Original Research Article

Efficacy of different fungicides and trichoderma isolates against leaf blight disease of *Piper longum, Tylophora indica* and *Hibiscus subdariffa* under in *vitro*.

Abstract:

Leaf blight of the medicinal plants (*Piper longum, Tylophora indica* and *Hibiscus subdariffa*) is one of the most significant diseases that severely damage these crops throughout the year in West Bengal .So, management of this disease is essential at now. A few chemical fungicides and trichoderma isolates were tested under *in vitro* against the disease-associated pathogens in order to identify efficient chemical fungicides and bio agents.It was found that the trichoderma isolates T-2 and T-3 were effective against *Colletotrichum gloeosporioides*, whereas the isolates T-1 and T-2 were efficient in inhibiting the growth of the test pathogen better against *Fusarium* sp. and *Sclerotium rolfsii*.With a higher percentage of inhibition (77.60%), copper oxychloride was the most effective fungicide against *Fusarium* sp. Conversely, carbendazim and dithane m-45 were more effective against *Sclerotium rolfsii* and *Colletotrichum gloeosporioides*, showing 78.33% and 77.93% of inhibition, respectively, and less effective against *Fusarium* sp at higher concentrations (400 ppm). To determine whether or not these chemical fungicides and trichoderma isolates are effective under *in vivo*, they will be tested in the field against the corresponding pathogens associated with the disease.

Key words: *Colletotrichum gloeosporioides, Fusarium* sp, leaf blight, management, *Sclerotium rolfsii*, trichoderma isolates.

Introduction:

India has a rich culture of medicinal herbs and spices, which includes more than 2000 species and has a vast geographical area with high potential abilities for Ayurvedic, Unani, and Siddha traditional medicines but only very few have been studied chemically and pharmacologically for their potential medicinal value.

Human beings have used plants for the treatment of diverse ailments for thousands of years. According to the World Health Organization, most populations still rely on traditional medicines for their psychological and physical health requirements since they cannot afford the products of Western pharmaceutical industries. Rural areas of many developing countries still rely on traditional medicine for their primary health care needs and have found a place in day-to-day life. These medicines are safer and cheaper than synthetic or modern medicine. People living in rural areas from their personal experience know that these traditional remedies are valuable sources of natural products to support human health, but they may not understand the science behind these medicines-, but know that some medicinal plants are highly effective only when used at therapeutic doses.

Herbal medicines are in great demand in both developed and developing countries as a source of primary health care owing to their attributes having wide biological and medicinal activities, high safety margins, and lower costs. Herbal molecules are safe and overcome the resistance produced by pathogens as they exist in a combined form or in a pooled form of more than one molecule in the protoplasm of the plant cell (Lai and Roy, 2004; Tapsell *et al.,* 2006). Even with the advent of modern or allopathic medicine, Balick and Cox (1996) have noted that a number of important modern drugs have been derived from plants used by Indigenous people.

Increasing demand for herbal medicines has resulted in the "slaughter harvesting" of medicinal plants from wild sources. At present 90% of the medicinal plants come from wild. These trends do not promise sustainable use of medicinal plants, which fulfills health care needs of majority of the population. In India 300 plant species become exceedingly rare and endangered and 150 of these species are endemic. So, at this point of crisis increased, organized cultivation of medicinal plants become necessity and inevitable.

West Bengal government has recommended some medicinal plants like Aswagandha, Sarpagandha, Senna, Tulsi, Pipul, etc. for commercial cultivation in different zones that suffer from root rots, cankers, wilts, leaf spots, scabs, blights, anthracnose, rusts, mildews, smuts, mosaics, yellows, root knots, etc throughout the year. Among them, leaf blight disease of a few medicinal plants (*Piper longum, Tylophora indica* and *Hibiscus subdariffa*) seems to be very important which causes a drastic reduction in crop production. So, management of this disease is very essential in the present situation. In this present dissertation, attempts have been made to manage the disease with different chemical fungicides and bio agents under *in vitro* conditions.

Methods materials:

Isolation of causal organism causing the leaf blight disease of medicinal plants:

For the isolation of the pathogens from the three host plants, infected leaves are collected and preserved. Isolation was carried out in a sterilized zone of the laminar air flow. The diseased specimens already washed with tap water were taken and with the help of a sterilized scissor, small pieces of the leaf were cut into small pieces which contained the diseased portion as well as the healthy tissue. The pieces were dipped in $HgCl_2$ solution for 1 min. and were later rinsed three times with sterile distilled water. With the help of sterilized forceps, each piece was placed aseptically on the solidified PDA on the sterilized plates depending upon the diseased specimen. About 3-4 such pieces were placed on each plate maintaining some distance from each other and the Petri plates were incubated at 28 $\pm 1^0$C. After 5 days, the growing fungus was examined under a micro-scope for sporangial production. The isolates were maintained on potato dextrose agar medium. All the isolates were preserved at 5 ^{0}C. Sub cultures were made at 15 days intervals. This procedure was followed for all the mentioned medicinal plants. Three replications were maintained for each plant specimen and kept in an incubator. Three pathogens namely *Colletotrichum gloeosporioides* causing Blight of leaves in *Piper longum*, *Sclerotium rolfsii* causing blight in *Tylophora indica* and *Fusarium* sp. causing blight of leaves in *Hibiscus subdariffa* were isolated.

Pathogenicity test:

a) Preparation of spore suspension and artificial inoculation of plant:

For artificial inoculation, the culture of inoculum was prepared in PDA media poured petri plate. The plate was incubated for 4 days in a BOD incubator at 28± ºC. After getting the culture of the test fungi, spore suspension was prepared with sterile distilled water. The spore suspension was sprayed on the healthy plants; control plants were also maintained by spraying only sterile distilled water and then covered with the poly-propylene packet to maintain the humidity and maintain the favourable condition for disease development.

b) Confirmation of pathogens:

15-20 days after inoculation on the test plants, disease symptoms were developed. The diseased leaves were collected and again re-isolated the pathogens to compare with the previously isolated pathogens and to get confirmation about the disease-causing pathogens.

Antagonistic potential of *Trichoderma* isolates:

The antagonistic properties of Trichoderma isolate were tested on PDA medium by Dual Culture Plate Technique. 5 days old culture of the fungi under study was plated aseptically at the edge of Petri plates 2 days before the placement of *Trichoderma sp*. Paired cultures were observed for a total of 9 days before being discarded. All the ratings were done after contact between pathogens and antagonists using a modified Bell's (Bell *et al.* 1982) scale (1-5) developed as follows:

Class I (R_1) – The antagonist completely overgrew the pathogen (100% overgrowth).

Class II (R_2) – The antagonist overgrew at least 2/3 $^{rd.}$ of pathogen surface (75% overgrowth).

Class III (R_3) – The antagonist colonized on half the growth of the pathogen (50% overgrowth).

Class IV (R_4) – The pathogen and antagonist locked at the point of contact.

Class V (R_5) – The pathogen overgrew the mycoparasite.

Bio-assay of some chemical fungicides against the pathogens:

The fungicidal solutions were prepared on the basis of active ingredients (ai) of the products and to determine the fungicidal effect on hyphal growth, the poisoned food technique was followed using CAM as a food base. After autoclaving and cooling (45–50 ^{0}C) different concentration of fungicides as per treatment were incorporated/mixed into the molten carrot agar media. This sterile molten media containing fungicide was poured aseptically into sterile Petriplates. In control only molten media without fungicides was poured. Each treatment as well as the control was replicated thrice. Each plate was aseptically inoculated with mycelial disc and incubated at 28 ± 1 ^{0}C. The colony diameter of the fungus was measured when in control full plate growth was observed.

Per cent inhibition of growth was calculated by the formula:

$$\% \text{ growth inhibition} = \frac{\text{Growth in control} - \text{Growth in treatment}}{\text{Growth in control}} \times 100$$

Result and discussion:

Colony Characters of the Bio-Control Agents:

Three isolates of *Trichoderma* sp were isolated and their colony characters were described after growing in PDA media for four days after inoculation at 24hours interval (Table 1).

Table no.1: Colony characters of the three isolates of *Trichoderma* sp.

Isolates	24 hours	48 hours	72 hours	96 hours
T_1	White cottony appearance	Sparse growth, covers 2/3 of the medium	Light greenish appearance from the center to the periphery of the plate	Deep green sporulation
T_2	Bright and white mycelial growth	Cottony, white mycelial growth covers 2/3 of the plate	Full plate compact, cottony growth, sporulation more or less entire plate	Deep green, compact sporulation
T_3	White sparse growth	White fluty mycelial growth	Whitish green sporulation at 2/3 of the plate	Compact deep greenish fully plate growth

Antagonistic potentiality of *Trichoderma* isolates:

The three isolates of *Trichoderma* were evaluated against *Colletotrichum gloeosporioides, Sclerotium rolfsii* and *Fusarium sp* by dual plate technique, described in the part of methods and materials. Rating of antagonism was presented in Table 2 according to the modified Bell's ranking. From the table-2, it is found that T-1 is moderately antagonistic on *C. gloeosporioides* where T-2 and T-3 are highly antagonistic on it. The T-1and T-2 isolates were comparatively higher antagonist against *Fusarium* sp and *Sclerotium rolfsii* but T-3 was moderately effective against these pathogens. The similar type of result was obtained by Satapathy and Beura (2020) where the maximum growth inhibition of *Colletotrichum gloeosporioides* was noted in case of *Trichoderma viride* (84.9%) and *T. harzianum*(77.4%).

Similarly Gadhave *et al* (2020) reported that *Trichoderma harzianum* and *Trichoderma virens* were the most effective against *Fusarium oxysporum f. sp. lycopersici* with highest mycelial inhibition.

Table no.2: Screening of *Trichoderma* isolates against pathogens:

Pathogen	Isolate of Trichoderma sp	Point of contact (day)	Bell's ranking
Colletotrichum gloeosporioides,	T-1	3	R2
	T-2	3	R1
	T-3	3	R1
Sclerotium rolfsii	T-1	3	R2
	T-2	3	R2
	T-3	3	R3
Fusarium sp	T-1	3	R2
	T-2	3	R2
	T-3	3	R3

Effect of copper oxychloride on different fungal isolates of medicinal plants:

In the table 3, *in vitr*, a test of copper oxychloride revealedthat this fungicide was not effective against *Colletotrichum gloeosporioides***,** at a lesser concentration (50ppm) as well as higher concentration (100ppm) where per cent inhibition was 5.94% and 21.28% respectively. But with the increasing of concentrations, its inhibition was increased. At 200ppm and 400ppm, it showed comparatively higher inhibition (41.58% and 52.47%) over control. Similar type of result was noticed against *Sclerotium rolfsii* where at lower concentrations (5ppm and 100ppm) it showed 7.04% and 32.86% inhibition and at higher concentrations (200ppm and 400ppm) per cent inhibition was 43.66% and 55.89% respectively over control. Although this fungicide was not effective against *Fusarium* sp at lower concentrations (5ppm and 100ppm). But it showed higher efficacy i.e., 55.60% and 77.60% inhibition at 200ppm and 400ppm over control. This result basically effect of copper oxychloride on *Fusarium* sp was similar with the result obtained by KL and Huilgol (2021).They reported that the non-systemic fungicides copper oxychloride was effective in inhibiting the mycelial growth with 82.97% and 86.02% at 2000 and 3000 ppm concentration.

Table no. 3: Effect of copper oxychloride on different fungi isolated from medicinal plants:

Concentra-tions	***Colletotrichum gloeosporioides***		***Sclerotium rolfsii***		***Fusarium* sp**	
	Average growth(cm.)	**% Inhibition**	**Average growth(cm.)**	**% Inhibition**	**Average growth(cm.)**	**% Inhibition**
400ppm	3.2	52.47	3.1	55.89	1.8	77.60
200ppm	3.9	41.58	4.0	43.66	3.6	55.60
100ppm	5.3	21.28	4.8	32.86	5.2	35.27
50ppm	6.3	5.94	6.6	7.04	7.4	8.30
Control	6.7		7.1		8.0	

SEm (±)	**0.077**		**0.042**		**0.044**	
CD (P=0.05)	**0.237**		**0.129**		**0.136**	
CV (%)	**3.02**		**1.62**		**1.69**	

Effect of carbendazim on different fungal isolates of medicinal plants:

According to Table 4, carbendazim was found to be ineffective against *Sclerotium rolfsii* and *Colletotrichum gloeosporioides* at concentration of 50ppm and 100ppm, respectively with percentage inhibition of 198.75%, 37.58% and 20.00%, 33.79%. However, it was found that it was most effective against both the pathogens as its concentration increased.It showed the highest inhibition (57.32% and 78.33 %) against *Colletotrichum gloeosporioides* at 200 ppm and 400 ppm, respectively, over the c ontrol. It also showed a comparable outcome against *Sclerotium rolfsii*, exhibiting inhibition of 55.17% and 77.93% at the same concentrations, respectively.This was also not be found as effective against *Fusarium* sp at lower concentrations (5ppm and 100ppm). But it comparatively showed higher inhibition of 53.805 and 64.56% at 200ppm and 400ppm respectively over control. The efficacy of test fungicide (carbendazim) is similar to studies conducted by Machenahalli *et al* (2021) where the fungicides Carbendazim 50 WP, Mancozeb 75 WP and Carbendazim 12% + Mancozeb 63% WP were effective and inhibited cent per cent mycelial growth of *C. gloeosporioides* in all the tested concentrations. Another experiment conducted by Bashir *et al* (2018) against *Fusarium oxysporum* f. sp. *capsici* causing Fusarium wilt of chilli pepper in Pakistan and found that Carbendazim at 700 ppm expressed significant reduction in fungal growth.

Table no. 4: Effect of carbendazim on different fungal isolates from medicinal plants:

Concentra-tions	***Colletotrichum gloeosporioides***		***Sclerotium rolfsii***		***Fusarium* sp**	
	Average growth(cm.)	**% Inhibition**	**Average growth(cm.)**	**% Inhibition**	**Average growth(cm.)**	**% Inhibition**
400ppm	1.1	78.33	1.0	77.93	1.9	64.56
200ppm	2.2	57.32	2.2	55.17	2.4	53.80
100ppm	3.2	37.58	3.2	33.79	3.0	43.03
50ppm	4.2	19.75	3.8	20.00	3.8	28.48
Control	5.2		4.8		5.3	
SEm (±)	**0.029**		**0.041**		**0.052**	
CD (P=0.05)	**0.089**		**0.126**		**0.160**	
CV (%)	**1.80**		**2.69**		**3.21**	

Effect of Dithane M-45 on different fungal isolates of medicinal plants:

The result of the in vitro assessment of Dithane M-45 against *Fusarium* sp., *Sclerotium rolfsii* and *Colletotrichum gloeosporioides* are shown in Table 5, where it was found to be most effective against both of these organisms at 100% inhibition at all fungicide concentrations (50ppm,100ppm,200ppm and 400ppm) relative to the control. However, it was in effective against *Fusarium* sp. At lower concentrations (50ppm and 100ppm) due to less inhibition (13.53% and 21.80%, respectively), as well as at 200ppm with less inhibition (36.09%).In an *in vitro* study by Javaid *et al.* (2007), the four fungicides (Acrobat MZ, Ditha neM45, Aliette, and Ridomil Gold) were tested against *Colletotrichum gloeosporioides*. The r esultsshowed that Dithane M-45 was the most effective with all used doses significantly reducing the fungal bio mass by roughly 60-66% when recommended and at various lower doses.In a similar vein, Yaqub and Shahzad (2006) found that at higher concentrations, Senco zeb and Dithane M-45 significantly inhibited the growth of *Sclerotium rolfsii*.

Table no.5: Effect of Dithane M-45 on different fungal isolates of medicinal plants:

Concentra-tions	*Colletotrichum gloeosporioides*		*Sclerotium rolfsii*		*Fusarium* sp	
	Average growth(cm.)	% Inhibition	Average growth(cm.)	% Inhibition	Average growth(cm.)	% Inhibition
400ppm	0	100	0	100	4.3	51.50
200ppm	0	100	0	100	5.7	36.09
100ppm	0	100	0	100	6.9	21.80
50ppm	0	100	0	100	7.7	13.53
Control	5.5		5.7		8.9	
SEm (±)	-		-		**0.065**	
CD (P=0.05)	-		-		**0.200**	
CV (%)	-		-		**1.95**	

Conclusion:

As per the experiment results, Trichoderma isolates T-2 and T-3 were shown to be more successful against *Colletotrichum gloeosporioides*, while isolates T-1 and T-2 demonstrated greater efficacy against *Fusarium* sp. and *Sclerotium rolfsii*. The efficacy of copper oxychloride was limited to *Fusarium* sp. Carbendazim, a different fungicide, worked well against *Sclerotium rolfsii* and *Colletotrichum gloeosporioides.* However, Dithane M-45 demonstrated 100% inhibition at all concentrations, making it more effective against both of these infections.

Future scope:

In order for farmers to obtain and use effective chemical fungicides and bio agents to protect their medicinal plants, all effective trichoderma isolates and chemical fungicides must be evaluated against the respective pathogen causing leaf blight disease under field conditions to test their efficacy under *in vivo*.

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