategies for management of fungal diseases is very essential in the changing climate scenario. In this regard, plant products appear to be an in-exhaustive source showing potential fungicidal activity which serves as harmless pesticides. In this study, use of *Azadirachta indica, Datura stramonium, Ocimum sanctum, Polyalthia longifolia and Vinca rosea* were used which were fungitoxic to *C. capsici*. Among the five fermented leaf extracts tested against *C. capsici, Azadirachta indica extract* at 20% concentration highly inhibited the growth of *C. capsici in vitro* condition. And *in vivo* the application of fermented leaf extract of *Azardiracthta indica* alone reduced the fruit rot incidence (@ 3%) and increased plant height, number of fruits and yield significantly.

Key words: *Capsicum annum*, Fruit rot, *Colletotrichum capsici*, Fermented leaf extracts.

Introduction

India is "The home of spices" and its spices are world famous for their medicinal values. Chilli (*Capsicum annum* L.) is one of the major spice crops of India and it stands 3rd in its production (Saxena *et al*., 2016). It is grown throughout the world for its green and red ripe fruits as it is lucrative. Green chillies are rich source of vitamins, especially vitamin A, C, B1 and B2. Pungency in chilli, which is due to the presence of capsicin, a digestive stimulant and a cure for rheumatic troubles. Chilli is an important economic crop worldwide and uncompromising component of the cuisines of tropical and subtropical countries (Saxena *et al.,* 2016). One of the important constraints for low productivity of chilli is the biotic stress caused by fungi, bacteria and viruses, the major being the fungal disease (Kumar and Venkateswarlu, 2011). The fungus *Colletotrichum capsici* infects both unripe (green) and ripe (red chilli) fruits. Disease symptoms can occur on leaves, stems and both pre- and post-harvest fruits and survives on seed as acervuli and micro sclerotia (Raj and Christopher, 2009).

Typical anthracnose symptoms on chilli fruit include sunken necrotic tissue with concentric rings of acervuli. Infection of *C. capsici* is higher at the mature fruit stage than in the early fruit stage. The fungal pathogen is both seed borne and air borne and affects seed germination and vigour to a greater extent. Its damage is not only in the field but also during storage and is one of the main cause for post-harvest decay of chilli.

In India during 2016-17, area under chilli cultivation was recorded as 8.31 million ha with a total production of 18.72 million tons (Anon, 2018). The estimated loss

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due to this disease ranged from 8 to 60% in different parts of India, although different chemical fungicides are being recommended and used to combat the disease. However, continuous use of chemical fungicides has negative effects on biodiversity, environment and human health (Avinash and Hosmani, 2012; Suthin Raj *et al*., 2013b). To overcome these undesirable effects, one of the approaches could be the use of plant based biological products to control fruit rot of chilli. Use of plant extract is reported to be safe due to its easy decomposition, non – residual activity and non- phytotoxic properties. The antifungal activity of plant extracts could be due to the presence of secondary plant metabolites like trepinoides, phenols, flavonoids and alkaloids (Mohamed and El-Hadidy, 2008; Suthin Raj *et al*., 2013a). Therefore, in present study, attempts were made to manage this disease using botanicals such as *Azadirachta indica, Datura stramonium, Ocimum sanctum, Polyalthia longifolia and Vinca rosea* which were, fungitoxic against *C. capsici* under *in vitro* condition.

Materias and Methods

Isolation of Pathogen

The pathogens were isolated from various localities of Tamil Nadu on potato dextrose agar (PDA) medium from the diseased specimen showing typical symptoms. The infected portion of the fruit was cut into small bits, surface sterilized in 0.1% mercuric chloride solution for 30 sec., washed in repeated changes of sterile distilled water and plated on to PDA medium in sterilized petri plates. They were then incubated at room temperature (28±2°C) for five days and were observed for fungal growth. The fungus was purified by single spore isolation technique (Rangaswami, 1958).

Preparation of Fermented Leaf Extracts

Plant materials such as *Polyalthia longifolia, Azadirachta indica, Pongamia pinnata,* dried leaves and weeds were collected. A pot was filled with as much as plant material. Enough quantity of water was poured until the leaves were fully immersed in it. Then it was sealed with the help of a khada cloth and was allowed to ferment. The pot was the placed out of direct sunlight and was allowed to ferment for a period of 3 months. After 3 months, the pH was tested. The solutions pH should be between 3.2 to 3.7. Then the liquid was strained off into an another container and it which is called as the fermented leaf extract. These extracts were used as foliar sprays to prevent diseases in plants. They can also be stored in a dark place with a relatively uniform temperature and can be stored upto 150 days.

Table 1: Evaluation of different fermented leaf extracts against chilli fruit rot caused by *Colletotrichum capsici* under *in vitro*.

S.No.	Fermented leaf extract	Inhibition zone (mm)															
		Poison food technique (mm)						Agar well method						Paper disc assay			
		Mycelial growth			% decrease over control			Mycelial growth			% decrease over control			Mycelial growth			% decrease over control		
		5 %	10 %	20 %	5 %	10 %	20 %	5 %	10 %	20 %	5 %	10 %	20 %	5 %	10 %	20 %	5 %	10 %	20 %
1.	*Polyalthia longifolia*	10.5^{c}	9.8^{c}	8.6^{c}	88.3	89.1	90.4	8.9^{c}	9.8^{c}	12.5^{c}	90.1	89.1	86.1	8.2^{c}	9.4^{c}	10.7^{c}	90.8	89.5	88.1
2.	*Azadirachta indica*	8.9^{a}	7.6^{a}	6.4^{a}	90.1	91.5	92.8	11.8^{a}	13.1^{a}	15.3^{a}	86.8	85.4	83.0	10.5^{a}	12.1^{a}	14.2^{a}	88.3	86.5	84.2
3.	*Pongamia pinnata*	9.7^{b}	8.8^{b}	7.7^{b}	89.2	90.2	91.4	10.6^{b}	12.7^{b}	14.9^{b}	88.2	85.8	83.4	9.4^{b}	10.3^{b}	12.6^{b}	89.5	88.5	86.0
4.	Dried leaves	14.3^{e}	12.6^{e}	10.3^{e}	84.1	86.0	88.5	6.4^{e}	7.3^{e}	8.7^{e}	92.8	91.8	90.3	6.1^{e}	7.2^{e}	8.2^{e}	93.2	92.0	90.8
5.	Weeds	12.4^{d}	11.9^{d}	9.9^{d}	86.2	86.7	89.0	7.8^{d}	8.2^{d}	10.1^{d}	91.3	90.8	88.7	7.3^{d}	8.1^{d}	9.5^{d}	91.8	91.0	89.4
	Control	90.0	90.0	90.0				90.0	90.0	90.0				90.0	90.0	90.0			

Poison food technique

PDA medium was prepared in a 100 ml conical flask and was autoclaved. Fermented leaf extracts at quantities of 5, 10, 15 and 20 ml were added to 45, 40, 35 and 30 ml aliquots respectively in flasks so as to get final concentrations of 5, 10, 15 and 20 percent. The incorporation of fungicide Hexaconazole @ 0.2% in the medium was used for comparison. PDA medium without extract served as the control. Each plate was inoculated at the centre with a ten days old culture disc (8 mm) of pathogen and incubated at room temperature (28±2°C). Three replications were maintained for each treatment. The diameter of the mycelial growth of the pathogen was measured after seven days.

Agar well method

Fermented leaf extracts and resistance inducing chemicals were tested using agar well method. Twenty ml of PDA medium was seeded with 3 ml of spore suspension (5×10^{-5}). Wells were made on agar surface with a 5mm cork borer. 1 ml of fermented leaf extract was poured separately into the wells using a sterile syringe at different concentrations, *viz*., 5, 10, 15 and 20 percent. Hexaconazole @ 0.2 percent was used for comparison. The plates were incubated at 28±2°C for seven days and observed for fungal growth. Three replications were maintained for each treatment. The plates were observed for zone formation around the wells. The zone of inhibition was calculated by measuring the diameter of the inhibition zone around the well (in mm) including the well diameter.

Paper disc method

Desired concentration of fermented leaf extracts were impregnated into sterile filter paper disc (5 mm dia). The extract discs were then placed on seeded agar plates and incubated at room temperature (28±2°C). Three replications were maintained for each treatment. The inhibition zone of fungal growth around the treated sterile filter paper discs were measured and recorded.

***In vitro* evaluation of fermented leaf extracts by paper towel method**

This method was used to know the growth promoting effect of seed treatments with fermented leaf extract on chilli. Randomly selected 100 seeds were treated with fermented leaf extracts and they were placed on two layers of moist germination paper and rolled carefully to avoid any excess pressure on seeds. These towels were incubated in a seed germinator at 20±2°C for 15 days. Similarly, the control set was maintained using non-treated seeds of chilli. Three replications were maintained for each treatment separately. The first count was taken on 15th day. All morphologically normal seedlings were counted and germination was expressed in percentage. The seedling vigour was recorded by taking ten normal seedlings randomly from the germination test. The root length was recorded at collar region up to tip of the primary root. The mean root length was recorded in cm. Same seedlings were used for the measurement of shoot length. The measurement of shoot length was taken from the collar region to the point of junction of cotyledons. The mean shoot length was recorded in cm. Vigour index was calculated by the following formula given by Abdul Baki and Anderson, (1973):

Vigour index = (Shoot length + Root length) × Germination %

Evaluation of fermented leaf extract for the management of fruit rot diseases in the field

The field experiment was conducted to test the efficacy of fermented leaf extract against fruit rot diseases on the variety K2 during October 2018 to March 2019 in the farmer's field at Sivapuri, Chidambaram, Cuddalore district, Tamil Nadu. The experiment was laid out in randomized block design with three replications with a plot size of 25 percent area. Thirty days old seedlings were planted into field plots in rows with a spacing of 35 × 45 cm. Three replicate plots were maintained for each treatment. Treatment application details and experimental observations were the same as in green house experiments with the below mentioned treatment schedule:

T_1 - Application of *Polyalthia longifolia* fermented leaf extract alone @ 2% (seedling dip + spraying @ 60 and 90 DAT).

T_2 - Application of *Polyalthia longifolia* fermented leaf extract alone @ 3% (seedling dip + spraying @ 60 and 90 DAT).

T_3 - Application of *Polyalthia longifolia* fermented leaf extract alone @ 4% (seedling dip + spraying @ 60 and 90 DAT).

T_4 - Application of *Azadirachtia indica* fermented leaf extract alone @ 2% (seedling dip + spraying @ 60 and 90 DAT).

T_5 - Application of *Azadirachtia indica* fermented leaf extract alone @ 3% (seedling dip + spraying @ 60 and 90 DAT).

T_6 - Application of *Azadirachtia indica* fermented leaf extract alone @ 4% (seedling dip + spraying @ 60 and 90 DAT).

T_7 - Application of *Pongamia pinnata* fermented leaf extract alone @ 2% (seedling dip + spraying @ 60 and 90 DAT).

T_8 - Application of *Pongamia pinnata* fermented leaf extract alone @ 3% (seedling dip + spraying @ 60 and 90 DAT).

T_9 - Application of *Pongamia pinnata* fermented leaf extract alone @ 4% (seedling dip + spraying @ 60 and 90 DAT).

T_{10}- Application of Dried leaves fermented leaf extract alone @ 2% (seedling dip + spraying @ 60 and 90 DAT).

T_{11} - Application of Dried leaves fermented leaf extract alone @ 3% (seedling dip + spraying @ 60 and 90 DAT).

T_{12} - Application of Dried leaves fermented leaf extract alone @ 4% (seedling dip + spraying @ 60 and 90 DAT).

T_{13} - Application of Weeds fermented leaf extract alone @ 2% (seedling dip + spraying @ 60 and 90 DAT).

T_{14} - Application of Weeds fermented leaf extract alone@ 3% (seedling dip + spraying @ 60 and 90 DAT).

T_{15} - Application of Weeds fermented leaf extract alone@ 4% (seedling dip + spraying @ 60 and 90 DAT).

T_{16} - Application of mancozeb (0.2%) (seedling dip + spraying @ 50 and 75 DAT).

T_{-17} – Control.

The fruit rot incidence was assessed on the 150th day after transplanting. The intensity of fruit rot was calculated as Percent Disease Index (PDI) following the grade chart proposed by Ravinder Reddy, (1982) and using the formula given by Mc Kinney, (1923) as described as earlier. Biometric assessment *viz.*, mean shoot length, mean no. of flower/plant, mean no. of fruits/ plant, mean no. of fruit length (cm) and fruit yield t/ha were assessed and recorded.

Results and Discussion

Various fermented leaf extracts were selected and evaluated for their antifungal activity. The extracts of *Azadirachta indica* at a highest concentration (20%) recorded minimum mycelial growth (6.4 mm) which was followed by *Pongamia pinnata* recording a mycelial growth of 7.7 mm. All dried leaf extracts at a highest concentration of 20% recorded maximum mycelial growth (10.3 mm) (Table 1).

Various fermented leaf extracts were selected and evaluated for their antimicrobial activity by two methods such as agar well method and paper disc assay (Table 1). *Azadirachta indica* at a highest concentration of 20% maximally reduced the mycelial growth in both methods which recorded 15.3 and 14.2 percent inhibition respectively. It was followed by *Pongamia pinnata* which recorded 14.9 and 12.6 percent inhibition in both methods respectively. All the concentration of dried leaf extracts recorded a minimum percent inhibition than all the other extracts.

Results of table 2 revealed that treatment T_6 showed a maximum seed germination 95.70% and was siginificantly superior over all the other treatments. A lowest percent germination 59.45% was reported in T_{-17}.

Application of *Azardiracta indica* fermented neem leaf extract alone @3% (seedling dip + spraying @ 60

Table 2: Effect of fermented leaf extract on seedling growth parameters of chilli by paper towel method.

Tr. No	Treatment	Germination %	Mean shoot length (cm)	Mean root length (cm)	Vigour index
T_1	*Polyalthia longifolia* fermented leaf extract alone @ 2%	75.50^{gh}	12.54^{ef}	7.30^{efg}	1497.92^{j}
T_2	*Polyalthia longifolia* fermented leaf extract alone @ 3%	76.33^{g}	13.32^{e}	7.70^{def}	1604.46^{i}
T_3	*Polyalthia longifolia* fermented leaf extract alone @ 4%	81.67^{f}	15.11^{d}	8.00^{cde}	1887.39^{h}
T_4	*Azadirachtia indica* fermented leaf extract alone @ 2%	91.40^{c}	18.30^{bc}	9.00^{ab}	2495.22^{c}
T_5	*Azadirachtia indica* fermented leaf extract alone @ 3%	94.10^{b}	19.10^{b}	9.20^{ab}	2663.03^{b}
T_6	*Azadirachtia indica* fermented leaf extract alone @ 4%	95.70^{a}	21.60^{a}	9.90^{a}	3014.55^{a}
T_7	*Pongamia pinnata* fermented leaf extract alone @ 2%	86.30^{e}	15.11^{d}	8.30^{bcd}	2020.28^{g}
T_8	*Pongamia pinnata* fermented leaf extract alone @ 3%	88.70^{d}	17.41^{c}	8.40^{bcd}	2289.35^{e}
T_9	*Pongamia pinnata* fermented leaf extract alone @ 4%	89.87^{d}	19.40^{b}	8.90^{bc}	2543.32^{d}
T_{10}	Dried leaves fermented leaf extract alone @ 2%	65.55^{l}	7.12^{jk}	5.10^{h}	801.02^{p}
T_{11}	Dried leaves fermented leaf extract alone @ 3%	67.12^{k}	8.34^{j}	5.30^{h}	915.52^{o}
T_{12}	Dried leaves fermented leaf extract alone @ 4%	69.88^{j}	9.64^{i}	5.60^{h}	1064.97^{n}
T_{13}	Weeds fermented leaf extract alone @ 2%	70.43^{j}	10.12^{hi}	6.70^{g}	1184.63^{m}
T_{14}	Weeds fermented leaf extract alone @ 3%	72.38^{i}	10.92^{gh}	6.80^{fg}	1282.57^{l}
T_{15}	Weeds fermented leaf extract alone @ 4%	74.33^{h}	11.89^{fg}	7.00^{fg}	1404.09^{k}
T_{16}	Mancozeb (0.2%)	85.54^{e}	17.34^{c}	8.50^{bcd}	2210.35^{f}
T_{17}	Control	59.45^{m}	6.33^{k}	4.70^{h}	655.73^{q}

Table 3: Effect of fermented leaf extract on seedling growth parameters of chilli by paper towel method.

Tr. No	Treatment	Mean plant height (cm)	Mean no. of flowers / plant	Mean no. of fruits / plant	Mean fruit length (cm)	Fruit yield (g/plant)	Fruit rot incidence	% Increase over control
T_1	*Polyalthia longifolia* fermented leaf extract alone @ 2%	95.21^{h}	169^{f}	93^{g}	8.31^{abcd}	342^{f}	7.87^{f}(16.29)	50.40
T_2	*Polyalthia longifolia* fermented leaf extract alone @ 3%	97.56^{g}	171^{e}	95^{g}	8.49^{abcd}	348^{ef}	6.51^{e}(14.78)	74.60
T_3	*Polyalthia longifolia* fermented leaf extract alone @ 4%	99.21^{f}	175^{d}	97^{f}	8.53^{abcd}	351^{e}	6.18^{e}(14.39)	84.89
T_4	*Azadirachtia indica* fermented leaf extract alone @ 2%	107.47^{c}	179^{c}	107^{b}	8.71^{abc}	369^{bc}	6.78^{c}(15.09)	57.27
T_5	*Azadirachtia indica* fermented leaf extract alone @ 3%	109.31^{b}	181^{b}	111^{a}	8.83^{ab}	373^{b}	5.77^{b}(13.89)	77.48
T_6	*Azadirachtia indica* fermented leaf extract alone @ 4%	111.08^{a}	185^{a}	112^{a}	9.01^{a}	377^{a}	4.99^{a}(12.90)	87.80
T_7	*Pongamia pinnata* fermented leaf extract alone @ 2%	101.68^{e}	171^{e}	100^{e}	8.59^{abcd}	355^{e}	7.32^{e}(16.29)	53.87
T_8	*Pongamia pinnata* fermented leaf extract alone @ 3%	104.83^{d}	175^{d}	102^{d}	8.63^{abcd}	360^{d}	6.02^{d}(14.79)	76.11
T_9	*Pongamia pinnata* fermented leaf extract alone @ 4%	106.96^{c}	178^{c}	105^{e}	8.65^{abcd}	364^{c}	5.91^{c}(14.39)	85.55
T_{10}	Dried leaves fermented leaf extract alone @ 2%	87.32^{k}	155^{l}	72^{m}	6.21^{e}	251^{k}	9.73^{k}(18.17)	38.68
T_{11}	Dried leaves fermented leaf extract alone @ 3%	89.56^{j}	157^{k}	77^{l}	6.57^{e}	281^{j}	9.21^{j}(17.66)	64.06
T_{12}	Dried leaves fermented leaf extract alone @ 4%	90.12^{j}	159^{j}	80^{k}	6.71^{e}	297^{i}	8.86^{i}(17.31)	78.34
T_{13}	Weeds fermented leaf extract alone @ 2%	92.77^{i}	161^{i}	85^{j}	7.87^{d}	312^{h}	8.68^{h}(17.13)	45.30
T_{14}	Weeds fermented leaf extract alone @ 3%	93.52^{i}	164^{h}	87^{i}	7.99^{cd}	320^{gh}	8.16^{h}(16.59)	67.10
T_{15}	Weeds fermented leaf extract alone @ 4%	93.11^{i}	167^{g}	90^{h}	8.11^{bcd}	333^{g}	7.38^{g}(15.76)	81.96
T_{16}	Mancozeb (0.2%)	105.42^{d}	174^{d}	104^{c}	8.61^{abcd}	362^{d}	5.48^{d}(13.53)	86.80
T_{17}	Control	80.32^{l}	149^{m}	62^{n}	5.03^{f}	233^{l}	40.92^{l}(39.76)	

and 90 DAT) (T_6) significantly reduced the fruit rot incidence to 87.80 percent increase over control at 150 days after planting respectively than other treatments. All treatments significantly enhanced growth and fruit yield when compared to control. Among the treatments, application of *Azardiracta indica* fermented neem leaf extract alone @3% (seedling dip + spraying @ 60 and 90 DAT) was found to significantly increase the mean plant height (111.08 cm), number of flowers/plant (185 nos), mean number of fruits/plant (112 nos), mean fruit length (9.01 cm) and fruit yield (377 g/plant) when compared to all other treatments (Table 3).

The use of the plant products for the management of plant diseases has a special significance in the context of avoiding environmental pollution, accumulation of toxic substances in the produce and development of resistance by the plant pathogens.

Screening of fermented leaf extracts *(Polyalthia longifolia, Azadirachta indica, Pongamia pinnata,* dried leaves and weeds) for their antifungal property against *C. capsici* revealed that plant species possessed a very high antifungal activity. Yadav *et al.,* (2017) obtained maximum reduction in disease (69.30%) with minimum percent disease (17.24) and maximum fruit yield (8.23 q/ha) with the application of propiconazole (0.1%) as seed treatment and foliar spray with neem leaf extracts (5%) resulting in decreased PDI and increased fruit yield. These findings were in agreement with the results of

Hedge *et al.,* (2001) who reported the efficacy of plant extracts (chilli, ocimum, neem, onion, *clerodendron*) and fungicides (0.05% carbendazim, 0.2% mancozeb) in controlling *C.capsici* causing fruit rot of chilli and reported an inhibitory activity of all the plant extracts and fungicide tested against the pathogen. Musakhan and Zacharia, (2017) revealed that two neem based plant products *viz*., Neem seed kernel extract (NSKE) and neem oil could inhibit the radial growth and sporulation of *C. capsici*. This effect of neem extract on mycelial growth and sporulation of *C. capsici* was also reported by Singh and Korpraditskul, (1999) and Suthin Raj *et al*., 2014 which supports their findings. According to Begum *et al*., (2016), garlic and neem showed a complete inhibition of mycelial growth of *C. capsici* at 0.1% and 0.2% concentrations. This findings is in accordance with the findings were Bharadwaj and Sahu, (2014) who reported that plant extracts of Ocimum, ginger, garlic, turmeric and onion extracts inhibited the growth and sporulation of *Colletotrichum spp*.

In conclusion, this study shows that fermented leaf extracts of neem have strong antifungal activities against *Colletotrichm capsici*. The extracts can be used as protective pesticides since mycelia inhibition of the pathogen was effective and also easily biodegradable and eco- friendly compared to chemicals. This may suggest their potential for future formulation into products for controlling anthracnose diseases of chilli, papaya and mango.

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