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Research Article

A study on diseases of *Vanilla planifolia* and their management through Biocontrol Agents.

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Abstract

Ten new diseases affecting Vanilla in Kerala have been reported and described. They are Anthracnose (*Colletotrichum gleosporoides*), Leaf blight (*C. gleosporoides, F. oxysporum*), Leaf scar (*C. gleosporoides*), Foot rot (*S. rolfsii, Verticillium*), Stem rot (Fusarium spp), Bean rot (C. gleosporoides, F. oxysporum) , Bean spot (*Fusarium sp*), Thread blight (*Pellicularia filamentosa*), Red rust (*Cephaleurosa parasiticus*) and mosaic (Virus). All these diseases are new reports on Vanilla. Antagonistic studies with *Pseudomonas fluorescens* against the fungal pathogens of Vanilla revealed almost complete inhibition under in vitro conditions. In dual culture technique the mycelial growth of all the pathogenic fungi was completely inhibited. In *S.rolfsii*, the sclerotial production was also inhibited to a satisfactory level. Antagonistic studies with *T.viridae* and *T. harzianum* also yielded positive results both species of the antagonists were very effective in inhibition of mycelial growth and sporulation by the pathogenic fungi. *T.viridae* was found to be more competitive than *T. harzianum* because of its metabolite production. Coconut water supplemented with 1% peptone was found to be cheap and effective medium for mass multiplication of *P. fluorescens* when compared to the standard KB medium. *P. fluorescens* was found to be compatible with all the nine pesticides tried. The best combination was obtained in the case of Imidacloprid and Mancozeb with synergistic effect. This also is a new attempt in the case of *P.fluorescens.*

Keywords: Vanilla, Antagonistic studies , *Pseudomonas fluorescens* , inhibition of mycelial growth.

Introduction

Vanilla (*Vanilla planifolia*) is the second most expensive spice on the world market after saffron and a valuable source of foreign exchange for several countries. In Kerala also its cultivation is attracting the attention of farmers recently. For the time being it`s the most profitable crop in Kerala and hence, the area under Vanilla cultivation is increasing very rapidly. In fact it`s very much suitable for organic farming. It`s generally believed that the crop is free from any major pests and diseases, which warrants pesticides application. But recent reports by several Vanilla growers and consequents survey conducted in some fields of leading Vanilla growers in Kottayam, Idukki and Ernakulam districts revealed the incidence of several fungal diseases responsible for moderate to heavy crops loss. In addition sporadic incidences of mosaic virus like symptoms were also noticed in some gardens in Kottayam district (Bibin, 2003).

Preliminary studies conducted at the Regional Agricultural Research Station Kumarakonam, showed that fungi like *Sclerotium rolfsii*, *Colletotrichum*

gleosporoides, Fusarium oxysporum, Fusarium semitectum, Pellicularia filamentosa and *Verticillium sp* are involved as causal organism of different diseases on Vanilla. The recommended control measures are application of 1% Bordeaux mixture, 0.2% Fytolan, 0.3% mancozeb, 0.1% carbondazin etc. However the commercial often growers complain that these fungicides are not always successful in managing different disease problems. The increasing incidence of different pests and diseases on Vanilla may attract the use of several chemical pesticides in future and any report on the residue may cause concern and reduced international demand for our product. This will definitely affect the growers who started Vanilla cultivation recently.

A perusal of literature revealed that the diseases recorded on Vanilla are new and much information's are not available. Hence, it was felt necessary to carry out detailed studies on the diseases and their management through biological control is necessary.

Materials and Methods

A survey was conducted in some Vanilla growing areas, viz; Moovathupuzha, Vazhakulam, Ramamangalam (Ernakulam Dt); Thodupuzha, Udumbannoor (Idukki Dt); Erattupea, Mundakayam and Koruthod (Kottayam Dt). Vanilla plants showing infections on different parts, viz; stem, root, leaves and beans were collected in polythene bags and brought to the laboratory for microbiological studies. The disease symptoms were described and specific names were given to each disease based on symptoms. Altogether eight fungal diseases, one algal disease and one virus disease were collected and described.

Isolation of the pathogens

The pathogens were collected on potato dextrose agar (PDA) medium. The medium was prepared by boiling 200mg of pealed and shied potato in one liter of distilled water. The extract was filtered and mixed with 20g each of Dextrose (sugar) and agar. The medium was then sterilized in conical flasks at 121º C (15 lbs pressure) for 20 minutes. 5 ml sterilized medium was poured into sterile Petri plates in a laminar flow chambers and allowed to solidify. The infected portions along with a little healthy area were cut into 5mm discs; surface sterilized with 0.1% mercuric chloride for 30 sec to one minute and after washing with three changes of sterile distilled water placed on the media taken in sterile Petri plates. The plates were then incubated at room temperature for about a week. The fungal colonies developed from the infected tissues were sub cultured in PDA slants and used for further studies on identification and pathogenicity trials.

Identification of the pathogens

The pathogenic fungi were identified based on morphological characters, standard keys and by slide culture technique. A loopful of the fungal culture was taken from the P.D.A slants and placed on a clean glass slide. It was then stained with methylene blue, covered with a cover glass and observed under the microscopic first under low power (40x) and then under high power (100x). Slide culture unit was prepared by placing a microscopic slide over two supporting glass rods. A filter paper was placed at the bottom of the plate earlier. The unit was then steam sterilized in an autoclave for 20 minutes plain agar melted and slightly cooled was poured into sterile Petri plates at the rate of 6 ml per plate. When it was solidified small quarter bits of agar were cut out and placed at the center of the microscopic slide in the sterilized slide culture unit. The fungal culture to be studies was then inoculated at four corners of the agar block using a sterile inoculation needle. A cover slip was dropped over the inoculated fungal after flaming and cooling the
Cover glass.

Then the filter paper at the bottom of the plate was moistened by using sterile distilled water with a sterile pipette. The unit was then incubated for 24-48 hours at room temperature. The slides were then taken out, mounts were prepared stained and observed under the microscope for further studies.

Pathogenecity Studies – Koch`s Postulates

For conducting pathogenicity trails healthy Vanilla plants were maintained in polythene bags at the research station. The isolated fungal cultures were inoculated on the stem, leaves, base of the plants, apex and beans; Inoculations were made with each fungal culture after providing pinpricks on the plant parts and also without providing any injury. After inoculation the plants were covered with polythene bags to provide sufficient humidity to initiate infection. The

polythene bags were removed after five days of inoculation. After the expression of symptoms the fungi were against reisolated on P.D.A. and identified.

In vitro Studies with Antagonistic Microorganisms

Antagonistic organisms, viz; *Pseudomonas fluorescens* (*bacteria)*, *Trichoderma viridae* and *T. harzianum (fungi)* maintained at Kumarakonam were utilized for the studies. The following pathogenic fungi were tested in vitro against these antagonists.

Colletotrichum gleosporoides
Fusarium oxysporum
Fusarium semitectum
Fusarium spp
Verticillium spp
Sclerotium rolfsii

Pseudomonas culture maintained on King`s B medium utilized for the studies. The pathogenic fungi were maintained on P.D.A slants. In vitro antagonistic studies were carried out on P.D.A in sterile Petri plates. Five mm discs of the pathogenic fungi were cultured on one end of the P.D.A in Petri plates and the antagonist *P. fluorescens* was streaked on the opposite end. Both organisms were inoculated in the same Petri plate simultaneously. In other set of experiment the fungus was cultured at the centre and *P. fluorescens* was streaked surrounding the fungi at the centre. The plates were then incubated at room temperature and observations were taken on the sixth day. The width of the inhibition zone between bacterial growth and edge of the fungal growth was recorded in the first case. In the second case the ability of the fungus to grow and cross the circular inhibition zone was recorded.

The antagonistic potential of *T. viridae* and *T. harzianum* also studied against the same six pathogens. In vitro antagonistic effect of *Trichoderma spp* was studied by using the dual culture technique on P.D.A. It consists of growing the antagonist and pathogenic fungus on the same P.D.A plate. 7 – 10 days old cultures of both the organisms were poured to sterilized Petri plates at 6 ml per plate and allowed to solidify in a laminar flow chamber. The test organism (8mm disc) was inoculated on one end of the plate and the antagonist was placed at the opposite end. The plates were then incubated at room temperature and periodical observations were taken on the development of inhibition zone. Paired cultures were observed for a total period often days. All the ratings were made after contact between pathogen and the antagonist using Bell`s scale (Class 1 – 5). The different ratings according to the Bell`s scale are given below. Suitable controls were also kept in each set of experiment on antagonistic studies.

Alternative Media for Mass Multiplication of *P. fluorescens*

Usually King`s B medium is used for mass multiplication of *P.fluorescens*. On this medium the bacteria produce a characteristic green fluorescent pigment after 48 hrs of incubation. The media requires costly chemicals and hence, it was felt necessary to think about some cheap media. Hence an alternate medium, coconut water alone (mature nut water) and coconut water with 1% peptone were used in the study. The media were sterilized at 121 º C for 20 minutes, cooled and loopful of 48 – 72 hrs old bacterial culture was inoculated. The culture bottles were then shaken on a rotary shaker for 48 – 72 hrs. The development of green fluorescent pigment and the colony forming units / ml (c.f.u /ml) were recorded by serial dilution plate technique on King`s B medium.

Dilution Plate Technique

9 ml sterile water blanks were prepared in test tubes. 1 ml of the culture was added to 9 ml sterile distilled water and mixed well to get 10^{-1} dilution. From this one ml was added to 9 ml sterile distilled water to get 10^{-2} dilution and repeated up to 10^{-8} dilution.

One ml of 10^{-8} dilution was added to a sterile Petri plate, rotated well and melted and cooled King`s B medium was poured over this. The Petri plate was again rotated well and incubated at room temperature for 48 – 72 hrs. Fluorescent colonies of Pseudomonas developed in the plates were counted by using a bacterial colony count to get the cfu/ml.

Compatibility of *P. fluorescens* with commonly used pesticides

Some times combined application of pesticides is warranted in crop cultivation. Hence, some in vitro experiments were tried out to study the compatibility of *P. fluorescens* with the following pesticides.

1. Carbaryl 50 wp medium	@ 2g/ litre King`s B
2. Mancozeb medium	@ 3g/ litre of King`s B
3. Carbendazin medium	@ 1g/ litre King`s B
4. Hexaconazole medium	@ 2 ml/ litre of King`s B
5. Triazophos medium	@ 2 ml/litre King`s B
6. Quinalphos medium	@ 2 ml/ litre King`s B
7. Imidacloprid medium	@ 0.5 ml/ litre King`s B
8. Chlorpyriphos medium	@ 2 ml/ litre King`s B
9. Etopenprox medium	@ 1 ml/ litre King`s B

Liquid King`s B medium was prepared and sterilized at 121º C for 20 minutes. The above mentioned pesticides were added at the said dosage to cooled King`s B liquid medium in a laminar flow chambers and mixed well. 48 hrs old culture of *P. fluorescens* was then added to each bottles and they were shaken on a rotary shaker at room temperature for 48 – 72 hrs. Afterwards observations on the development of fluorescent green pigment and cfu / ml were taken by serial dilution plate technique.

Results and Discussion

Survey

Vanilla cultivation is being popularized in the States of Kerala, Tamil Nadu & Karnataka. When the crop was introduced about five years back on a commercial scale the crop was almost free from any pests and diseases. Now a days farmers are facing problems in cultivating the crop especially due to the attack of several diseases. Hence, a survey was undertaken in some of the plantations in Moovattupuzha, Vazhakulam, Thodupuzha, Erattupetta, Ramamangalam, Kottayam, Koruthod and Mundakayam. Vanilla crop; in all these areas were found to be affected by the following diseases (Table 1) and their symptoms are described (Plate 1 – 7).

Anthracnose disease

The disease usually affects the vines, leaves and beans. Water soaked lesions appear on the vines here and there and later results in rotting of the vines. The rotten vines later dried up and pinhead like acervuli appear on the dried up portion of the vine. The disease symptoms on the leaves also appear as water-soaked lesions followed by brown colouration of the spots. The centre of the spots was ash in colour with pinhead like acervuli. The disease also attacked young beans, which become brownish at the tip or at the base and finally fall down in large numbers. The incidence of the disease is more during rainy season.

Leaf Blight

The disease was characterized by the appearance of brownish water- soaked lesions on leaves followed by decaying of the infected portion. The disease mainly sets in during rainy season and after the cessation of rains the decayed leaves dry up resulting in leaf blight symptoms.

Leaf Scar

The disease was characterized by the appearance of brownish black scars on the leaf surface either on the upper or lower surface. Very rarely infection was scar on both the surfaces of the leaf.

Foot Rot

Rotting of the vines and leaves at the base was the main disease symptom noticed in the field. The disease was mainly seen during rainy season. Occasionally the disease was noticed in plantations where there was too much mulching at the base. Too much moisture at the base of the plants was the pre-disposing factor for the disease. Under such situations silky white mycelial growth and mustard like *Sclerotia* could be seen at the base of the infected plant and leaves. This was found to be a very severe disease in several Vanilla plantations.

Stem Rot

The symptoms were similar to that of the anthracnose disease. The disease was more when lack of drainage and free air circulation. The disease sets in during the rainy season and continuous in the garden even after

the rains. The disease affected the stem, leaves and tip of the Vanilla vines. When the growing tip is affected it is known as tip rot. The decayed vines and leaves dried up after the rains. On the dried up portion black pinhead like pyenidia of the fungus can be seen. Any injury to the vines while cockling on the support plant also pre- disposes the plant to infection. The disease was very severe in Vazhakulam and Ramamangalam.

Bean Rot

The disease was initiated as light browning of the beans at the tip or base followed by rotting and shedding. This was very severe in certain plantations in Koruthod. The disease was responsible for heavy loss to the farmers and usually seen during rainy season.

Bean Spot

Brown colored, narrow eye shaped sunken spots appear on the immature beans. The growth of the beans was badly affected and they fall down in advanced stages of infection. If they remain attacked to the vines the quality of such beans will be poor.

Thread Blight

The characteristic symptom of the disease was the appearance of white thread like mycelial growth of the fungus on mature leaves and vines. In advanced stages of infection the leaves and vines dried up and such leaves remain attached to the vine without failing. For the time being this is found to be a minor disease of Vanilla. Sometimes the fungal mycelium extends to the branches and leaves of the support plant also. If can also attack crops like coffee, tea, jasmine, etc.

Red Rust

This is an algal disease caused by *Cephaleuros parasiticus*. Red colored raised spots appear on the surface of mature leaves on either side resulting in loss of photosynthetic area. In advanced stages the spotted region showed shot hole like symptoms. The disease was noticed in plantations with excess shade. For the time being this also is a minor disease of Vanilla.

Mosaic Virus

The disease was noticed in some of the plantations at Erattupetta. This disease was characterized by light yellow raised streaks on mature leaves. On tender leaves the symptoms were mild and when such leaves matured the symptoms became clearer. This is in contrast to other viral diseases of crop plants where severe symptoms are seen on younger leaves. In Vanilla the difference may be due to the texture of the leaves, reduction in the size of the leaves, pale yellow coloration of the plant and gradual stunting. The virus was sap transmissible to healthy Vanilla plants. For the time being this also is a minor disease of the crop.

Isolation of disease causing organisms

The pathogens responsible for different diseases were isolated on P.D.A and are listed below.

Anthracnose disease - *Colletotrichum gleosporoides*
Leaf blight - 1) *C. gleosporoides*
Fusarium oxysporum
Leaf scar - *C. gleosporoides*
Foot Rot - 1) *Sclerotium rolfsii*
Verticillium spp
Stem Rot - *Fusarium oxysporum*
F. semitectum. Fusarium sp
Bean Rot - 1) *C. gleosporoides*
F.oxysporum
Bean Spot - *Fusarium sp*
Thread Blight - *Pellicularia filamentosa*
Red Rust - *Cephaleuros parasiticus (Algae)*
Mosaic disease - Virus

Culture characteristics and identification

All the fungal pathogens isolated from diseased plant parts were identified by standard keys and microscopic observations. The fungi responsible for anthracnose, leaf scar, leaf blight and bean rot were *C. gleosporoides*. The mycelial growth was initially white and turned grey after a week`s growth. Dark pinhead like *Sclerotia* also developed in the culture after incubating at room temperature for 7 days. On an average the spores measured 1.5 x 10 µm.

The fungi responsible for foot rot in Vanilla were isolated and identified as *S. rolfsii* and *Verticillium sp. S.rolfsii* was the predominant pathogen in all the foot rot affected samples whereas *Verticillium* was isolated

from only one sample. *S.rolfsii* was originally isolated by plating both infected stem bits and *Sclerotium* on P.D.A. In both cases pure white coloured fungal growth was obtained which was very much similar to the white silky mycelial growth observed on the infected base of the stem in the field. The colonies were fast growing on P.D.A completely covering the petriplate within five days after incubation at 28 - 30º C. The mycelium was white and thick with many hyphal strands running along the sides of the petriplates on culture flasks. *Sclerotia* were smooth and globose and by themselves could initiate infection as evidenced by pathogenicity tests. The creamy white *Sclerotia* increased in size and changes its colour to light brown or chocolate brown on further incubation. They measured 1- 3 mm in diameter. More than 80 *Sclerotia* were formed in a single P.D.A Petri plate culture. The fungus was identified as *Sclerotium rolfsii* sacc. (Telemorph = Athelia rolfsii (Curzi) Tu and Kimbrough, sy. *Corlicium rolfsii* (Domseh et al., 1980), which is an important plant pathogenic state of Alhelic species, it belongs to class Deutromycetes of order mycelia sterile (Ainsworth et al., 1973).

Colony of *Verticillium* of PDA was smooth and white and its growth was slow when compared to *S. rolfsii*. It was mainly isolated from the roots of foot rot affected plants.

Three different species of *Fusarium* were isolated from Vanilla leaves (Leaf blight), stem (Foot rot) and beans (Bean rot, Bean spot). Two culture namely *F.oxysporum* and *F. semitectum* produced pink coloured pigment or exudates on PDA after three to five days of incubation. This species was responsible for stem rot and Bean spot in vanilla. The spores of *F.oxysporum* had a measurement of 4.95 x 38.48 µm. The septation varied from 6 -15 numbers (7 -16 celled spores) (Table 5). *F. semitectum* spores measured 3.3 x 24.68 µm with 0 – 4 septations (single celled to four celled spores) (Table 6).

The fungus responsible for thread blight could not be cultured on PDA but was identified as *Pellicularia filametosa* based on standard keys.

Pathogenecity studies

The pathogenecity was proved by inoculating the isolated fungal cultures on healthy leaves, stem and beans of Vanilla under controlled conditions. The inoculated portions were kept moist by keeping a wet cotton pad and covering with a polythene cover to provide enough humidity. The symptoms appeared on the inoculated parts within a week`s time and the symptoms were comparable to those appeared in the field. The fungi were re-isolated from the infected parts and re-identified, thus proving the Koch`s postulates. The symptoms developed on both the plant parts with and without injuries. But symptoms appeared faster on plants provided with injuries and then inoculated.

Antagonistic studies

With *P.fluorescens*

The study revealed that *P. fluorescens* was almost completely inhibitory to the different pathogenic fungi tested viz. *C. gleosporoides* (plate 8*) Fusarium oxysporum*, *F. semitectum, S. rolfsii and Verticillium*. *P.fluorescens* initiated the mycelial growth and *Sclerotium* formation of *S. rolfsii* in dual culture on PDA to the extent of 90%. The bacterium showed consistenly good antagonistic effect against all the five pathogens tested (Table 2).

With *Trichoderma spp*

Both *Trichoderma viridae* and *T. harzianum* were able to inhibit all the pathogens tested. However, their extent of antagonism varied according to the nature of the pathogens. Both species of *Trichoderma* were able to inhibit the growth of *C. gleosporoides* by totally overgrowing it within 7 days of incubation and were categorized in class I as per Bell`s scale (Table 3, Plate 12, 13). *T. viridae* and *T. harzianum* also were antagonistic to all the three species of *Fusarium* tested showing 75% overgrowth. It can be included in class II of Bell`s scale (Table 3). Both species of *Trichoderma* were also totally antagonistic to *Verticillium* and *S. rolfsii*. These isolates were catagorised in class I of Bell`s scale (Table 3).

Alternate media for mass multiplication of *P. fluorescens:*

P. fluorescens could grow satisfactory in the media tried, viz. coconut water along and coconut water with 1% peptone. In coconut water alone a light

yellow green pigmentation was produced whereas in coconut water supplemented with 1% peptone dark green pigmentation was produced after 48- 72 hrs of incubation. The colony forming units in all the three media were calculated as 3×10^8, 1×10^8 and 5×10^8 respectively for King`s B , coconut water, and coconut water with 1% peptone.

Compatibility of *P. fluorescens* with commonly used pesticides

The studies revealed that *P. fluorescens* was compatible with all the 9 pesticides, viz, Carbaryl, Quinalphos, Trizophos, Imidacloprid, Chlorphyriphos, Etofenprox (Insecticides), Mancozeb, Carbandazines, Hexaconazole (Fungicides). Bright green pigmentation was obtained with Imidacloprid and Mancozeb. In the case of other pesticides only week pigmentation was observed. The colony forming units / ml of the combinations were calculated and presented in Table 4.

Discussion

Vanilla is a commercial crop belonging to the orchidaceae family. Hence, it is susceptible to all the diseases affecting orchids especially fungi and viral diseases. A survey carried out on the incidence of Vanilla diseases in three districts of Kerala (Kottayam, Ernakulam and Idukki) revealed that the crop is affected with eight fungal diseases, one algal disease and one virus disease. Eight diseases of Vanilla were caused by 6 different fungi, viz, *C. gleosporoides, F. oxysporum, F. semitectum, Fusarium sp, Verticillium sp* and *S. rolfsii*. A perusal of literature revealed that all the diseases reported on Vanilla are new to the crop and they are the first report from India. However, two diseases, viz, *Phytophthora* rot and *Sclerotium* rot affecting Vanilla in Kerala were reported by Bhai and Thomas (2000) respectively. The *Phytophthora* rot infected beans, leaves and stem of Vanilla plants. But, the *Sclerotium* rot was reported as the causal organism. In the present study *S. rolfsii* was found to infect only the basal portion of the stem and adjacent leaves resulting in foot rot symptoms. *S. rolfsii*, a soil- borne pathogen causes blight, root and stem rot in tropical and subtropical countries on more than 50 plants species (Aycock, 1966). It thrives well at 25-35^o C with high moisture and attacks crowded plants in shady condition. In dry soil the infection tends to occur below the soil surface and the principal propagates are the *Sclerotia* produced by the fungus. *S. rolfsii* has been earlier reported as a common pathogen in ginger and turmeric. (Nair and Menon, 1983). In ginger, the pathogen causes thread blight and it causes basal rot in the field and storage rot at the post harvest stage. Quipping (1995) reported a type of Vanilla bean rot caused by *S. rolfsii* in China. However, this is the first record of the disease causing foot rot in Vanilla caused by *S. rolfsii.*

In the present study three different pathogenic species of *Fusarium*, viz,*: F. oxysporum, F. semitectum* and an un-identified *Fusarium* were isolated from Vanilla. All the three isolated caused severe disease symptoms on beans, leaves and stem of Vanilla plants. These are new reports from India. However, Tombe et al., (1994) reported a severe disease of Vanilla in Indonesia caused by *F. oxysporum*, *Fusarium species.*

P. meadii was reported as causative factor of *Phytophthora* rot in Vanilla. (Bhai and Thomas, 2000). Cornol (1953) reported a similar type of bean rot in Vanilla caused by *P. parasitica*. Blight or mildew attack in developing fruits of vanilla caused by *P. jatrophae*. Hence was reported by Bouriquet (1954) from Malagasy republic and was observed in all Vanilla growing regions of the world. Tsao and Mu (1987) reported three species of *Phytophthora, viz, P. palmivora, P. parasitica and P. capsici* responsible for root rot disease in French Polynesia. In the present study *Phytophthora* was not isolated from any part of the diseasesd Vanilla plant. However, fungi like *C. gleosporoides, Fusarium sp and Verticillium sp* were found to be consistently associated with bean rot, stem rot and root rot respectively. Similarly the red rust caused by *Cephaleuros parasiticus* and thread blight caused by *Pellicularia filamentosa* is new reports on Vanilla. *C. parasiticus* also attack other crops like pepper, mango, guava etc in Kerala and Tamil nadu. *P.filamentosa* also attack crops like nutmeg, tea, coffee, cocoa, jasmine etc in Kerala and Tamil nadu. For the time being these two are minor diseases on Vanilla in Kerala. The sap transmissible mosaic virus reported is also a new record on Vanilla in India. However, detailed studies on this virus are warranted.

Antagonistic studies with *P. fluorescens* against the six pathogenic fungi revealed that they were almost completely inhibited under in vitro conditions. It has been experimentally proved by several researchers that *P. fluorescens* can be very effectively used for

Table – 1 Results of survey on Vanilla diseases

S. No	Name of the place	Name of disease
1)	Moovattupuzha	Leaf Blight, Stem Rot, Leaf Scar
2)	Vazhakulam	Leaf Blight, Stem rot, Foot Rot, Leaf Scar
3)	Ramamangalam	Leaf Blight
4)	Thodupuzha	Anthracnose, Stem Rot, Leaf scar
5)	Udumbanrioor	Leaf Scar
6)	Erattupetta	Stem Rot, Leaf Blight, Mosaic virus
7)	Mundakayam	Anthracnose, Red Rust
8)	Koruthod	Bean rot, Bean Spot, Red Rust, Leaf Scar.

Table – 2 Antagonistic effect of *P. fluorescens* against different pathogenic fungi on Vanilla

S.No	Pathogenic fungi on Vanilla	Mycelial inhibition on PDA
1)	*Colletotrichum gleosporoides*	100%
2)	*Fusarium oxysporum*	75%
3)	*F. semitectum*	75%
4)	*Fusarium sp*	75%
5)	*Verticillium sp*	90%
6)	*Sclerotium rolfsii*	90%

Table -4 Colony forming units of *P.fluorescens* (per ml of KB medium) in Combined inoculation with different pesticides

S.No	Pesticides	Cfu/ ml at 10^8
1)	Carbaryl 50	1.2
2)	Quinalphos	2.0
3)	Triazophos	2.5
4)	Chlorpyriphos	2.5
5)	Imidacloprid	5
6)	Carbendazin	1.4
7)	Mancozeb	6
8)	Hexaconozole	1.6
9)	Etopenprex	2.5
10)	Control – KB medium alone	5

Table – 5 Microscopic identification of *Fusarium spp* isolated from Vanilla stem

S.No	Length of the spore in µm	Breadth of the spores in µm	No of the septa/ spore	End cell shape of the spore
1	46.4	3.3	8	Pointed
2	42.9	4.95	9	Bend
3	42.9	4.95	6	Foot shape
4	44.5	6.6	7	Bend
5	36.3	6.6	9	Bend
6	49.5	3.3	11	Pointed
7	36.3	6.6	10	Pointed
8	36.3	6.6	8	Bend
9	56.1	4.95	6	Pointed
10	42.9	4.95	13	Bend
11	49.5	3.3	7	Bend
12	42.9	6.6	8	Bend
13	19.8	4.95	8	Bend
14	39.6	3.3	12	Pointed
15	52.8	3.3	14	Bend
16	56.1	4.95	15	Pointed
17	39.6	6.6	13	Bend
18	36.3	4.95	9	Pointed
19	36.3	6.6	9	Bend
20	26.4	4.95	13	Pointed
21	29.7	3.3	7	Pointed
22	36.3	3.3	8	Pointed
23	42.9	4.95	5	Bend
24	46.2	4.95	12	Bend
25	29.7	4.95	6	Pointed
Average	38.48	4.95	9	

The fungus has been identified as *Fusarium oxysporum.*

Table – 6 Microscopic Identification of *Fusarium spp* isolated from Vanilla Leaf

S.No	Length of the spores in µm	Breadth of the spores in µm	No of septa/ spore	End cell shpe of the spore
1	29.7	3.3	3	Bend
2	26.4	3.3	3	Pointed
3	23.1	3.3	4	Pointed
4	26.4	4.95	3	Bend
5	19.8	4.95	3	Bend
6	23.1	3.3	4	Pointed
7	26.4	3.3	0	Foot shaped
8	19.8	3.3	0	Bend
9	29.7	4.95	3	Pointed
10	23.1	4.95	4	Pointed
11	33	3.3	3	Pointed
12	26.4	4.95	4	Bend
13	39.6	4.95	1	Bend
14	23.1	3.3	0	Pointed
15	29.7	4.95	0	Pointed
16	16.5	4.95	3	Bend
17	23.1	3.3	2	Bend
18	29.7	4.95	1	Pointed
19	26.4	3.3	2	Pointed
20	6.6	4.95	3	Pointed
21	23.1	4.95	3	Pointed
22	29.7	3.3	4	Pointed
23	26.4	3.3	2	Pointed
24	19.8	4.95	1	Pointed
25	16.5	4.95	2	Pointed
Average	24.68	3.3	2	

The fungus has been identified as Fusarium semitectum.

controlling several pathogenic fungi like *Rhizoctonia solani, Macrophonina phaseolina, Pyricularia oryzae, Colletotrichum gleosporoides, Pythium, Phytophthora etc* (Elad and Baker, 1985; Ganesan and Gnanamanickam 1987; Dube 2001). Ganesan and Gnanamanickam (1987) reported that *S.rolfsii* infection in Pea nut was controlled by inoculating with *P. fluorescens*. In the present study also *S.rolfsii* was completely inhibited with *P. fluorescens*. Similarly all the other pathogenic fungi on Vanilla, viz, *C. gleosporoides, Fusarium* (3 species) , *Verticillium sp* were also inhibited successfully by *P. fluorescens*. Sasidharan (2002) reported that fungal pathogens like *R.solani* (Rice isolate) *Phytophthora capsici* (pepper), *C. gleosporoides* (pepper) and *Pythium aphamidermatum* (Ginger) were completely inhibited by *P. fluorescens.*

It was also found that the same pathogenic fungi were also inhibited completely by *T.viridae* and *T.harzianum*. *T. viridae* also produced a yellow metabolite in the growing medium and hence it was found more promising in biocontrol effect. Several researchers have already reported on the biocontrol potential of *Trichoderma*; Elad et al., 1986, Jayarajan et al., 1991, Kehri and Chandra 1991, Manzali et al., 1993, Krishnamoorthy et al., 1999) Renymol (2002) reported that two different isolates of *T.viridae* were able to inhibit *R. solani, P. capsici, P. apahanidermatum* and *C.gleosporoides*. In the present study also both the species of *Trichoderma* were able to successfully inhibit the pathogens tested.

In the present study it was found that coconut water supplemented with 1% peptone was the best medium for mass multiplication of *P.fluorescens*. It gave maximum intensity of fluorescent green pigmentation and more colony forming units per ml of the medium. Sasidharan (2002) reported several alternate media for mass multiplication of *P.fluorescens* and found that coconut water alone supported good growth of the bacterium. The results obtained as per the study indicated that this is a new report of such a cheap medium for mass multiplication of *P.fluorescens*.

Compatibility studies of *P.fluorescens* with commonly used pesticides revealed that it is compatible with all the nine pesticides tested. However, maximum compatibility based on pigmentations and Cfu was obtained in the case of Imidaclopride (insecticide) and Mancozeb (fungicide). Moreover, in these cases a synergistic effect was also noticed when compared to the control.

These findings are also new to the science and will be helpful to reduce the cost of cultivation in Vanilla and several other crops were combined applications of pesticides are warranted.

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