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Efficacy of Plant Extracts and Chemicals against *Ascochyta phaseolorum*, Causal Agent of Ascochyta Blight of *Vigna anguiculata* (L.) Walp.

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A B S T R A C T

During survey, cowpea [*Vigna anguiculata* (L) Walp.] was found severely affected by ascochyta blight and disease was found well distributed in the surveyed areas of Meghalaya. The per cent disease incidence was found maximum in Mawlai (52.34%) under East Khasi Hills followed by RRTC (45.57%) under Ri-Bhoi district. In average, PDI was found highest in Ri-Bhoi district (37.50%) and least in West Khasi Hills district (15.47%). *Ascochyta phaseolorum* was consistently isolated from symptomatic leaves. Plant extracts and fungicides were evaluated *in vitro* against the growth of the pathogen.Among the fungicides, propiconazole and carbendazim at 0.1 and mancozeb at 0.2 per cent concentrations gave 100% inhibition of mycelial growth. Among the plant extracts, garlic extract at 5% showed the highest per cent inhibition (86.91±1.07%) of mycelial growth followed by turkey berry at 15% (82.33±0.44%). These could be further evaluated under *in vivo* condition to confirm its efficacy and could be included as components for integrated disease management for ascochyta blight.

Introduction

Cowpea [*Vigna anguiculata* (L.) Walp.] synonymous as lobia, barbati and black eyed pea is assumed to be the principal ancient pulse crop of India. Cowpea grain provides a cheap and nutritious food for relatively poor urban communities. They are source of protein (22-24%), calcium (0.08-0.11%), iron (0.005%) and essential amino acids (Lysine, leucine and phenylalanine) (Tiwari and Shivhare, 2016). It leaves 30-40 kg N/ha in the soil for the succeeding crop. The share to world production cowpea dry was 8.45 million metric tons (Singh, 2011). Ascochyta blight is a major disease of cowpea (and many other legumes) under humid conditions often devastating, causing extensive defoliation (Singh and Allen, 2006). Ascochyta blight causes more than 50 per cent or 75 per cent of yield loss in pea (Salam *et al.*, 2011). A wide variability of French bean, cowpea and Indian bean is found in the various parts of the NE region (Asati and Yadav, 2009). Prevailing

weather conditions in this region is very favorable for the occurrence and disease severity. Until now, little effort has been directed towards the occurrence and management of ascochyta blight in this area. In the present study, attempt has been made to ascertain the occurrence of ascochyta blight on cowpea in Meghalaya and efficacy of locally available plant extracts and fungicides were evaluated for their efficacy against *A.phaseolorum*.

Materials and Methods

Survey and assessment of incidence of cowpea ascochyta blight

Surveys were conducted in East Khasi hills, West Khasi hills and Ri-Bhoi districts of Meghalaya during July to August 2017. The leaves per cent disease incidence was assessed by collecting leaves from randomly selected cowpea plants per field surveyed, applying standard 0-9 grade disease rating scale (Mayee and Datar, 1986) as detailed below.

Based on numerical ratings or scale observed per cent disease index (PDI) was calculated using the formula given below:

$$PDI = \frac{\text{Total numerical ratings}}{\text{No. of leaves examined X Maximum disease grade}} \times 100$$

Samples collection, isolation and maintenance of *A. phaseolorum* isolates

The leaves showing typical leaf blight symptoms collected from various places of Meghalaya were brought to the laboratory. The selected infected leaf samples were cut into small pieces from the advancing margins of lesions and the healthy portions. The pieces were surface sterilized with 1% sodium hypochlorite for one minute followed by three serial washing with sterile distilled water. Surface sterilized leaf bits were then aseptically plated on solidified and cooled PDA (Potato Dextrose Agar) medium in 90 mm Petri plates under aseptic conditions of Laminar-air-flow cabinet. Inoculated plates were incubated at 27±1°C for seven days. Developing fungal colonies were purified by hyphal tip cut method to obtain pure culture of the isolates. The pathogen was identified by comparing with the cultures in the laboratory and relevant literatures. The fungal isolates were then sub cultured periodically and preserved in PDA slants at 4°C.

Pathogenicity test

Pathogenicity test was done on detached healthy leaves of *Vigna unguiculata,* leaves were surfaced sterilized with 1% sodium hypochlorite solution followed by rinsing in sterile distilled water 2-3 times and then air-dried. Leaves were placed in chambers lined with moist filter paper and wounded by pressing slightly with a pipette tip, and 5mm disks of actively growing mycelium (5 days old) were applied on the wounded leaves. Each of the leaf were moistened with wet balls of absorbent cotton wool to create a humid condition and incubated at room temperature for 7 days and observed for disease appearance. Controls were inoculated only with PDA disks. The pathogen was re-isolated from lesions developed on the cowpea leaves.

In vitro evaluation of botanicals (plant extracts) and fungicides against *A. phaseolorum*

Several plant extracts / botanicals and fungicides have been found antifungal against many *Ascochyta* spp. Therefore, four botanicals and four fungicides reported earlier as effective against many phytopathogens and which are locally available were evaluated for their biopesticidal effects on growth of

pathogen applying poisoned food technique (Nene and Thapliyal, 1979) and using PDA as basal medium. The plant extracts and chemicals that were used are given in Table 1.

Preparation of plant extracts

Aqueous leaf extracts of the test botanicals were prepared by taking 100 gm of the desired plant material and washing them two to three times in running tap water. Then they were subjected to washing again thrice with sterile distilled water and air dried and crushed in 100 ml distilled (w/v). The crushed extracts were squeezed through two layers of muslin cloth to extract the juice and further filter it through Whatman No. 1 filter paper. The filtrate was centrifuge at 15,000 rpm for 10mins. The supernatant was sterilized finally through bacteria proof membrane syringe filter (0.22µ) under laminar air flow. The final clear extracts prepared was the standard plant extracts of 100% concentration and were stored at 4ºC in refrigerator.

Poisoned food technique

An appropriate quantity of each plant extract (100%) and fungicides was mixed thoroughly with autoclaved and cooled (40ºC) PDA medium in conical flasks (250 ml cap.) to obtain desired concentrations. The plates containing PDA without any plant extract was maintained as untreated control. Upon solidification of PDA, all the plates were aseptically inoculated by placing in the centre a 5 mm mycelial disc obtained from a five days old actively growing pure culture. All these plates were then incubated at 27±1°C. Three replications were maintained for each treatment.

The mean colony growth of the test fungus was recorded when the control plate achieved full growth. The mean mycelial growth and standard deviation were calculated as described below. Percentage inhibition (I) of the pathogen was calculated by following the formula described by Vincent (1927):

$$\text{Inhibition (\%)} = \frac{C-T}{T} \times 100$$

Where,

I = per cent inhibition of mycelial growth,
C = Growth in control plate (cm) and
T = Growth in treated plate (cm)

Results and Discussion

Survey and assessment of incidence of cowpea ascochyta blight

A survey was conducted at different localities under three districts of Meghalaya for assessing the disease incidences ascochyta blight on cowpea, the details of which are mentioned in (Table 2). The ascochyta blight disease was found well distributed in all surveyed areas. Highest disease incidence was recorded at Mawlai (52.34%) under East Khasi Hills, followed by RRTC (45.57%) under Ri-Bhoi district. In average, disease incidence was found highest in Ri-Bhoi district (37.50%), followed by East Khasi Hills (30.85%) and least in West Khasi Hills district (15.47%).

Sample collection, isolation and maintenance of *A. phaseolorum* isolates

The affected plant parts were collected and standard tissue isolation technique was followed to obtain the pathogen causing ascochyta blight in cowpea (Figure 1). A globose light brown pycindia and numbers of hyaline pycnidiospores was observed on microscopic dissection of the disease samples (Figure 2A, B and C). On repeated isolation, it was found that *A. phaseolorum* was

consistently associated with ascochyta blight of cowpea. The purified pathogen culture was sub-cultured periodically on PDA slants and stored in refrigerator at 4ºC.

Pathogenicity test

Pathogenicity was carried out by inoculating 5mm disks of actively growing mycelium on the healthy leaves of *Vigna unguiculata* which was wounded by pressing slightly with a sterile pipette tip and maintained in a moist chamber. Seven days after inoculation irregular circular pale lesions with grey centre and less prominent zonation of spots appeared on inoculated leaves (Figure 3). The pathogen was found similar on re-isolation from these artificially infected leaves.

In vitro efficacy of plant extracts and fungicides on growth of *A. phaseolorum*

Four different plant extracts and four fungicides were evaluated for their efficacies on mycelium growth of *A. phaseolorum* at different concentrations by following poisoined food technique (Figure 4). The per cent inhibition was worked out when the mycelium growth of the pathogen in control plate cover full growth. The data obtained were statistically analysed and presented in Table 3.

Survey and assessment of incidence of cowpea ascochyta blight

Rating scale	Description
0	No symptoms on the leaf.
1	Small, irregular brown spots covering one percent or less of the leaf area.
3	Small, irregular, brown spots with concentric rings covering 1-10 per cent of the leaf area.
5	Lesions enlarging, irregular, brown with concentric rings covering 11-25 per cent of the leaf area.
7	Lesions coalescing to form irregular brown patches with concentric rings covering 26-50 percent of the leaf area.
9	Dark brown patches lesion with concentric rings covering 51 percent or more of the leaf area.

Table.1 Treatments of botanicals and fungicides

Sl. No.	Treatments		Part to be used	Concentration (%)
1	Garlic	*(Allium sativum)*	Clove	5
2	Ginger	*(Zingiber officinale)*	Rhizome	15
3	Turmeric	*(Curcuma longa)*	Rhizome	15
4	Turkey berry	*(Solanum torvum)*	Leaf	15
5	Propiconazole	(Tilt 25 EC)		0.1
6	Carbendazim	(Bavistin 50% WP)		0.1
7	Copper hydroxide	(Kocide 2000 35% WG)		0.2
8	Mancozeb	(Indofil M-45 75% WP)		0.2
9	Control	Media without plant extract/chemicals		

Table.2 Per cent disease incidence (PDI) of ascochyta blight in various regions of Meghalaya

District	Locality	Disease Incidence (%)	Mean Disease Incidence (%)
Ri-Bhoi	Umiam,	44.72	37.5
	Mawpun Kshaid	29.72	
	RRTC	45.57	
	Syadrit	30	
West Khasi Hills	Nongstoin	40.57	15.47
	Riangdo	0	
	Balat	0	
	Mairang	21.34	
East Khasi Hills	Mawlai	52.34	30.85
	Malki	33.33	
	Upper Shillong	37.74	
	Sohryngkham	0	

Table.3 Efficacy of plant extracts and fungicides against *A. phaseolorum*

Sl. No.	Treatments	Concentration (%)	Mean colony diameter (cm)	Per cent Inhibition
1	Garlic	5	1.18 ± 0.09 (1.08)	86.91 ± 1.07 (9.32)
2	Ginger	15	3.28 ± 0.07 (1.81)	63.58 ± 0.86 (7.97)
3	Turmeric	15	4.38 ± 0.06 (2.09)	51.37 ± 0.69 (7.16)
4	Turkey berry	15	1.59 ± 0.03 (1.24)	82.33 ± 0.44 (9.07)
5	Propiconazole	0.1	0.00 ± 0.0 (0.70)	100.00 ± 0.00 (10.00)
6	Carbendazim	0.1	0.00 ± 0.0 (0.70)	100.00 ± 0.00 (10.00)
7	Copper hydroxide	0.2	2.69 ± 0.04(1.64)	70.15 ± 0.48 (8.37)
8	Mancozeb	0.2	0.00 ± 0.00 (0.70)	100.00 ± 0.00 (10.00)
9	Control	-	9.00 ± 0.00 (3.00)	0.00 ± 0.00(0.70)

(Note: Values within the parentheses indicate square root transformed values)

Fig.1 Typical ascochyta blight symptoms on cowpea leaves in field

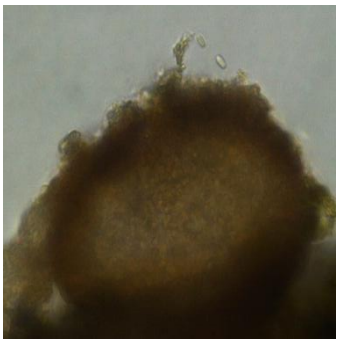

Fig.2 (A) Pycnidium (40X)

Fig.2 (B) Pycnidiospores extruding from pycnidium (10X)

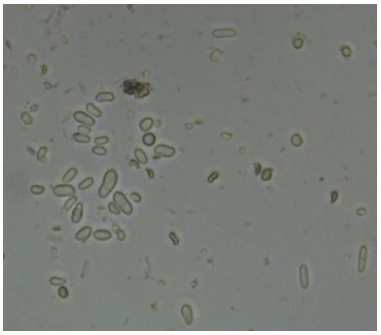

Fig.2 (C) Pycnidiospores (40X)

Fig.3 Disease symptoms on artificial inoculation of *A.phaseolorum* on cowpea leaves

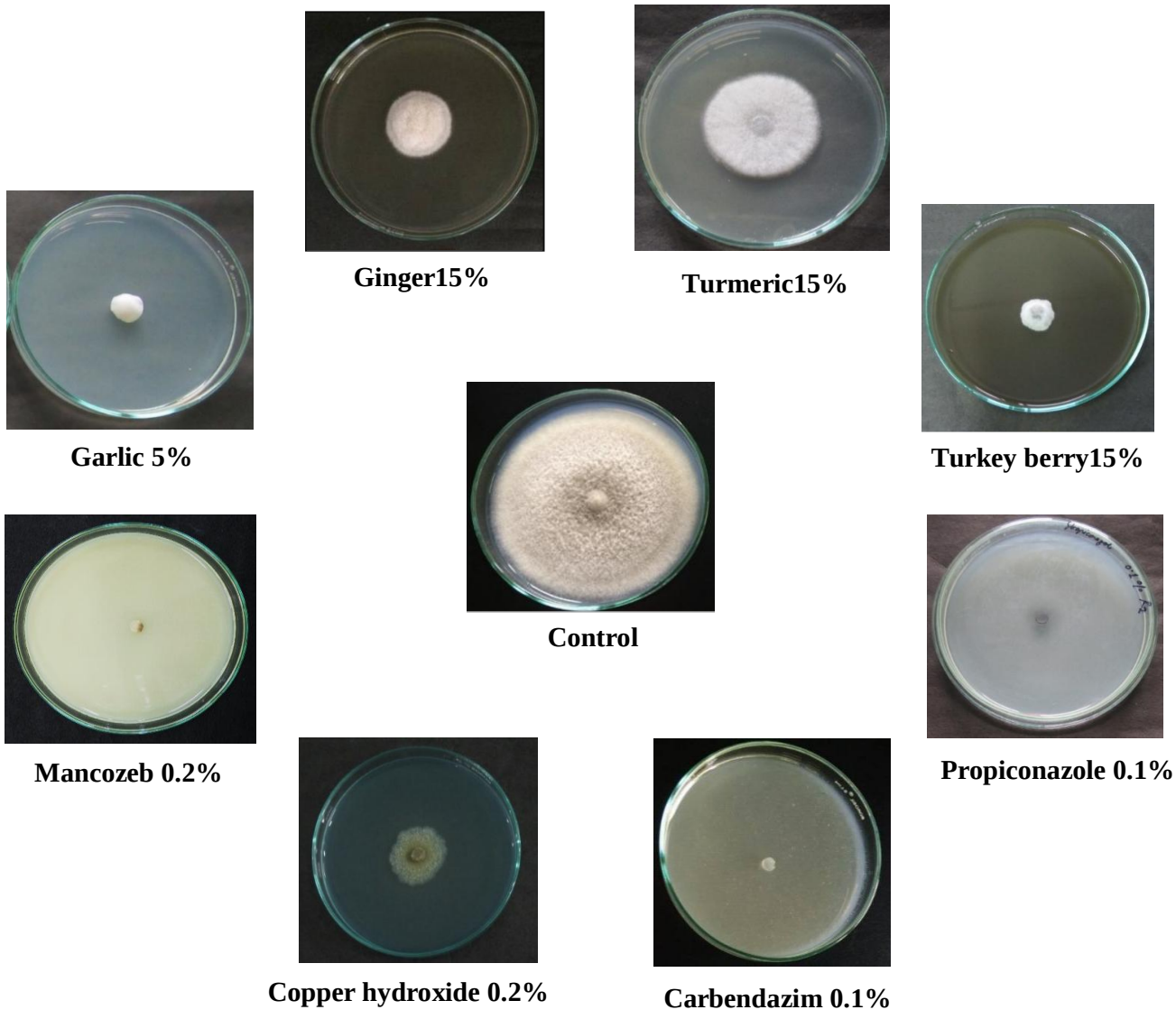

Fig.4 *In vitro* efficacy of different plant extracts and fungicides at different concentrations on growth of *A. phaseolorum*

Plant extracts displayed varying degrees of effectiveness against *A. phaseolorum* at different concentrations. The study showed that the garlic clove extract at 5% potentially inhibited (86.91%) the growth of the pathogen followed by extracts of turkey berry at 15%(82.33%), ginger 15% (63.58%)and turmeric 15% (51.37%).

Among the four different fungicides tested against *A. phaseolorum*, it was found that propiconazole and carbendazim each at 0.1 per cent concentration gave 100% inhibition of mycelial growth. However, mancozeb at 0.2 per cent concentration could inhibit 100% while copper hydroxide showed 70.15% inhibition at 0.2 per cent concentration.

Survey and assessment of incidence of cowpea ascochyta blight

The disease was found well distributed in all surveyed areas during the month of July and August. Highest disease incidence was recorded at Mawlai (52.34%) under East Khasi Hills, followed by RRTC (45.57 %) under Ri-Bhoi district. In average, disease incidence was found highest in Ri-Bhoi district (37.50%), followed by East Khasi Hills (30.85%) and least in West Khasi Hills district (15.47%).

The present finding is in agreement with those of Sutton and Waterston (1966) who reported that *A. phaseolorum* was severe in rainy seasons and had caused considerable losses to pulses in mountainous regions of the Punjab and Rhodesia. Similarly, Singh and Sharma (1998) observed that the disease occurs at all physiological stages ranging from seedling to maturity and Tadesse *et al.,* (2017) also observed that the disease was found more prevalent during flowering/ pod setting stage in surveyed fields of Ethiopia and recorded disease incidence of chickpea blight ranged from 0 to 46.6%.

In vitro efficacy of plant extracts and fungicides on growth of *A. phaseolorum*

Out of four plant extracts and fungicides evaluated for their efficacy against the growth of *A. phaseolorum*, garlic clove at 5% gave maximum average inhibition (86.91%) of mycelial growth. This finding was on par with the findings of Jargees *et al.,* (2010) who reported that plant extracts of garlic have an inhibitory activity at 2, 4, and 6 g/l concentrations against *A. rabiei in vitro*. The fungal property of garlic might be due to presence of sulphur compounds such as ajoene and allyl propyl on the growth of pathogen. In the present investigation leaf extracts of turkey berry at 15% (82.33%) was found effective against growth of *A. phaseolorum*. The effectiveness of turkey berry might be due to the presence of alkaloids. However, ginger 15% (63.58%) and turmeric 15% (51.37%) also displayed satisfactory effect. The efficacy of turkey berry for its antagonistic potential against *A. phaseolorum* was found next to garlic. The fungicides propiconazole and carbendazim each at 0.1 per cent concentration gave 100% inhibition of mycelial growth. However, mancozeb and copper hydroxide showed 100% and 70.15 %inhibition at 0.2 per cent concentration respectively. The present findings were in agreement with Pande *et al.,* (2009) who reported that benomyl and carbendazim were effective against ascochyta blight. Shtienberg *et al.,* (2006) reported adequate suppression of ascochyta blight using mancozeb and chlorothalonil. Lepcha (2015) further observed that carbendazim and propiconazole at 0.1% gave 100% inhibition of *A.phaseolorum.*

Summary

The ascochyta blight of cowpea was found well distributed in the surveyed areas of Meghalaya. Disease incidence was found

maximum in Mawlai (52.34%) under East Khasi Hills followed by RRTC (45.57%) under Ri-Bhoi district. In average, disease incidence was found highest in Ri-Bhoi district (37.50%) and least in West Khasi Hills district (15.47%). *Ascochyta phaseolorum* Sacc. was found to be consistently associated with ascochyta blight of cowpea. The disease was found more prevalent on leaves than stems and pods. Garlic 5% (86.91±1.07%) showed the highest per cent inhibition of mycelial growth followed by turkey berry 15% (82.33±0.44%).Fungicides propiconazole and carbendazim at 0.1% concentration gave 100% inhibition of mycelial growth and mancozeb at 0.2% concentration also gave 100% inhibition.

Ascochyta blight was found to be widely distributed on cowpea in different regions of Meghalaya. The chemical carbendazim and turkey berry plant extracts were found effective in inhibiting the growth and may be further evaluated under *in vivo* condition to confirm its efficacy and could be included as components for integrated disease management for ascochyta blight.

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