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In vitro evaluation of various antagonists against *Cercospora malayensis* causing leaf spot of okra

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Abstract
Okra (*Abelmoschus esculentus* L.) is one of the foremost vegetable crop grown extensively in south Gujarat during *Kharif* as well as summer seasons. *Cercospora* leaf spot incited by *Cercospora malayensis* (Stev. and Solh.) is a minor disease which was less significant earlier but is now considered as one of the emerging disease in south Gujarat region. Out of seven antagonists evaluated against *C. malayensis* under *in vitro*. *T. viride* appeared as strong and potent antagonism against *C. malayensis*. *T. viride* showed maximum antagonistic effect against the pathogen and had highest growth inhibition (86.86%) followed by *T. harzianum* (59.74%).

Keywords: *Bioagents*, *Cercospora malayensis*, dual culture method

Introduction
Okra (*Abelmoschus esculentus* L.) is a nutritious and delicious annual vegetable crop grown in the tropical and sub-tropical regions. It belongs to the family Malvaceae. There are about eight *Abelmoschus* species available in India. Out of these, *A. esculentus* is the only known cultivated species.

In India, total grown in an area of 511 hectares having total production of 6219 million tons with the productivity of 12.0 million tons per hectare (Anon., 2019a) [1]. Andhra Pradesh is the leading producer followed by West Bengal. In Gujarat total area under okra is 36740 hectares and the total production 480690 million tons with the productivity of 11.6 million tons per hectare (Anon., 2019b) [2].

In Gujarat, it is mainly grown in the districts of Vadodara, Surat, Junagadh, Bhavnagar, Valsad, Gandhinagar, Anand and Navsari. The okra crop occupies an area of 6500 hectares, having production 82225 million tons in Navsari district. (Anon., 2019b) [2].

In India, two species of *Cercospora viz.*, *Cercospora malayensis* Stev. And Solh. And *C. abelmoschi* EII. And Ev. Were found to be the cause of leaf spots in okra (Sridharan and Rangaswamy, 1968) [7]. These species differ in production of symptoms. In India, mainly two species of *Cercospora* produce leaf spots in okra *C. malayensis* causes brown, irregular spots and *C. abelmoschi* causes sooty black, angular spots. The affected leaves roll, wilt and fall. The leaf spots cause severe defoliation and are common during humid seasons.

The specific fungal diseases of okra are, *Cercospora* leaf spot caused by *Cercospora abelmoschi* and *C. malayensis*, Damping-off caused by *Pythium* sp. and *Rhizoctonia solani*, Powdery mildew caused by *Erysiphe cichoracearum*, Southern blight caused by *Sclerotium rolfsiii*, Wilt disease caused by *Verticillium alboatrum*, wet rot caused by *Choanephora cucurbitarum* (Raid and Palmateer, 2006) [6] and Alternaria leaf spot caused by *Alternaria chlamydospora* (Atia and Tohamy, 2004) [3].

During the rainy season, sooty leaf spot disease of okra caused by *Cercospora ablemoschi* (Ellis and Everh.) and *C. malayensis* (Stev. and Solh.) is now becoming one of the most important diseases of okra. Now a days, this disease incited by *C. malyensis* and *C. abelmoschi* becomes more severe in southern transition zone. The disease progress upward from lower and upper leaves and infects stem and fruits and produces similar symptoms (Dhancholia and Singh, 1992) [5].

Materials and Methods
The experiment was carried out in the laboratory of Department of Plant Pathology, N. M. College of Agriculture, Navsari Agricultural University, Navsari (Gujarat) during 2018-19. The trial was laid out in completely randomized design under laboratory condition.

Different bioagents *viz.*, *Trichoderma viride*, *T. harzianum*, *T. virens*, *T. asperellum*, *T. koningii*, *Pseudomonas fluorescens and Bacillus subtilis* obtained from Department of Plant

Pathology, Anand Agricultural University, Anand were tested against *C. malayensis* by using dual culture method (Dennis and Webster, 1971) [4].

Seven days old culture of the bioagents and 10 days old culture of the test fungus (*C. malayensis*) were used for the antagonistic studies. Twenty milliliter of sterilized melted V 8 juice agar was poured aseptically into Petri plates of 90mm diameter. Mycelial disc of 90mm diameter was cut from the edge of actively growing 10 days old culture of the pathogen and mycelial disc (5mm) of bioagents was cut from actively growing growth of the respective fungal sp. with help of cork borer were placed in the Petri plates at opposite side and 4cm apart from each other. Incase of bacterial antagonist, the bacterium was streaked on the one side of the Petri plate. On the other side of Petri plate 5mm mycelial discs of test pathogen was placed at the opposite side. Three repetitions of each treatment were kept and Petri plate with only pathogen served as control. All the plates were incubated at temperature 28 ± 1˚C in BOD incubator.

Observations on linear mycelial growth of the test fungus and bioagent were recorded at an interval of 24 hours and continued till untreated control plates were fully covered with mycelial growth of the test fungus. Per cent inhibition of the test fungus by the bioagents over untreated control was calculated by applying following formula:

The per cent inhibition of pathogen species was calculated as suggested by Vincent (1927) [10].

$$PGI = \frac{DC-DT}{DC} \times 100$$

Where,

PGI = Per cent growth inhibition

DC = Average Diameter of mycelial colony in control treatment (mm)

DT = Average Diameter of mycelial colony in treated set (mm)

Results and Discussion

Seven different antagonists *viz., Trichoderma viride, T. harzianum, T. virens, T. asperellum, T. koningii, Pseudomonas fluorescens* and *Bacillus subtilis* were obtained from different sources were used and studied for their antagonistic potential against *C. malayensis* by dual culture method as explained in materials and methods.

The result presented in table 1 and and Plate 1 revealed that all the antagonists against *C. malayensis* were significantly superior showing growth inhibition in the range of 25.00 to 86.86 per cent over the control. Out of seven antagonists, *T. viride* showed significantly maximum per cent growth inhibition (86.86 per cent) with the lowest fungal colony diameter of pathogen 10.33mm. The next better antagonists in order of merit were *Trichoderma harzianum* with 59.74 per cent and *T. virens* with 57.20 per cent growth inhibition, respectively with 31.67mm and 33.67mm fungal colony diameter. *Trichoderma asperellum* and *T. koningii* exhibited moderate inhibition of 51.27 and 49.57 per cent, respectively with fungal colony diameter of 38.33 and 39.67mm of *C. malayensis.*

Evaluation of antagonists indicated a significant difference between the fungal and bacterial antagonists on per cent inhibition of mycelia growth of *C. malayensis.* Among bacterial antagonists 36.86 and 25.00 per cent growth inhibition was recorded in *Bacillus subtilis* and *Pseudomonas fluorescens*, respectively. Our findings are in agreement with the results obtained by Swamy (2010) [9] who recorded a significant difference between the fungal and bacterial antagonists on per cent inhibition of mycelial growth of *C. capsici* under *in vitro* condition. Maximum inhibition (83.22%) was recorded in *Trichoderma viride* followed by *T. harzianum* (82.22%) and *T. virens* (79.44%) which were on par with each other. Among the bacterial antagonists *Bacillus subtilis* gave maximum inhibition of mycelial growth (58.70%). *Pseudomonas fluorescencs* (23.70%) showed least inhibition. The present results are in conformity with finding of Suresh (2013) [8] measuring the colony diameter of *C. capsici*, it was noticed that maximum reduction in colony growth was observed in *T. harzianum* (73%) which was significantly superior to all other bioagents tested. Next best was *T. viride* IIHR isolate (72.77%) followed by *T. viride* (71.33%) and *T. harzianum* IIHR isolate (71.11%). Least inhibition was notice in *B. subtilis* (23.33%).

Table 1: *In vitro* evaluation of bio agents against *C. malayensis* causing leaf spot of okra

Treatment No.	Bioagents	Colony diameter of pathogen (mm)	Growth inhibition over control (%)
T_1	*Trichoderma viride*	10.33	86.86
T_2	*Trichoderma harzianum*	31.67	59.74
T_3	*Trichoderma virens*	33.67	57.20
T_4	*Trichoderma asperellum*	38.33	51.27
T_5	*Trichoderma koningii*	39.67	49.57
T_6	*Pseudomonas fluorescens*	59.00	25.00
T_7	*Bacillus subtilis*	49.67	36.86
T_8	Control (Test pathogen only)	78.67	0.00
S Em±		0.80	
CD at 5%		2.40	
CV (%)		3.25	

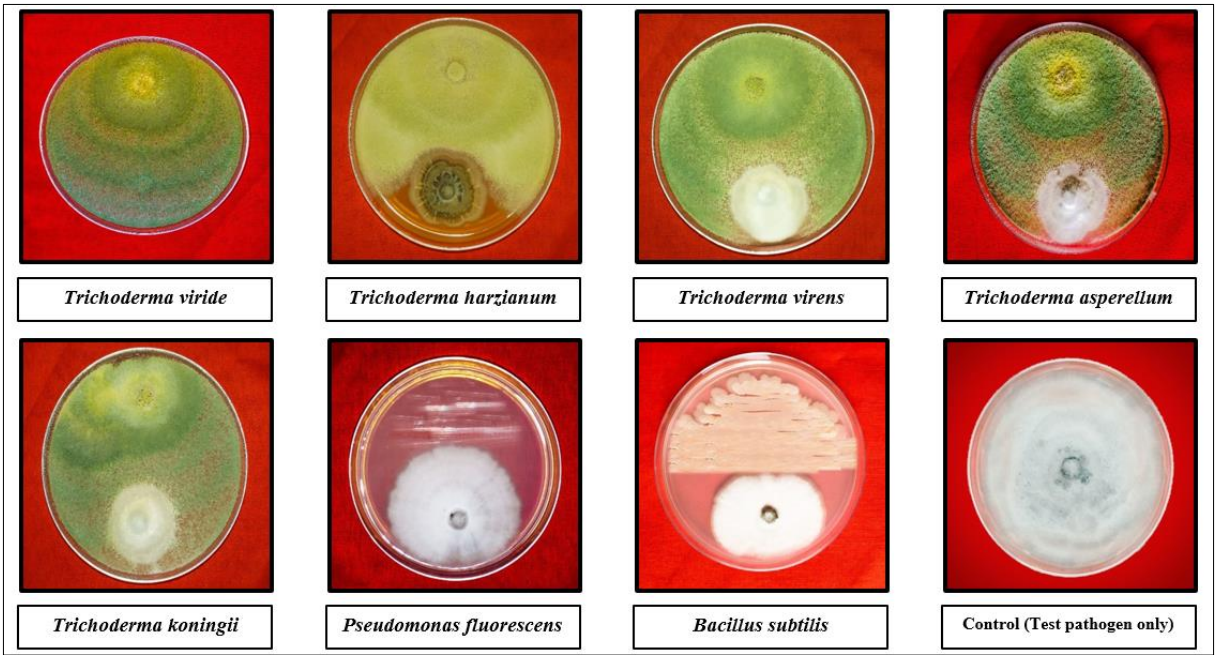

Plate 1: Antagonism of different bio agents against *C. malayensis* under dual culture technique

Conclusion

Out of seven antagonists evaluated against *C. malayensis* under *in vitro* by dual culture technique. All the *Trichoderma* isolates were more effective than bacterial isolates. Among these *T. viride* appeared as strong and potent antagonism against *C. malayensis*. *T. viride* showed maximum antagonistic effect against the pathogen and had highest growth inhibition (86.86%) followed by *T. harzianum* (59.74%).

From this experiment, it is very clearly shown that bioagents *T. viride* and *T. harzianum* having a different mode of action i.e., Antibiosis Produce several fungi toxic cell wall degrading enzymes (Chitinases, β-1-3 glucanases, proteases and cellulase) which can cause the inactivation of the pathogens enzymes.

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