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Major diseases of Ber (*Zizyphus mauritiana*) in West Bengal and *in vitro* interactions of associated fungal pathogens with bioinoculants

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Ber is a perennial, tropical and subtropical minor fruit crop belongs to the family Rhamnaceae. Due to extension of its commercial cultivation, importance of different diseases and their effects are also increasing. Therefore under this experiment the major emphasis was given to develop an efficient management strategy of the specific disease problems. The major diseases of Ber were Black dusty growth on ber (*Neopestalotiopsis clavispora*), Fruit and Leaf Canker (*Nectria mauritiicola*), Anthracnose (*Colletotrichum gloeosporioides*) and Leaf spot of Ber (*Cladosporium zizyphi*). Several antagonists viz, *Trichoderma viride*, *Trichoderma harzianum*, *Trichoderma* sp., *Pseudomonas fluorescens, Bacillus amyloliquefaciens* and also some fungicides - Mancozeb 75% WP, Carbendazim 12% + Mancozeb 63 % (SAAF-75%WP) and Fosetyl-aluminium 80%WP (Alliette) were tested against these diseases under in vitro condition. Among the antagonists *Trichoderma viride* and *Trichoderma harzianum* were found to be most effective against *Neopestalotiopsis clavispora* and *Nectria mauritiicola* respectively while Trichoderma sp. (unidentified) was effective against both *Colletotrichum gloeosporioides* and *Cladosporium zizyphi*. The fungicide Carbendazim 12% + Mancozeb 63% (SAAF-75%WP) was observed as most effective against *Neopestalotiopsis clavispora* and *Nectria mauritiicola* with ED_{50} value 3-4 ppm and 56 ppm respectively followed by mancozeb75%WP with ED_{50} value 4.97 ppm and 185 ppm and Fosetyl-aluminium 80%WP (Alliette) was inefficient to check the mycelia growth of these pathogens. Hence Carbendazim 12% + Mancozeb 63% WP was recommended to apply in the field to control these diseases successfully.

Key words: Antagonists, $ED_{50,}$ fungicides, *Zizyphus mauritiana*

INTRODUCTION

Among the all minor fruits, ber (*Zizyphus mauritiana*) is one of the most important fruit, belongs to the family Rhamnaceae. It has been grown in India since antiquity and Indo-Malaysian region of South East Asia is regarded as centre of origin of ber. In India ber cultivation is extensively done in Madhya Pradesh, Bihar, Uttar Pradesh, Punjab, Assam, Maharashtra, Gujrat (Singh *et al.* 2016). Ber is known as Poor man's fruit or King of Aridfruits.Now-a-days its cultivation is getting more importance but nature of damage and different diseases reduced the attention on its cultivation. The major diseases of Ber are Black Dusty growth on (80-90% fruit part & 40-50% leaf part affected), Fruit and Leaf Canker(50-100% fruit part & 20-30% stem affected), Anthracnose (60-70% leaf & 30-40% fruit part affected) and Leaf spot of Ber (affected leaf part 20-30 %). Therefore an attempt was made to isolate and identify the pathogens causing different diseases of ber and simultaneously to develop an effective management strategy under *in vitro* condition which will assist for controlling of these diseases under field condition resulting higher yield and better economical return to the farmers.

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MATERIALS AND METHODS

Survey of major diseases of ber

An extensive survey was done on major diseases of ber (Black mildew, fruit and leaf canker,

anthracnose and leaf spot) at different locations viz, Mandouri orchard (north 24 pgs dist., 22°57' N latitude and 88°20' E longitude with an altitude of 9.75 m above MSL) and Haringhata ber orchard (Nadia dist., 21.5° N latitude and 85° E longitude with an altitude 11.7 m above MSL), Cooch Behar district (26.3357° N, 89.4459° E) and Damanpur (Alipurduar dist., 26.4919° N, 89.5271° E) to study on their characteristic symptom and collection of the infected parts (leaf, fruit and stems) which ultimately used for isolation of their associated pathogens using PDA (Potato Dextrose Agar media).

Isolation of Trichoderma sp.

Trichoderma sp. which was used as antagonist, isolated from *Pleurotus* sp. (Oyster mushroom) and others (*Trichoderma viride* and *Trichoderma harzianum*) were from the air dried, grinded and sieved soil sample. Serial dilution was done upto 10^{-6}using sterilized distilled water. For each dilution one ml of suspension was first plated in each Petridis which was immediately rotated well in both directions (clockwise and anticlockwise) after pouring *Trichoderma* specific media (TSM). Three replications were made for each dilution. These plates were then incubated at 27±1°C for 6 days and maintained by sub-culturing on PDA slants.

Isolation of Pseudomonas and Bacillus sp.

Pseudomonas sp. was isolated from root through enrichment culture. *Bacillus* sp. was isolated using nutrient agar medium (NA).

In vitro assay of Trichoderma sp.

Antagonistic activities were measured through dual culture technique against pathogens on standard laboratory media PDA. From this observation the percent growth inhibition was calculated as {(Control- Treatment)/Control} ×100. Observation was also made as to whether the *Trichoderma* isolate was suppressive, competitive or inhibitory towards the test pathogen. The antagonistic ability of each isolate was measured through modified Bell's scale [Bell *et al.,* 1982] developed follows-

$S_{1=}$Antagonist completely overgrew the pathogen (100% overgrowth)
$S_{2=}$ Antagonist overgrew at least 2/3 growth of the pathogen (75% overgrowth)
$S_{3=}$ Antagonist colonized on half of the growth of the pathogen (50% overgrowth)
$S_{4=}$ Antagonist and pathogen locked at the point of contact.
$S_{5=}$ Pathogen starts overgrowing the antagonist.

In vitro fungicidal bioassay

For *in vitro* fungicidal bioassay, Mancozeb75% WP as contact fungicide, combination of Carbendazim 12% + Mancozeb 63% WP (SAAF 75%WP), Fosetyl-aluminium 80%WP (Alliette) as systemic fungicide were used at 500 ppm, 100 ppm, 50 ppm and 5 ppm concentration against two important pathogens *Neopestalotiopsis clavispora* and *Nectriamauritiicola* on Potato Dextrose Agar media. After inoculation of 9 mm mycelial disc at the centre of the petriplates, the plates were incubated at 28 ± 1^0C temperature for 2days. Extent of inhibition of mycelial growth by each fungicide was calculated by estimating the percent reduction in mean mycelial radial growth over that of control (Vincent, 1947).Effective concentration for 50% growth inhibition (ED_{50}) by the fungicides for each isolate was determined by plotting the log values of the fungicide concentration against the probit values of percent inhibition on a log- probit scale (Horsefall, 1956).A regression equation Y = a + bx (Y = antilog of concentration of the fungicide, x = probit value of percent inhibition, b = Regression co- efficient/slope, a = intercepts) was worked out and fitness of the equation was judged comparing the level of significance with the simple correlation coefficient (r) value at 5% level. Toxicity index is a screening tool to assess the potential toxicity of the fungicide. Toxicity index for each isolate fungicide combination was calculated by summation of the percent inhibition figures for four doses of fungicides.

RESULTS

Characteristic symptoms of the important diseases of ber and their associated pathogens

Black dusty growth

The infection appears as small, dirty black speaks on the leaf surface which quickly enlarges in size and coalesce to each other to form large patches and ultimately covers the whole leaf surface. Symptoms also appeared on developing fruits and in severe cases whole fruit surface is covered with

black powdery growth of fungal spores. Isolated organism from the infected part was earlier identified as *Pestalotiopsis* sp. (Hoque *et al.,* 2008). Pathogen, isolated from same disease sample has now been identified as *Neopestalotiopsis clavispora* which causes 80-90% infection in fruits and leaves of ber (Fig 1A-C).

Cankerous spot

The infection initially appears as small, dark black, completely circular elevated lesion (0.2-0.5cm dia.) on the affected fruits which rapidly become bigger in size and coalesce to each other. In some cases greyish black lesions are depressed, marked by raised prominent circular margin which can easily be defined. Small numerous white dot-like fungal structures can be seen on the spot of the fruits. In advanced stage of the disease the whole fruit become dry and mummified. Isolated organism from the infected part was *Nectria mauritiicola* (Fig 2 A-C).

Anthracnose or Fruit rot

Small circular to irregular corky, rough dark reddish brown spots appear with yellowish margin (2-4 mm diameter) on the upper surface of the leaves. Water soaked lesions were visible on fruits. At the centre of the spot a depressed zone or sporulating zone. Infected areas of the fruit peel become discoloured and rapidly loosen the tissues which give a characteristic rotting appearance. Due to production of numerous blackish structures (acervulli) the fruit body of the pathogen can be seen with naked eyes in this zone. Isolated pathogen from the infected part was *Colletotrichumgloeosporioides* (Fig 3 A-D).

Table 1: Antagonistic effect of *Trichoderma* sp. against *Neopestalotiopsis clavispora*

Treatment	Radial growth of pathogen at 3DAI (mm)	Per cent inhibition over control	Bell's scale
T. viride	21	53.33	S_1
T. harzianum	22	51.11	S_2
T. sp	25	44.44	S_2
Control	45	-	-
SE(m)	0.962	-	-
CD at 5%	00	-	-
CV	7.317	-	-

Leaf spot

Symptoms appear on the both surfaces of leaves. Dark black circular, regular spots can be seen on the upper surface of the leaves. These spots are separated from each other generally but sometimes coalesce to each other. On the corresponding lower surface black coloured circular spots were observed but on this side spots are intervenal. 2-5 spots were on the lower surface measuring 1-3mm in diameter. Disease was not so severe and associated pathogen was confirmed as *Cladosporium zizyphi* (Fig 4 A-C).

Antagonistic effect of different fungal and bacterial bio-agents against the pathogen isolates

Trichoderma viride, *Trichodermaharzianum* and *Trichoderma* sp.(Fig5 A- D) as well as *Pseudomonasfluorescens* and *Bacillus amyloliquefaciens* (Fig 6 A-C).were used to test their antagonistic potentiality against *Neopes-talotiopsis clavispora, Nectria mauritiicola, Colletotrichum gloeosporioides* and *Cladosporium zizyphi*.

Neopestalotiopsis clavispora

Maximum radial growth of this pathogen (25mm) was recorded with *Trichoderma* sp. (unidentified) at 3days after incubation (DAI) and minimum (21mm) with *T. viride* (Fig 7A)resulting highest inhibition with T. *viride* (53.33%) (Table 1).The bacterial bio agent *P. fluorescens* was more effective as it caused highest inhibition (31.37%) in comparison to *B. amyloliquefaciens*. which causes 23.52% inhibition of the target pathogen.(Table 5, Fig.8A).

Nectria mauritiicola

Most effective result was obtained in *T. harzianum* which showed 63.49% inhibition to the growth of the targeted pathogen and lowest antagonistic effect was found in *T. viride* where inhibition was 59.79% (Table 2 , Fig 7B). In case of bacterial antagonists highest inhibition (40%) was observed in *B. amyloliquefaciens* compared to *P. fluorescens* which exhibited 35.48% inhibition. (Table 5, Fig. 8B)

Colletotrichum gloeosporioides

Among the three *Trichoderma* species the most effective was *Trichoderma* sp. against this pathogen where inhibition was 65.38% (Fig 7 C).On the other hand *T. harzianum* was the least effective

Table 2: Antagonistic effect of *Trichoderma* sp. against *Nectria mauritiicola*

Treatment	Radial growth of pathogen at 3DAI (mm)	Per cent inhibition over control	Bell's scale
T. viride	31	50.79	S_1
T. harzianum	23	63.49	S_2
T. sp	24	61.90	S_2
Control	63	-	-
SE(m)	2.117	-	-
CD at 5%	00	-	-
CV	13.983	-	-

Table 3: Antagonistic effect of *Trichoderma* sp. against *Colletotrichum gloeosporioides*

Treatment	Radial growth of pathogen at 3DAI (mm)	Per cent inhibition over control
T. viride	20	61.53
T. harzianum	23	55.76
T. sp	18	65.38
Control	52	-
SE(m)	0.509	-
CD at 5%	2.053	-
CV	4.314	-

Table 4: Antagonistic effect of *Trichoderma* sp. against *Cladosporium zizyphi*

Treatment	Radial growth of pathogen at 3DAI (mm)	Per cent inhibition over control	Bell's scale
T. viride	23	32.35	S_2
T. harzianum	19	44.11	S_1
T. sp	14	58.82	S_2
Control	34	-	-
SE(m)	1.122	-	-
CD at 5%	4.524	-	-
CV	10.666	-	-

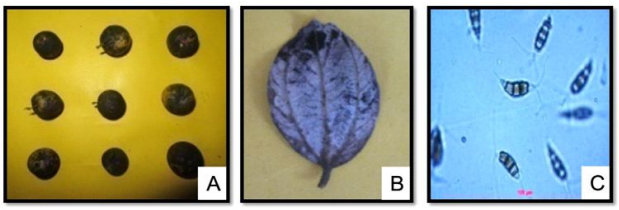

Fig. 1:(A-C). Symptoms of Black Dusty growth on Ber Fruit **(A)** and Leaf **(B)**, Spores of *Neopestalotiopsis clavispora*(C)

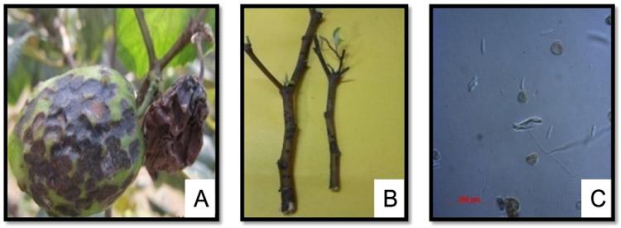

Fig. 2: (A-C) Symptoms of Cankerous infection on Fruits (A) and Stems (B), Spores of *Nectriamauritiicola* (C).

due to less inhibition (55.76%) (Table 3).*B. amyloliquefaciens* exhibited highest inhibition (54.83%) at 4 DAI and in case of *P. fluorescens* it was 35.48% inhibition (Table 5, Fig 8 C).

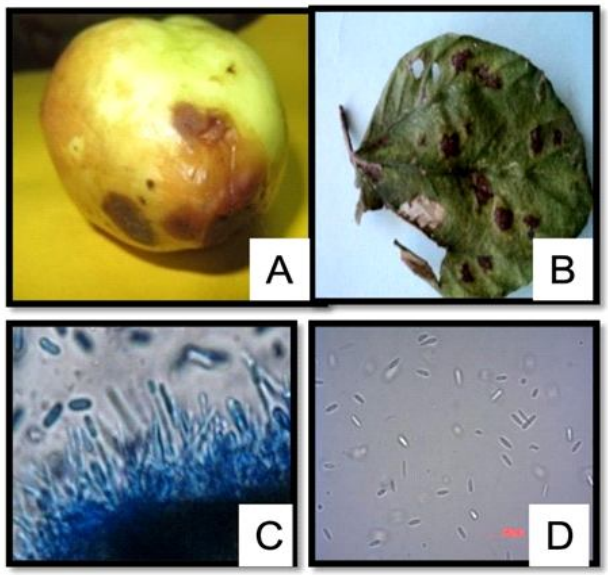

Fig 3: (A-D) Symptoms of Fruit Rot or Anthracnose on Fruit (A) and Leaf (B) , Acervuli (C) and Spores of *Colletotrichum gloeosporioides* (D)

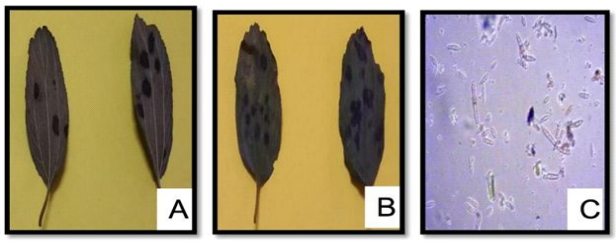

Fig 4: (A-C) Symptoms of Leaf spot of Ber on both side (A,B),Spores of *Cladosporium zizyphi* (C)

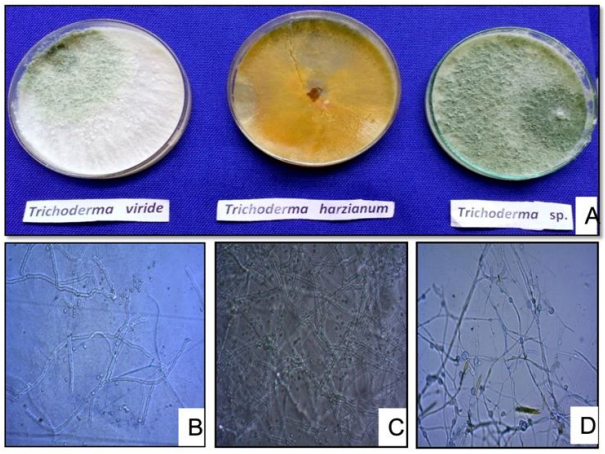

Fig 5: Cultures of various *Trichoderma* species (A),Microscopic view of *T. viride* (B), *T.harzianum* (C) and *Trichoderma* sp. (D).

Cladosporium zizyphi

Cladosporium zizyphi is a comparatively slow growing pathogen like *Colletotrichum* sp. Its radial growth is checked effectively by *Trichoderma* sp. (unidentified) with inhibition (58.82%) (Fig. 7 D).*T.viride* is comparatively least effective against

Table 5: Antagonistic effect of bacterial antagonist against *Neopestalotiopsis clavispora*, *Nectria mauritiicola*, *Colletotrichum gloeosporioides* and *Cladosporium zizyphi*

Treatment	*Neopestalotiopsis*		*Nectria*		*Colletotrichum*		*Cladosporium*	
	Radial Growth of Pathogen	Per cent inhibition over control (%)	Radial Growth of Pathogen	Per cent inhibitio-n over control (%)	Radial Growth of Pathogen	Per cent inhibition over control (%)	Radial Growth of Pathogen	Per cent inhibition over control (%)
Bacillus amyloliquefaciens	39	23.52	38	40	28	54.8	31	36.7
Pseudomonas fluorescens	35	31.37	42	35.48	40	35.4	37	24.5
Control	51	-	64	-	62	-	49	-
SE(m)	0.236	-	0.408	-	0.236	-	0.624	-
CD at 5%	1.118	-	2.675	-	1.544	-	4.085	-
CV	1.544	-	1.735	-	1.201	-	3.192	-

Table 6: *In-vitro* sensitivity of fungicides against *Neopestalotiopsis clavispora*

Fungi-cides	Growth of pathogen isolateat 2 days after incubation (mm)				Growth of pathogen isolateat 4 days after incubation (mm)				Growth of pathogen isolateat 6 days after incubation (mm)			
	5 ppm	50 pmm	100 ppm	500 ppm	5 ppm	50 ppm	100 ppm	500 ppm	5 ppm	50 ppm	100 ppm	500 ppm
Mancozeb 75%WP	19	12	2.0	00	22	14	8.0	00	29	16	10	00
SAAF 75% WP	1.0	0.6	00	00	13	11	9.0	00	25	18	16	00
Alliete 80% WP	35	30	25	16	60	55	43	35	75	58	48	40
Control	40.0				63.0				82.0			
SE(m)	0.808				0.615				0.854			
CD at 5%	2.385				1.815				2.522			
CV	11.838				4.727				5.316			

Table 7: *In-vitro* sensitivity of fungicides against *Nectria mauritiicola*

Fungicide	Growth of pathogen isolateat 2 days after incubation (mm)				Growth of pathogen isolateat 4 days after incubation (mm)			
	5ppm	50ppm	100ppm	500ppm	5ppm	50ppm	100ppm	500ppm
Mancozeb 75% WP	33	31	25.5	14	89	79	56	21
SAAF 75% WP	51	43.5	13	00	84	79	40	00
Alliete 80% WP	50	46	40	34	85	79	70	57
Control	56				89			
SE(m)	0.694				1.076			
CD at 5%	2.050				3.177			
CV	3.789				3.021			

Table 8: Effective dose of different fungicides for 50% Radial growth inhibition of target pathogens

Pathogen	Treatment	Mancozeb 75% WP Conc. of fungicide (ppm)				Carbendazim 12% + Mancozeb 63% WP (SAAF 75%WP) Conc. of fungicide (ppm)				Fosetyl-aluminium 80%WP (Aliette 80% WP) Conc. of fungicide (ppm)			
		5	50	100	500	5	50	100	500	5	50	100	500
	Log of conc.	0.69	1.69	2.00	2.69	0.69	1.69	2.00	2.69	0.69	1.69	2.00	2.69
	% growth inhibition	64.63	80.48	87.80	100	69.51	78.04	80.48	100	8.53	29.26	41.46	51.21
N. clavispora	Probit value of % inhibition	5.3745	5.8560	6.1650	8.0900	5.5101	5.7722	5.8560	8.0900	3.6278	4.4524	4.7879	5.0301
	Regression equation	Y=1.279x + 4.109 R^2 = 0.797				Y=1.177x + 4.225 R^2 = 0.667				Y=0.723x + 3.195 R^2 = 0.965			
	ED_{50} (ppm)	4.97				3-4				313			
	Log of conc.	0.69	1.69	2.00	2.69	0.69	1.69	2.00	2.69	0.69	1.69	2.00	2.69
	% growth inhibition	00	11.23	37.07	76.40	4.49	11.25	55.05	100	2.24	11.23	21.34	35.95
N. mauritiicola	Probit value of % inhibition	-	3.7893	4.6681	5.7192	3.2940	3.7840	5.1257	8.0900	2.9859	3.7840	4.2039	4.6389
	Regression equation	Y=2.928x – 1.631 R^2 = 0.954				Y=2.322x + 0.968 R^2 = 0.802				Y=0.842x + 2.413 R^2 = 0.989			
	ED_{50}	185				56				1181			

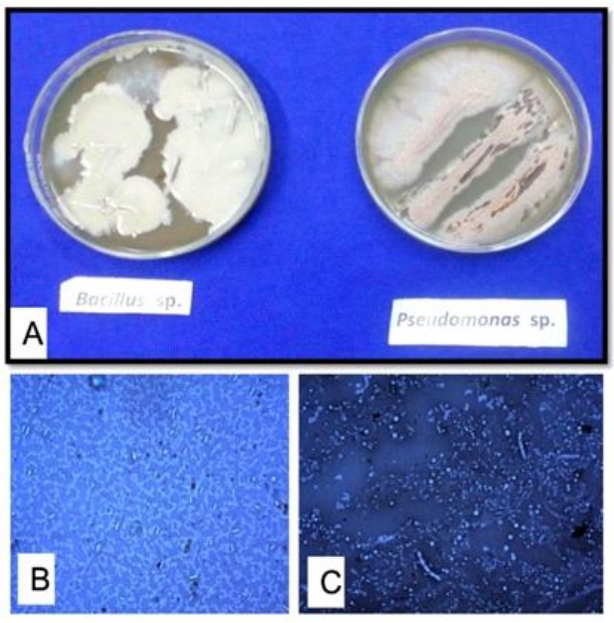

Fig. 6 : Culture of *Bacillus amyloliquefaciens* and *Pseudomonas fluorescens* (A), Microscopic view of *B.amyloliquefaciens* (B) and *P. fluorescence* (C).

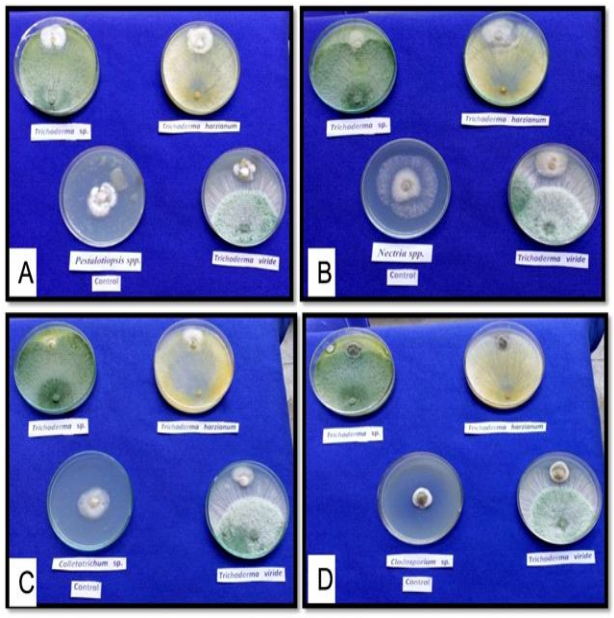

Fig. 7 : *In vitro* pairing of *Trichoderma* against *Neopestalotiopsis clavispora* (A), *Nectria mauritiicola* (B) *Colletotrichum gloeosporioides* (C) and *Cladosporium zizyphi* (D)

this pathogen due to less inhibition of mycelia (32.35%) (Table 4).*B. amyloliquefaciens* proved itself comparatively more effective against *C. zizyphi*as compared to *P. fluorescens B. amyloliquefaciens* causes 36.73% inhibition over the target pathogen (Table 5, Fig 8 D).

Sensitivity test of different fungicides against pathogen isolates collected from Be

Before application to the field, it is mandatory to test the chemical fungicides under the *in-vitro* condition to obtain a truthful result. So, to find out an effective chemical control measure, bioassay of three fungicides was done usingpoisoned food technique. The Contact fungicide-Mancozeb75% WP and systemic fungicide -Combination of Carbendazim 12% + Mancozeb 63% WP (SAAF 75%WP) and Fosetyl-aluminium 80%WP (Alliette) were tested at different concentrations viz, 500ppm, 100 ppm, 50 ppm and 5 ppm against two important pathogens *Neopestalotiopsis clavispora* and *Nectria mauritiicola.* .

Neopestalotiopsis clavispora

This is a fast growing pathogen and requires only 7 days to cover the whole petriplate (90 mm). Here observations were taken at 48 h interval from the day of incubation up to 6 DAI. In case of Mancozeb 75%WP, growth inhibition (64.63%) was recorded at 5 ppm concentration as compared to control. Complete inhibition of linear growth (100%) was found at 500 ppm. Combined product of carbendazim 12% + mannose 63% WP (SAAF 75%WP) showed 69.5% inhibition at 5ppm and complete inhibition of linear growth (100%) at 500ppm. Similar result was reported by Amrutha *et al.* (2018)that above 70% leaf blight disease of strawberry caused by *Neopestalotiopsis clavispora* was controlled using this combination of fungicides (Fig 9 A-C).Aliette was least effective against this pathogen. (Table 6).This result was similar with the work of Sanjay *et al.,* 2014who observed that systemic fungicide, combined product of Carbendazim 12% + Mancozeb 63% WP (SAAF 75%WP) @ 0.05% is highly effective against *N. clavispora* followed by Mancozeb 75% WP.

Nectria mauritiicola

Like *Neopestalotiopsis clavispora* both fungicides Mancozeb75%WP and carbendazim 12% + mancozeb 63% WP (SAAF 75% WP) were effective against *Nectria mauritiicola.* Carbendazim 12% + Mancozeb 63% WP (SAAF 75% WP) exhibited complete inhibition of mycelial growth (100%) at 500 ppm while Mancozeb 75% WP exhibited 76.40% inhibition. Aliette was least effective against this pathogen as it showed less inhibition (35.95%) at same concentration (Table 7, Fig 10 A-C).

Determination of ED_{50} value ofdifferent fungicides towards mycelium growth of the pathogens

Effective concentration for 50% inhibition (ED_{50}) of radial growth of the pathogen were calculated based on Regression equation of high correlation value for each fungal isolates and fungicide combination to assess the degree of sensitivity of the pathogen against fungicides. Regression value and correlation value were obtained from excel sheet by a scatter graph putting the value of log of concentration vs Probit value of percentage inhibition (Table 8, Figs. 11, 12).Pathogens showed discriminating sensitivity to Mancozeb75%WP and combination of carbendazim 12% + mancozeb 63% WP (SAAF 75%WP). ED_{50} value of mancozeb75%WP and carbendazim 12% + mancozeb 63% WP (SAAF 75%WP) against *Pestalotiopsis* sp. is 4.97ppm & 3-4ppm respectively whereas ED_{50} value of fosetyl-aluminium 80%WP (Alliette) is 313ppm Against *Nectria* sp. ED_{50} value of mancozeb75%WP and carbendazim 12% + mancozeb 63% WP (SAAF 75%WP) are 185ppm & 56ppm respectively whereas ED_{50} value of fosetyl-aluminium 80%WP (Alliette) is 1181ppm (Fig.13).

DISCUSSION

Various fungal diseases of ber (*Zizyphus mauritiana*) have been reported earlier by several authors (Banerjee *et al.,* 2021; Misra *et al.*, 2013).In the present study also the pathogens of the fungal diseases were isolated and identified. For management of the above mentioned diseases under field conditions first *in vitro* interaction study was carried out against their causal organisms with few bio control agents, where *T. viride* and *T. harzianum* were found to be most effective against *Neopestalotiopsis clavispora* and *Nectria mauritiicola* respectively and *Trichoderma* sp. was effective against *Colletotrichum gloeosporioides* and *Cladosporium zizyphi.* Similar results were also

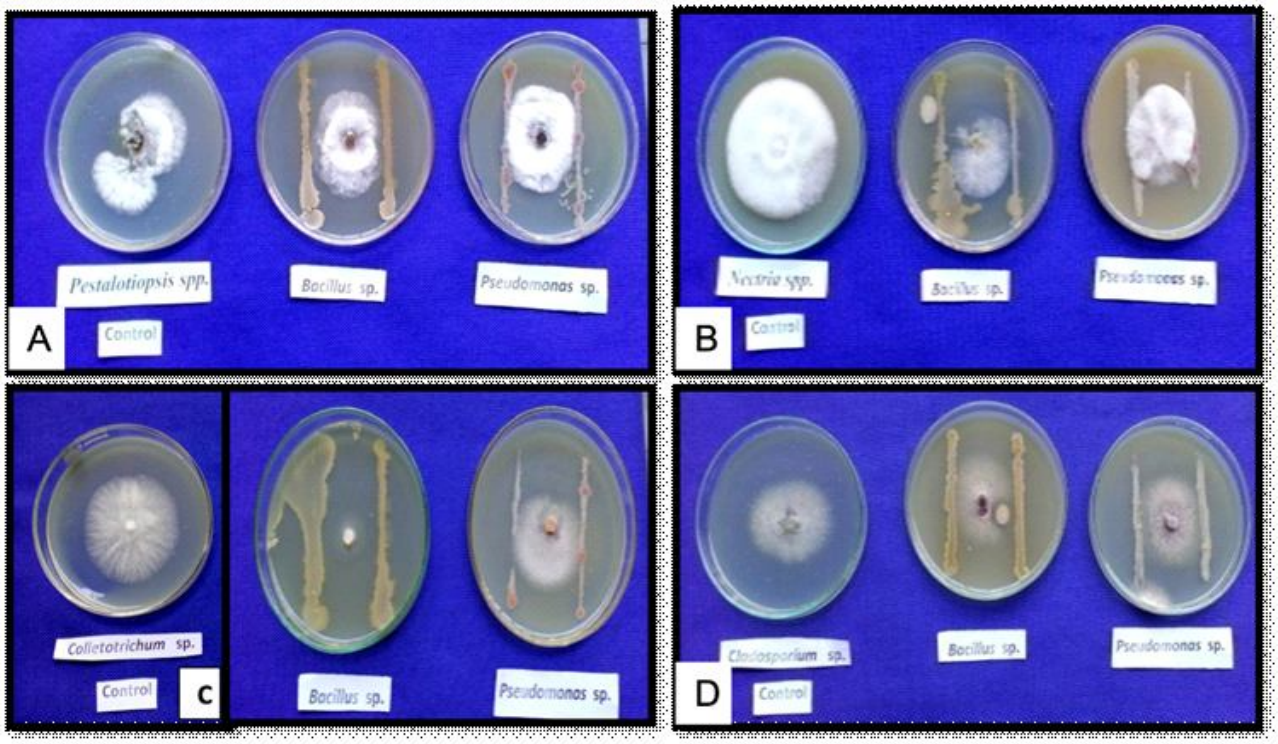

Fig. 8 : Antagonistic effect of *Bacillus amyloliquefaciens* and *Pseudomonas fluorescence* (Bacterial antagonists) against *Neopestalotiopsis clavispora* (A) *Nectria mauritiicola* (B), *Colletotrichum gloeosporioides* (C), *Cladosporium zizyphi* (D)

Fig. 9 : Effects of Mancozeb (A), SAAF (B) and Aliette (C) on mycelial growth inhibition of *N. clavispora*

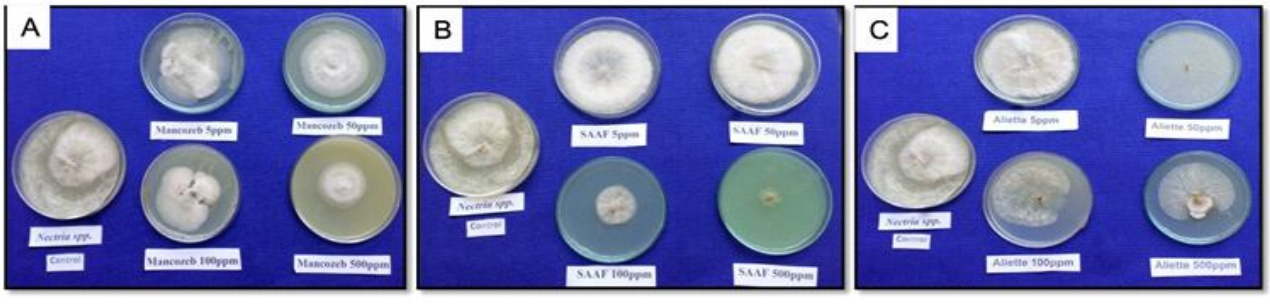

Fig. 10 : Effects of Mancozeb (A), SAAF (B) and Aliette (C) on mycelial growth inhibition of *N. mauritiicola.*

reported by many researchers (Rahman *et al.,* 2013) where *Trichoderma* strains effectively inhibited the mycelial growth of the fungi. Among the bacterial antagonists, *Pseudomonas fluorescens* was found effective only against *Neopestalotiopsis clavispora* while *Bacillus amyloliquefaciens* was effective against *Nectria mauritiicola*, *Colletotrichum gloeosporioides* and *Cladosporium zizyphi***.** Application of biocontrol agents against pathogenic fungi or bacteria is a good alternative of conventional disease manag-ement system (Kumar. 2008).In a simila work, Linu

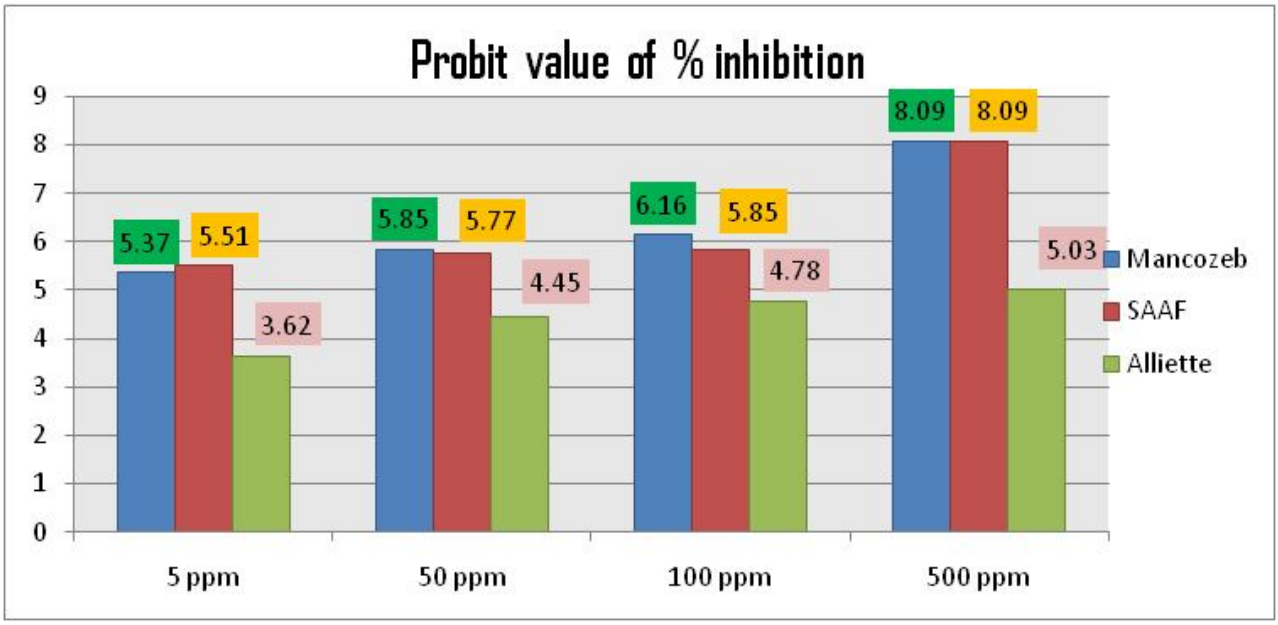

Fig. 11 : Effects of different concentrations of Mancozeb, SAAF and Alliette on growth inhibition(%) of *N. clavispora*

et al. (2013) who found that 40% inhibition of the radial growth against *Colletotrichum capsici* due to antagonistic effect of *Pseudomonas* sp. The SAAF was the most effective fungicide due to its lowest ED50value compared to other fungicides because lowest ED50 value indicate that fungicide is highly efficiency in controlling of the diseases. This property of SAAF may be due to combination of two molecules (carbendazim 12% + mancozeb 63% WP) whereas other fungicides are single molecule. Similar result was reported by Amrutha *et al.* (2018)that above 70% leaf blight disease of strawberry caused by *Neopestalotiopsis clavispora* was controlled using this combination of fungicides. Therefore combined product is better and recommended to control the diseases of ber compared to single product.

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