CHAPTER III

Isolation, characterization and screening of potential antagonists against causal organisms

3.1. Introduction

Throughout the history of agriculture humans have struggled to reduce the adverse effects of plant disease on their crops. Early agriculturists realized the benefits of cultural practices such as crop rotation and the use of organic soils amendments in promoting plant productivity. Interest in biological control has increased recently fuelled by public concerns over the use of chemicals in the environment in general and the need to find alternatives to the use of chemicals for disease control. The key to achieving successful, reproducible biological control is the gradual appreciation that knowledge of the ecological interactions taking place in soil and root environments is required to predict the conditions under which biocontrol can be achieved (Deacon, 1994; Whipps, 1997). Management of diseases are difficult in developing countries, where fungicides are very expensive, where as biocontrol agents are cheap, effective and can be used without much skill or additional infrastructure (Jayalashmi *et al.*, 2003). Isolation of rhizospheric bacteria are easy because they are ubiquitous but selection is difficult since only few of them will be disease suppressor (Cook, 1981; Vidhyasekaran, 1998).

Antagonist is an umbrella term for parasites, predators, pathogens, competitors and other organisms that repel, inhibit or kill plant parasites. Microorganisms that can grow in the rhizosphere are ideal to exploit as a biocontrol agents, since the rhizosphere provides the front line of defense for roots against pathogenic attack. Pathogens encounter antagonism from rhizosphere microorganisms before and during primary infection as well as in secondary infection (Weller, 1988). Representative of a range of bacterial (*Pseudomonas, Burkholderia, Bacillus, Serratia, Actinomycetes*) and fungal (*Trichoderma, Penicillium, Gliocladium, Sporodesmium,* non pathogenic *Fusarium* spp.) groups have been identified as antagonists of soil borne plant pathogen. The mechanism by which these microorganisms make soil suppressive can be divided into several categories; nutrient competition, microbial antagonism, parasitism and systemic induced resistance. The potential strains of *Pseudomonas* sp. for suppression of plant

disease in agriculture has been demonstrated in hundreds if not thousands, of experiments world wide, since they represent not only a dominant bacterial group in the rhizosphere ecosystem but are also metabolically and functionally most versatile (Lutenberg and Dekkers, 1999). The worldwide interest in this group of bacteria was sparked by studies initiated at the University of California, Barkley, during the 1970s (Weller, 1988). In 1978 Burr *et al.* reported that strain of *Pseudomonas fluorescens* and *P. putida* applied to seed pieces, improved the growth of potatoes. These strain and similar strains were given the name plant growth promoting rhizobacteria (PGPR). The term rhizobacteria was coined for those bacteria which have the ability to colonize roots aggressively (Schroth and Hancock, 1981). Numerous examples of plant growth stimulation by fluorescent *Pseudomonas* species have been reported (Kloepper *et al.*, 1980b; Howell and Stipanovic, 1980; Suslow and Schroth.1982; Gardner *et al.*, 1984; Bashan, 1986; Weller and Cook, 1986 a; Keel *et al.*1989; Preston, 2004). Summarizing the results from field test, Schroth and Hancock (1982) reported that the fluorescent pseudomonads increased the yield of potato 5-33%, of sugarbeet 4-8 tones per hectors and root weight of radish 60-144%. Van Peer and Schippers (1988) documented increases in root and shoot fresh weight for tomato, cucumber, lettuce, and potato as a result of bacterization with *Pseudomonas* strains. These bacteria are ideally suited as soil inoculants because of their potential for rapid and aggressive colonization. This feature alone was suggested as a disease control mechanism by preventing the invasion of detrimental soil microorganisms onto the root surface (Lucy, 2004). Several antibiotic producing *Pseudomonas* sp. were isolated from soil suppressive to disease such as take-all of wheat, black rot of tobacco (Keel *et al.*, 1989), and fusarium wilt (Kloepper *et al.*, 1980 a; Scher and Baker, 1982; Sneh *et al.*, 1984). Fluorescent *Pseudomonas* spp. have also been implicated in the control of *Phytophthora* root rot of soybean (Lifshitz *et al.*, 1986), potato seed decay due to *Erwinia carotovora* (Xu and Gross 1989), and fungal diseases of orange and lemon citrus roots (Gardner *et al.*1984) and ornamental plants (Yuen and Schroth, 1986). The control of various

Pythium fungi by specific fluorescent *Pseudomonas* strains has also been documented. Howell and Stipanovic (1980) isolated a fluorescent pseudomonad which could inhibit *Pythium ultimum,* an important pathogen of cotton seedlings (Gaber *et al.,* 1979; Johnson *et al.*, 1978).

3.2. Selection of efficacious organism

In vitro screening is frequently used to select prospective antagonists, even though *in vitro* results may not be related to biocontrol in the field. Because of the time and expense required for field testing, both methods are needed to select potential field effective strains (Fravel, 1988). Rhizosphere bacteria with the ability to provide biological control appear to comprise less than 10% of the total population of bacteria in the rhizoplane (Scworth and Hancock, 1981; 1982; Weller and Cook, 1986 b). There is no shortage of methods and techniques for isolating and testing putative antagonists of plant pathogens (Merriman and Russell 1990). *In vitro* tests, such as bioassays conducted on nutritive media in petiplates that identify zones of antagonistic activity by microbials against plant pathogens, remains popular as at least a preliminary screening method to identify potential antagonists of pathogens, this is despite the fact that studies have demonstrated that the test medium greatly sways the results of *in vitro* antagonism bioassays (Schisler *et al.,* 1997) and that correlations between *in vitro* results and the level of biological control observed *in situ* are frequently inconsistent (Reddy *et al.,* 1993). Conversely, a bioassay of putative antagonists that involves testing microbial agents immediately after isolation using a whole plant system is enormously time consuming. It can also result in settling for mediocre biocontrol performance due to limitation on number of strains that can be processed in this manner.

There are several reports of correlation between assays *in vitro* and biocontrol *in vivo.* Production of the antibiotic chetomin by *Chaetomium globosum in vitro* was positively correlated with antagonism of *Venturia inequalis* on apple seedlings in a growth chamber (Cullen and Andrews, 1984). Similarly, the presence of zones of inhibition *in vitro* was good predictors of field performance of *Agrobacterium radiobacter* in controlling *A.*

tumifaciens on peaches (Alconero, 1980). *In vitro* and *in vivo* tests showed that antagonism to *Drechslera dictyoides* by bacteria isolated from the phylloplane was related to the *in vitro* production of inhibitory metabolites by these bacteria (Austin *et al.*, 1977).

Many published studies employ *in vitro* tests in which the pathogen and the antagonist are inoculated at opposing sides of petri-dishes filled with nutrient-rich laboratory medium, and inhibition of pathogen growth is measured. Most laboratory media are not representative of natural, nutrient-limited substrates. Such *in vitro* interaction have been criticised for over 30 years (Huber and Watson, 1966). Unfortunately, despite of modern reiterations of the warning (Sandys-Winsch *et al.*, 1994; Whipps, 1987), *in vitro* interactions are still widely used in the selection of biocontrol agents. This chapter deals with isolation, characterization of a large number of fluorescent pseudomonads and study were undertaken to identify the antagonistic activity of the isolates by *in vitro* screening against *Erwinia carotovora, Fusarium oxysporum* and *Mucor hiemalis* f. *hiemalis* and finally selection of one putative biocontrol agent by comparing its level of antagonism with other screened fluorescent pseudomonads.

3.3. Materials and Methods

3.3.1. Isolation of fluorescent pseudomonads

Fluorescent pseudomonads were isolated from potato rhizosphere and healthy *Cymbidium* rhizosphere during 2003-2004 from five potato fields, viz., Darjeeling, Rimbik, Srikhola, Bijonbari and Singtam and five orchid nurseries such as Darjeeling, Kurseong, Kalimpong, Mirik and Ging. Each field samples consisted of roots of the plants and soils from the surrounding plants were taken in polythene bags and brought to the plant pathology laboratory. Roots of both potato and *Cymbidium* were shaken vigorously to remove loosely adhering soil prior to bacterial isolation. Roots and soil (1 gm) were washed in 10 fold (w/v) quantity of sterile phosphate buffer (pH 7.1) by agitation for 30 min on a rotary shaker at 150 rpm (Xu and Gross, 1986). Aliquots from appropriate serial dilutions were plated in duplicate on King's medium B agar (King, *et al.*, 1954). After incubation at 28°C for 48 hours, counts were made of total fluorescent colonies under UV light on King's B medium.

3.3.2. Characterization of fluorescent pseudomonads

3.3.2.1. Morphological characters

Young cultures (18 to 24 hours) were used for morphological characterization and the following morphological characters were evaluated.

3.3.2.1.1. ***Gram morphology:*** - A drop of bacterial suspension was placed on a clear grease free slide and made smear, dried and then heat fixed, slides were flooded with crystal violet solution for 30-60 sec. Poured off strain and applied iodine solution to washed away the crystal violet . Then slides were covered with fresh iodine solution for 30-60 sec. Washes the iodine solution with absolute alcohol until colour ceases to come out of preparation .Then saffranin was put on the slide for 1-2 min, washed with water and dried with blotting paper (Skerman, 1967).

3.3.2.1.2. ***Fluorescence and Motility:*** - Bacterial cultures were inoculated on King's B medium and incubated at 28°C for 48 hours. The bacterial colony appears on the plate were seen under UV light to check for fluorescence (Shinde and Lukezie, 1974).

Motility was checked by making hanging drop preparation of young broth and agar cultures (Skerman, 1967)

3.3.2.1.3. **Diffusible pigment:** - Pigment productions by fluorescent pseudomonads were determined by streak inoculation on King's B medium. Cultures were examined for pigmentation after 1, 2, 3 and 6 days of incubation at 28°C. Ultraviolet light used to check for fluorescence (Shinde and Lukezie, 1974).

3.3.2.1.4. **Size and Shape:** Leica DMSL research microscope was used to determine the size and shape of the fluorescent pseudomonads native isolates.

3.3.2.2. Cultural characteristics

Different cultural parameters like growth, form, elevation, colour, surface edges etc. were tested in King's B slant and plates after incubation at 28°C for 48-72 hours (Seeley and Vandenask, 1962).

3.3.2.2.1. ***Slant characters:*** - Fluorescent pseudomonads were grown on King's B slants and incubated at 28°C and there cultural characteristics were recorded as;

Growth: Scanty, moderate, abundant

Form: Beaded, arborescent, effuse, echinulate, rhizoid, filiform

Elevation: Flat or raised

3.3.2.2.2. ***Plate characters:*** - Fluorescent pseudomonads were point inoculated on King's B plate and incubated at 28°C and their cultural characteristics were recorded as;

Growth: Slow, moderate, rapid

Form: Punctiform, Circular, rhizoid, irregular

Surface: Smooth, rough, dry, moist, dull, glistening

Elevation: Flat, raised, convex, umbonate, pulvinate

Edges: Entire, undulate, lobate.

3.3.2.3. Biochemical characteristics

3.3.2.3.1. ***Levan production*****:** -Plates of Difco nutrient agar containing 5% sucrose was streaked with the isolates. Two days after inoculation, isolates that produced large, white, domed mucoid colonies were considered to be levan producers (Lelliott *et al.*, 1966; Misaghi *et al.*, 1969).

3.3.2.3.2. ***Oxidase production*****:** - Kovac's method (Kovacs, 1956) was used to test for the presence of oxidase. Loopful of bacteria from 48 hours old cultures grown on nutrient agar and King's B medium was smeared on filter paper previously soaked with 1% (w/v) aqueous solution of N,N-dimethyl-p-phenylene-diamine with a platinum needle. Production of dark purple colour in 10 sec indicated the presence of oxidase.

3.3.2.3.3. ***Arginine dihydrolase production*****:** - About 10 ml of Thornley's arginine medium 2A (Shinde and Lukezie, 1974) in screw capped test tubes were sterilized by autoclaving. The pH was adjusted to 7.2 with NaOH. Duplicate tubes were stab inoculated with 48 hours old cultures. One was sealed with sterile, melted and cooled vaseline to a depth of 5 mm. The screw cap of the other tube was left loose. The anaerobic formation of alkali from arginine was detected by a change in colour of the indicator after 4 days. This change suggested the presence of all or part of the arginine dihydrolase system (Lelliott *et al.*, 1966; Misaghi *et al.*, 1969).

3.3.2.3.4. ***Amylase production*****:** - Plates of nutrient agar containing 1% soluble starch were streaked once and incubated at 28°C. After 48 hours iodine solution were poured in the plate. The presence of a clear area around the bacterial growth indicated starch hydrolysis (Shinde and Lukezic ,1974).

3.3.2.3.5. ***Protease production*:** - Gelatin liquifaction was tested by stabbing tubes of Difco nutrient gelatin and checking 1,5,10 and 14 days after inoculation. The cultures were placed at 4°C for 30 min before recording the results. Liquifaction was positive if the medium flowed readily when tubes were tilted (Lelliott *et al.*, 1966; Misaghi *et al.*, 1969).

Gelatin hydrolysis was confirmed by streaking duplicate plates of nutrient agar with 0.4% gelatin with each isolate and testing with an $HgCl_2$/Tri Chloro Acetic Acid (TCA) (Skerman, 1967).

Protease activity was also performed by inoculating the isolates on 5X minimal media supplemented with 5% of skim milk and 1% agar; incubate at 28°C for 24-48 hours and testing with TCA solution. Presence of clear zone around the colonies indicated protease activity (Gaur *et al.*, 2004).

3.3.2.3.6. ***Pectinase production*:** - Plates of 5X minimal media supplemented with 1% pectin were inoculated with the isolates. After 2-4 days plates were flowed with cetrimide solution. The presence of clear zone around the colonies indicated pectin hydrolysis (Gaur *et al.*, 2004).

3.3.2.3.7. ***Cellulase production*:** - Plates of 5X minimal media supplemented with 1% carboxy methyl cellulose (CMC) were inoculated with the isolates. After 2-4 days plates were flowed with cetrimide solution. The presence of clear zone around the colonies indicated cellulose hydrolysis (Gaur *et al.*, 2004).

3.3.2.3.8. ***Catalase activity*:** - Colony of bacteria from a 24 hours old nutrient agar slants were transfer on a glass slide and few drops of H_2O_2 (3% w/v) were poured over the culture. The release of bubble within 20 sec indicates positive catalase activity (Skerman, 1967).

3.3.2.3.9. ***Indole production*:** - Tubes of 1% tryptone were inoculated with one drop of bacterial suspension. Strips of dry filter paper previously soaked in a saturated solution of oxalic acid were inserted with the cotton plugs in such a position that they did not touch the surface of the medium and incubate it

at 28°C for 48 hours. The presence of indole was indicated by the development of pink colour on the filter paper strip (Shaffer, 1967).

3.3.2.3.10. ***Nitrate reduction*****:** -Tube of Nitrate broth (KNO_3-0.1%; peptone-1%; beef extract-0.3%) were inoculated with bacterial culture and incubated at 28°C for 48 hours. Three drops of reagent (Zink chloride-20 gm; starch -4 gm; potassium iodide-2 gm; powdered zinc metal; water-1 lit) and 1 drop of sulphuric acid were poured to a drop of 48 hours old bacterial culture. Appearance of blue colour indicates nitrate production (Shaffer, 1967).

Nitrate reduction was also performed by adding few drops of solution A (sulphanilic acid) and same amount of solution B (dimethyl-alpha-napthyalamine) in 48 hours old nitrate broth culture. Appearance of red colour indicates the nitrate reduction (Dubey and Musheshwari, 2002).

3.3.2.3.11. ***Acid and gas formation from carbohydrates*****:** - To detect acid and gas formation from carbohydrates, thirteen different types of carbohydrates (Glucose, sucrose, glycerol, maltose, manitol, rhaffinose, xylose, ribose, mannose, galactose, arabinose, rhamnose and fructose) were used. Fermentation broth (peptone- 10 gm; Carbohydrate- 5 gm; NaCl- 5 gm; Phenol red- 0.018 gm, water – 1 lit.) of thirteen different sugars were taken in fermentation tube and inoculated with fluorescent pseudomonad isolates, whereas uninoculated tubes were served as control. All the inoculated and uninoculated tubes were incubated at 28°C for 24 to 48 hours. Change in colour (due to production of acid) or change in colour and appearance of gas (due to production of acid from gas) were recorded by comparing with the uninoculated (control) tube (Dubey and Musheshwari, 2002).

3.3.2.3.12. ***Methyl red (MR) test*** **:-** The ability of different isolates to produce acid from glucose in amount sufficient to reduce the pH to 4.2 or less

and to maintain this low pH for at least 4 days was determined by using the glucose-phosphate-peptone-water method outlined by Skerman (Skerman, 1967). Cultures were tested for acid production 2 and 4 days after inoculation by adding a few drops of methyl red solution. The presence of red colour was taken as a positive indication and yellow colour as a negative indication.

3.3.2.3.13. *Voges-Proskaner (VP) test*: - The ability of different isolates to produce acid from glucose and subsequently to convert it to acetylmethylcarbinol or 2:3-butylene glycol, both neutral substances, was determined by the method outlined by Skerman (Skerman, 1967). Cultures grown in glucose-phosphate-peptone-water were tested for the presence of a neutral substance with 1 ml of 10% KOH solution, after 2 and 4 days of incubation. The presence of pink colour was scored positive and no colouration as negative.

3.3.3. Preliminary screening of antagonists

All the rhizospheric fluorescent pseudomonads were preliminary screened for antagonistic activity by using the petriplate assay as described by Anderson et al. (1980). *In vitro* antagonistic activities of the isolated fluorescent pseudomonads were performed against both the fungal pathogens i.e., *Fusarium oxysporum* and *Mucor hiemalis* f. *hiemalis* as well as against bacterial pathogen i.e. *Erwinia carotovora* by inoculating four isolates 2-3 cm apart from each other on Peptone Glucose and Agar (PGA) medium. Mycelial plugs of 5 mm in diameter of the fungal pathogens were transferred at the centre of the four inoculated fluorescent pseudomonads on PGA plate whereas for bacterial pathogen two fluorescent pseudomonads were streaked straight to an nutrient agar plate and *E. carotovora* was streaked aseptically perpendicular to the two streaks in such a way that they do not touch each other. Plates were replicated and repeated thrice. They were incubated at

30°C and antagonistic activities of different isolates were recorded after 72 hours. Isolates, which showed antagonism, were subcultured and maintained on King's B slant at 4°C for further use.

3.3.4. *Interaction of potential (screened) fluorescent pseudomonads against Cymbidium rot pathogens by dual culture technique*

3.3.4.1. Dual plate culture technique

The antagonistic effects of screened fluorescent pseudomonads were tested against fungal pathogens on Peptone Glucose Agar (PGA) plate by following the method of Skidmore and Dikinson (1970). This was done by streaking the test fluorescent *Pseudomonas* from 24 hours old culture (containing 10^7 cfu/ml) in a circle / O and semicircle / U shaped pattern, where as fungal pathogens (*Fusarium oxysporum* and *Mucor hiemalis* separately) were point inoculated at the centre of the plate. Uninoculated sets were served as control. The plates were incubated at 30°C for 5 days and inhibitions of colony growth were measured.

To test the inhibitory effect of screened fluorescent pseudomonads against the bacterial pathogen (*Erwinia carotovora*), the pathogen was streaked straight to an agar plate and incubate it at 30°C for 24 hours. After incubation and appearance of growth, the test fluorescent *Pseudomonas* was streaked perpendicular to the pathogenic bacteria in such a way that they do not touch each other. Then inoculated plates were again incubated at 30°C.

3.3.4.2. Dual liquid culture technique:

To test the antifungal activity of the screened fluorescent pseudomonads, dual liquid culture method was employed (Basha and Ulaganathan, 2002). One ml of freshly grown (24 hours old) fluorescent pseudomonad culture (containing 10^7 cfu / ml) and an agar block (5mm diameter) of fungal pathogen was inoculated onto 50 ml of peptone, glucose medium in 250 ml conical flask. The

culture was incubated on a rotary shaker at 30°C for 72 hours. For the control experiment the fungal pathogens were inoculated alone in the peptone glucose broth. Experiments were performed thrice taking triplicate for every set.

The difference in dry weights between the fungal culture grown with the test isolates or the control culture grown without any bacterium were recorded. For this 72 hours old dual liquid culture were passed through the pre weighed Whatman No 1 filter paper. It was dried for 24 hours at 70°C and weights were measured.

3.4. Results and Discussion

A total of fifty-four fluorescent pseudomonad strains were isolated from rhizosphere of potato and *Cymbidium*. Of which thirty-six strains were isolated from potato rhizosphere. Among them Darjeeling contributes - 6, Rimbik- 3, Srikhola- 3, Bijonbari- 9 and Singtam- 15. Eighteen strains were isolated from healthy *Cymbidum* rhizosphere, of which, from Darjeeling- 5; Kurseong- 8 and Kalimpong -5. Morphological, cultural, physiological and biochemical parameters of these native isolates were further evaluated.

3.4.1. Morphological characterization of fluorescent pseudomonads

All the isolates of fluorescent pseudomonads were non spore forming, fluoresces under UV light, gram negative, medium size rods, size ranges from 0.5 to 0.8 × 1 to 3 μm, with round to slightly pointed ends, motile in hanging drop preparation. On King's B medium they produce greenish-yellow to yellow fluorescent pigment when seen under UV light.

3.4.2. Biochemical and physiological characterization of fluorescent pseudomonads

All the fluorescent pseudomonad native isolates were aerobic as they grew in the upper layer and on the medium surface. They were levan positive (Table 3.1). The isolates were oxidase positive on nutrient agar and King's B medium. All the isolates were able to show reduction of nitrate after 48 hours of incubation. Most of the isolates were catalase positive, strong liquefiers of gelatin. A clear zone of 1 to 12 mm around each colony within 48 to 72 hours of incubation, indicating rapid hydrolysis of gelatin by the isolates (Table 3.1). The organisms did not produce arginine dihydrolase, indole production negative but few isolates showed faint reaction which was indicated by the development of pink colouration on saturated oxlic acid strip. In most of the cases methyl red (MR) test were negative as well as Voges-Proskauer (VP) test results were negative till after 72 hours of incubation. There were no cellulase

activity in most of the cases but protease, pectinase, amylase activities of the isolates were recorded as moderate to wide clear zone around several colonies (Table 3.1).

Table 3.1. Biochemical characterization of fifty-four fluorescent pseudomonads

Strains	Amylase	Gelatin hydrolysis	Cellulase	Pectinase	Protease	Catalase	Oxidase	Nitrate Reduction	Arginine Dihydrolase	Indole Production	MR test	VP test-	Levan production
BRL-1	-	+	-	-	+	++	+	+	-	+	-	-	+
BRL-2	-	++	-	+	-	+	+	+	-	-	-	-	+
BRL-3	-	-	-	+	+	++	+	+	-	-	-	-	+
BRL-4	+	+	-	-	-	+++	+	+	-	-	-	-	+
BRL-5	-	-	-	-	-	+	+	+	-	-	-	-	+
BRL-6	++	+++	+	-	-	++	+	+	-	-	-	-	+
BRL-PR-1	-	+	++	-	-	-	+	+	-	-	-	-	+
BRL-PR-2	+	+	++	-	++	+	+	+	-	-	-	-	+
BRL-PR-3	-	+	++	+	+	-	+	+	-	+	-	-	+
BRL-Slg-1	+	++	++	+++	+	++	+	+	-	+	-	-	+
BRL-Slg-2	+	++	++	++	-	-	+	+	-	-	-	-	+
BRL-Slg-3	++	++	-	++	-	-	+	+	-	-	-	-	+
BRl-Slg-4	+	-	-	+++	-	+	+	+	-	-	-	-	+
BRl-Slg-5	++	++	-	+	++	++	+	+	-	-	-	-	+
BRl-Slg-6	+	-	-	+++	+	++	+	+	-	-	-	-	+
BRl-Slg-7	+++	-	-	-	-	+++	+	+	-	-	-	-	+
BRL-Slg-8	+++	++	-	-	-	+	+	+	-	-	-	-	+
BRL-Dj1	-	+++	-	-	+++	+	+	+	-	-	-	-	+
BRL-DJ2	-	+	-	+	-	+++	+	+	-	-	-	-	+
BRL-Dj3	-	++	-	+	-	+	+	+	-	-	-	-	+
BRL-DJ4	+	++	-	+	-	+	+	+	-	-	-	-	+
BRL-DJ5	+	++	-	-	-	+++	+	+	-	-	-	-	+
BRL-Rim-1	-	+	-	-	+	+	+	+	-	+	-	-	+
BRL-Rim-2	-	++	-	-	-	++	+	+	-	-	-	-	+
BRL-Rim-3	-	++	-	+	+	+	+	+	-	-	-	-	+
BRL-BB1	+	++	-	+	++	-	+	+	-	-	-	-	+
BRL-BB2	-	+	-	-	-	-	+	+	-	-	+	-	+
BRL-BB3	+	++	-	+	+++	-	+	+	-	-	-	-	+
BLR-BB4	-	-	-	+	-	++	+	+	-	+	-	-	+
BRL-BB5	+	+	+	+	-	+++	+	+	-	-	-	-	+

Strains	Amylase	Gelatin hydrolysis	Cellulase	Pectinase	Protease	Catalase	Oxidase	Nitrate Reduction	Arginine Dihydrolase	Indole Production	MR test	VP test-	Levan production
BRL-BB6	+	++	-	+++	+	-	+	+	-	-	-	-	+
BRL-BB7	+	++	-	+	-	+	+	+	-	+	-	-	+
BRL-BB8	+	+	+	-	-	++	+	+	-	-	-	-	+
BRL-BB9	++	+	-	-	-	++	+	+	-	+	-	-	+
BRL-Sg-1	+	+	-	+	-	+	+	+	-	+	-	-	+
BRL-Sg-2	+	+	-	+	+	++	+	+	-	-	-	--	+
BRL-Sg-3	-	-	-	+	-	+++	+	+	-	-	-	-	+
BRL-Sg-4	-	-	-	-	-	-	+	+	-	-	-	-	+
BRL-Sg-5	+	+	-	-	-	++	+	+	-	+	-	-	+
BRL-Sili1	-	-	-	-	-	+	+	+	-	+	+	-	+
BRL-Sili2	+++	-	-	+	+	-	+	+	-	-	+	-	+
BRL-Sili3	++	+	-	++	++	++	+	+	-	+	+	-	+
BRL-Sili4	-	+	-	-	+	++	+	+	-	+	-	-	+
BRL-Sili5	-	+++	+	-	++	+	+	+	-	-	+	-	+
BRL-Sili6	-	+++	-	-	+++	+	+	+	-	-	+	-	+
BRL-Sili7	-	++	-	-	+++	-	+	+	-	-	+	-	+
BRL-Sili8	-	+++	-	-	++	-	+	+	-	-	+	-	+
BRL-Sili9	++	+	-	+	+	++	+	+	-	-	+	-	+
BRL-Sili10	-	-	+	-	-	-	+	+	-	-	+	-	+
BRL-Sili11	++	+	-	+	+	+	+	+	-	-	+	-	+
BRL-Sili12	-	+++	-	-	++	-	+	+	-	-	+	-	+
BRL-Sili13	-	+++	+	-	+++	++	+	+	-	-	+	-	+
BRL-Sili14	+++	++	-	+	+	+++	+	+	-	+	+	-	+
BRL-Sili15	++	+	-	++	++	++	+	+	-	+	-	-	+

The columns containing '+++', '++', '+' and '-' denoting High, Moderate, Low and Negative activity respectively. The columns containing '+' and/or '-' denoting positive and negative activity against that biochemical test.

Acid and gas formation by the isolates against thirteen different carbohydrate sources showed a wide variation on the level of their acid production during growth after 48 hours of incubation. Gas formations by the isolates from different carbohydrate sources were recorded as negative (Table 3.2).

Table 3.2. Acid and gas formation by the isolates using thirteen different carbohydrate sources.

Strains	Glucose	Sucrose	Glycerol	Maltose	Manitol	Rhaffinose	Xylose	Ribose	Mannose	Galactose	Arabinose	Rhamnose	Fructose
BRL-1	+++	+++	++	+	++	+	+	++	+++	+	-	-	+++
BRL-2	++	+	+	+	-	-	-	+	-	-	-	+	+++
BRL-3	+++	+	+	+	-	+	+	+	+	+	+	+	+++
BRL-4	+++	+	+	+	++	+	+	+	++	-	-	+	+++
BRL-5	++	+++	++	++	++	++	++	+	+++	+++	++	+	+++
BRL-6	+++	+++	++	+	+	-	-	+	++	++	-	-	+++
BRL-PR-1	++	+	++	++	++	+	-	+	+	+	-	+	+++
BRL-PR-2	+++	++	+	++	+	+	+	+	-	++	-	++	++
BRL-PR-3	+++	+++	+	++	++	++	+	++	++	++	-	+	+++
BRL-Slg-1	++	+	-	-	-	+	-	+	-	-	+	+	+++
BRL-Slg-2	++	+	-	-	+	+	-	+	-	-	+	+	++
BRL-Slg-3	++	+++	+	++	++	++	+	++	+	+	+	+	+++
BRl-Slg-4	+++	++	+	+	-	+	++	+	-	+	++	+	+++
BRl-Slg-5	++	+	-	+	-	+	+	++	+	+	+	+	+++
BRl-Slg-6	+	++	-	+	+	++	+	++	+	-	-	-	++
BRl-Slg-7	+++	+++	+	+	-	+	+	+	++	+	+	+	+++
BRL-Slg-8	+++	++	+	++	+	+	+	+	+	+	-	+	++
BRL-Dj1	+++	+++	+	++	+	++	-	++	+	+	+	+	+++
BRL-DJ2	+++	++	+	++	++	+++	+	++	+	+	+	+	++
BRL-Dj3	+++	+++	+	+	+	-	+	+	+++	+	+	+	+
BRL-DJ4	++	-	-	+	-	+	-	+	-	-	-	-	+
BRL-DJ5	+++	+++	++	+	+++	+	++	++	+++	+	+	+	++
BRL-Rim1	++	-	-	++	-	-	-	-	+	+	-	-	++
BRL-Rim2	+++	++	-	+	+	+	+	+	+++	+	-	-	++
BRL-Rim3	+++	++	-	+	+	+	+	+	+++	+	+	+	+++
BRL-BB1	+	+++	-	-	++	-	-	-	++	-	-	+	++
BRL-BB2	+++	++	-	-	+	-	-	-	+	-	-	-	+++
BRL-BB3	+++	+++	+	+	+	+	+	+	++	+	+	+	+++
BRL-BB4	+++	+++	+	+	++	+	+	+	+++	+	+	+	+
BRL-BB5	++	+	-	-	-	+	-	-	+	-	+	-	++
BRL-BB6	+++	+	+	++	-	+	++	+	++	-	+	+	+++
BRL-BB7	+++	+++	++	+	+	+	+	+	+	+	+	++	+++
BRL-BB8	+	+++	-	+	-	-	-	-	++	-	-	+	+
BRL-BB9	+++	+++	+	++	++	+	-	+	+++	+	-	++	+++
BRL-Sg-1	+++	+++	+	+	+++	+	+	++	+++	+	+	++	+++
BRL-Sg-2	+++	+++	-	-	+	+	-	+	+	-	-	-	++
BRL-Sg-3	+++	+	-	-	-	+	-	+	+	-	+	-	++
BRL-Sg-4	+++	++	+	++	++	+	++	+	++	+	-	-	++
BRL-Sg-5	+++	+++	+	++	+++	+	++	+	+++	+	-	+	+++
BRL-Sili1	++	+	-	-	-	-	+	-	+	-	-	-	+++

Strains	Glucose	Sucrose	Glycerol	Maltose	Manitol	Rhaffinose	Xylose	Ribose	Mannose	Galactose	Arabinose	Rhamnose	Fructose
BRL-Sili2	+++	++	+	++	+	++	+	+	+	+	+	+	+++
BRL-Sili3	++	+	+	++	+	+	++	++	++	+	++	+	++
BRL-Sili4	+	+	+	+	+	+	+	+	++	++	+	+	+
BRL-Sili5	+++	+	+	++	+	+	-	+	-	-	+	-	+++
BRL-Sili6	+++	+	+	+	+	+	+	++	++	+	++	+	+++
BRL-Sili7	+++	+++	+	+++	+	-	-	+++	+++	+	+	+	+++
BRL-Sili8	+++	+	+	+	+	+	+	++	+	+	+	+	+++
BRL-Sili9	+++	+++	+	+	+	-	+	++	+++	+	+	+	+++
BRL-Sili10	+++	+++	+	++	+	-	-	++	++	+	+	+	+++
BRL-Sili11	+++	+++	+	+++	+	-	+	+++	+++	+	+	+	+++
BRL-Sili12	+++	+	++	++	+	+	+	++	++	-	+	+	+++
BRL-Sili13	+++	+	+	+++	+	+	+	+++	+++	+	+	+	+++
BRL-Sili14	+++	+	+	++	+	+	+	+++	+++	-	+	+	+++
BRL-Sili15	+++	+++	+	+++	+	-	+	+	+++	+	+	+	+++

'-' = *No change*
'+' = *Minor change (reddish yellow)*
'++' = *Moderate change (Yellowish red)*
'+++' = *High (Yellow)*

3.4.3. Cultural characteristics

Different cultural parameters were tested in King's B slant and plate after incubation at 30°C for 48-72 hours and their mode of growth on plate as well as in slants were recorded (Table 3.3).

Table 3.3. Cultural Characteristics of fifty four fluorescent pseudomonads

	Slant				Plate				
Strain	**Growth**	**Form**	**Elevation**	**Colour**	**Growth**	**Form**	**Surface**	**Elevation**	**Edges**
BRL-1	Abundant	Echinulate	Raised	Whitish	Rapid	Irregular	Smooth	Raised	Undulate
BRL-2	Moderate	Beaded	Flat	Whitish	Moderate	Irregular	Rough	Raised	Undulate
BRL-3	Moderate	Filiform	Flat	Yellowish	Rapid	Circular	Smooth, Glistening	Flat	Entire
BRL-4	Abundant	Echinulate	Raised	Whitish	Rapid	Irregular	Rough, Dry	Raised	Entire
BRL-5	Scanty	Beaded	Raised	Whitish	Slow	Circular	Smooth, Glistening	Raised	Undulate
BRL-6	Abundant	Filiform	Flat	Whitish	Moderate	Irregular	Rough, Dull	Flat	Lobate
BRL-PR-1	Abundant	Effuse	Raised	Orange	Rapid	Irregular	Glistening	Raised	Undulate
BRL-PR-2	Abundant	Beaded	Raised	Whitish	Rapid	Circular	Smooth	Raised	Entire
BRL-PR-3	Abundant	Echinulate	Raised	Orange	Rapid	Circular	Smooth, Glistening	Raised	Circular
BRL-Slg-1	Moderate	Beaded	Flat	Whitish	Rapid	Circular	Smooth	Raised	Entire
BRL-Slg-2	Scanty	Filiform	Flat	Whitish	Moderate	Irregular	Rough, Dry	Flat	Entire
BRL-Slg-3	Abundant	Echinulate	Raised	Whitish	Rapid	Circular	Smooth	Raised	Entire
BRL-Slg-4	Abundant	Filiform	Flat	Whitish	Rapid	Punctiform	Smooth, Dry	Raised	Undulate
BRL-Slg-5	Moderate	Echinulate	Flat	Whitish	Rapid	Circular	Rough	Flat	Entire
BRL-Slg-6	Abundant	Echinulate	Flat	Whitish	Moderate	Circular	Smooth, Glistening	Convex	Entire
BRL-Slg-7	Abundant	Beaded	Raised	Whitish	Rapid	Irregular	Rough	Raised	Undulate
BRL-Slg-8	Abundant	Effuse	Raised	Whitish	Rapid	Circular	Smooth, Glistening	Flat	Entire
BRL-Dj-1	Abundant	Echinulate	Flat	Whitish	Rapid	Irregular	Rough	Raised	Lobate
BRL-Dj-2	Scanty	Beaded	Raised	Whitish	Moderate	Circular	Smooth, Glistening	Raised	Entire
BRL-Dj-3	Moderate	Beaded	Flat	Whitish	Moderate	Circular	Smooth	Raised	Entire
BRL-Dj-4	Abundant	Beaded	Raised	Whitish	Rapid	Irregular	Rough	Raised	Entire
BRL-Dj-5	Moderate	Filiform	Flat	Whitish	Rapid	Irregular	Rough	Raised	Undulate
BRL-Rim-1	Moderate	Effuse	Raised	Orange	Moderate	Circular	Smooth, Glistening	Raised	Undulate
BRL-Rim-2	Abundant	Echinulate	Flat	Yellowish	Moderate	Circular	Smooth, Glistening	Raised	Entire
BRL-Rim-3	Moderate	Filiform	Flat	Whitish	Rapid	Circular	Rough, Dry	Raised	Undulate
BRL-BB1	Abundant	Arbores cent	Raised	Whitish	Moderate	Circular	Glistening	Convex	Entire
BRL-BB2	Abundant	Beaded	Raised	Reddish	Rapid	Irregular	Rough	Umbonate	Undulate
BRL-BB3	Scanty	Effuse	Flat	Whitish	Slow	Punctiform	Rough	Flat	Undulate

	Slant				Plate				
BRL-BB4	Abundant	Effuse	Flat	Whitish	Moderate	Irregular	Rough	Flat	Undulate
BRL-BB5	Moderate	Beaded	Raised	Yellowish	Moderate	Circular	Smooth	Flat	Entire
BRL-BB6	Abundant	Beaded	Raised	Whitish	Moderate	Irregular	Rough	Flat	Undulate
BRL-BB7	Abundant	Beaded	Raised	Grayish	Moderate	Irregular	Rough	Flat	Undulate
BRL-BB8	Scanty	Arbores cent	Flat	Whitish	Slow	Rhizoid	Rough, Moist	Flat	Lobate
BRL-BB9	Abundant	Echinulate	Flat	Whitish	Moderate	Irregular	Smooth, Glistening	Raised	Undulate
BRL-Sg1	Moderate	Beaded	Raised	Yellowish	Slow	Circular	Dry, Rough	Flat	Undulate
BRL-Sg2	Moderate	Echinulate	Flat	Yellowish	Slow	Circular	Smooth, Glistening	Raised	Entire
BRL-Sg3	Abundant	Echinulate	Flat	Whitish	Moderate	Circular	Smooth	Flat	Entire
BRL-Sg4	Scanty	Filiform	Flat	Whitish	Rapid	Irregular	Rough	Raised	Lobate
BRL-Sg-5	Abundant	Beaded	Flat	Whitish	Moderate	Irregular	Rough	Raised	Lobate
BRL-Sili-1	Abundant	Effuse	Flat	Whitish	Moderate	Circular	Smooth, Dry	Flat	Entire
BRL-Sili-2	Abundant	Filiform	Raised	Whitish	Rapid	Circular	Smooth, Glistening	Raised	Entire
BRL-Sili-3	Moderate	Effuse	Raised	Whitish	Rapid	Circular	Smooth, Glistening	Raised	Entire
BRL-Sili-4	Moderate	Echinulate	Raised	Yellowish	Slow	Punctiform	Rough, Dull	Flat	Entire
BRL-Sili-5	Abundant	Filiform	Flat	Whitish	Rapid	Circular	Smooth, Glistening	Raised	Undulate
BRL-Sili-6	Abundant	Echinulate	Raised	Whitish	Rapid	Irregular	Rough	Flat	Undulate
BRL-Sili-7	Abundant	Echinulate	Raised	Whitish	Rapid	Circular	Glistening	Flat	Undulate
BRL-Sili-8	Abundant	Filiform	Raised	Whitish	Moderate	Punctiform	Rough, Dull	Flat	Undulate
BRL-Sili-9	Abundant	Filiform	Raised	Whitish	Rapid	Circular	Smooth, Glistening	Raised	Entire
BRL-Sili-10	Scanty	Beaded	Flat	Whitish	Slow	Punctiform	Rough, Dry	Flat	Entire
BRL-Sili-11	Scanty	Echinulate	Flat	Whitish	Rapid	Circular	Smooth, Glistening	Convex	Entire
BRL-Sili-12	Scanty	Filiform	Flat	Whitish	Moderate	Irregular	Dry, Rough	Flat	Undulate
BRL-Sili-13	Scanty	Beaded	Flat	Whitish	Rapid	Circular	Smooth, Glistening	Raised	Entire
BRL-Sili-14	Scanty	Filiform	Flat	Whitish	Moderate	Circular	Smooth	Raised	Entire
BRL-Sili-15	Abundant	Beaded	Flat	Whitish	Moderate	Irregular	Rough	Raised	Undulate

3.4.4. Preliminary screening of potential antagonists

During preliminary screening of potential antagonist it has been found eleven strains out of fifty-four native isolates showed antagonistic activity, but considering their effectiveness only six isolates viz., BRL-1, BRL-Dj5, BRL-Sg-5, BRL-BB-9, BRL-Sg-1 and BRL-Sili-15 showed antagonism against all three pathogens (Table 3.4). All the fluorescent pseudomonad isolates tested, reacted similarly in almost all the physiological and biochemical tests to which they were subjected. Slight variations in reactions to a few of the tests are not unusual, since these isolates were collected from various localities and at different times. Similar variation among the isolates of other bacteria has been reported by others (Dye, 1968; Lelliott *et al.*, 1966; Misaghi and Grogan 1969; Sands *et al.*, 1970).

Among the fifty four fluorescent pseudomonad native isolates, though there were similarities in their physiological and biochemical parameters, but only six of them have been found to be the most potent antagonists. *In vitro* assay of these six screened antagonists were evaluated against *Erwinia carotovora, Fusarium oxysporum* and *Mucor hiemalis* f. *hiemalis* by dual plate and dual liquid culture technique.

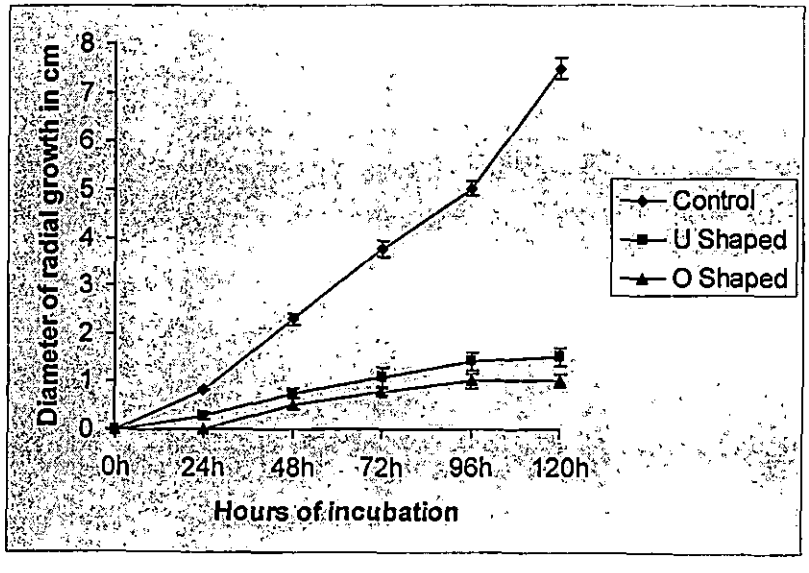
Table 3.4: Screening of fifty-four fluorescent pseudomonads against three pathogens of *Cymbidium* rot

Strains	*Erwinia carotovora*	*Fusarium oxysporum*	*Mucor hiemalis* f. *hiemalis*
BRL-1	+	+	+
BRL-2	-	-	-
BRL-3	-	-	-
BRL-4	-	-	-
BRL-5	-	-	-
BRL-6	-	-	-
BRL-PR-1	-	-	-
BRL-PR-2	-	-	-
BRL-PR-3	-	+	+
BRL-Slg-1	-	-	-
BRL-Slg-2	-	-	-
BRL-Slg-3	-	-	-
BRl-Slg-4	-	-	-
BRl-Slg-5	-	-	-
BRl-Slg-6	-	+	-
BRl-Slg-7	-	-	-
BRL-Slg-8	-	-	-
BRL-Dj1	-	-	-
BRL-DJ2	-	-	-
BRL-Dj3	-	-	-
BRL-DJ4	-	-	-
BRL-DJ5	+	+	+
BRL-Rim-1	-	-	-
BRL-Rim-2	-	-	-
BRL-Rim-3	-	-	-
BRL-BB1	-	-	-
BRL-BB2	-	-	-

Strains	*Erwinia carotovora*	*Fusarium oxysporum*	*Mucor hiemalis f. hiemalis*
BRL-BB3	-	-	-
BLR-BB4	-	-	-
BRL-BB5	-	-	-
BRL-BB6	-	-	-
BRL-BB7	-	-	-
BRL-BB8	-	-	-
BRL-BB9	+	+	+
BRL-Sg-1	+	+	+
BRL-Sg-2	-	-	-
BRL-Sg-3	-	-	-
BRL-Sg-4	-	-	-
BRL-Sg-5	+	+	+
BRL-Sili1	-	-	-
BRL-Sili2	-	-	-
BRL-Sili3	-	-	-
BRL-Sili4	+	-	-
BRL-Sili5	-	-	-
BRL-Sili6	-	-	-
BRL-Sili7	-	-	+
BRL-Sili8	-	-	-
BRL-Sili9	-	-	-
BRL-Sili10	+	-	-
BRL-Sili11	-	-	-
BRL-Sili12	-	-	-
BRL-Sili13	-	-	-
BRL-Sili14	-	-	-
BRL-Sili15	+	+	+

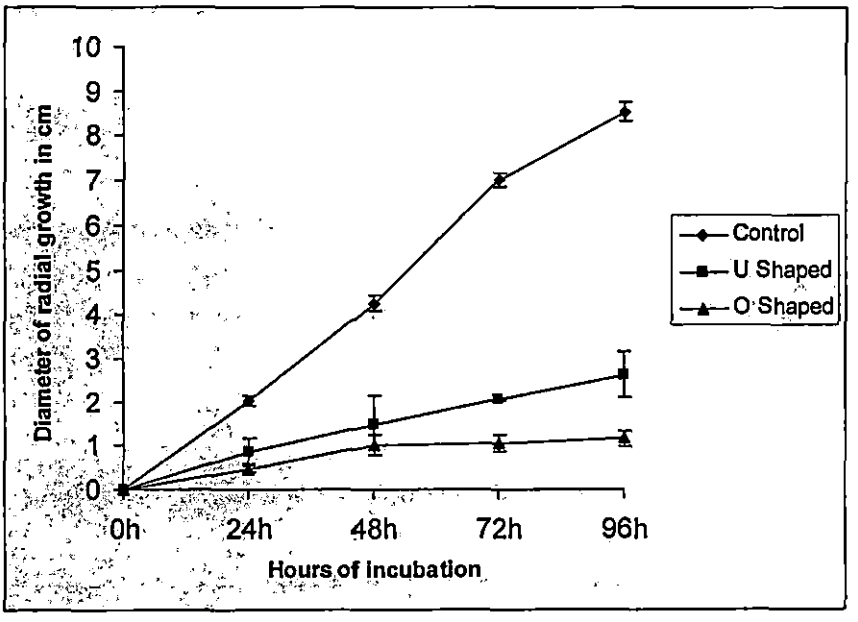
3.4.5. *In vitro antagonistic activity of fluorescent Pseudomonas BRL-1 against Fusarium oxysporum, Mucor hiemalis* f. *hiemalis and Erwinia carotovora*

In dual culture, significant growth inhibition of the fungal pathogens i.e. *F. oxysporum* and *M. hiemalis* f. *hiemalis* occurred due to antagonistic effect of fluorescent *Pseudomonas* BRL-1, which was evident from inhibition of colony diameter. The results are presented in Figure 3.1(A and B). At 48 hours of incubation, pathogens were strongly inhibited by fluorescent *Pseudomonas* BRL-1 and simultaneous increase in incubation time corresponded escalation in percent growth inhibition (Figure 3.2). There was more than 68% and 60% reduction in dry weight of the *F. oxysporum* and *M. hiemalis* f. *hiemalis* culture respectively grown with fluorescent *Pseudomonas* BRL-1 strain when compared to the control. In nutrient agar plate, the growth inhibition of the bacterial pathogen i.e. *Erwinia carotovora* was clearly seen after 48 hours of incubation which shows no growth in the interacting zone where as luxuriant growth away from the interaction (Figure 3.13a).



A

Figure 3.1A. In vitro inhibitory effect of fluorescent Pseudomonas BRL-1 cultured as circular and semicircular streak, around Fusarium oxysporum, the fungal pathogen was point inoculated at the center of PGA plates, incubated as 30°C. Data represents the mean of triplicate sets of experiments.



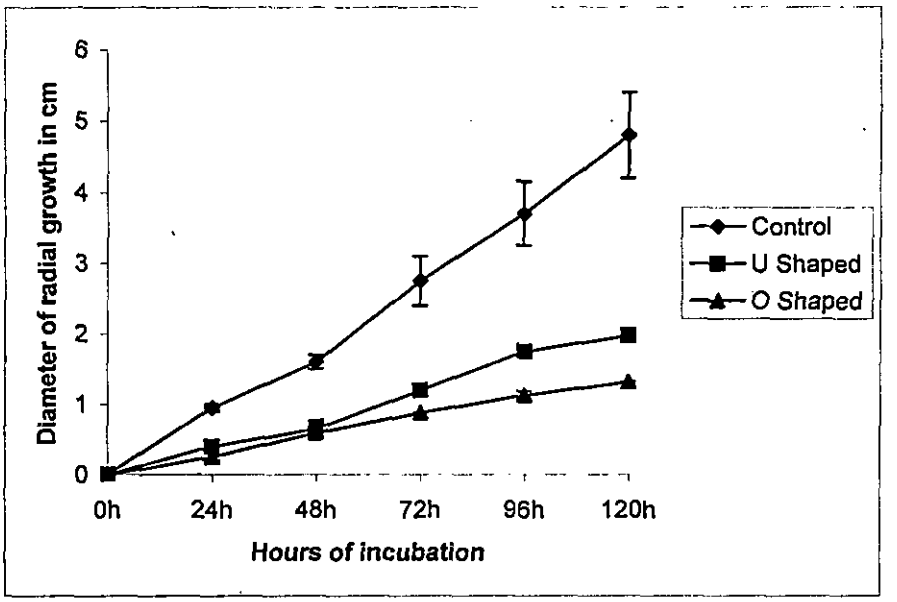
B

Figure 3.1B: In vitro inhibitory effect of fluorescent Pseudomonas BRL-1 cultured as circular and semicircular streak around Mucor hiemalis f. hiemalis, the fungal pathogen was point inoculated at the center of PGA plates, incubated as 30°C. Data represents the mean of triplicate sets of experiments.

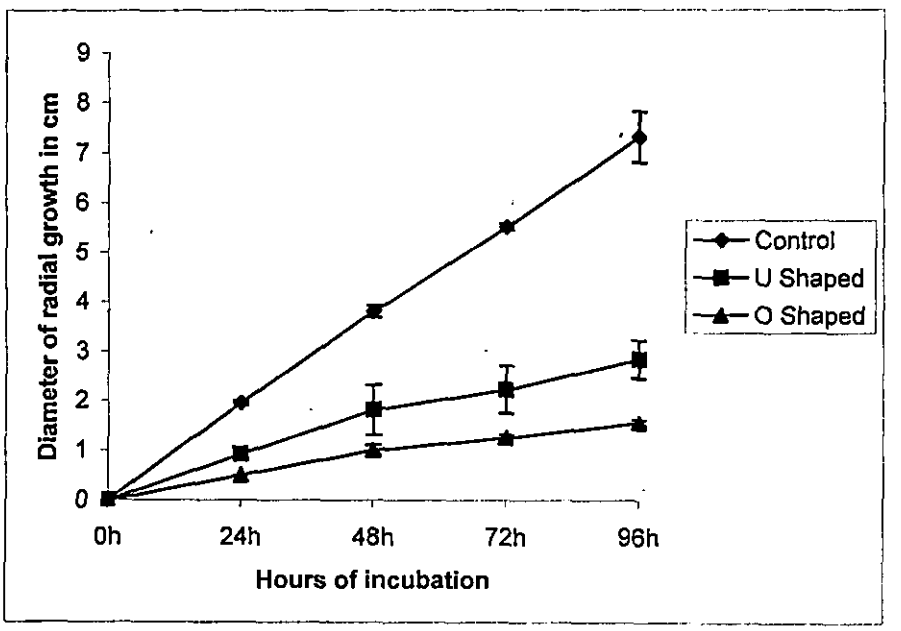
3.4.6. In vitro antagonistic activity of fluorescent Pseudomonas Dj-5 against Fusarium oxysporum, Mucor hiemalis f. hiemalis and Erwinia carotovora:

Fluorescent *Pseudomonas* Dj-5 is also a potent organism, shows satisfactory growth inhibition of the fungal pathogens which was evident in dual plate culture. The results are presented in Figure 3.3 (A and B). At 48 hours of incubation, pathogens were well inhibited by fluorescent *Pseudomonas* Dj-5 and the percent growth inhibitions were increased with rise in incubation time (Figure 3.4). It was shown that there were more than 62 and 57 percent reduction in dry weight of the *F. oxysporum* and *M. hiemalis* f. *hiemalis* culture respectively. The growth inhibition of the bacterial pathogen was also observed after 48 hours of incubation indicates inadequate growth at the interacting zone (Figure 3.13b).

Figure 3.2. Inhibitory effect of fluorescent Pseudomonas BRL-1 on F. oxysporum and M. hiemalis f. hiemalis: (A and D) Control set (A) F. oxysporum and (D) M. hiemalis f. hiemalis colony in pure culture showing more rapid growth; (B and E) Colony showing restricted growth, but growing freely towards the top end away from the U shaped streak culture of the antagonist; and (C and F) Colony of (C) F. oxysporum and (F) M. hiemalis f. hiemalis showing restricted growth due to the antagonist inoculated as a circular streak



A



B

Figure 3.3: In vitro inhibitory effect of fluorescent Pseudomonas Dj-5 cultured as circular and semicircular streak, around A. Fusarium oxysporum and B. Mucor hiemalis f. *hiemalis, where as the fungal pathogens were point inoculated at the center of PGA plates, incubated as 30°C. Data represents the mean of triplicate sets of experiments.*

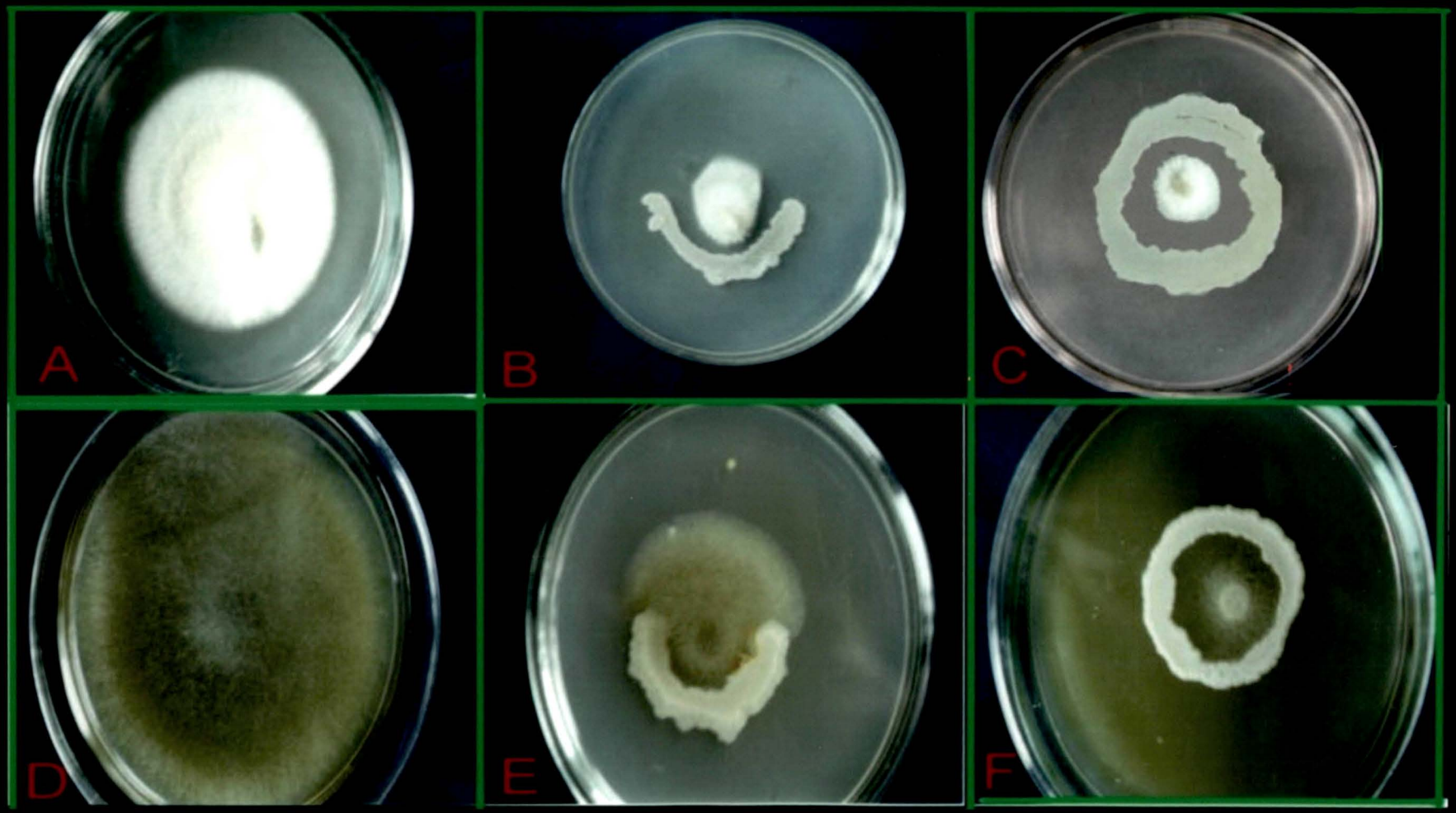
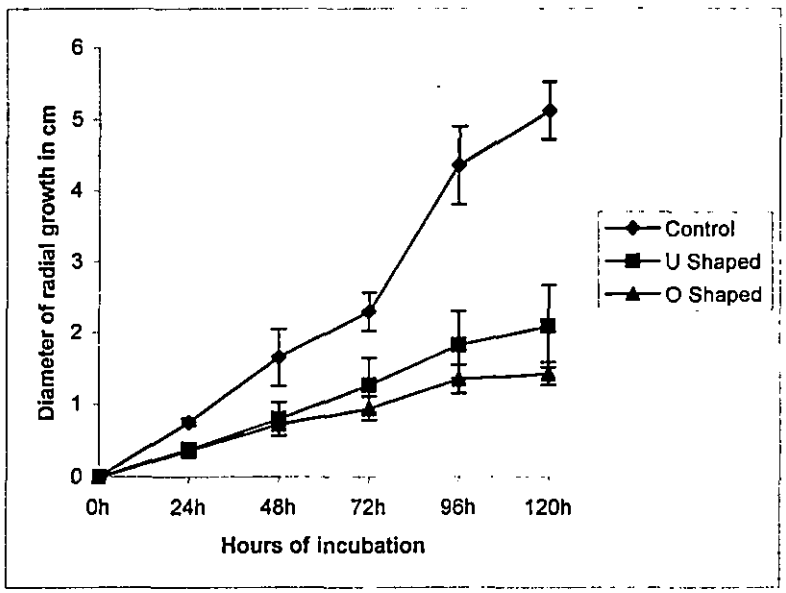


Figure 3.4. Inhibitory effect of fluorescent Pseudomonas Dj-5 on F. oxysporum and M. hiemalis f. hiemalis: (A and D) Control set (A) F. oxysporum and (D) M. hiemalis f. hiemalis colony in pure culture showing more rapid growth; (B and E) Colony showing restricted growth, but growing freely towards the top end away from the U shaped streak culture of the antagonist; and (C and F) Colony of (C) F. oxysporum and (F) M. hiemalis f. hiemalis showing restricted growth due to the antagonist inoculated as a circular streak.

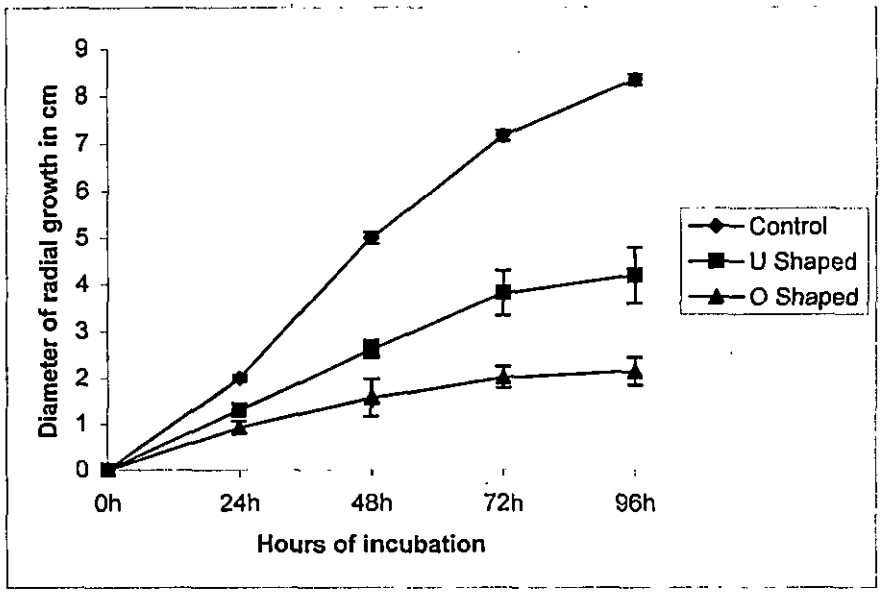
3.4.7. In vitro antagonistic activity of fluorescent Pseudomonas BB-9 against Fusarium oxysporum, Mucor hiemalis f. *hiemalis and Erwinia carotovora:*

From dual culture it was quite visible that the isolate fluorescent *Pseudomonas* BB-9 was also responsible for considerable growth inhibition of the fungal pathogens as was visualized with clear inhibition of colony diameter (Figure 3.5 A and B). The fungal pathogens were inhibited by fluorescent *Pseudomonas* BB-9 and with the simultaneous increase in incubation time, the percent growth inhibition increased respectively (Figure 3.6). Reduction in dry weights of about 59 percent in *F. oxysporum* and 58 percent in case of *M. hiemalis* f. *hiemalis* was noted when compared to the control. The isolate showed marked antagonistic activity against *Erwinia carotovora* at 48 hours of incubation, the growth of *Erwinia carotovora* was restricted in the interacting zone where as abundant growth away from the interaction (Figure 3.13c).



A

Figure 3.5A: In vitro inhibitory effect of fluorescent Pseudomonas BB-9 cultured as circular and semicircular streak, around Fusarium oxysporum, the fungal pathogen was point inoculated at the center of PGA plates, incubated as 30°C. Data represents the mean of triplicate sets of experiments.



B

Figure 3.5B: In vitro inhibitory effect of fluorescent Pseudomonas BB-9 cultured as circular and semicircular streak, around Mucor hiemalis f. hiemalis, the fungal pathogens was point inoculated at the center of PGA plates, incubated as 30°C. Data represents the mean of triplicate sets of experiments.

3.4.8. In vitro antagonistic activity of fluorescent Pseudomonas Sg-5 against Fusarium oxysporum, Mucor hiemalis f. hiemalis and Erwinia carotovora:

The antagonist strain Sg-5 of fluorescent *Pseudomonas* showed moderate inhibitory effect against both fungal and bacterial pathogen. The results were shown in Figure 3.7 (A and B). The growths of the fungal pathogens were moderately inhibited after 48 hours of incubation. The percent growth inhibitions were also increased with increase in time when compared to control (Figure 3.8). There were more than 54 percent reduction in dry weight of *F. oxysporum* and about 57 percent in case of *M. hiemalis* f. *hiemalis* were recorded. *Erwinia carotovora* was also inhibited by the antagonist. It is clear from the figure (3.13d) that the growth of the pathogen was checked at the interacting zone and ample growth away from the interaction.

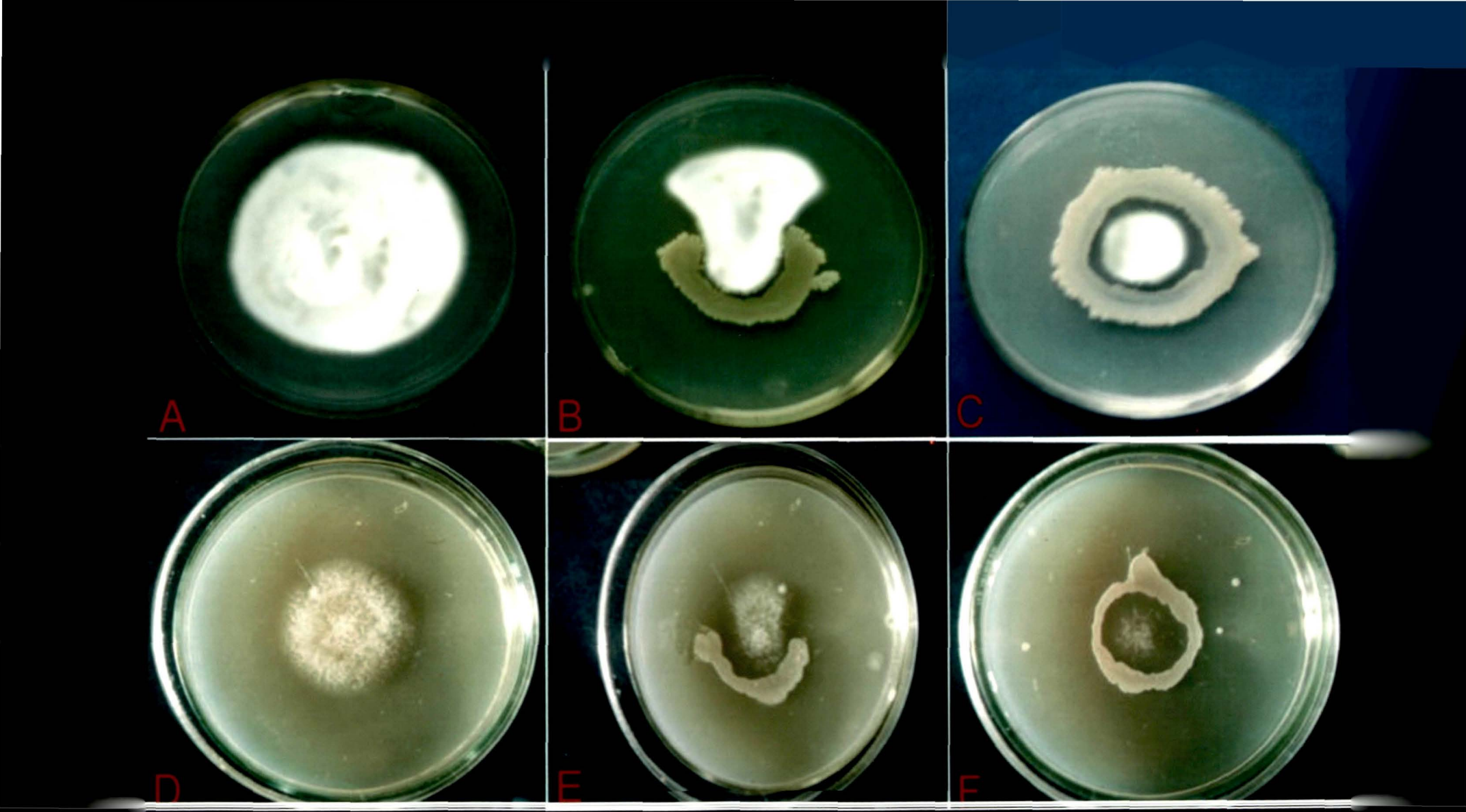
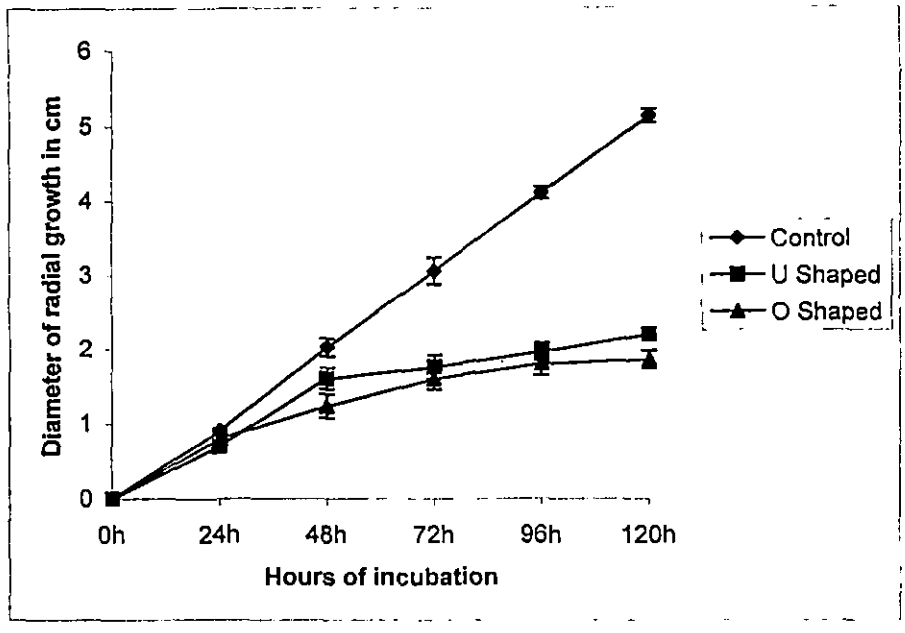
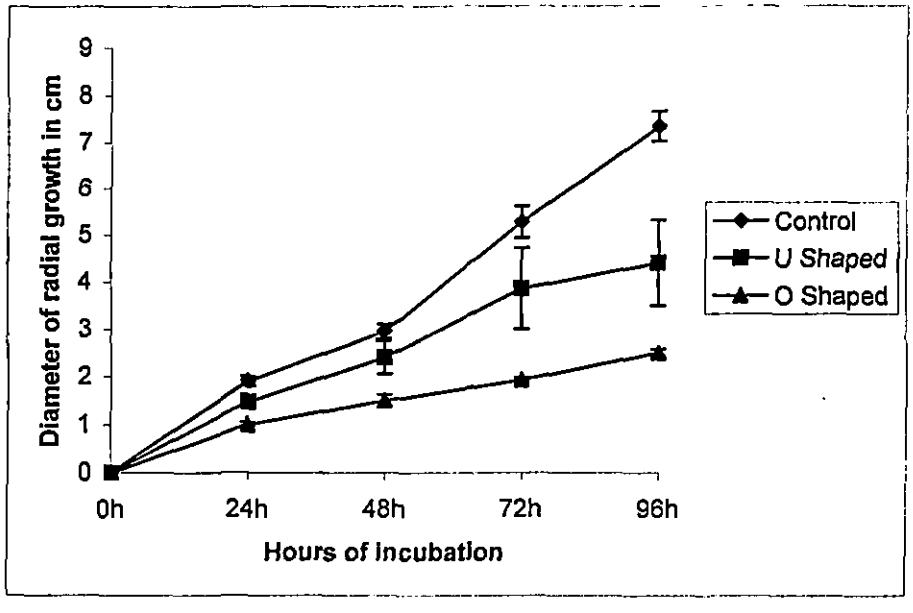


Figure 3.6. Inhibitory effect of fluorescent Pseudomonas BB-9 on F. oxysporum and M. hiemalis f. hiemalis: (A and D) Control set (A) F. oxysporum and (D) M. hiemalis f. hiemalis colony in pure culture showing more rapid growth; (B and E) Colony showing restricted growth, but growing freely towards the top end away from the U shape streak culture of the antagonist; and (C and F) Colony of (C) F. oxysporum and (F) M. hiemalis f. hiemalis showing

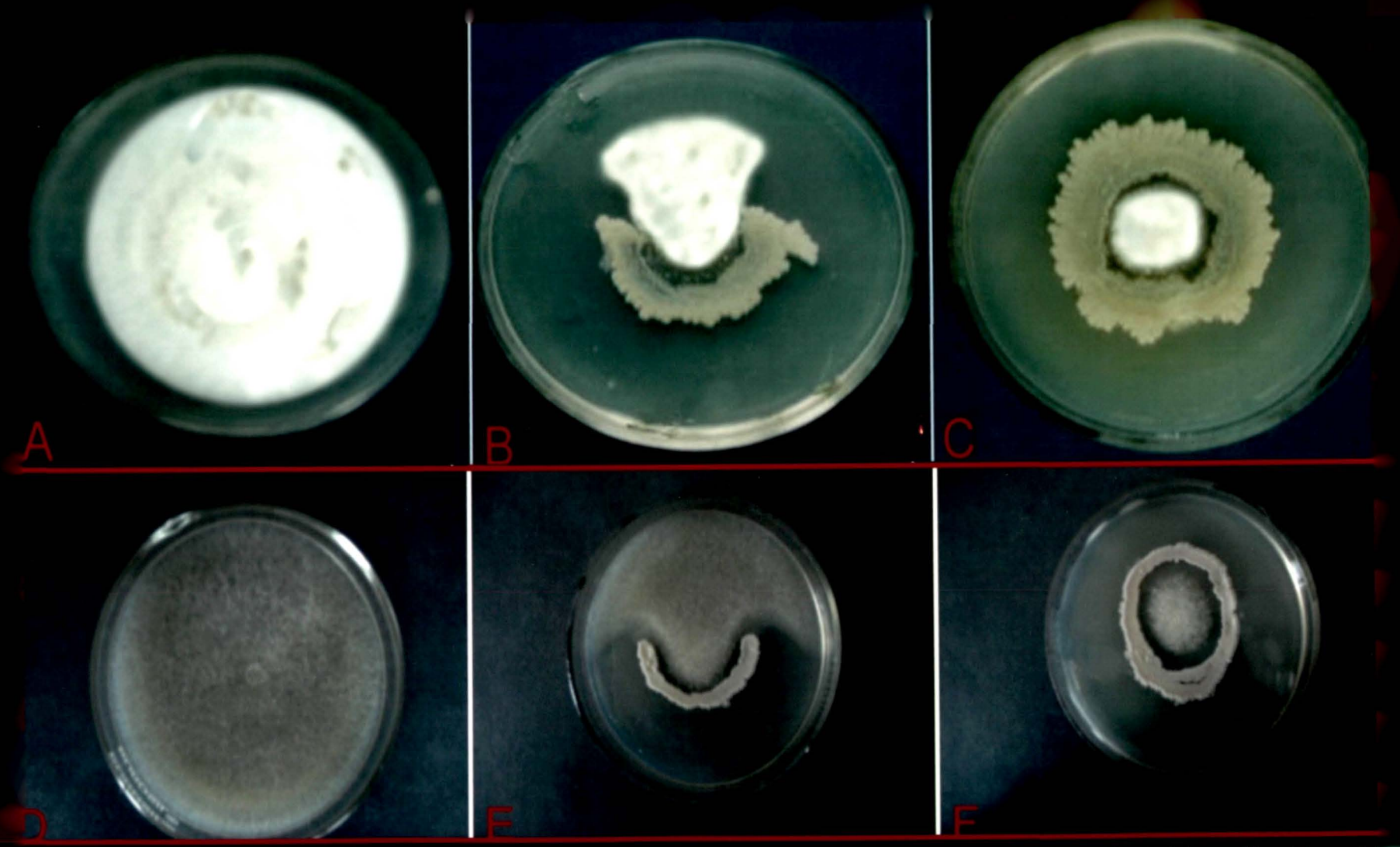


A



B

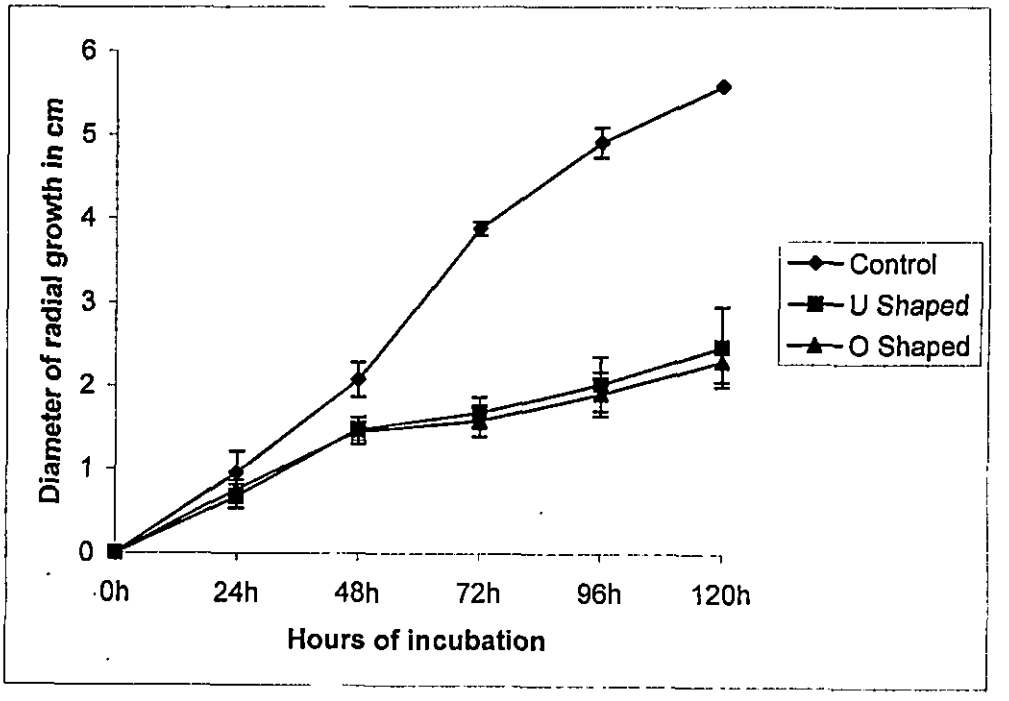
Figure 3.7: In vitro inhibitory effect of fluorescent Pseudomonas Sg-5 cultured as circular and semicircular streak, around A. Fusarium oxysporum and B. Mucor hiemalis f. hiemalis, where as the fungal pathogens were point inoculated at the center of PGA plates, incubated as 30°C. Data represents the mean of triplicate sets of experiments.



gure 3.8. Inhibitory effect of fluorescent Pseudomonas Sg-5 on F. oxysporum and M. hiemalis f. hiemalis: (A and ntrol set (A) F. oxysporum and (D) M. hiemalis f. hiemalis colony in pure culture showing more rapid growth; and E) Colony showing restricted growth, but growing freely towards the top end away from the U shaped streak c the antagonist; and (C and F) Colony of (C) F. oxysporum and (F) M. hiemalis f. hiemalis showing restricted grov

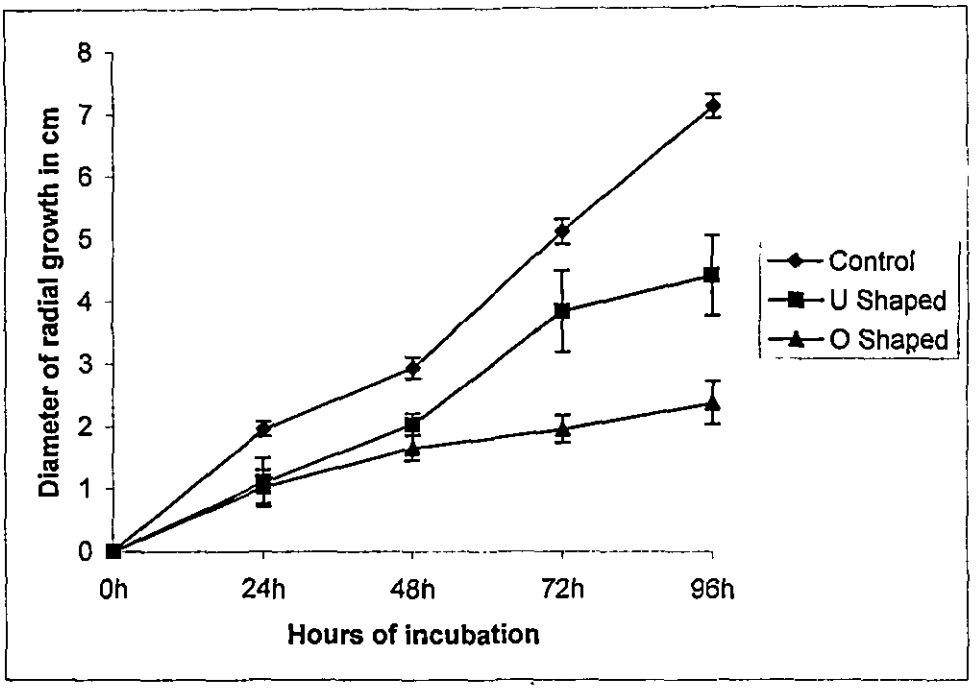
3.4.9. In vitro antagonistic activity of fluorescent Pseudomonas Sg-1 against Fusarium oxysporum, Mucor hiemalis f. hiemalis and Erwinia carotovora:

Inhibitory effect of Sg-1 was also found to be moderate against the fungal pathogens. The results were depicted in Figure 3.9 (A and B). After 48 hours of incubation pathogens were inhibited by the antagonistic strain (Figure 3.10). There were more than 54 and 56 percent reduction in dry weight of *F. oxysporum* and *M. hiemalis* f. *hiemalis* culture respectively grown with strain Sg-1 when compared to the control. The antagonist shows moderate growth inhibition of the bacterial pathogen which was visualized form the figure (3.13e).



A

Figure 3.9A: In vitro inhibitory effect of fluorescent Pseudomonas Sg-1 cultured as circular and semicircular streak, around Fusarium oxysporum, the fungal pathogen was point inoculated at the center of PGA plates, incubated as 30°C. Data represents the mean of triplicate sets of experiments.



B

Figure 3.9B: In vitro inhibitory effect of fluorescent Pseudomonas Sg-1 cultured as circular and semicircular streak, around Mucor hiemalis f. hiemalis, the fungal pathogen was point inoculated at the center of PGA plates, incubated as 30°C. Data represents the mean of triplicate sets of experiments.

3.4.10. In vitro antagonistic activity of fluorescent Pseudomonas Sili-15 against Fusarium oxysporum, Mucor hiemalis f. hiemalis and Erwinia carotovora:

In dual culture fluorescent *Pseudomonas* Sili-15 showed meager antagonistic effect against the fungal as well as the bacterial pathogen. Results were furnished in the Figure 3.11 (A and B). Increasing incubation period moderately inhibit the percent growth inhibition (Figure 3.12). More than 53 and 55 percent reduction in dry weights were recorded for *F. oxysporum* and *M. hiemalis* f. *hiemalis* respectively. Bacterial pathogen was also moderately inhibited by this antagonist (Figure 3.13f).

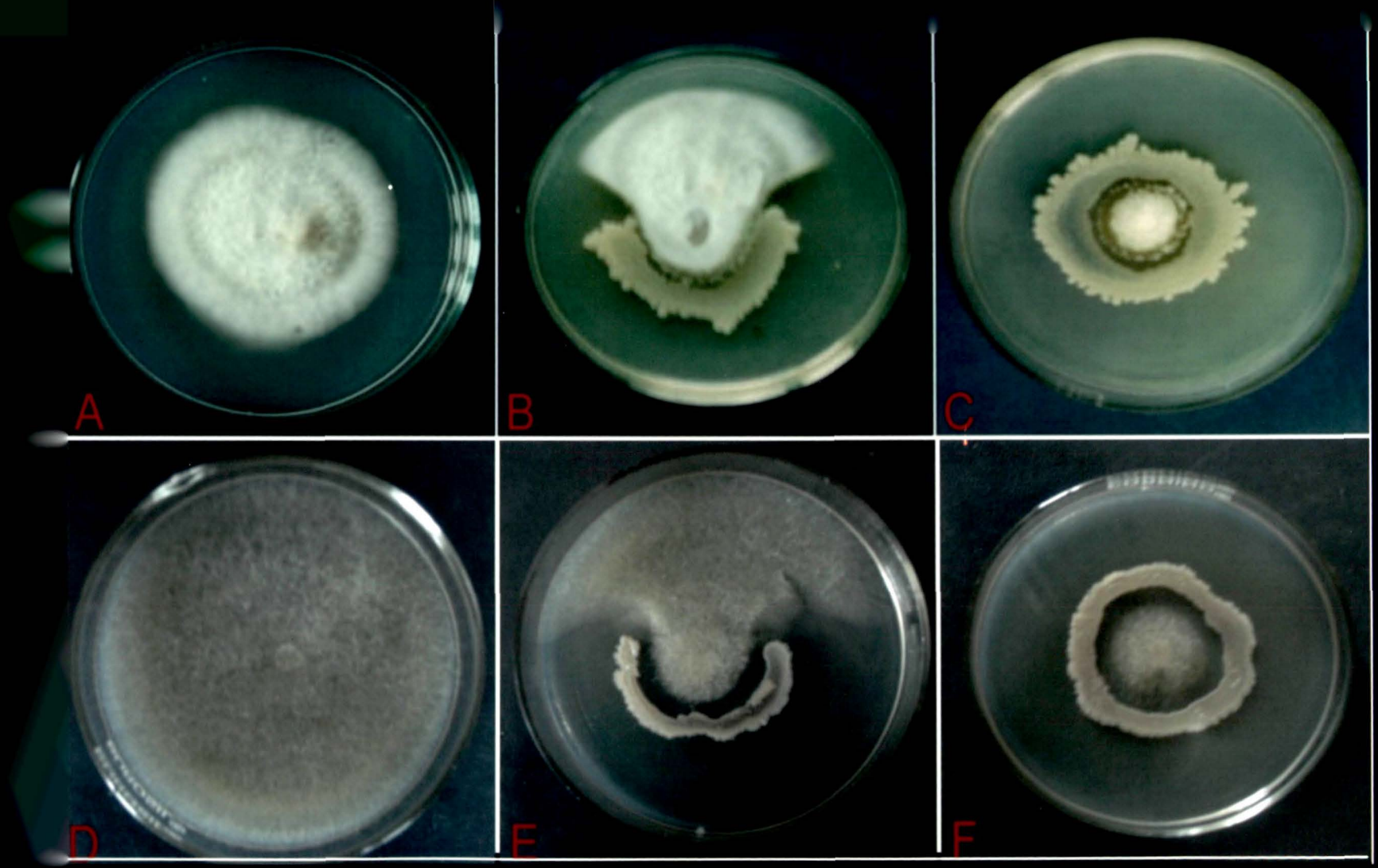
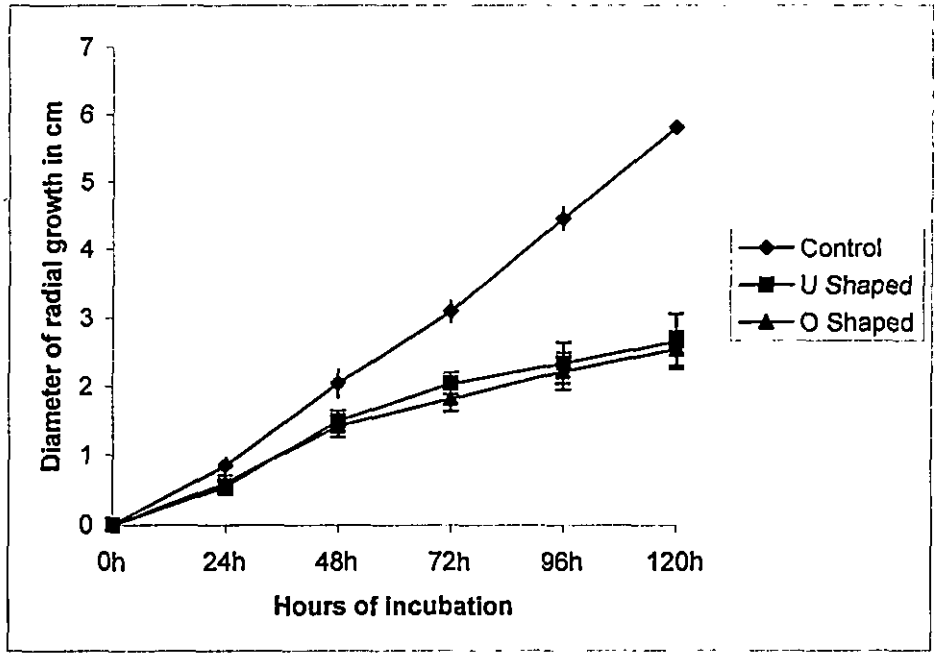
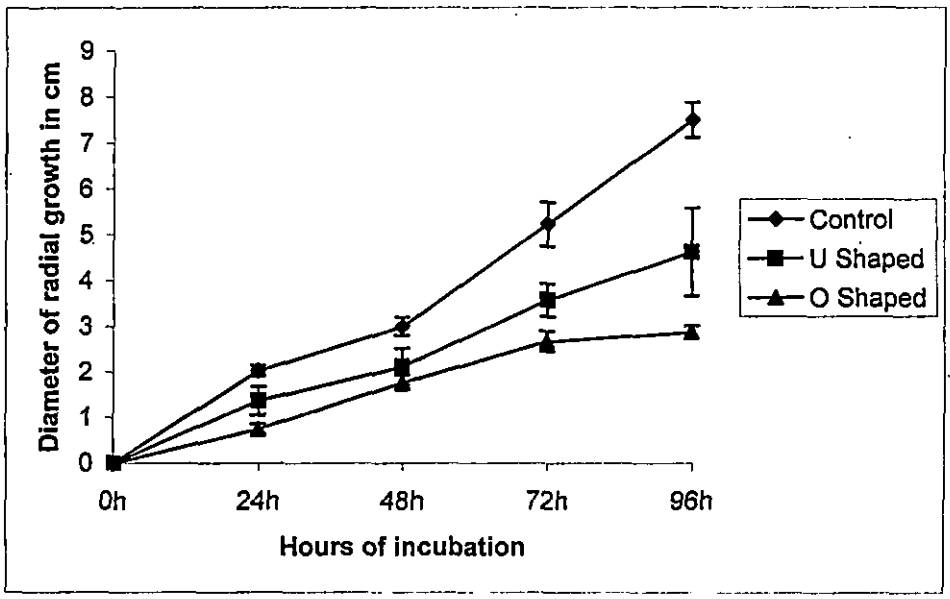


Figure 3.10. Inhibitory effect of fluorescent Pseudomonas Sg-1 on F. oxysporum and M. hiemalis f. hiemalis: (A and D) Control set (A) F. oxysporum and (D) M. hiemalis f. hiemalis colony in pure culture showing more rapid growth; (B and E) Colony showing restricted growth, but growing freely towards the top end away from the U shaped streak culture of the antagonist; and (C and F) Colony of (C) F. oxysporum and (F) M. hiemalis f. hiemalis showing restricted growth due to the antagonist inoculated as a circular streak



A



B

Figure 3.11: In vitro inhibitory effect of fluorescent Pseudomonas Sili-15 cultured as circular and semicircular streak, around A. Fusarium oxysporum and B. Mucor hiemalis f. hiemalis, where as the fungal pathogens were point inoculated at the center of PGA plates, incubated as 30°C. Data represents the mean of triplicate sets of experiments.

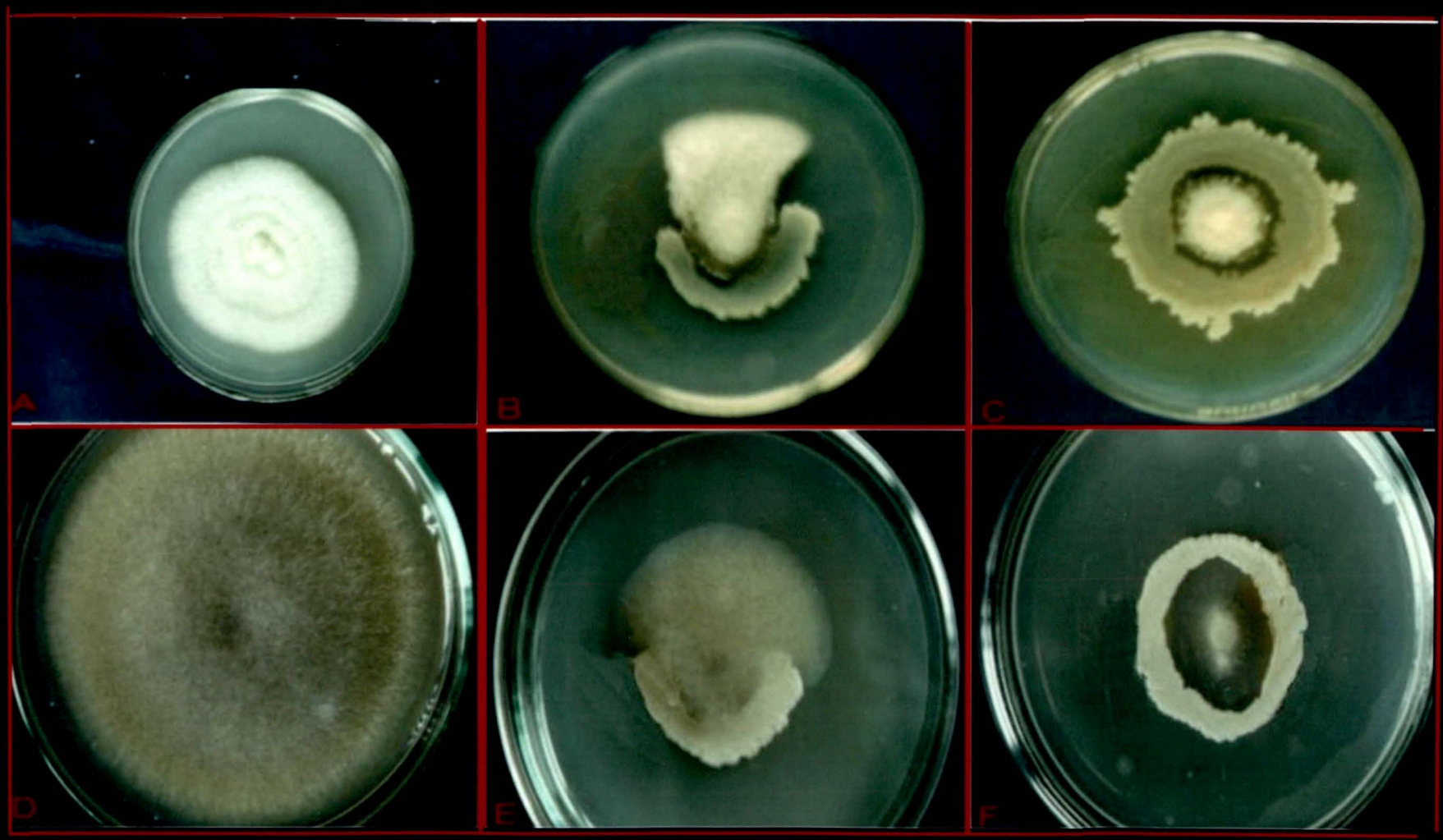


Figure 3.12. Inhibitory effect of fluorescent Pseudomonas Sili-15 on F. oxysporum and M. hiemalis f. hiemalis: (A and D) Control set (A) F. oxysporum and (D) M. hiemalis f. hiemalis colony in pure culture showing more rapid growth; (B and E) Colony showing restricted growth, but growing freely towards the top end away from the U shaped streak culture of the antagonist; and (C and F) Colony of (C) F. oxysporum and (F) M. hiemalis f. hiemalis showing restricted growth due to the antagonist inoculated as a circular streak.

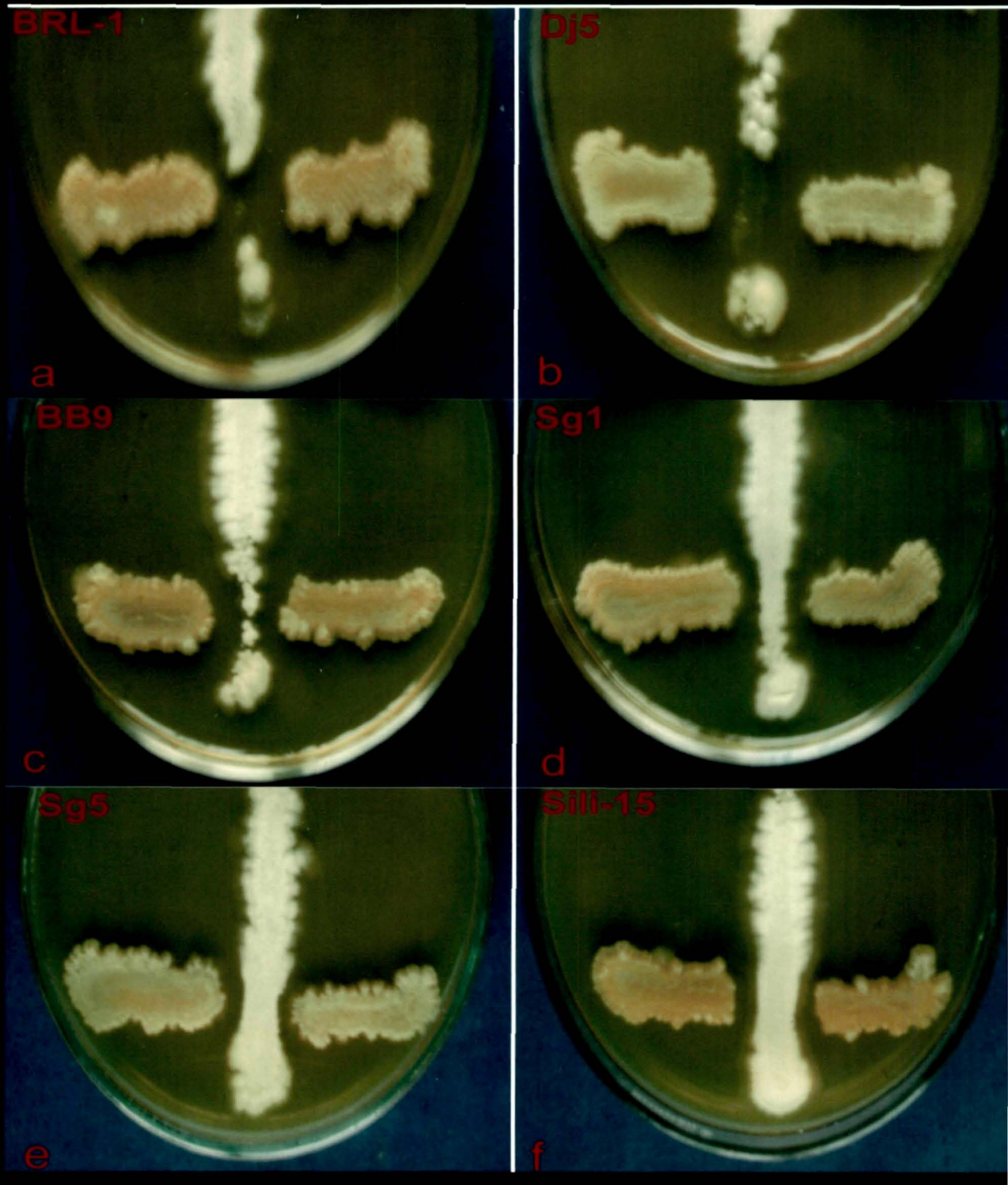


Figure 3.13: Growth inhibition of Erwinia carotovora by the six screened fluorescent pseudomonads. (a) Growth in inhibition by fluorescent Pseudomonas BRL-1 showing no growth of the pathogen in the interacting zone; (b) Inhibition by Dj-5 where faint growth of the pathogen was visualized; (c and d) Moderate growth inhibition by the isolates BB-9 and Sg-1; and (e and f) Meager growth inhibition by Sg-5 and Sili-15.

In dual culture, growth inhibition of *Fusarium oxysporum* and *Mucor hiemalis* f. *hiemalis* were variably inhibited by all the six strains which were evident with clear inhibition of colony diameter. In all cases it was observed that after 72 hours of incubation the mycelia growing towards the interaction zone, which was prominently evident in semicircular/ U-shaped streak stopped, and the mycelia gradually lost vigor. Among these six screened isolates three strains viz., BRL-1, Dj-5 and BB-9 showed effective antagonism as compared to other, but the effect was more significant in case of fluorescent *Pseudomonas* BRL-1, than that of any other strain investigated (Figure 3.14). Maximum reduction of mycelial dry weight in dual liquid culture after 72 hours of incubation was furnished by strain BRL-1 which was 68% and 61% in case of *Fusarium oxysporum* and *Mucor hiemalis* f. *hiemalis* respectively (Table 3.5).

Table 3.5: Percent reduction in dry weight of *Fusarium oxysporum* and *Mucor hiemalis* by the six screened fluorescent *Pseudomonas*

Name of the Screened Isolates	Fungal Pathogen (s)	% reduction in dry weight
Fluorescent *Pseudomonas* BRL-1	*Fusarium oxysporum*	68.8
	Mucor hiemalis	60.72
Fluorescent *Pseudomonas* Dj-5	*Fusarium oxysporum*	62.3
	Mucor hiemalis	57.4
Fluorescent *Pseudomonas* BB-9	*Fusarium oxysporum*	59.71
	Mucor hiemalis	58.62
Fluorescent *Pseudomonas* Sg-5	*Fusarium oxysporum*	54.23
	Mucor hiemalis	57.2
Fluorescent *Pseudomonas* Sg-1	*Fusarium oxysporum*	54.0
	Mucor hiemalis	56.25
Fluorescent *Pseudomonas* Sili-15	*Fusarium oxysporum*	53.4
	Mucor hiemalis	55.5

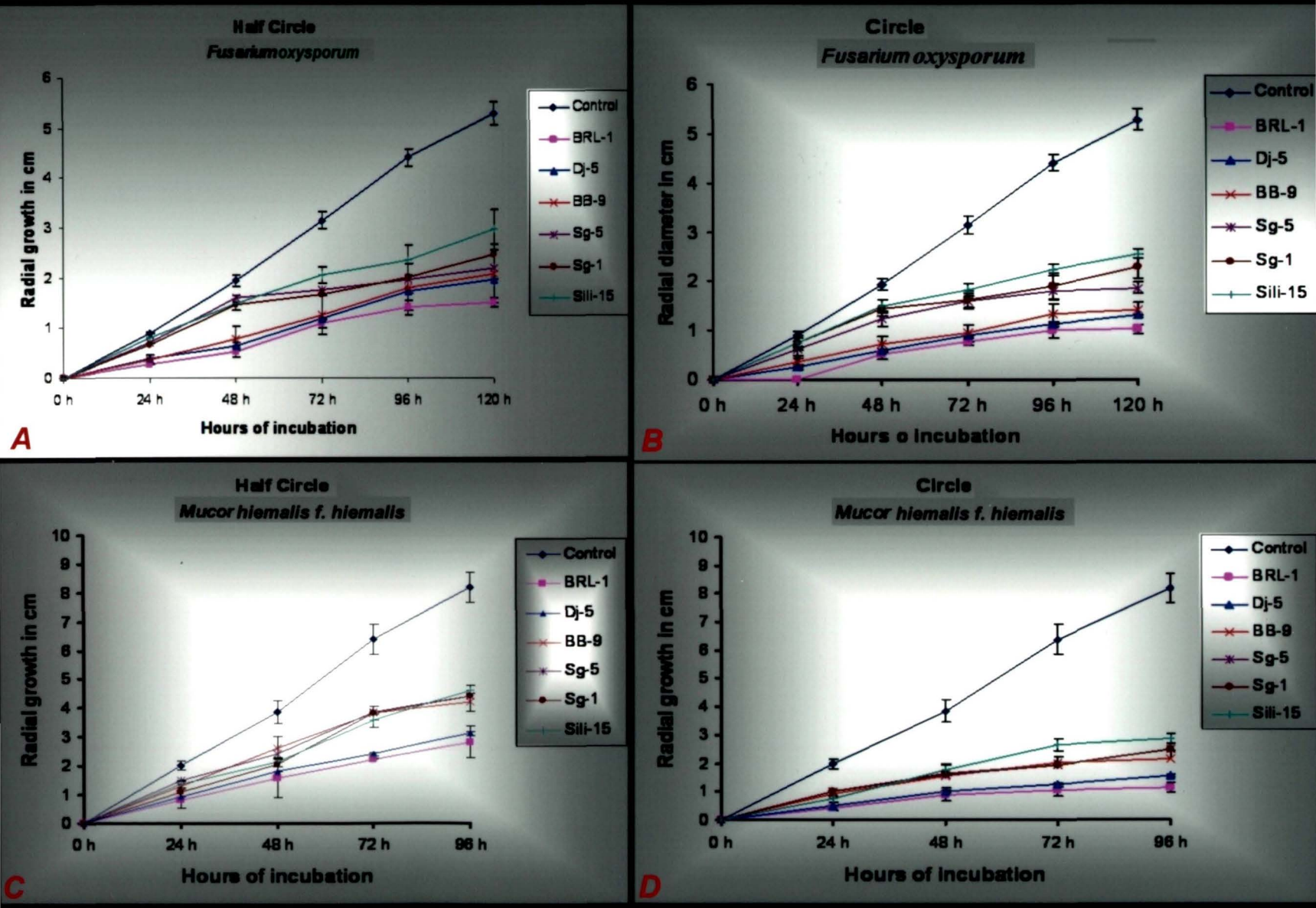


Figure 3.14: Comparative analysis of antagonistic activity of six fluorescent Pseudomonas strains A and B showing the inhibitory effect of all six strains against Fusarium oxysporum and C and D showing inhibitory effect of all six strains against Mucor hiemalis f. hiemalis.

The growth inhibition of the bacterial pathogen (*Erwinia carotovora*) was clearly seen after 48 hours of incubation (Figure 3.13). All the six isolates showed positive result to check the growth of the pathogen, but it is more prominent in case of fluorescent *Pseudomonas* BRL-1 strain which shows no growth in the interacting zone and abundant growth away from the interaction.

The next chapter of this thesis will investigate the possible mechanism behind the antagonistic activity of fluorescent *Pseudomonas* BRL-1.

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