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Invitro evaluation of Biological and Chemical agents against *Fusarium* wilt of Tobacco crops of Rajamahendravaram rural areas, Andhra Pradesh, India

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A B S T R A C T

Tobacco is one of the commercially important plants commonly affected by Fusarium wilt caused by *Fusarium* sp. It is vascular or fungal wilt causes major damage to the plant, in turns crop yield and productivity. It can be controlled by both fungicides and bio agents with various environmental impacts. Further, biocontrol agents play a significant role in the fungal diseases management of various plants predominantly *Trichoderma* sp. It is a saprophytic filamentous fungus of rhizospheric soil widely used as bio pesticides, bio fertilizers and also as soil fertility enhancer. The present work was intended to assess the possibilities of using *Trichoderma* sp. to inhibit the pathogenicity of Fusarium wilt by using *invitro* methods like dual culture technique, dual plate technique. At the same time efficacy of chemical agents – fungicides were studied by using poisoned food technique against the pathogen. *Trichoderma* isolates has the most effective antagonism activity based on *invitro* evaluations. Similarly, fungicides Benfil at 10 ppm and 50 ppm concentrations were effective in the inhibition of pathogen growth whereas Kavach was more potent at 100 ppm and 200 ppm dilutions in the of selected pathogen isolates. It was concluded that both bio control agent and fungicides were effective in the control of Fusarium wilt of tobacco plants either individual or in combine. Further studies may also require for usage of both biological and chemical agents in commercial formulations.

Introduction

Tobacco is one among commercial crops of the India (Jayalakshmi *et al.,* 2017) belongs to genus *Nicotiana tabacum*. It is cultivated all over the world which plays a major role in global economy. It is an herbaceous, annual or perennial plant with thick, hairy stem and large, simple leaves with oval in shape grows up to 1.2-1.8 m (4-6 ft). It is having 20 to 30 leaves, grows up to 60 cm in size, depending on the type of variety and environmental conditions. It is seriously damaged by tobacco bacterial wilt (Yun Hu1a *et al.,* 2021).

Fusarium sp. is vascular wilt widely seen in tobacco. It causes infection, damage and significant losses to quality of plants, flowers, in turns yield. It is a destructive pathogen of both green house and field grown tobacco plants especially in the hot areas of the world (Lucas,

1975; Gopalachari, 1984). A variety of methods are in use for the control of plant pathogens includes usage of fungicides and bio control agents. Among these fungicides may have negative environmental impacts but biocontrol agents are not in fungal diseases management. Bio control agents also involves in plant growth enhancement, reducing physiological and abiotic stress (Olumayowa Mary Olowe *et al.,* 2022).

In the fungal disease management of different crops Trichoderma sp. are majorly used as biocontrol agents due to its wide range of mycoparasitism against plant pathogens. It is a common saprophytic filamentous fungus of rhizospheric soil being commercialized as biopesticides, biofertilizers, soilfertility enhancer, growth promoter (Nazia Manzar *et al.,* 2022) and promote high yields in crops (Peteira *et al.,* 2001), These are non-pathogenic microbes gives protection from fungal diseases caused by Phytophthora, Rhizoctonia, Sclerotium, Pythium and Fusarium genera (Ezziyyani *et al.,* 2004). They also have ability to produce antifungal metabolites, release of hydrolytic enzymes and the production of plant growth enhancing substances (Suárez-Mesa *et al.,* 2008). In the present work an attempt was done to assess the antagonistic activity of Trichoderma isolates against Fusarium sp. by dual culture technique and dual plate technique. Further, it was intended to verify the efficacy of various fungicides against pathogen by poisoned food technique.

Materials and Methods

Soil survey and sampling

Soil samples were collected from the root regions of cultivated maize plant in and around 5 selected rural areas of Rajamahendravaram in dry air tight plastic bags used for isolation of Trichoderma sp. Moist soil samples were immediately stored in sealed plastic bags at 4°C. Fungal isolations was done within one week of sampling. At the same time, disease plant samples of tobacco crops (both root and leaf) were collected from 5different locations of Rajamahendravaram rural for isolation of pathogen in clean air-dried containers.

Isolation of *Fusarium* sp

Plant leaves were chopped into small pieces and surface sterilized in 0.5% NaOCl for two minutes then rinsed twice with triple distilled water, placed on Potato dextrose agar (PDA) medium widely used for fungal isolations and incubated at 27°C under sterile, dark conditions. After five days of incubation, small colonies of fungus appeared, which were picked with a sterilized tooth pick and transferred to fresh PDA plates and further identified by using morphological characters.

Isolation of *Trichoderma* sp.

Isolation of Trichoderma were done by using serial dilution technique for which soil samples were collected, air dried, and ground into powder. Stock solution of sample was prepared by dissolving 1 g of powdered soil sample into 9 mL of distilled water. Next, serial dilutions of samples were prepared as 10−1, 10−2…10−5. In a Petri plate with suitable medium 1 mL of each dilution was poured uniformly and incubated at 28 ± 1°C for 7 days.

Identification of pathogen isolates

Fusarium isolates were identified based on the major morphological characters like phialid type presence and absence of microconidia and chlamydospores, colony colour, macroconidia size, shape etc (Booth, 1971; Leslie and Summerell, 2008; Aoki *et al.,* 2012 and Crous *et al.,* 2021).

Identification of antagonistic isolates

Identification of Trichoderma sp. involves in the observation of branching of conidiophores containing conidia with bright green colour (Gams and Bissett, 1998). It includes organization of conidiophores, conidia and phialides. While cultural features involve in linear growth, growth pattern, colony colour and hyphae pigmentation. Usage of different cultivation media gives rise to different morphologies of fungi.

Dual culture method

In this method two discs (5 mm) each obtained from one-week old culture on PDA, the first one was Trichoderma isolates -antagonistic agents TS1, TS2, TS3, TS4 and TS5while the other was Fusarium sp. pathogenic agents F.sp1, F.sp2, F.sp3, F.sp4 and F.sp5, were placed towards the periphery of 5 different petri plates at a distance of 9 cm diameter. One disc of each pathogenic agent was maintained as control (alone culture). Each replicates have three plates. Both the dual and alone

cultures were incubated at 27±3°c for one week and measurement of radial mycelial growth of the fungus was taken every 24 hours. The percentage inhibition of growth (I) was calculated using the formula given below:

[I (%) = (1 – T /C) x 100]

Where I= Inhibition Percentage of pathogen growth by antagonists.

C=Radial growth in control.

T=Radial growth in the treatment (Mokhtar and Aid, 2013).

Dual plate method

The effect of volatile metabolites produced by the antagonistic fungi on pathogens, mycelial growth was determined by dual plate method described by Dennis and Webster (1971); Goyal *et al.,* (1994). Both antagonistic fungi and pathogen mycelia disc (5 mm) one-week old cultures were inoculated on PDA plates at the centre separately. The two plates of both Tricoderma and Fusarium samples with different combinations were sealed with paraffin tape and incubated for 5 days at 27±3°C. Another PDA plate was prepared with Fusarium sp. which was marked as control. Colonies diameter of each plate was recorded at every 24 hrs. for 5 days after incubation and the inhibition percentage of mycelial growth was calculated by using the equation: (Dolatabadi *et al.,* 2011).

PI = C-T/C X 100

Where PI= Percent inhibition of mycelial growth,

C= Radial growth of the pathogen in control plates (cm) and

T= Radial growth of pathogen in dual culture (cm)

Poisoned food Method

This technique was used to identify the fungicide efficiency against the pathogen (Shravelle, 1961). In this PDA medium was prepared with different concentrations of the formulation include 10, 50, 100, 200 ppm by using different types of fungicides they were presented in the Table – 01 along with their common name and manufacturing company.

Mycelial discs of Fusarium sp. were placed at the centre of petri plate containing PDA with different concentrations of various listed fungicides and incubated at 27±3°C for 5 days.

The control plate was prepared without any formulation. The radial growth (mm) of mycelium was measured. All the experiments were carried out in triplicates and percent reduction of mycelial growth over control was calculated using the following formula (Vincent, 1947).

The percentage of growth inhibition (%) = X-Y/ X x 100

Where, X = diameter of colony in control sample.

Y= diameter of colony in treated sample.

Results and Discussion

Morphological Characterization of *Fusarium* sp.

Collected Fusarium sp. samples were cultured on PDA and the colonies were identified using morphology and morphological characteristic of macro and micro-conidia which were thin walled 3-5 septate, fusoid falcate macro conidia with somewhat hooked apex and pediculate base were observed (Mamta Joshi *et al.,* 2013 and Kai-Li *et al.,* 2019). Further, isolated colonies appear woolly to cottony shape, flat structure and spreading (Narayan Prasad Verma *et al.,* 2017). The colour and pigmentation of the isolates were creamish white to light pink is a general characterization of Fusarium sp.

Morphological Characterization of *Trichoderma* Isolates

Total 5 Trichoderma samples were cultured in PDA medium and the colonies were distinct in colours from each other. Isolate (TS-4) was observed in fluorescent yellowish green pigmentation, while remaining other four isolates were found to be green to dark green pigmentation. Strain TS-1, TS-2 and TS-5 were very fast growers and produced luxuriant mycelia, compared to TS-3 and TS-4.

Evaluation of Antagonistic activity of Trichoderma Isolates by Dual culture method

The dual culture study revealed strong antagonistic activity of Trichoderma sp. by the inhibition of pathogen

growth. Among the 5 biocontrol agents *TS3* exhibits minimum growth inhibitions *while TS5 recorded* maximum pathogen growth inhibition of the selected samples. Therefore, *TS5* regulates the growth of pathogen at higher rate with respect to other isolates by occupying the measurable surface of the medium limiting the space for Fusarium sp.

Microscopic observations were reported prominent coiling around the hyphae of pathogens. However, *TS5 marked with significant pathogen growth inhibition indicates that more potent biocontrol agent against selected* Fusarium sp. after 5 days of incubation. Moreover, 120 hrs old culture pathogen growth inhibition percentages were calculated and presented in the Table - 02 followed by Histogram 01.

Determination volatile metabolites effect by Dual Plate Technique

This technique was used to study the inhibitory actions of Trichoderma isolates against the pathogen growth by the production of volatile compounds. Out of all isolates *TS 1presented lowest* and *TS 5 given highest* mycelial growth inhibition rates of the pathogen.

The rate of growth inhibition was observed by the growth patterns of mycelia. Thus, *TS 5* isolate significantly reduced the mycelial growth of the pathogen evident it will be a strong biocontrol agent against 5 Fusarium sp. in the study. At the same time percentages of antagonistic activity on pathogens were denoted in the Table -03 followed Histogram 02.

Poisoned food technique

In in vitro conditions efficacy of fungicides against pathogens were screened by using of poisoned food technique. In this technique, control of pathogen growth percentages was reported at different dilutions ranging from 10 ppm to 200 ppm of various fungicides which were represented respectively.

The percentage of growth inhibition at 10ppm dilution Kocide fungicide reported low rate of growth control and Benifil exhibited pathogen growth control rate as presented in Table - 04 followed by Histogram 03.

At 50ppm concentration Kocide fungicide reported minimum control of growth and Benifil exhibited maximum pathogen growth control. However, based percentages of pathogen growth controlled by fungicides were presented in the Table -05 and Histogram 04.

In case of 100 ppm dilution percentage growth control of pathogen was observed significantly in Kavach at higher levels instead of Benifil at 10 ppm and 50 ppm but Kocide reported low rate of pathogen growth inhibition which were presented in the Table- 06 with Histogram-05.

Based on 200 ppm dilution pathogen growth control percentage was reported high in case of Kavach fungicide and Kocide reported low pathogen growth percentage which were similar to that of 100 ppm as presented in the Table – 07and Graph -06.

The effectiveness of fungicides in controlling of mycelial growth of pathogen and were observed at 200 ppm dilutions as presented in the table.

However, Kavach was effective in controlling of selected pathogens at 100 ppm and 200 ppm dilutions while Benfil was more potent chemical agent against isolated pathogens at 10 ppm and 50 ppm.

Hence, they may be strong fungicides which can be used for the control of the *Fusarium wilt* in tobacco plants at various concentrations.

In the present study Trichoderma isolates shows varying degrees of antagonism against the soil borne plant pathogens. There was variability in the average antagonistic abilities of the Trichoderma isolates against plant pathogen Fusarium sp.

Based on the dual culture test antagonistic fungi *TS5* isolates were having strong antagonistic activity against the pathogens.

In vitro assay of dual plate reveals that all the strains are having inhibition effects on the mycelial growth and development of the pathogen but significant effect was shown by *TS5*. Hence, it was observed that *TS 5* isolate was more efficient biocontrol agent against selected Fusarium sp. In poisoned food assay both Kavach and Benfil was effective fungicides in the control of isolated pathogens. Therefore, t may be preferred as chemical agent in controlling of *Fusarium wilt* in tobacco plants at different concentrations.

Table.1 List of fungicides

S. No.	Trade Name	Technical Name	Company
1.	Kavach	Chlorothalonil	Syngenta
2.	Kocide	Copper hydroxide	E.I Dupont de nemurs
3.	Matco	Metalaxy l8%+Mancozeb 64%	Indofil
4.	Indofil M-45	Mancozeb	Indofil
5.	Antracol	Propineb	Bayer
6.	Benfil	Carbendazim	Indofil

Table.2 Percentages of pathogen growth Inhibition by bio agent using Dual Culture Technique

Bioagent➔ Pathogen ↓	**TS 1**	**TS 2**	**TS 3**	**TS 4**	**TS 5**
F.sp 1	37.03%	25.39%	***18.51%***	21.16%	**41.79%**
F.sp 2	33.96%	24.52%	***11.05%***	16.44%	**38.81%**
F.sp 3	32.00%	24.00%	***17.33%***	21.33%	**38.13%**
F.sp 4	30.43%	10.14%	***07.24%***	14.20%	**33.62%**
F.sp 5	34.23%	23.45%	***13.78%***	19.13%	**36.11%**

*Trichoderma Isolates as TS -1,2,3,4 and 5 #Fusarium sp. as F.sp. 1,2,3, 4 and 5

Table.3 Percentages of pathogen growth Inhibition by bio agent using Dual Plate Technique

Bioagent➔ Pathogen ↓	**TS 1**	**TS 2**	**TS 3**	**TS 4**	**TS 5**
F.sp 1	***02.50%***	29.03%	07.16%	21.14%	**32.25**%
F.sp 2	***0.546%***	29.32%	05.10%	17.48%	**29.87**%
F.sp 3	***03.50%***	29.94%	08.23%	21.36%	**35.37%**
F.sp 4	***01.96%***	31.25%	07.14%	19.82%	**35.89**%
F.sp 5	***02.82%***	32.15%	11.30%	20.49%	**37.45**%

*Trichoderma Isolates as TS -1,2,3,4 and 5 #Fusarium sp. as F.sp. 1,2,3, 4 and 5

Table.4 Table representing pathogen growth control (%) at 10 ppm Concentration

Fungicide	PPM	F.sp 1	F.sp 2	F.sp. 3	F.sp 4	F.sp 5
Kavach	**10**	**28.28%**	**28.02%**	**22.85%**	**28.74%**	**20.30%**
Kocide	**10**	**13.29%**	**11.02%**	**12.06%**	**13.38%**	**15.83%**
Matco	**10**	**34.38%**	**31.82%**	**31.07%**	**38.03%**	**35.10%**
Indofil M45	**10**	**22.92%**	**20.29%**	**31.07%**	**28.96%**	**33.96%**
Antracol	**10**	**34.09%**	**29.78%**	**27.09%**	**28.88%**	**33.09%**
Benfil	**10**	**36.13%**	**32.84%**	**33.88%**	**29.25%**	**34.54%**

Table.5 Table representing pathogen growth control (%) at 50 ppm Concentration

Fungicide	PPM	F.sp 1	F.sp 2	F.sp 3	F.sp 4	F.sp 5
Kavach	50	38.99%	38.90%	36.24%	35.92%	23.22%
Kocide	50	18.91%	18.28%	11.92%	16.59%	18.39%
Matco	50	24.92%	21.31%	21.06%	26.29%	29.31%
Indofil M45	50	34.67%	32.99%	31.64%	29.33%	33.25%
Antracol	50	46.99%	47.15%	44.18%	42.96%	47.16%
Benfil	50	48.42%	47.15%	46.83%	36.22%	46.87%

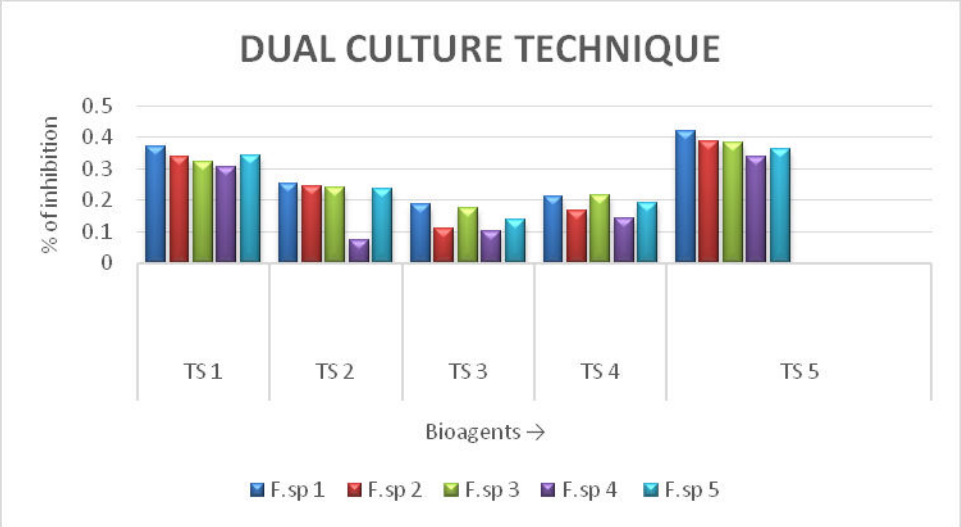
Table.6 Table representing pathogen growth control (%) at 100 ppm Concentration

Fungicide	PPM	F.sp 1	F.sp 2	F.sp 3	F.sp 4	F.sp 5
Kavach	100	50.42%	49.05%	47.56%	48.14%	46.38%
Kocide	100	26.93%	29.27%	27.09%	28.00%	31.04%
Matco	100	24.64%	23.21%	22.23%	25.77%	30.04%
Indofil M45	100	33.81%	31.09%	32.53%	38.59%	34.84%
Antracol	100	46.27%	46.27%	43.29%	43.70%	46.62%
Benfil	100	49.28%	48.17%	47.86%	33.25%	40.21%

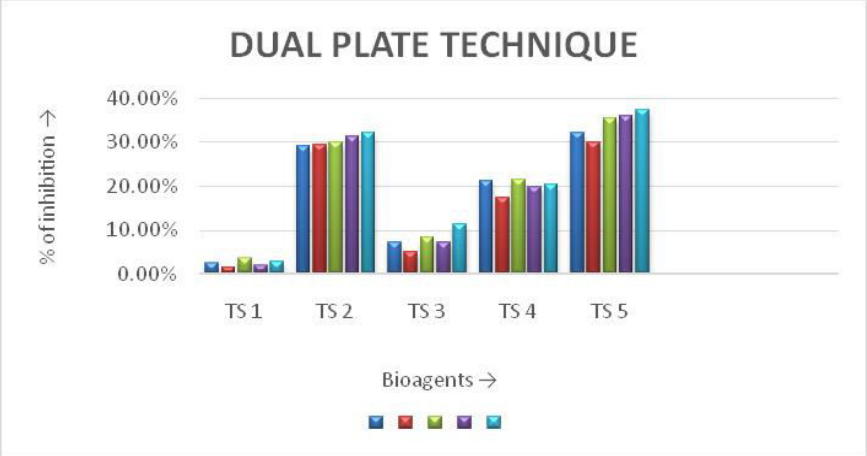
Table.7 Table representing pathogen growth control (%) at 200 ppm Concentration

Fungicide	PPM	F.sp 1	F.sp 2	F.sp 3	F.sp 4	F.sp 5
Kavach	200	51.86%	49.48%	48.60%	48.74%	49.92%
Kocide	200	31.86%	46.00%	42.96%	41.03%	32.36%
Matco	200	37.24%	34.16%	36.81%	39.77%	38.02%
Indofil M45	200	42.69%	38.54%	34.02%	37.62%	48.62%
Antracol	200	50.71%	48.90%	47.42%	47.11%	50.65%
Benfil	200	51.28%	50.51%	50.66%	37.03%	50.79%

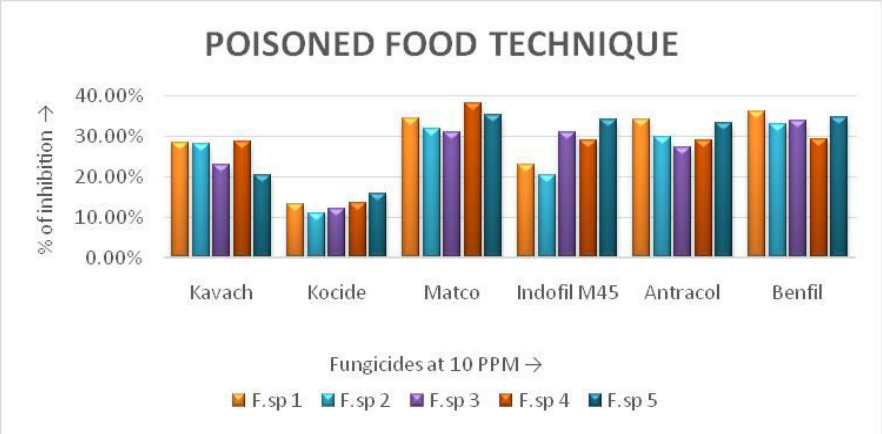
Histogram.1 Dual Culture Technique



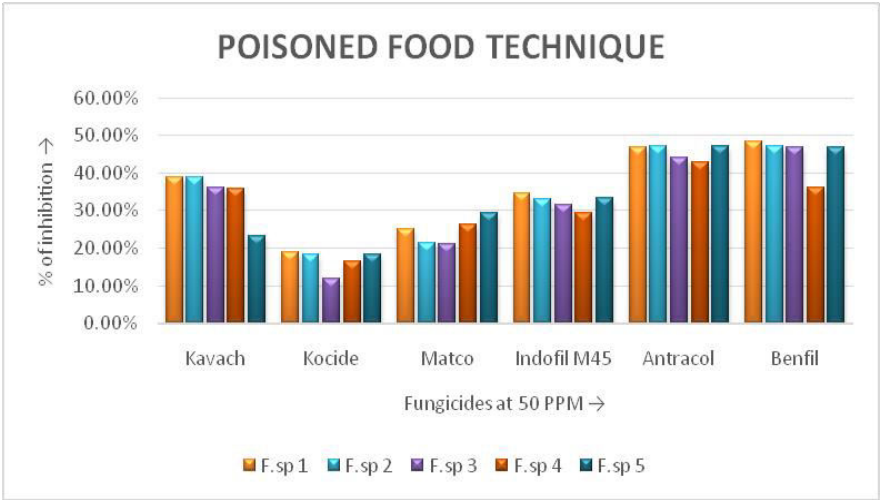
Histogram.2 Dual Culture Technique



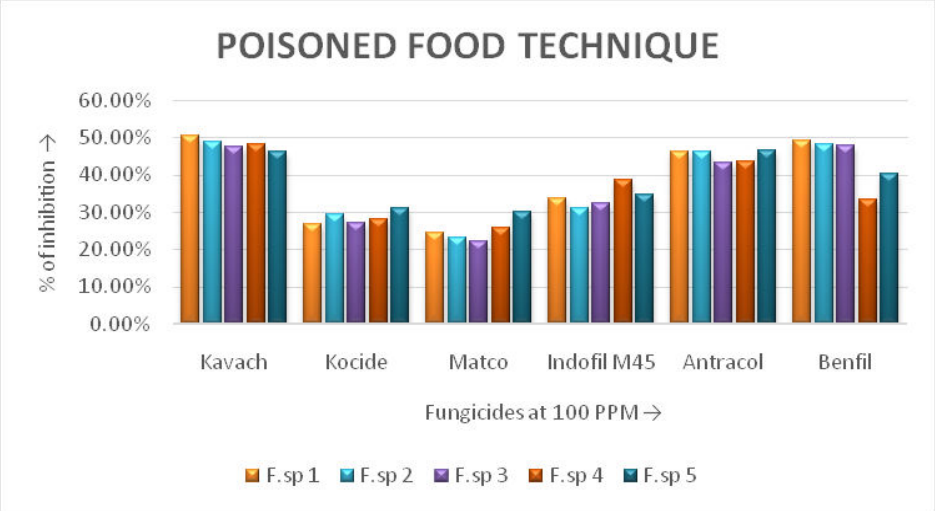
Histogram.3 Poisoned Food Technique representing pathogen growth control (%) at 10 ppm Concentration



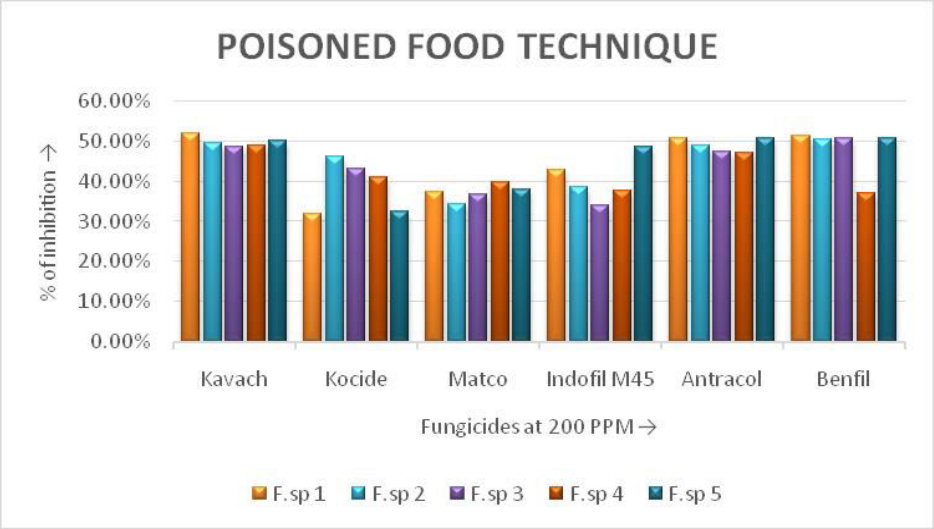
Histogram.4 Poisoned Food Technique representing pathogen growth control (%) at 50 ppm Concentration



Histogram.5 Poisoned Food Technique representing pathogen growth control (%) at 100 ppm Concentration



Histogram.6 Poisoned Food Technique representing pathogen growth control (%) at 200 ppm Concentration



In conclusion, Trichoderma sp. was found to be as potential biocontrol agent and Benfil as robust fungicide against Fusarium sp. of selected areas. The effective bioagent and chemical agent concluded from the study should be tested for their efficacy in in vivo conditions. Thus, both agents may be strong in the growth inhibitions of Fusarium sp. which can be used in agricultural practices of tobacco crop for the benefit to the farmers. They may be used individually or in combination depending up on the severity of the disease condition of the wilt. Further, it may also necessary to identify the close linkage between research and pesticide companies to use as potential control agents in commercial formulation.

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Author Contributions

P. Lakshmi Vasavi: Investigation, formal analysis; J. Balaji Chandra Mouli: Validation, methodology, writing-original draft & reviewing..

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest Authors are declaring any conflict of interest regarding research work and publishing material in the article.

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