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Research Article

Exploitation of epiphytic microorganisms and organic preparations for the management of Choanephora pod rot of cowpea

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ABSTRACT: Exploration of epiphytic microorganisms from different plant parts and their exploitation for the management of plant pathogens is a relevant approach in view of greater awareness of pollution free environment. The aim of the present study was to analyze the microbial communities with special focus on antagonists isolated from the fructosphere of cowpea (*Vigna unguiculata* L. Walp.) and the use of organic preparations such as panchagavya, jeevamruth, compost tea, vermiwash and fish amino acid for suppression of *Choanephora cucurbitarum,* the pathogen inciting pod rot in cowpea. A collection of six isolates of bacteria and fungi were isolated through serial dilution technique, and their efficacy in suppressing the pathogen were tested under *in vitro* conditions. Among the six isolates, the bacteria and fungi with maximum inhibitory activity against the targeted pathogen were selected for further identification and *in vivo* assay. Based on the cultural, morphological and biochemical characters, the bacterial and fungal antagonists were identified as *Pseudomonas fluorescens* and *Trichoderma virens*, respectively. *In vitro* assay of the organic preparations revealed that vermiwash (5% and 10%), jeevamurth (10%) and panchagavya (10%) completely inhibited the growth of pathogen. Application of effective dose of organic preparations and the selected antagonists on the excised cowpea pods revealed that, among organic preparations jeevamurth (10%) exhibited maximum suppression of pod rot by 60.64%, however the selected bacterial antagonist, *i.e., P. fluorescens* gave complete suppression of the pathogen. Under *in vivo* conditions, jeevamurth (10%), vermiwash (5%), *T. virens* (10^6cfu/ml), and *P. fluorescens* (10^6cfu/ml) showed the maximum suppression of the pathogen and the percentage suppression was recorded as 87.33, 75.22, 75.27 and 72.31% respectively. Therefore, the present study revealed that the organic preparations such as jeevamurth (10%), vermiwash (5%), and the indigenous species of *Pseudomonas fluorescens* and *Trichoderma virens* obtained from the fructosphere can be used in integrated disease management strategies against Choanephora pod rot of cowpea.

KEYWORDS: Antagonist, *Choanephora cucurbitarum,* panchagavya, *Pseudomonas fluorescens, Trichoderma virens*

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INTRODUCTION

Cowpea (*Vigna unguiculata* L. Walp.), a dicotyledonous plant belonging to the order Fabaceae is of major importance to the livelihood of millions of people in the tropics and is a good source of protein, vitamins and minerals (Quin, 1997). Due to the favourable agro climatic conditions and its higher biological value of proteins, the crop has gained much importance in Kerala and occupied a prime position among the vegetable crops raised in the state. However, the cultivation of this remunerative and nutritious crop is hampered by the incidence of several diseases. A wet pod rot disease has been noticed on the cowpea pods around the time of harvest, especially during the rainy season. The disease has been found to be more serious when the crop is raised for seed purpose. The cultural, morphological and molecular characterization of the pathogen revealed that the pathogen associated with this disease is *Choanephora cucurbitarum* (Berk. & Ravenel) Thaxter. Wet rot or pod rot of cowpea caused by *C. cucurbitarum* has been reported to affect the yield significantly (Bashir *et al.,* 1985). The crop loss due to this disease is estimated to be 7 - 20% (Oladiran, 1980). The disease was found to be aggravated during the period of high temperature and humidity (Hussein and Ziedan, 2013). The pathogen is a facultative saprophytic fungus belonging to sub division Zygomycotina, with a wide host range encompassing families such as Amaranthaceae, Cucurbitaceae, Malvaceae, Solanaceae and Nyctaginaceae (Abel – Mortal *et al.,* 2010). The pathogen under favourable conditions is capable of reproducing sexually by zygospore and asexually by sporangia and conidia.

The pathogen has been reported to be managed by spraying fungicides such as mancozeb (Chahal and Grover, 1974) after pod set. Since the disease is noticed in the edible pods, the dependence on fungicides alone for management poses a threat to human health and also enhances the risk of environmental pollution. These concerns along with the cost involved necessitate the disease to be managed by cheaper and ecofriendly strategies. Biological control is one among the safest method to reduce the incidence and severity of plant diseases without causing collateral damages to the environment as well as to the human health. The surfaces of the plant parts nests many epiphytic microorganisms, many of which are capable of influencing the growth of the pathogens based on an array of mode of action. Also the use of indigenous organic preparations such as panchagavya, jeevamruth, fish amino acid and vermiwash have emerged as promising biological control strategies in suppression of pest and disease incidence in several agricultural and horticultural crops. Hence, the present study is taken up with the objective managing the pathogen by exploiting the epiphytic microorganism obtained from the fructosphere of cowpea and the use of indigenous organic preparations.

MATERIALS AND METHODS

The epiphytic microorganisms present in the fructosphere were isolated, identified, and its potential in suppressing the growth of the pathogen under *in vitro* and *in vivo* conditions were studied using the standard protocols along with the indigenous organic preparations.

Isolation, purification and *in vitro* evaluation of antagonistic microflora against Choanephora pod rot of cowpea

Isolation of the antagonistic microflora from fructosphere

The saprophytic microflora present on the healthy fructosphere collected from the diseased field was studied. Serial dilution technique described by Johnson and Curl (1972) was used for the isolation of antagonist microflora from the healthy cowpea pods. The plates at room temperature were examined for the growth of fungal and bacterial colonies after 3-4 days of incubation. Observations were recorded on colony counts in Petri plates and expressed as the number of colony-forming units (cfu) per gram of sample. The colonies were observed for colour and shape, which were then transferred to PDA slant/nutrient agar slants.

In vitro evaluation of antagonistic microflora by dual culture technique

The fungal isolates obtained through serial dilution technique were evaluated for suppression of the pathogen *Choanephora cucurbitarum* by dual culture method described by Skidmore and Dickinson (1976). The mycelial disc of 7 mm diameter cut from seven day old culture of the saprophytic fungus and the pathogen were placed on two opposite sides of Petri plates containing sterilized PDA and incubated at room temperature. Three replications were maintained for the experiment. Control plates contained only the pathogen. Observations were recorded on the radial growth.

The bacterial antagonists obtained through serial dilution were tested for antagonism against *C. cucurbitarum* by dual culture technique (Utkhede and Rahe, 1983). The nutrient agar medium was melted and poured into sterile petri plates. After solidification, culture bits of 7 mm size of the pathogen was placed at the centre of each dish. The respective bacterial isolate was then streaked 2.5 cm away on both sides perpendicular to the pathogen placed at the centre. The nutrient agar plates with the pathogen served as the control.

The percentage inhibition of the pathogen over the control was calculated by the formula (Vincent, 1927):

$$I (\%) = R1- R2/ R1 \times 100$$

I - Percentage growth inhibition

R1 - Growth of pathogen in control

R2 – Growth of pathogen in treatment

Purification of the antagonistic microflora

The selected fungal and bacterial isolates were subjected to purification and further identification. The single colonies of fungus were purified by the hyphal tip method. Purification of bacteria was done by streaking on nutrient agar plates, and the single colonies of the bacteria transferred were then stored under refrigerated conditions for identification and subsequent studies of antagonism.

Identification of the antagonistic microflora

The fungal antagonist selected was subjected to a single spore isolation technique for purification and slide culture for morphological identification.

The single spore isolation technique by Dhingra and Sinclair (1985) was used for the purification of the selected fungal antagonist. A small bit of mycelium containing the conidia was transferred to a test tube containing sterile water and made into a spore suspension. From this spore suspension, a loopful was taken and streaked on water agar in a zig-zag manner. The plates were sealed using paraffin film and were incubated at room temperature for 24 h. The colonies developed from the single conidia were sub-cultured into PDA slants.

The method described by Riddell (1950) was used for the identification of the fungal isolate. The slide culture unit,

which consisted of Petri plates containing filter paper, two pieces of glass rods, two coverslips and one microscopic slide were autoclaved in a hot air oven. A five mm plain agar block was cut out using a sterile blade and using a sterilised inoculation needle, it was placed on the microscopic slides kept on glass rods. The mycelium of the fungal isolate was then inoculated at four corners of the agar block, and a coverslip was placed over the agar block. The filter paper was moistened with sterile water. The slide culture units were then sealed and incubated at room temperature. The slides were examined under low and medium power objectives of a Leika DM750 microscope, and micro-morphological characters of mycelia and conidia were observed.

Identification of bacteria

The bacterial isolates obtained through serial dilution were streaked on nutrient agar medium contained in sterile Petri plates. The bacterial isolates obtained were subjected to Gram staining technique to identify the bacterium. Gram staining was done based on the technique devised by Gram (1884). A bacterial smear was prepared on a glass slide. The smear was fixed by heating and a drop of crystal violet was poured on the heat-fixed smear. The excess stain was washed off in water after 2- 4 min. Gram's iodine was poured and waited for 1-2 min the excess stain was washed off in running water. The bacterial cells were stained with 95% ethanol so as to decolourise the stain, then the bacterial cells were counter-stained using safranin, and after 2 min excess stain was washed out. A drop of cedar oil was dropped into the slide and was observed under the oil immersion objective (100X).

The bacterial isolate that showed maximum inhibition of the mycelia of *C. cucurbitarum* was identified based on biochemical characters by outsourcing the culture at the Cashew Export and Promotion Council of India (CEPC), Kollam.

***In vitro* evaluation of organic preparations on fungal growth**

The organic preparations like compost tea, panchagavya, jeevamruth, vermiwash and fish amino acid were evaluated for *in vitro* suppression of the pathogen *C. cucurbitarum* by poisoned food technique described by Nene and Thapliyal (1993). Three different concentrations, *i.e.,* 2.5ml, 5ml and 10ml of each organic preparations were initially filtered through Whatman No.1 filter paper. It was then further sterilized by passing through bacterial proof filter before adding to the media. The measured quantity, *i.e.,* 2.5 ml, 5 ml and 10 ml of the organic preparations were then added into 47.5 ml, 45 ml and 40 ml of the distilled water under aseptic condition and mixed thoroughly. This mixture of different concentrations was then added to 50 ml of molten double strength PDA contained in three separate conical flasks to get desired concentration. The amended medium (20 ml) was then poured into the sterile petri plates and was allowed to solidify. The 7 mm mycelial disc from three-day old *C. cucurbitarum* culture was inoculated at the centre of the amended medium under aseptic conditions. The PDA plates with pathogen at the centre were served as control. The percentage inhibition of the pathogen over the control was calculated using the formula given previously.

***In vitro* suppression of Choanephora pod rot on excised pods using selected antagonists and organic preparations**

Fresh and healthy cowpea pods were collected from the field. The pods were washed under running water and then surface sterilized using 70% ethanol. The desired concentration of the selected antagonist and the organic preparations were sprayed on excised pods. The pods were then kept in a polythene cover and were incubated at room temperature for 24 h. The pods without any treatment served as the control. Three replications of each treatment were maintained. The percentage suppression of the disease by each treatment was worked out based on the lesion size and using the formula given in 1.2.

Studies on *in vivo* suppression of Choanephora pod rot using selected antagonists

A pot culture experiment was conducted in CRD to evaluate the efficacy of the selected antagonists in order to study the suppression of Choanephora pod rot under field conditions. The susceptible bush type cowpea variety Bhagyalakshmi was used for evaluation. The seeds were sown in pro - trays filled with potting mixture consisting of vermicompost and coir pith in 1:1 ratio. The cowpea seedlings were transplanted to the pot filled with potting mixture (sand, soil and cow dung in 1:1:1 ratio) after the emergence of first true leaves. Each pot contained one plant and three replication of each treatment were maintained. The treatments were given as foliar application after the pods attained maturity followed by challenge inoculation of the pathogen at 10^6 cfu/ml.

The occurrence of pod rot was recorded at weekly intervals. Scoring of the disease was done using 0-4 disease scale (Ziedan *et al.,* 2012) (Figure 1).

Grade	Description
0	Healthy
1	0 - 25 % of the pod infected
2	26 - 50 % of the pod infected
3	51 – 75 % of the pod infected
4	> 75 % of the pod infected

The percentage of disease incidence was calculated by using the formula

$$\text{Percentage Disease Incidence} \frac{\text{No. of pods affected}}{\text{Total no. of pod}} = \times 100$$

The percentage disease index was calculated using the formula given by McKinney (1923).

$$\text{Percentage Disease Index} = \frac{\text{Sum of grades of each leaf}}{\text{No : of leaves assessed}} \times \frac{100}{\text{Maximum grade used}}$$

RESULTS AND DISCUSSION

Isolation of the antagonistic microflora from the fructosphere

The saprophytic microflora was isolated from the healthy pods of disease-free cowpea plants among the Choanephora pod rot-infected cowpea plants in the field. A total of six isolates were obtained from the fructosphere through serial dilution technique (Table 1). Among the six isolates, three isolates were fungi which were initially designated as FS1, FS2 and FS3 while the bacterial isolates were named as BF1, BF2 and BF3 respectively.

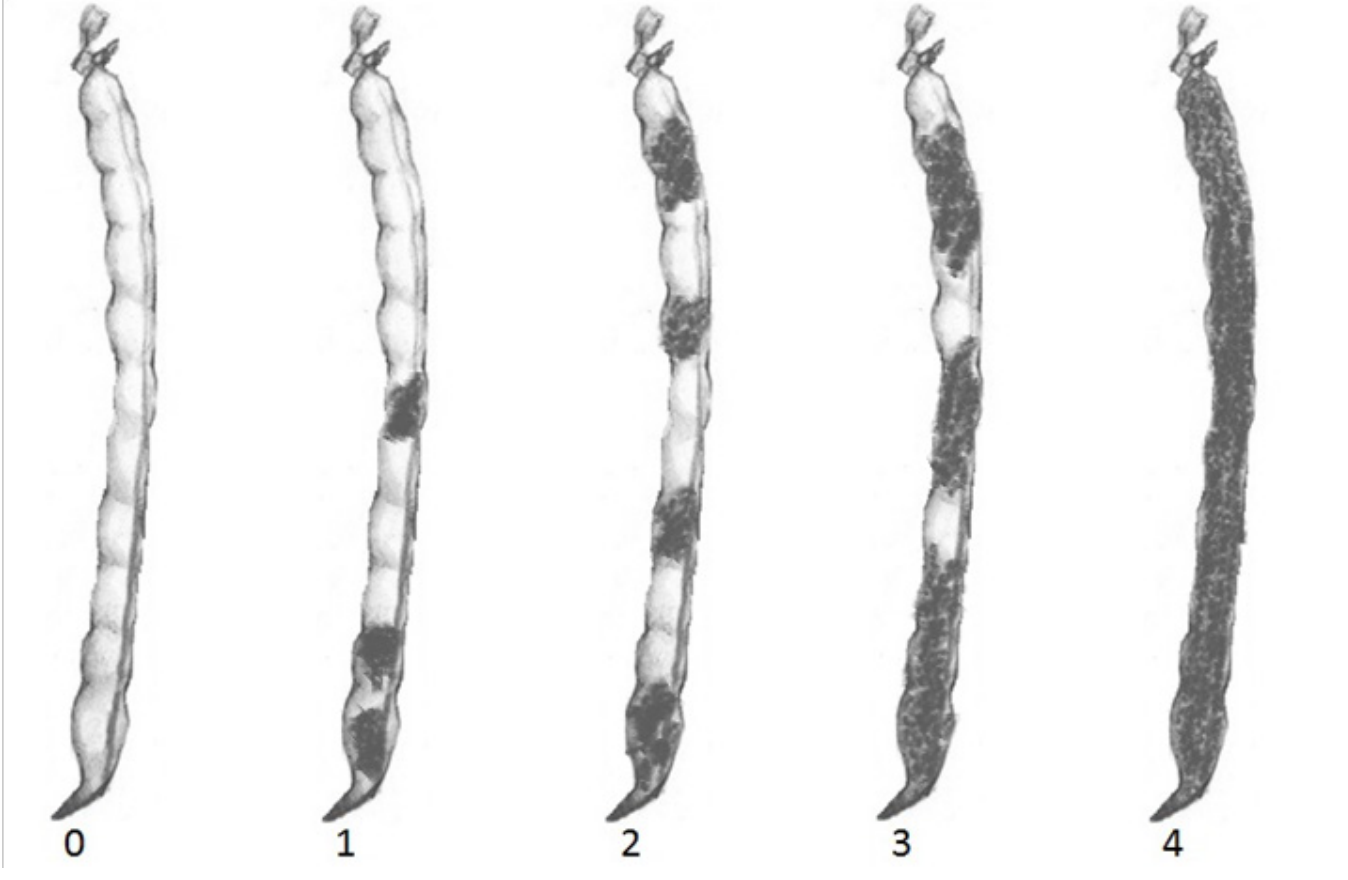
In vitro evaluation of the antagonistic fungi and bacteria against the pathogen

The three fungal isolates obtained through isolation *i.e.,* FS1, FS2 and FS3 were evaluated for their antagonistic efficacy against *Choanephora cucurbitarum* under *in vitro* conditions. Among these, FS1 was found to be most effective in controlling the growth of the pathogen with a percentage inhibition of 79.5% (Figure 2) and was significantly superior over the other two fungi while the antagonistic effect of FS2 and FS3 was found to be on par with each other (Table 2). Therefore, FS1 was selected for evaluating the efficiency of disease suppression under *in vivo* conditions.

Among the three bacterial isolates, *viz.,* BF1, BF2 and BF3, evaluated for their efficacy in suppressing the growth of pathogen under *in vitro,* BF1 was found to be effective in controlling the pathogen with a percentage inhibition of 55 % (Figure 3) followed by BF2 and BF3 (Table 3). Hence, among the six isolates the fungal and bacterial isolates with higher efficacy, *i.e.,* FS1 and BF1 in suppressing the growth of the pathogen were selected for further studies.

Identification of the selected antagonists

The fungal antagonist FS1 was identified as *Trichoderma virens* based on the cultural (Figure 4(a)) and morphological studies (Figure 4(b)) of the fungus. The fungus was fast

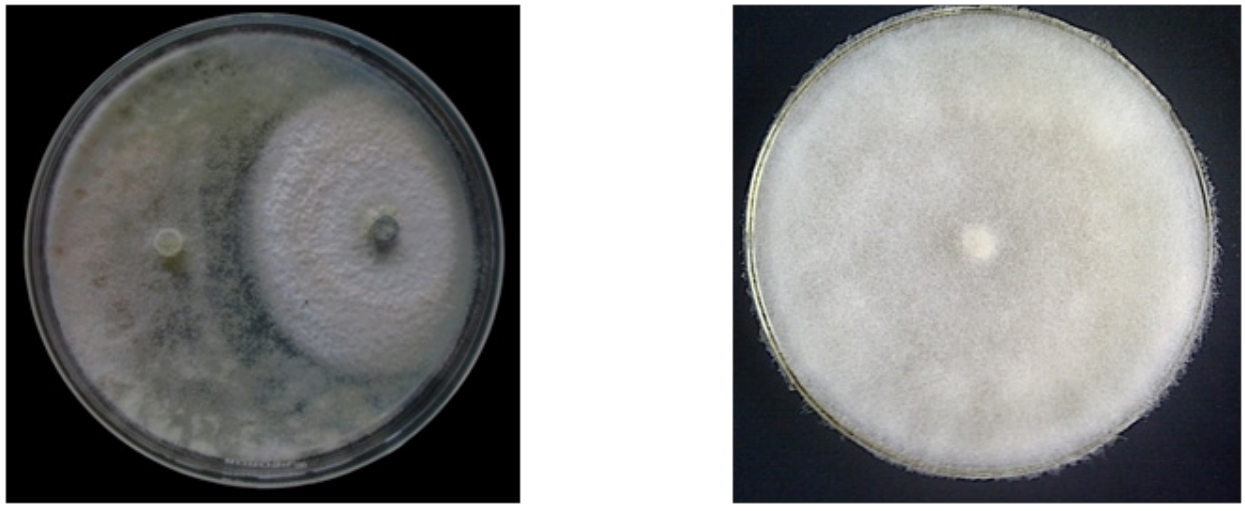


0: Healthy, 1 : 0 - 25 % of the pod infected, 2: 26 - 50 % of the pod infected, 3: 51 – 75 % of the pod infected, 4 : > 75 % of the pod infected

Figure 1. Score chart for assessment of intensity of Choanephora pod rot of cowpea.

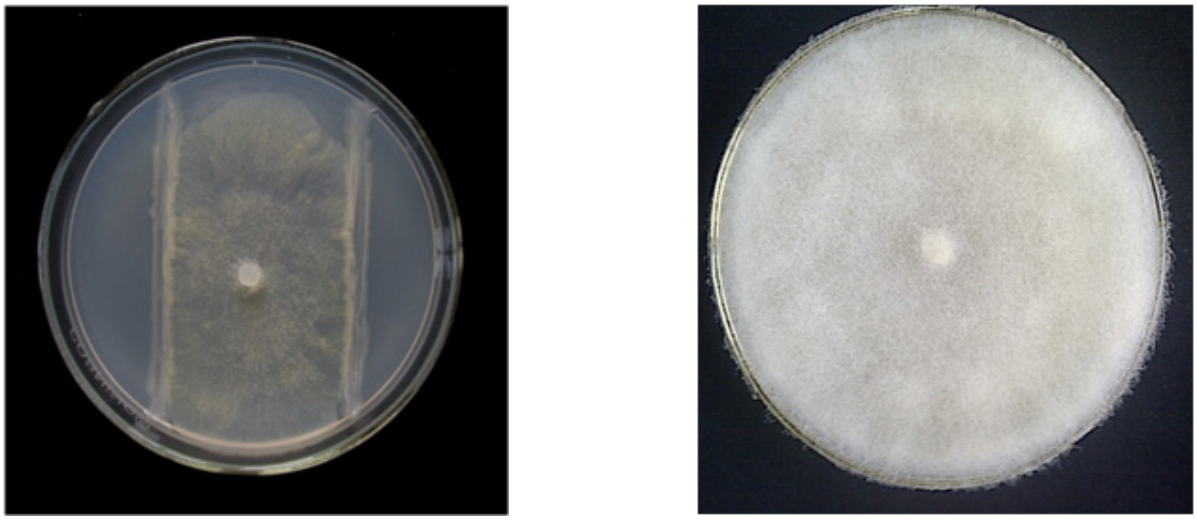
Table 1. Population of saprophytic micro flora obtained from fructosphere of cowpea

Source	Fungal population (cfu/g of plant material)		Bacterial population (cfu/g of soil or plant material)	
Fructosphere	FS1	19×10^4	BF1	12×10^6
	FS2	8×10^4	BF2	22×10^6
	FS3	8×10^4	BF3	11×10^6



FS1 x *C. cucurbitarum* Control (*C. cucurbitarum*)

Figure 2. Effect of FS1 on suppression of *Choanephora cucurbitarum* under *in vitro* conditions.



BF1 x *C. cucurbitarum* Control (*C. cucurbitarum*)

Figure 3. Effect of BF1 on suppression of *Choanephora cucurbitarum* under *in vitro* conditions.

Table 2. Effect of saprophytic fungi against *Choanephora cucurbitarum*

Treatments	Mycelial growth (cm)	Percentage inhibition *
FS1	1.83	79.50 (63.15)[a]
FS2	8.83	0.56 (4.30)[b]
FS3	8.60	2.82 (9.67)[b]
Control	9	0.000 (0.0)[b]
CD (0.05)		(8.78)

*Mean of three replications; Values in the parenthesis are arc-sine transformed; Treatments with same alphabets in the superscript, do not differ significantly

Table 3. Effect of saprophytic bacteria on the mycelial growth of *Choanephora cucurbitarum*

Treatments	Mycelial growth (cm)	Percentage disease suppression *
BF1	4.00	55.00 (47.87)[a]
BF2	8.26	4.07 (11.65)[b]
BF3	8.60	2.82 (9.67)[b]
Control	9.00	0.00 (0.00)[c]
CD (0.05)		(8.22)

*Mean of three replications; Values in the parenthesis are arc-sine transformed; Treatments with same alphabets in the superscript, do not differ significantly

growing in culture, the colony initially appeared white in colour, which later turned to greenish white. The conidiophores were sub-hyaline, measured 30-300 µm in length and 2.5-4.5 µm in diameter. The conidiophores were branched at right angles and each conidiophore terminated by a cluster of 3-6 closely appressed phialides. Conidia were ellipsoidal to ovoid 3.5-4.4 µm in size and dark green in color.

The gram staining techniques produced pink colour for BF1 isolate, thus revealing the Gram negative nature of the bacterial antagonist. . When grown on King's B medium, BF1 grew rapidly, and the colonies produced fluorescent pigment when observed under UV Transilluminator GeNei™ (Figure 5). Further identification of the BF1 based on biochemical analysis revealed that BF1 tested positive for gelatin liquefaction, fluorescent pigment, catalase test and oxidase test while negative for starch hydrolysis test. Based on the above observations the bacterial antagonist was tentatively identified as *Pseudomonas fluorescens.*

In vitro evaluation of organic preparations against *Choanephora cucurbitarum*

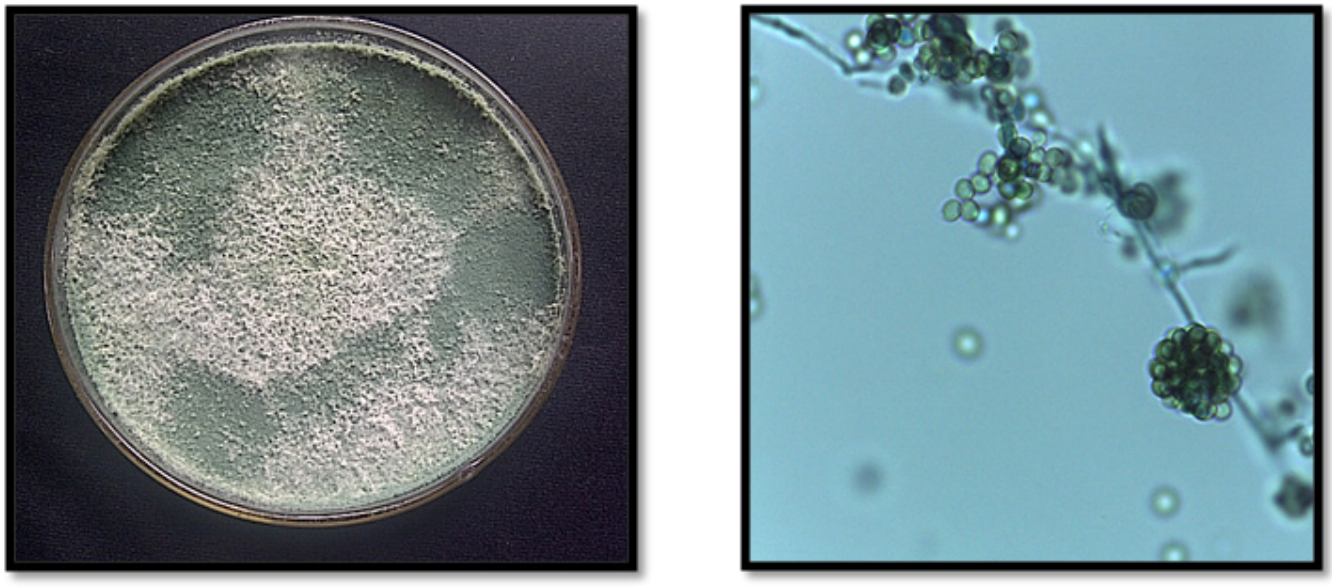
Organic preparations like panchagavya, jeevamruth, compost tea and fish amino acid were prepared and their effectiveness in suppression of mycelial growth of *C. cucurbitarum* on PDA under *in vitro* conditions was carried out at different concentrations. Vermiwash at 5 and 10 %, jeevamurth (10 %), and panchagavya (10 %) completely suppressed the mycelial growth of the pathogen. However, fish amino acid and compost tea were not effective in inhibiting the growth of the pathogen (Table 4).

In vitro suppression of pod rot of cowpea on excised pods using selected antagonists and organic preparations

The selected antagonist at 10^6cfu/ml and the effective dose of the organic preparations were sprayed on excised pods using the poison food technique. Out of the treatments, the application of *P. fluorescens* was found to be effective giving 100% inhibition and was significantly superior to other treatment by *Trichoderma virens,* which inhibited the growth of the pathogen by 63.32% , jeevamurth (10%) by 60.40 %, vermiwash (5%) by 55.54% and panchagavya (10%) by 45.29%. The effect of fish amino acid (10%) and compost tea (10%) was statistically inferior to other treatments but was better than the control (Table 5).

In vivo suppression of Choanephora pod rot of cowpea using the selected antagonists

The results revealed that application of all the treatments either organic preparations or biocontrol agent were effective in suppressing the pathogen when compared to the uninoculated control. After first week of application of the treatments, *Pseudomonas fluorescens* (10^6 cfu/ml), panchagavya (10%), jeevamurth (10%), fish amino acid (10%), vermiwash (5%) and KAU released *Trichoderma* (2%) showed 100% disease suppression. However, in general



(a) (b)

Figure 4. Cultural and morphological characters of *Trichoderma virens*.(a).Mycelial growth and sporulation. (b).Phialides and gleoid mass.

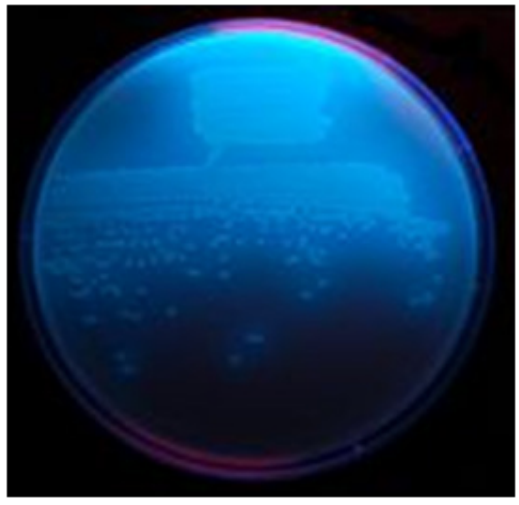


Figure 5. Fluorescent pseudomonads viewed under UV light.

Table 4. Effect of organic preparations on growth of pathogen under *in vitro* conditions

Treatments	Percentage disease suppression		
	2.5%	**5%**	**10%**
Panchagavya	0.00 (0.00)[b]	48.92 (37.38)[c]	100.00 (90.00)[a]
Fish amino acid	0.00 (0.00)[b]	0.00 (0.00)[d]	4.55 (12.32)[c]
Jeevamruth	34.75 (36.11)[a]	58.60 (49.95)[b]	100.00 (90.00)[a]
Vermiwash	23.96 (21.94)[a]	100.00 (90.00)[a]	100.00 (90.00)[a]
Compost tea	0.00 (0.00)[b]	0.00 (0.00)[d]	0.00 (0.00)[d]
Control	0.00 (0.00)[b]	0.00 (0.00)[d]	0.00 (0.00)[d]
CD(0.05)	(13.43)	(10.90)	(7.59)

*Mean of three replications; Values in the parenthesis are arc-sine transformed; Treatments with same alphabets in the superscript, do not differ significantly

Table 5. Effect of selected dose of organic preparations and selected antagonists for suppression of pod rot caused by *Choanephora cucurbitarum* on excised pods

Treatments	Lesion size (cm)	*Percentage disease suppression
Pseudomonas fluorescens (10^6cfu/ml)	0.00	100.00 (90.00)[a]
Trichoderma virens (10^6cfu/ml)	5.50	63.32 (52.72)[b]
Jeevamruth (10%)	5.93	60.40 (51.00)[b]
Vermiwash (2.5%)	6.66	55.54 (48.18)[c]
Panchagavya (10%)	8.20	45.29 (42.30)[d]
Fish amino acid (10%)	9.80	34.62 (36.05)[e]
Compost tea (10%)	10.73	28.37 (32.18)[f]
Control	20.00	0.000 (0.00)[g]
CD(0.05)		**(2.11)**

*Mean of three replications; Values in the parenthesis are arc-sine transformed; Treatments with same alphabets in the superscript, do not differ significantly

Table 6. Effect of selected antagonists and indigenous organic preparations on suppression of pod rot caused by *Choanephora cucurbitarum* under *in vivo* conditions

Treatments	*Disease index			*Percentage disease suppression		
	Week after inoculation					
	1ST Week	2ND Week	3RD Week	1ST Week	2ND Week	3RD Week
Trichoderma virens (10^6cfu/ml)	0.39 (3.59)[c]	22.15 (28.08)[d]	24.64 (29.76)[d]	99.08	75.59	75.27
Pseudomonas fluorescens (10^6cfu/ml)	0.00 (0.00)[c]	19.84 (26.45)[d]	27.61 (31.70)[d]	100.00	78.14	72.31
Compost tea (10%)	11.69 (20.00)[b]	22.80 (28.52)[d]	28.97 (32.57)[d]	72.64	74.87	70.95
Panchagavya (10%)	0.00 (0.00)[c]	19.84 (26.45)[d]	27.97 (31.92)[d]	100.00	78.14	71.95
Jeevamurth (10%)	0.00 (0.00)[c]	11.20 (19.56)[e]	12.63 (20.82)[e]	100.00	87.65	87.33
Fish amino acid (10%)	0.00 (0.00)[c]	32.74 (34.90)[c]	46.36 (42.92)[c]	100.00	63.93	53.50
Vermiwash (5%)	0.00 (0.00)[c]	11.38 (19.08)[e]	22.16 (18.46)[d]	100.00	87.38	75.22
KAU released *Trichoderma* (2%)	0.00 (0.00)[c]	34.65 (36.06)[c]	37.63 (37.83)[c]	100.00	61.76	62.26
KAU released *Pseudomonas* (2%)	2.37 (8.85)[b]	50.87 (45.50)[b]	60.62 (51.13)[b]	94.45	43.95	39.20
Untreated control	0.00 (0.00)[c]	0.56 (4.30)[g]	1.63 (7.34)[f]			
Inoculated check	42.74 (40.83)[a]	90.76 (72.30)[a]	99.72 (86.96)[a]			
CD(0.05)	**16.13**	**6.41**	**6.41**			

*mean of three replications, Values in the parenthesis are arc-sine transformed

in all the treatments there was a progressive increase in the development of disease over lapse of time (Table 6). At third week after the application of the treatments, among the organic preparations and biocontrol agents the lowest disease index recorded was 12.63 by jeevamurth (10%) which was significantly different from other treatments with the percentage of suppression of 87.33% followed by the selected fungal antagonist *T. virens* with a disease index of 24.64 and the percentage of suppression was 75.27%. This treatment was on par with the disease index of vermiwash, the selected bacterial antagonist *P. fluorescens*, panchagavya and compost tea, with disease index of 22.16, 27.61, 27.97 and 28.97 respectively. The application of fish amino acid (10%) and the KAU released talc-based *Trichoderma* formulation recorded the disease index of 46.36 and 37.63. The application of the KAU released talc based *Pseudomonas* formulations were least effective with a disease index of 60.62 but was better than the inoculated control.

Biological control solutions for the control of plant diseases are in high demand for many patho systems. Exploitation of epiphytic microorganisms and indigenous organic preparations for the control of many plant diseases has gained much importance as these represent sources of new potential biological control agents without causing harm to the environment and human population.

In the present study all the antagonistic epiphytes obtained were efficient in suppressing the pathogen compared to the control, however, one each fungal and bacterial antagonists were very effective and identified as *T. virens* and *P. fluorescens*, respectively based on the cultural and morphological characters Sharma and Singh (2014), Park *et al.* (2005) and Chaverri *et al.* (2001). Nepali *et al.* (2018) and Belkar and Gade (2012). The use of *T. virens* and *P. fluorescens* as biocontrol agents against plant diseases were well studied by Abou-Aly *et al.* (2015), Kar *et al.* (2014), Pandey and Chandel (2014), Junaid *et al.* (2013), Brewer and Larkin (2005) and Benı´tez *et al.* (2004). Several epiphytic and endophytic microorganisms has been tested for their efficacy in controlling many diseases such as bacterial wilt of sweet pepper (Mamphogoro *et al.,* 2020), Fusarium head blight in wheat (Rojas *et al.,* 2020), Exserohilum blight of maize (Sartori *et al.,* 2015), scab of citrus (Graham *et al.,* 2016), rice sheath blight (Haque and Khan, 2021) and anthracnose of papaya (Capdeville *et al.,* 2007). They reported that epiphytic and endophytic microorganisms are potential biological competitors as they occupy the same resources and ecological niche as that of plant pathogens.

The utilization of organic amendments for controlling soil-borne plant pathogens has often been considered as the best option for ecofriendly management of plant diseases without upsetting the sustainability of environment or health of humans. The use of organic preparations against Choanephora pod rot of cowpea revealed that the efficacy of panchagavya, jeevamurth, vermiwash and compost tea were on par with the efficacy of isolated antagonist bacteria and were effective in controlling pod rot disease both under *in vitro* and *in vivo* conditions. Similar findings were given by Sugha (2005), who reported the antifungal potential of panchagavya and jeevamurth against *R. solani, S. rolfsii, F. solani*, *S. sclerotiorum* and *Phytphthora colocasia.* Kamble *et al.* (2009) opined that vermiwash (30%) was effective in inhibiting the growth and sporulation of *A. solani.*

CONCLUSION

The present study concluded that the use of naturally occurring antagonist epiphytes and the organic preparations were effective in reducing the growth of the pathogen *C. cucurbitarum* in cowpea both under *in vitro* and *in vivo* conditions. Considering the stage of the crop at which the disease hits the crop and the environmental hazards caused by the chemical fungicides, exploiting the antagonistic potential of epiphytes and organic preparation in suppressing the disease is an alternative approach in order to complement or replace current approaches to sustain crop productivity.

REFERENCES

Abel- Mortal, F. F., El-Sayad, M. A., El-Zayat, A. S., Nassar, S. M., and Ito S. 2010. Leaf spot disease of *Hyoscyamus muticus. J Gen Plant Pathol,* **7**(6): 437-439. https://doi.org/10.1007/s10327-009-0200-2

Abou-Aly, H. E., Neweigy, N. A., Zaghloul, R. A., El-Sayed, S. A., and Bahloul AM. 2015. Evaluation of some biocontrol agents against soil pathogenic fungi. *Res J Pharma Biol Chem Sci,* **6**(1): 439-448.

Bashir, M., Qureshi, S. H., Alam, S. S., and Malik, B. A. 1985. *Choanephora* pod rot of cowpea – A new disease record in Pakistan. *Pakist J Agric Res,* **6**(3): 295.

Belkar, Y. K., and Gade, R. M. 2012. Biochemical characterization and growth promotion activities of *Pseudomonas fluorescens*. *J Pl Dis Sci*, **7**(2): 170-174.

Benitez, T., Rincon, A. M., Limon, M. C., and Codon, A. C. 2004. Biocontrol mechanisms of *Trichoderma* strains. *Int Microbiol,* **7**(4): 249–260.

Brewer, M. T., and Larkin, R. P. 2005. Efficacy of several potential biocontrol organisms against Rhizoctonia solani on potato. *Crop Prot,* **24**(11): 939-950. https://doi.org/10.1016/j.cropro.2005.01.012

Chahal, A. S., and Grover, R. K. 1974. Chemical control of soft fruit rot of chilies caused by *Choanephora cucurbitarum*. *J Hortic Sci*, **3**: 190-194.

Chaverri, P., Samuels, G. J., and Stewart, E. L. 2001. *Hypocrea virens* sp. nov., the teleomorph of *Trichoderma virens*. *Mycologia*, **93**(6): 1113-1124. https://doi.org/10.1080/00275514.2001.12063245

de Capdeville, G., Souza, M. T., Santos, J. R. P., de Paula, M. S., Caetano, A., Torres, F. A. G. 2007. Selection and testing of epiphytic yeasts to control anthacnose in post-harvest of papaya fruit. *Scientia horticulturae,* **111**(2): 179-185. https://doi.org/10.1016/j.scienta.2006.10.003

Dhingra, O. D., Sinclair, J. B. 1985. *Basic Plant Pathology Methods.* CBC Publications and Distributors, New Delhi, p. 335.

Graham, J. H., Johnson, E. G., Myers, M. E., Young, M., Rajasekaran, P., Das, S., and Santra, S. 2016. Potential of nano-formulated zinc oxide for control of citrus canker on grapefruit trees. *Plant Dis,* **100***(*12): 2442-2447. https://doi.org/10.1094/PDIS-05-16-0598-RE

Gram, C. 1884. Ueber die isolierte Farbung der Schizomyceten in Schnitt- und Trockenpraparaten. *Fortschr Med*, **2** (6): 185-189.

Haque, Z., and Khan, M. R. 2021. Identification of multi-facial microbial isolates from the rice rhizosphere and their biocontrol activity against *Rhizoctonia solani* AG1-IA. *Biol Control*, **161**: 104640. https://doi.org/10.1016/j.biocontrol.2021.104640

Hussein, E. S., and Ziedan, E. S. 2013. First report of pod blight of okra caused by *Choanephora cucurbitarum* in Egypt. *J Agric Technol*, **9**(1): 135-140.

Johnson, L. F., and Curl, E. A. 1972. Methods for Research on Ecology of Soil borne Plant Pathogen. Burgers Publishing Co. New Delhi, 435 p.

Junaid, J. M., Dar, N. A., Bhat, T. A., Bhat, A. H., and Bhat, M. A. 2013. Commercial biocontrol agents and their mechanism of action in the management of plant pathogens. *Int J Modern Pl Animal Sci*, **1**(2): 39-57.

Kamble, S. B., Pawar, D. R., Sankeshware, S. B., Arekal, J. S., and Sawant, U. K. 2009. *In vitro* efficacy of fungitoxicants against *Alternaria solani*. *Int J Agric Sci,* **5**(1): 137-139.

Kar, A. K., Dash, S. K., Sahu, and K. C. 2014. Efficacy of biocontrol agents on growth of some pathogenic seed borne fungi of groundnut and their management. *J Plant Prot Environ,* **11**(2): 59-63.

Mamphogoro, T. P., Babalola, O. O., and Aiyegoro, O. A. 2020. Sustainable management strategies for bacterial wilt of sweet peppers (*Capsicum annuum*) and other Solanaceous crops. *J Appl Microbiol,* **129**(3): 496-508. https://doi.org/10.1111/jam.14653

McKinney, H. H. 1923. Influence of soil temperature and moisture on infection of wheat seedlings by *Helminthosporium sativum. J Agric Res,* **26**: 195-217.

Nene, Y. L., and Thapliyal, P. N. 1993. *Fungicides in Plant Disease Control* (3rd Ed.). Oxford and IBH Publishing Co. Pvt. Ltd, p. 691.

Nepali, B., Bhattarai, S., and Shrestha, J. 2018. Identification of *Pseudomonas fluorescens* using different biochemical tests. *Int J Appl Biol*, **2**(2): 27-32. https://doi.org/10.20956/ijab.v2i2.5260

Oladiran, A. O. 1980. Choanephora pod rot of cowpea in Southern Nigeria. *Trop Pest Manag*, **26**(4): 396-402. https://doi.org/10.1080/09670878009414921

Pandey, S. K., and Chandel, S. C. R. 2014. Efficacy of *Pseudomonas* as biocontrol agent against plant pathogenic fungi. *Int J Curr Microbiol Appl Sci,* **3**(11): 493-500.

Quin, F. M. 1997. Introduction. In: Singh BB, Mohan Raj DR, Dashiell KE, Jackai LEN (eds). Proceedings, Advances in cowpea research ix-xv, Sayce Publishing, Devon, UK.

Riddell, R. W. 1950. Permanent stained mycological preparation obtained by slide culture. *Mycologia*, **42**: 265-270. https://doi.org/10.1080/00275514.1950.12017830

Rojas, E. C., Jensen, B., Jørgensen, H. J., Latz, M. A., Esteban, P., Ding, Y., and Collinge, D. B. 2020. Selection of fungal endophytes with biocontrol potential against Fusarium head blight in wheat. *Biol Control*, **144**: 104222. https://doi.org/10.1016/j.biocontrol.2020.104222

Sartori, M., Nesci, A., Formento, Á., and Etcheverry, M. 2015. Selection of potential biological control of *Exserohilum turcicum* with epiphytic microorganisms from maize. *Revista Argentina de Microbiologia*, **47**(1): 62-71. https://doi.org/10.1016/j.ram.2015.01.002

Sharma, K. K., and Singh, U. S. 2014. Cultural and morphological characterization of rhizospheric isolates

of fungal antagonist *Trichoderma*. *J Appl Nat Sci*, **6**(2): 451-456. https://doi.org/10.31018/jans.v6i2.481

Skidmore, A. M., and Dickinson, C. H. 1976. Colony interaction and hyphal inference between *Septoria nodorum* and phyllophene fungi. *Br Mycol Soc*, **66**: 57-64. https://doi.org/10.1016/S0007-1536(76)80092-7

Sugha, S. K. 2005. Antifungal potential of panchagavya. *Plant Dis Res*, **20**: 156-158.

Utkhede, R. S., and Rahe, J. E. 1983. Interactions of antagonist and pathogen in biological control of onion white rot. *Phytopathol,* **21**(3): 373-378.

Vincent, J. M. 1927. Distortion of fungal hyphae in the presence of certain inhibitors. *Nature*, **159**: 800.

Ziedan, E. H. E. 2012. First report of *Alternaria* pod blight of okra in Egypt. *J Agric Technol,* **8**(7): 2239-2243.