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Immunodetection of *Sphaerostilbe repens* causing Violet Root Rot disease of *Camellia sinensis*

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Among 11 varieties of tea tested against *Sphaerostilbe repens* causal agent of Violet Root Rot disease, TV-26 and Teenali-17/1/54 were found most susceptible while TV-23 and TV-25 were resistant.Cultural characteristic of the pathogen *in vitro* revealed optimum growth after 24 days of incubation at pH 5.0. Sucrose was found to be the best carbon source for growth of the pathogen. Optimum growth was recorded in yeast extract followed by casein hydrolysate as organic nitrogen sources while maximum growth occurred in calcium nitrate among inorganic nitrogen sources. Polyclonal antibody (PAb) was raised in white male rabbit against mycelial antigen of *S. repens.* The immunoglobulin (IgG) fraction of the antibody was purified by ammonium sulphate precipitation and Sephadex column chromatography. Effectiveness of raising antibody against the pathogen was confirmed by immunodiffusion test and dot immunobinding assay. Optimization of antigen and antibody concentration was done using plate trapped antigen coated enzyme linked immunosorbent assay (PTA-ELISA).Immunodetection of *S. repens* in soil samples using PTA-ELISA format was developed.Treatment of mycelia and conidia of *S. repens* with homologous PAb and reacted with fluorescein isothiocyanate (FITC) showed a strong apple green fluorescence that was more intense on young hyphal tips, while conidia showed a general fluorescence throughout the surface.

Key words: Polyclonal antibodies, *Sphaerostilbe repens*, tea, Violet root rot

INTRODUCTION

Tea (*Camellia sinensis*), one of the important perennial plantation crops grown in tropical agro climatic regions is considered as most popular hot beverage in the world being consumed for its stimulatory property, flavour and aroma. The tea bush, like any other living plant, during its various stages of growth and development is susceptible to attack by fungal diseases (Chakraborty and Chakraborty, 2018). Some of the existing soil conditions in the tea plantations predispose the plants to attacks by specific fungi causing some of the important diseases. Among root diseases of tea, Violet Root Rot disease caused by *Sphaero stilberepens* is often observed in stiff clay soil with water logging and poor aeration. The pathogen attacks all tea plants from above one year but the characteristic symptoms are produced on plants two year and above. Roots with enlarged lenticels are main targets of infection. Bushes at first turn yellow and leaves drop. The root turn inky Fig. 1a and the barks peel away. When the bark of affected roots is peeled off, the wood surface is found to be covered with thick, irregular, white to orange and mauve to purplish black, flattened strands, the rhizomorphs (Fig. 1b).

Immunodetection of fungal pathogens and diagnosis of plant diseases (Chakraborty and Chakraborty, 2002) is considered as an early step in developing management strategies which leads towards sustaining optimum yield (Chakraborty and Chakraborty, 2021). Some of these rapid serological techniques are enzyme linked

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immunosorbent assay (ELISA), dot immunobinding assay (DIBA) and indirect immunofluorescence (IIF). These techniques have been used to detect various foliar fungal pathogens viz. *Exobasidium vexans* (Chakraborty and Sharma, 2007); *Glomerella cingulata* (Chakraborty *et al.* 2008a); *Corticium invisum* (Chakraborty and Das Biswas, 2008); *Pestalotiopsis disseminata* (Acharya *et al.* 2015); *Bipolaris sorokininana* (Chakraborty *et al.* 2016); *Drechslera oryzae* (Khati and Chakraborty, 2019); *Alternaria alternata* (Das Biswas and Chakraborty, 2020) and *Curvularia lunata* (Acharya *et al.* 2021) as well as fungal root pathogens viz. *Fomes lamaoensis* (Chakraborty *et. al*, 2002a); *Ustulina zonata* (Chakraborty *et al*, 2002b) *Macrophomina phaseolina* (Chakraborty *et al.*, 2012) and *Sclerotium rolfsii* (Bhagat and Chakraborty, 2020).In the present study, polyclonal antibody based serological detection of *Sphaerostilbe repens* was developed using various immunological formats such as immunodiffusion, dot immunobinding assay, plate trapped antigen (PTA) coated enzyme linked immunosorbent assay (ELISA) and indirect immunofluorescence for early detection from soil and root tissues in order to develop management strategies of violet root rot disease of tea plant.

MATERIAL AND METHODS

Plant material

Tea clones (Teen Ali-17, TV-9, TV-18, TV-22, TV-23, TV-25, TV-26, TV-27, TV-28, TV-29 and TV-30) collected from Tocklai Experimental Station, Jorhat Assam were grown and maintained as suggested by Barbora (1988) in Tea Germplasm Bank and Experimental Garden of Immuno-Phytopathology Laboratory, Department of Botany, North Bengal University.

Fungal culture

The cultures of *Sphaerostilbe repens, Fomes lamaenosis, Poria hypobrunnea, Ustulina zonata,* and *Armillaria mellea* were obtained from Tocklai Tea Research Station, Jorhat. Other soil fungi (*Beauveria bassiana, Metarrhizium anisopliae, Trichoderma harzianum, T. viride, Fusarium oxysporum* and *Sclerotium rolfsii*) used in the serological experiment were obtained from Fungal culture collection centre, Division of Plant Pathology, Indian Agricultural Research Institute, New Delhi. These fungal cultures were maintained at room temperature and at 4°C.

Assessment of mycelial growth

Mycelial growth of *S. repens* was assessed both on solid media and liquid media. Seven different solid media viz. potato dextrose agar (PDA), potato sucrose agar (PSA), carrot juice agar (CJA), Richard's agar (RA), Eliot agar (EA), Czapek-Dox agar (CDA)and Yeast dextrose agar (YDA) were prepared to assess mycelial growth and sporulation. For assessment of growth in liquid media Richard'smedium was used in all experiments with desired modification. To record the maximum growth of mycelia liquid fungal culture were incubated up to 33 days and mycelial mats were collected after 3,6,9,12,15,18,21,24,27,30,33 days interval.Seven different carbon sources (starch, sucrose, fructose, maltose, dextrose, sorbitol and mannitol) were tested for their effect on the growth of *S. repens*. The effect of inorganic nitrogen sources (potassium nitrate, sodium nitrate,calcium nitrate, ammonium sulphate and ammonium nitrate) as well as complex organic sources (urea, peptone, beef extract, casein hydrolysate and yeast extract) were used. In every cases the cultures were incubated at 28 ± 2°C.

Artificial inoculation of tea plants

Rhizosphere of 2 years old tea plants (10 plants of each variety) were inoculated with *S. repens* in potted condition following the method Chakraborty *et al.* (2002b) and maintained in water logging condition. Disease incidence was made after 30 days of inoculation considering the following character:0-No symptoms; 1- plants look sick, root surface started roughening in patches; 2- most of the leaves withered or looking yellow, light black patches with rough surface appear on roots; 3 - defoliation starts with random yellowing, roots inky black with random patches; 4- random defoliation, upto70% roots become black; 5- total defoliation, 70 to 85% blackening of roots; 6- total defoliation with drying of shoots; 85 to 100% blackening and drying of roots.

Antigen preparation

Mycelial antigen was prepared following the method of Chakraborty and Purkayastha (1983) and antigens were further purified by 100% ammonium sulphate precipitation, dialyzed and stored at -20°C. Soil antigen for microplate trapping and blotting were prepared following the method of Walsh *et. al.* (1996). 1g of soil was crushed in 2 ml of 0.05ml

sodium carbonate bicarbonate buffer (pH 9.6) in mortar with pestle and kept overnight at 4^{0}C. Next day it was centrifuged at 10,000 rpm for 10 min. Supernatant was used as antigen for experimental purposes.

Raising Polyclonal antibodies

White male New Zealand rabbits were taken for immunization. Normal sera were collected from each rabbit before immunization. Separate rabbits were intramuscularly injected once a week with 1ml antigen mixed with 1ml of freund's complete adjuvant (Difco, USA) for first two injections followed by incomplete adjuvant up to 12 weeks. Blood samples were collected by marginal ear vein puncture, 3 days after the first six injections and once in every fortnight, kept at 37^{0}C for 1 h for clotting, followed by centrifugation at 5000 rpm for 10 min at room temperature. IgG was purified from serum as described by Clausen (1988).

Immunodiffusion

Agar gel double diffusion tests were performed following the method of Ouchterlony (1967). The antigens and undiluted antisera (100µl/well) were pipetted directly into the appropriate wells and diffusion allowed to continue in moist chamber for 27 h at 25^{0}C. After immunodiffusion, the slides were initially washed with sterile distilled water for 2h and then aqueous NaCl solution (0.9% NaCl+0.1% NaN2) for 72h with 6 hourly changes to remove unreacted antigens and antiserum widely dispersed in the agarose. Then slides were stained with Coomassie blue (R 250) for 10 min at room temperature. After staining, slides were destained with methanol: water: acetic acid (45:45:10) following changes until the background became clear. Finally, the slides were washed with distilled water and dried in hot air oven for 3h at 50^{0}C.

Plate trapped antigen coated enzyme linked immunosorbent assay (PTA-ELISA)

PTA-ELISA was performed following the method as described by Chakraborty *et al.* (1995). Plants and fungal antigens were diluted with coating buffer and loaded in 96 wells ELISA plate. Then plate was incubated at 25°C for 4 hours and washed with running tap water and twice with PBS tween. Afterwards 200 µl blocking regent was added per well and incubated at 25°C for 1 hourand washed again as mentioned earlier. Purified IgG was diluted (40µg/ml) and 200 µl loaded in each well and incubated at 4°C overnight. After a further washing, antirabbit IgG goat antiserum labeled with Alkaline Phosphatase (diluted 10,000 times in PBS) was added (200 µl) to each well and kept in dark for 1 hour. Colour development was stopped by adding 50 µl/well of NaOH (3N) solutions and the absorbance was determined in an ELISA Reader (Labsystem multiscan) at 405 nm. Absorbance values in well not coated with antigens antigens were considered as blanks.

Dot Immunobinding Assay

Mycelial antigen of *S. repens* as well as healthy and artificially inoculated (with *S. repens*) tea root antigens were loaded on nitrocellulose membrane filter using Bio-Dot apparatus (Bio-Rad). Dot immunobinding assay was performed using PAb of *S. repens* performed as outlined by Lange *et. al.* (1989).

Indirect Immunofluorescence

This process was performed following the method of Chakraborty *et al.* (1995). Four days old mycelia, 12 days old spore of *S. repens* were washed with PBS tween (pH-7.2) and treated with normal sera or antisera diluted and PBS (3:10) and incubated for 1 hour in room temperature. Then specimens were washed again as before and treated with goat antirabbit (whole molecule) conjugated with fluorescein isothiocyanate (FITC) (Sigma) diluted with PBS (1:40) and incubated in dark for 45 minutes at room temperature. After incubation specimens were washed thrice and mounted in 10% glycerol. Then slides were observed using a Biomed microscope (Leitz) equipped with an I-3 filter block ideal for FITC fluorescence under UV light in dark. Photographs were taken by Moticam Pro 285B.

RESULTS

Sphaerostilbe repens causes Violet Root Rot disease of tea in water logging condition. Presently tea plantations are extending in plain areas where this disease may cause tremendous loss of tea production. A survey of the Tea estates in the Terai and Dooars revealed the occurrence of Violet Root Rot disease of tea in water logged sections with

poor drainage in Bijoynagar, Matigara, Bentaguri and Hansqua Tea Estates.

Cultural conditions affecting growth of the pathogen in vitro

The cultural conditions affecting growth and sporulation of *S. repens* were studied in seven different solid media such as potato dextrose agar (PDA), carrot juice agar (CJA), potato sucrose agar (PSA), czapek dox agar (CDA), Richards agar (RA), elliots agar (EA) and yeast extract dextrose agar (YDA). In solid media *S. repens* generally exhibited white fluffy growth and within a few days submerged rhizomorphs started forming. These are at first white in colour, gradually changing to orange and later to brown. Maximum growth recorded in CJA followed by PSA and minimum growth was observed in EA (Fig. 2). Rhizomorphs were clearly visible on the back surface of the media which were projected out as small orange projections. Rhizomorph formation was observed mostly in PDA, PSA and YDA media and very few in CJA and CDA but not observed in RA and EA. While in most of the media fluffy growth was seen but in RA, EA and CDA crustaceous growth was observed. In liquid media rhizomorphs aggregates into small groups (Fig.3a & b), maximum growth occurred after 24 days of incubation after which it declined. Profuse sporulation (Fig.1c) was observed in YDA, followed by PSA, all other media also supported sporulation to a lesser or greater degree. Mycelial growth of the pathogen was recorded at different time intervals and incubated upto 35 days. Maximum mycelial growth was recorded on 24 days after incubation and then started decline (Fig. 4a). It grew over a wide range of pH (4.0-8.0) and showed optimum growth at pH 5.0. (Fig 4b).The pathogen showed maximum growth in medium containing containing sucrose, and maltose, while no growth was observed in sorbitol and mannitol. When pathogen was grown in different organic and inorganic nitrogen sources,organic sources were found to be better for growth than inorganic ones (Fig 5). Among organic sources maximum growth was recorded in yeast extract followed by casein hydrolysate while in the inorganic nitrogen sources maximum growth occurred in calcium nitrate. Very less growth was observed in basal medium without nitrogen.

Pathogenicity test of S. repens

Rhizosphere region of two year old potted plants of elevenTocklaitea varieties (Teen Ali-17/1/54, TV-9, TV-18, TV-22, TV-23, TV-25, TV-26, TV-27, TV-28, TV-29 and TV-30) were artificially inoculated with *S. repens*. Twenty plants of each variety were used. Disease assessment was done on the basis of visual observation of symptoms and disease index ranged from 1-6 and was calculated after 10, 20 and 30 days of inoculation. Results (Table 1) revealed that among the Tocklai varieties TV-26 and T-17 were the most susceptible while TV-23 and TV-25 were the most resistant. Plants of susceptible varieties were further inoculated in the field and appearance of the disease symptoms were noted in the field. Plants showed both above

Table 1. Varietal resistance test of tea against *Spherostilbe repens*

Tea varieties	Disease Index[a] Days after inoculation with *S. repens*		
	10 d	20 d	30 d
TeenAli-17/1/54	2.46	3.92	5.31
TV-9	1.46	3.08	4.10
TV-18	1.82	2.86	3.77
TV22	1.42	2.22	2.91
TV-23	0.34	0.84	1.02
TV-25	0.42	0.92	1.10
TV-26	2.90	4.10	5.55
TV-27	1.02	1.98	2.16
TV-28	1.45	1.98	2.25
TV-29	1.80	2.68	3.86
TV-30	2.02	3.12	4.14

[a] 0-No symptoms; 1- plants look sick, root surface started roughening in patches; 2- most of the leaves withered or looking yellow, light black patches with rough surface appear on roots; 3 - defoliation starts with random yellowing, roots inky black with random patches; 4- random defoliation, upto70% roots become black; 5- total defoliation, 70 to 85% blackening of roots; 6- total defoliation with drying of shoots; 85 to 100% blackening and drying of roots

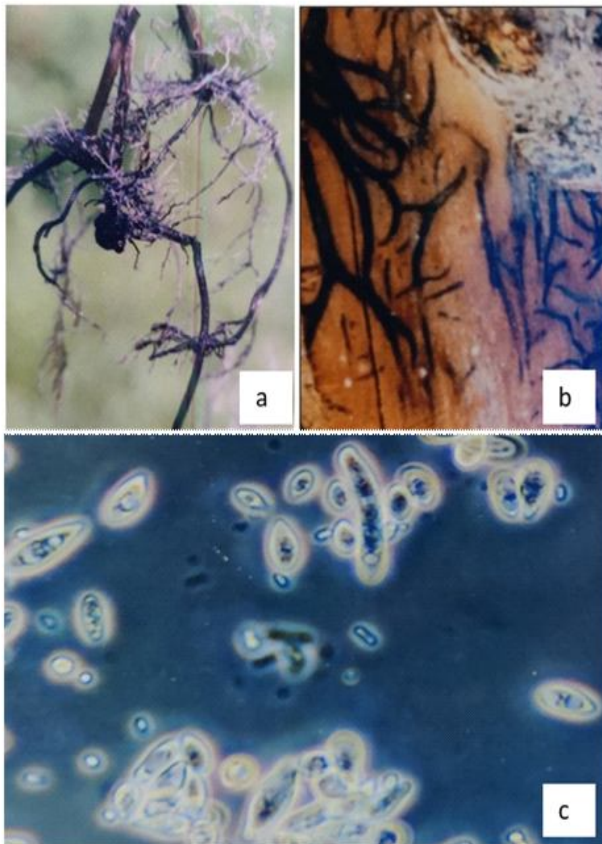

Figure 1 (a-c) Symptoms of Violet Root Rot disease of tea and causal organism,(a)Infected roots showing inky black and light violate colour, (b)Bark of affected root when peeled off, the wood surfacecovered with thick, irregular, purplish-black, flattened strands, the rhizomorphs, (c)Conidia of *Spherostilberepens* under phase contrast microscope

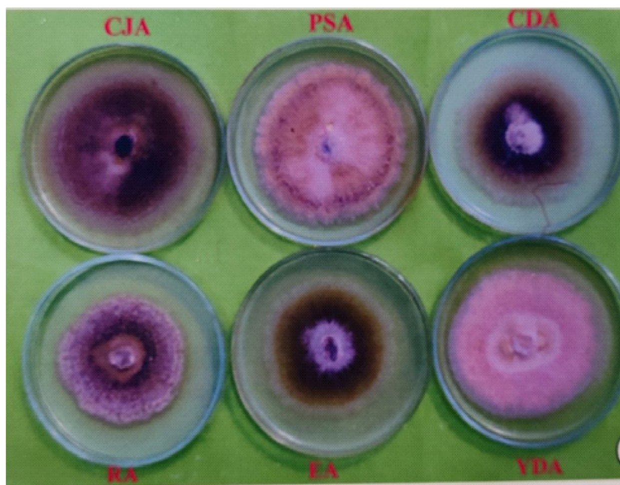

Fig. 2 : Mycelial growth of *Sphaerostilbe repens* on different media [CJA – Carrot Juice Agar; PSA – Potato Sucrose Agar; CDA – Czapek Dox Agar; RA – Richards Agar, EA – Elliot's Agar; YDA – Yeast Extract Dextrose Agar]

ground and underground symptoms ultimately leading to the death of the plants (Fig 6 a-f).

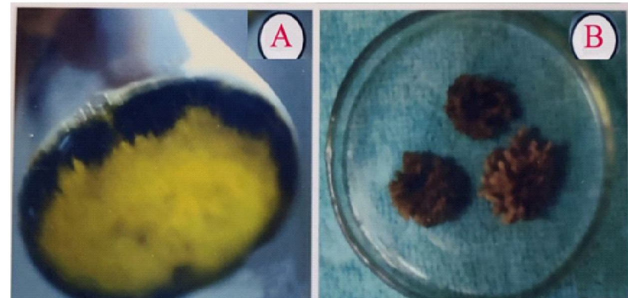

Fig. 3 : (A) Mycelial growth of *Sphaerostilbe repens* in potato dextrose broth (PDB) showing formation of rhizomorph; (B) Rhizomorphs taken out from PDB.

Immunodiffusion and dot immunobinding assay

The effectiveness of mycelial antigen preparations of *S. repens* for raising polyclonal antibodies (PAbs) were checked by homologous cross reaction following agar gel double diffusion test. Control sets involving normal sera and antigens of pathogen (*S. repens*) were negative. PAbs raised to mycelial extract immunogens and purified IgG for rabbits with five bleedings were used for immunodiffusion tests. Strong precipitin reactions (Fig.7a) were observed 1st to 4th bleedings. Precipitin reaction in agar gel double diffusion test was found to be week in 5th bleeding.

PAb raised against *S. repens* exhibited strong colouron nitrocellulose paper in its homologous reaction with mycelial antigen. In reciprocal cross reaction with PAb of *S. repens* and tea root antigen extracted from artificially inoculated highly susceptible tea variety (TV-26) also exhibited strong colour reaction. Whereas no such colour reaction could be observed on nitrocellulose paper when tea root antigens from healthy plants of TV-26 was reacted with PAb of the pathogen (Fig. 7b). When antigens prepared from soil amended with the violet root rot pathogen were probed with PAb of *S. repens,* dots of medium colour intensity developed. Mycelial antigens prepared from other tea root pathogens such as *Poriahypobrumea* and *Armillaria mellea* when reacted with PAb of *S. repens*, did not show cross reactivity, but antigens from *Ustulina zonata, Rosellinia arcuata* and *Fomes lamaoensis* showed very light colour intensity on the nitrocellulose paper, indicating a slight degree of cross reactivity.

Optimization of antigen and PAb concentrations using PTA-ELISA format

Polyclonal antibodies (PAbs) were raised against mycelial, and spore antigens of *S. repens* and

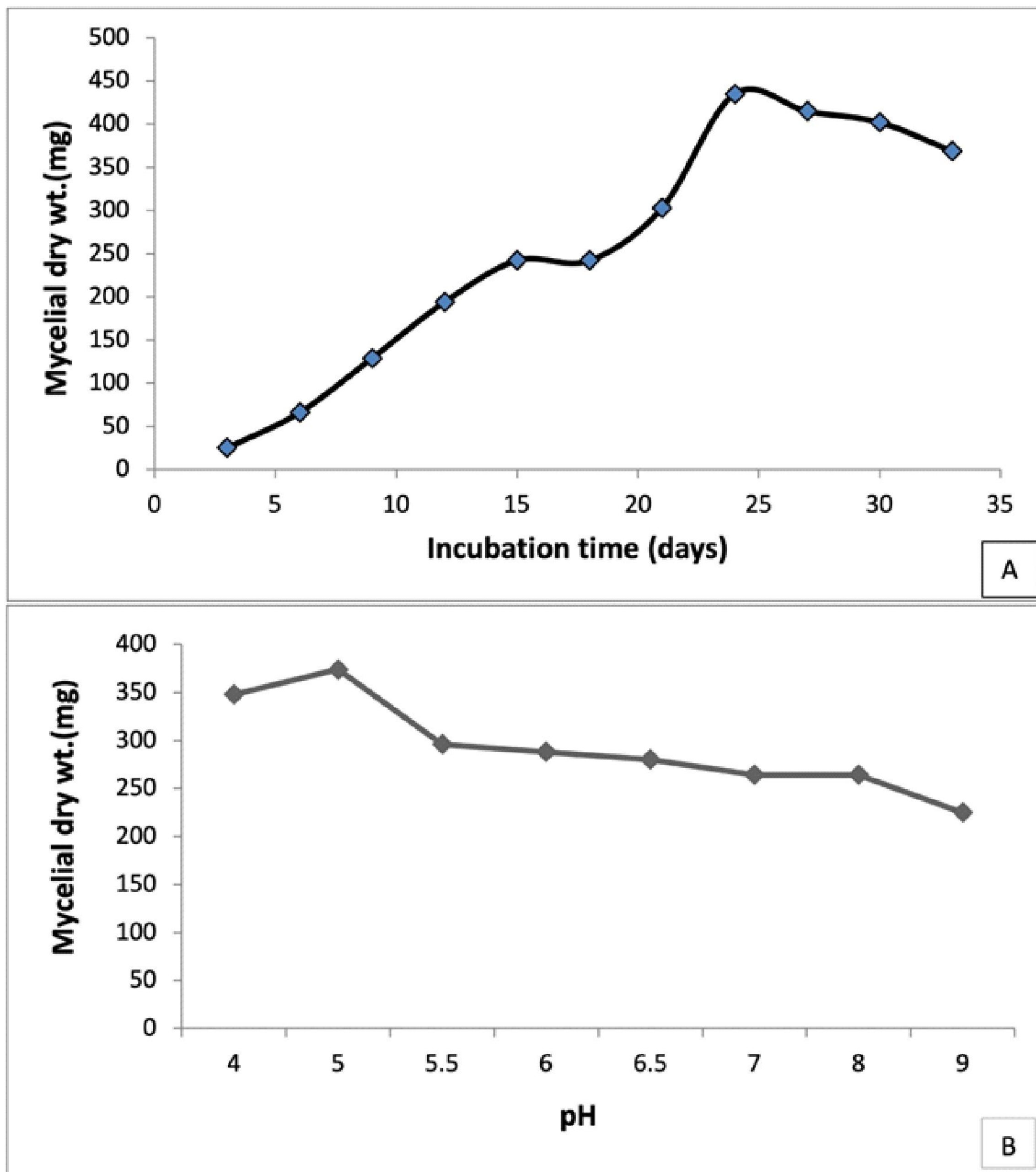

Fig 4. Effect of different incubation period (A) and pH (B) on mycelial growth of *Sphaerostilbe repens*

optimization PAbs through PTA-ELISA was done considering two variables i.e. concentration of antigen and concentration of PAb (IgG). In both cases, reactions were done with PAb obtained after 1st, 2nd, 3rd and 4th bleedings. Doubling dilution of *S. repens* mycelial antigen ranging from 40 to 0.312 µg/ml tested against IgG at a concentration of 40 µg/ml. ELISA values decreased with the decrease of antigen concentration but the values were still quite high indicating that the range of detection could be much lower. Double dilution of IgG purificd from PAbs obtained from all bleedings were also tested against homologous antigens at a concentration of 10 mg/ml. Absorbance values decreased from 40 to 0.312 µg/ml. A_{405} values on the other hand increased with the different bleedings (Fig 8a & b).

Immunodetection of S. repens in soil samples using PTA-ELISA format

In order to determine whether PAb of *S. repens* could detect the pathogen in soil samples collected

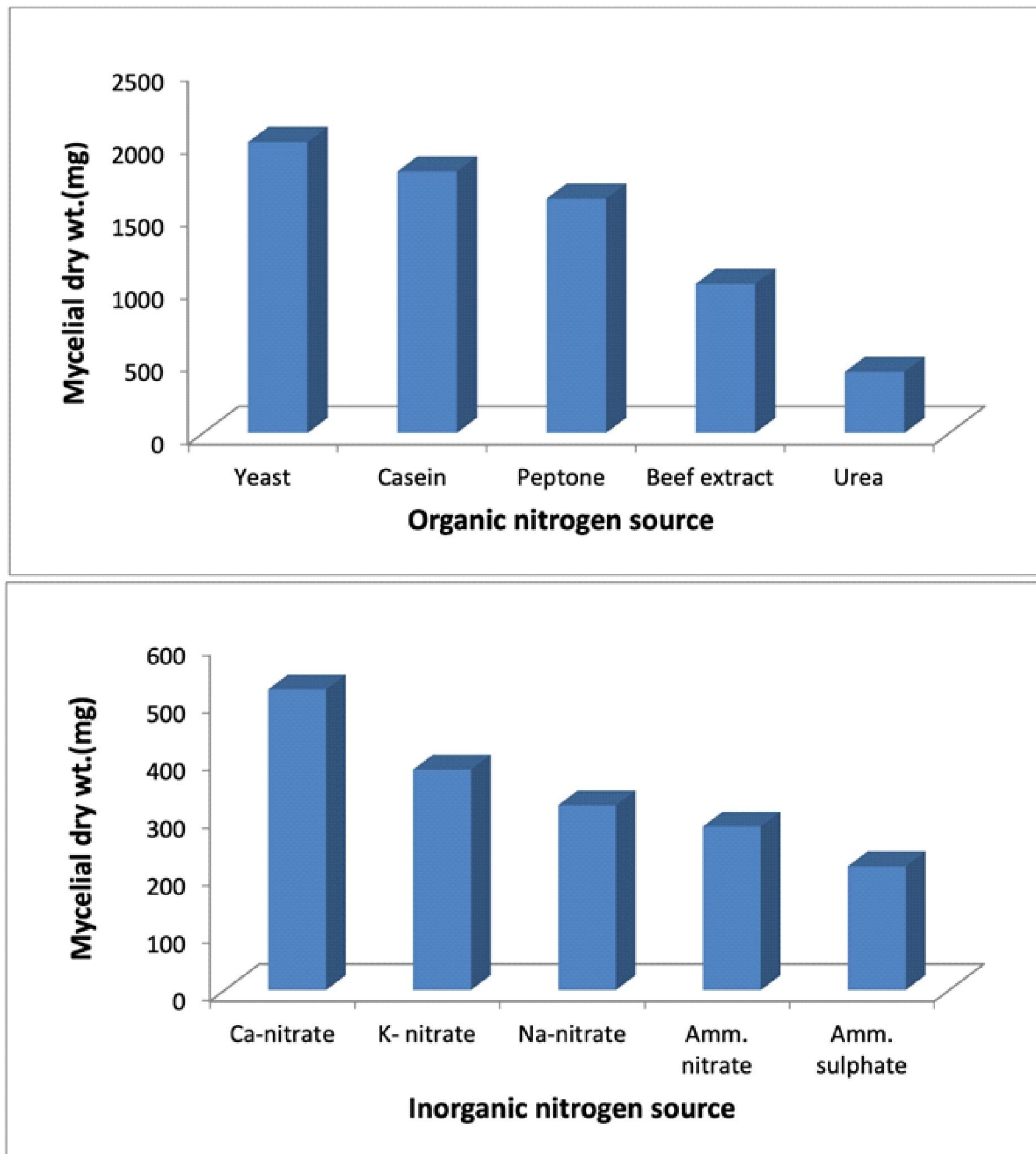

Fig 5. Effect of different organic and inorganic nitrogen sources on mycelial growth of *Sphaerostilbe repens*

from various tea estates. One set of the soil was spiked with spores of *S. repens.* Antigens from 26 soil samples were prepared and tested against PAb of *S. repens*. Spiked soil antigen gave very high values against Pab of the pathogen which were comparable to homologous mycelial antigen. Among the 26 soil samples tested, 4 samples [two from Matigara Tea Estate (Section 11 & 12), one from Bijoynagar Tea Estate (section 8) and other from Bentaguri TE] gave high absorbance in ELISA (ranging from 0.9 to 1.2), while other soil samples showed low A405 values in the range of 0.3-0.5.Cross reactivity of the PAb raised against *S. repens* was tested against a number of soil fungi some of which were pathogenic to tea roots *Fomes lamaenosis, Poria hypobrumia, Ustulina zonata, Armillaria mellea*) and others were non pathogenic (*Beauveria bassiana, Metarrhizium anisopliae, Trichoderma harzianum, T. viride, Fusarium oxysporum* and *Sclerotium rolfsii*). Antigens were

Fig. 6 (A-F) Roots of uprooted tea plants showing symptoms 20 days after inoculation with *S. repens* (A) TV-26, (B) TeenAli-17/1/54, (C) TV-23,(D) TV-28, (E) TV-25, (F) TV-29

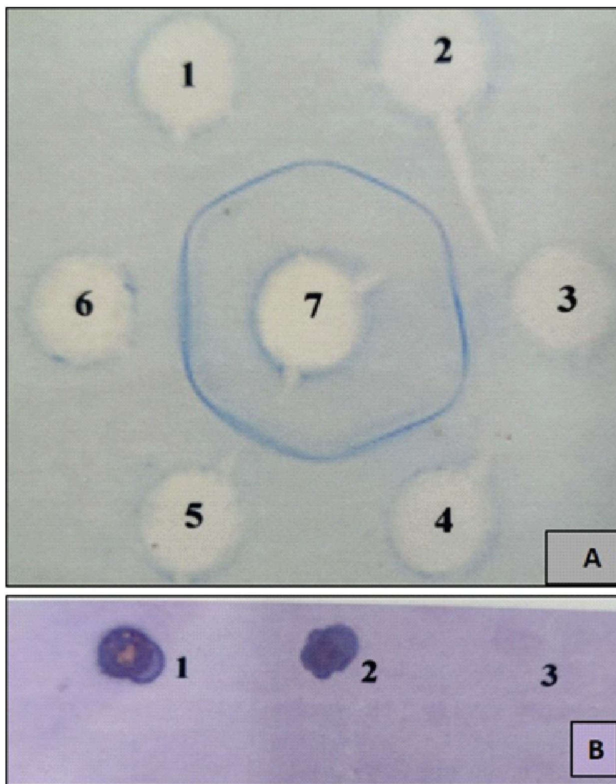

Fig. 7. (A)Agar gel double diffusion test with PAb of *S. repens*. Peripheral wells (1-6) were loaded with homologous fungal antigens and central well (7) loaded with PAb of pathogen.(B) Dot immunobinding assay onnitrocellulose paper loaded with mycelial antigen of *S. repens* (1), antigen from tea root (TV-26) artificially inoculated with*S. repens* (2) and health tea root (TV-26) antigen (3)reacted with PAb of *S. repens*.

in the range of 0.5-0.6 in comparison with high A405 value (2.1) in homologous reaction (Fig 9).

Indirect immunofleuroscenceof mycelia and conidia of S. repens

Mycelia and conidia of *S. repens* was not auto-fluorescent nor did it fluoresce when treated with normal serum followed by reaction withFITC. Treatment of mycelia and conidia of *S. repens* with homologous PAb and reacted with FITC showed a strong apple green fluorescence that was more intense on young hyphal tips (Fig 10)while conidia showed a general fluorescence throughout the surface (Fig 11) .

DISCUSSION

Appearance of Violet Root Rot disease of tea has been observed in water logged area with poor drainage systems. Since the growth and infectivity of the pathogen (*S. repens*) depends on a number of environmental factors, the effect of some of the factors on mycelial growth of this pathogen was investigated. Cultural characteristic of the pathogen *in vitro* revealed optimum growth after 24 days of incubation and at pH 5.0. Sucrose was found to be the best carbon source for growth of the pathogen. Optimum growth was recorded in yeast extract followed by casein hydrolysate as

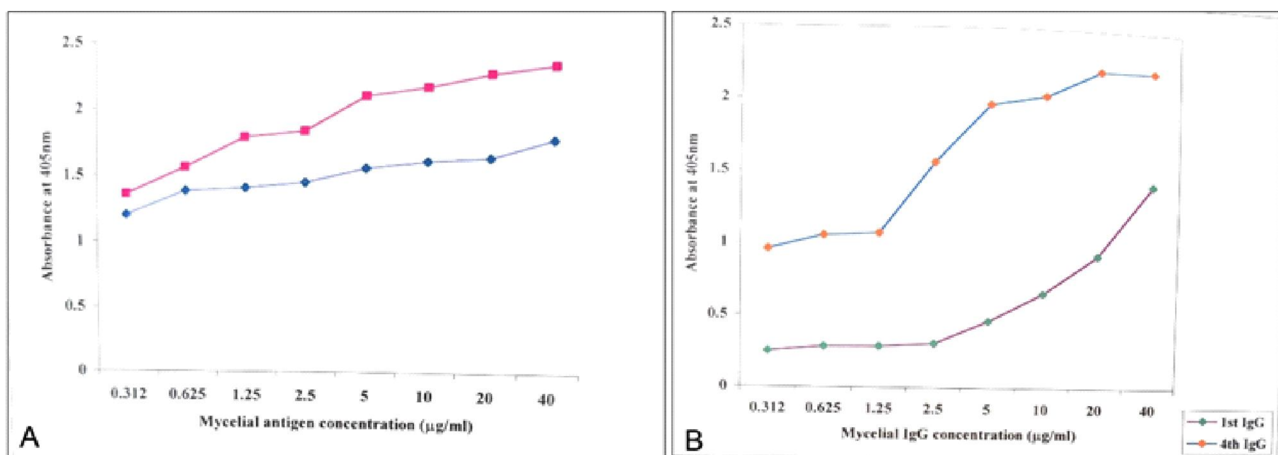

Fig 8. Optimization ofmycelial antigen concentrations (A) and PAb concentrations (B) of *S. repens* using PTA-ELISA format

prepared from the mycelia of all the above fungal isolates and were tested against PAb of *S. repens* using PTA-ELISA format. Result revealed that among all the fungi tested PAb of *S. repens* reacted to some extent with mycelial antigens of *T. harzianum, F. lamaoensis*, *P. hypobrunnea* and *B. bassiana* (isolate-135) showing low A405 values

organic nitrogen sources while maximum growth occurred in calcium nitrate among inorganic nitrogen sources. Rhizomorph formation which is an important event in the pathogen life cycle was also found to occur *in vitro* when the fungus was grown in certain media.

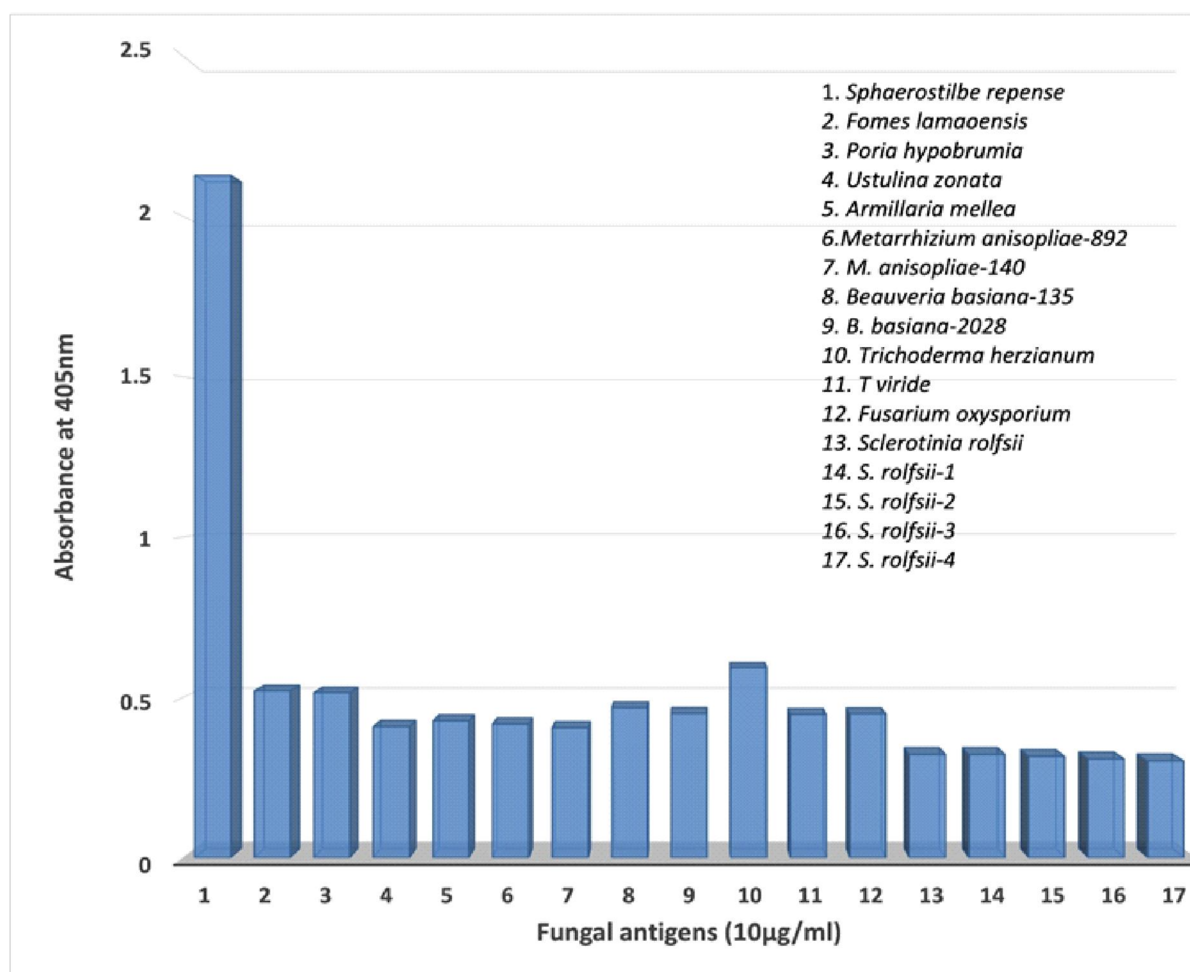

Fig 9. Cross reactivity of PAb of *S. repens* with other soil fungi using PTA-ELISA format.

Serological assay for detection of pathogens causing plant diseases are gaining ground in case of fungal diseases. Both disease detection and diagnosis of plant disease is rapidly increasing by using immunological formats (Chakraborty and Chakraborty, 2012). Effectiveness of mycelial antigen of *S. repens* in raising antibody was assessed using immunodiffusion and dot immunobinding assay. Acharya *et al.* (2021) have also raised polyclonal antibody against mycelial antigen of *Curvularia lunata* causing leaf blight of *Persea bombycina* and observed strong precipitin reactions in agar gel diffusion test when mycelial antigen was reacted with antibody of 4th and 5th bleeding. The dot immunobinding technique has been found to be rapid and sensitive method for detection of pathogens. Effectiveness of mycelial antigen of *Macrophomina phaseolina* (Chakraborty *et al.* 2012), *Bipolaris sorokiniana* (Chakraborty *et al.* 2016), *Drechslera oryzae* (Khati and Chakraborty, 2019) in raising antibody against these fungal pathogens were also evident as a positive reaction between the antigen and antibody on nitrocellulose membrane. Antigens prepared from healthy and artificially inoculated tea (TV 26) roots when reacted with PAb of *S. repens* on nitrocellulose membrane, development of strong colour reaction was evident only in inoculated root tissue indicating the establishment of infection in tea root. Early and rapid diagnosis of brown root rot disease of tea caused by *Fomes lamaoensis* (Chakraborty *et al.* 2002a) and charcoal stump rot disease of tea caused by *Ustulina zonata* (Chakraborty *et al.* 2002b) were also demonstrated using DIBA technique where infected samples depicted intense colour reaction on the nitrocellulose membrane due to the antigen-antibody reaction.

PTA- ELISA format has also been used for pathogen detection which has proved to be one of the most sensitive serological techniques. Since ELISA is a very sensitive technique and non-specific binding interferes with the actual antigen-antibody reaction, initially PAb raised against *S. repens* was purified and IgG fractions were used in all further

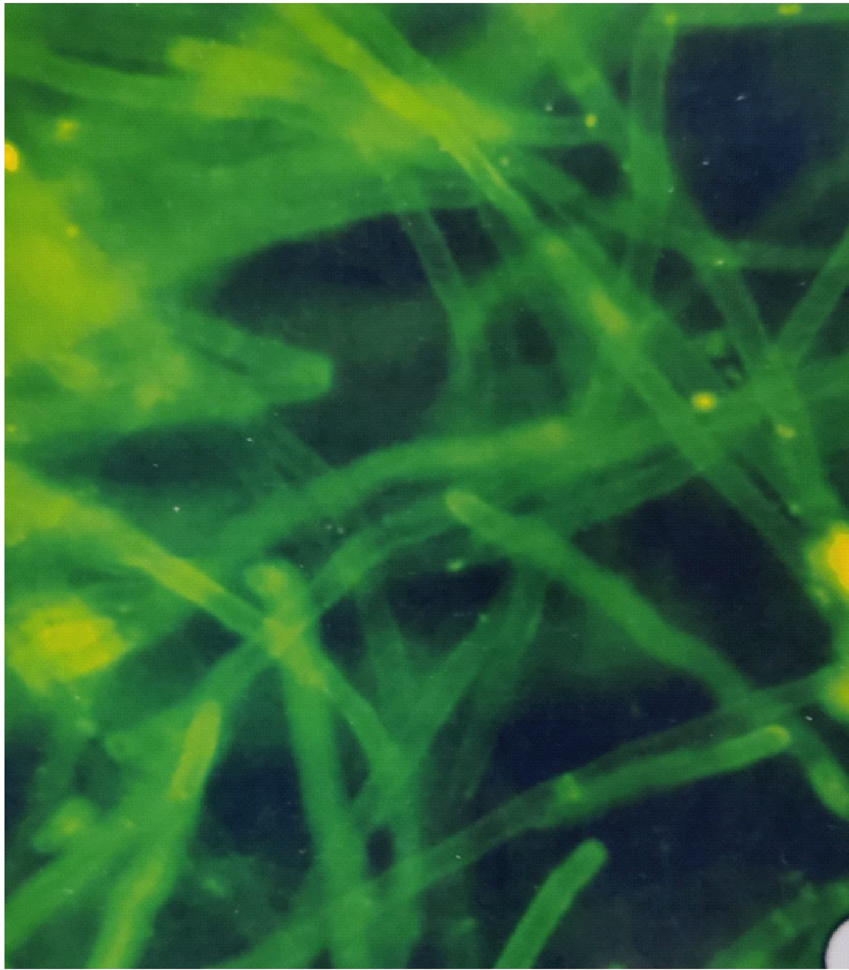

Fig 10. Immunofluorescence of hyphae of *S. repens* treated with PAb of *S. repens* and reacted with FITC labelled antibodies of goat specific for rabbit globulin.

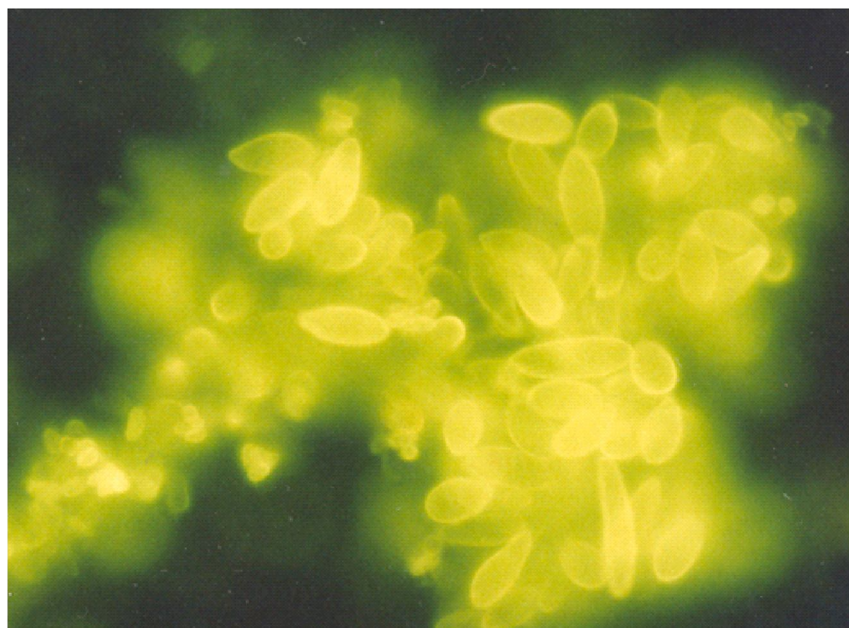

Fig 11. Immunofluorescence of conidia of *S. repens* treated with PAb of *S. repens* and reacted with FITC labelled antibodies of goat specific for rabbit globulin

tests. Prior to other tests, the sensitivity of assay was optimized and the minimum detectable antigen concentration and optimum IgG concentration were determined in homologous reactions. Positive results were obtained with very low concentration of both antigens and IgG. Efficacy ofIgG in detecting the specific pathogen in the soil was considered equally or more important thandetecting the pathogen in the root tissues. Detection of specific pathogens in soil requires very sensitive techniques which would make it possible to differentiate between the various microorganisms. Cross reactivity of the PAb raised against *S. repens* when tested with other soil fungi mycelial antigens of *T. harzianum, F. lamaoensis*, *P. hypobrunnea* and *B. bassiana* reacted to some extent and showed low A405 values (0.6) in comparison with high A405 value (2.1) in homologous reaction with antigen of *S. repens.* So it is clear that the PAb raised against *S. repens* was found to be specific to detect the violet root rot pathogen but notother soil fungi Thus PTA- ELISA showed potential for detection of *S. repens* in soil.

Treatment of mycelia and conidia of *S. repens* with homologous IgG and reaction with FITC labelled antibodies of goat specific for rabbit globulin showed a general fluorescence that was more intense on young hyphae and conidia. Chakraborty *et al.* (2012) conducted indirect immunofluorescence with young hyphae and sclerotia of *M. phaseolina* with homologusPAb to obtain apple green fluorescence confirming the pathogen. Similarly, treatment of mycelia and spore of *Exobasidium vexans* causing blister blight (Chakraborty and Sharma, 2007) and *Glomerellacin gulata* causing brown blight (Chakraborty *et al*. 2008a); mycelia and sclerotia of *Corticium invisum* causing black rot of tea (Chakraborty and Das Biswas, 2008) with their respective homologous PAbs followed by FITC labelling developed a general fluorescence that was more intense on young hyphal tips and spore wall. Immunodetection of *Aspergillus flavus* in stored seeds of Cowpea, Lentil and Mungbean have also been reported (Chakraborty *et al*. 2008b). In case of *Pestalotiosis disseminata* spores only the setulae and appendages showed apple green fluorescence as the conidia are dark septate, confirming the identity of the pathogen (Acarya *et al*. 2015). Different test formats including DIBA, PTA-ELISA and indirect immunofluorescence were assessed for their potential to detect *Sclerotium rolfsii* causing Sclerotial blight disease of tea (Bhagat and Chakraborty, 2020). These techniques can be used for early detection of fungal pathogens of different crops for developing disease management strategies. Early detection of phytopathogens and diagnosis of plant diseases using serological and molecular techniques (Chakraborty and Chakraborty, 2021) have opened up a step in developing management strategies which leads towards sustaining crop production.

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