

## **Isolation and Characterization of Bacterial Antagonist to Plant Pathogenic Fungi (*Fusarium* Spp.) from Agro Based Area of Bilaspur**

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**Abstract:** A variety of fungi are known to cause important plant diseases, resulting in a significant lost in agricultural crops. The plant diseases need to be controlled to maintain the level of yield both quantitatively and qualitatively. Farmers often rely heavily on the use of synthetic fungicides to control the plant diseases. However, the environmental problems caused by excessive use and misuse of synthetic fungicide have led to considerable changes in people's attitudes towards the use of synthetic pesticides in agriculture. Today, there is an **increase** in the awareness of the the healthy food and healthy environment. In response to this need, some researchers have focused their effort to develop alternative measures to synthetic chemicals for controlling plant diseases. Among these, is that people about referred to as biological control using microbial antagonists. Many microbial antagonists have been reported to posses antagonistic activities against plant fungal pathogens, such as *Pseudomonas fluorescens*, *Agrobacterium radiobacter*, *Bacillus subtilis*, *B. cereus*, *B. amyloliquefaciens*, *Trichoderma virens*, *Burkholderia cepacia*, *Saccharomyces* spp., *Gliocadium* spp. Antagonistic bacteria commonly inhabit soil which can be used as biological control agent for the management of soil borne diseases of various crops. In this study a total of three (03) composite soil samples were collected from the agro-based areas at the bank of Arpa river of Bilaspur city and 15 isolates of bacteria were isolated using Nutrient agar medium. The morphological characteristics and bio-chemical characteristics of all bacterial isolates were documented. The objective of this study is to isolate indigenous bacteria from agricultural soils and screen them for their growth inhibition of phyto-pathogenic fungus isolated from infected plants and seeds.

**Keywords:** Antagonism, phytopathogenic fungi, soil borne bacteria.

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### **1. INTRODUCTION**

The rhizosphere is considered as the hot spot of biodiversity. Competition for nutrient sources in the rhizosphere is very high. Therefore, different microorganisms have developed distinct strategies, ranging from antagonistic to synergistic interactions, both among themselves and with the plant (Perotto and Bonfante 1997). The highest portion of microorganisms which inhabit the rhizosphere are fungi and bacteria. The most common method for isolation of soil bacteria is the serial dilution method which allow the enumeration of only viable living cell in the soil. Bacterial population is one-half of the total microbial biomass in the soil ranging from 1,00,000 to several hundred millions per gram of soil, depending upon the physical chemical and biological condition of the soil. In few decades a large number of bacteria from the rhizosphere soil have been isolated and identified among which the common species of *Pseudomonas*, *Azospirillum*, *Azotobacter*, *Enterobacter*, *Bacillus* and *Serratia* have reported (Kloepper et al., 1989; Okon and Labandera-Gonzalez, 1994 ;Glick, 1995). The bacteria isolated from rhizosphere soils, have proved to be beneficial to the plants by directly having an effect on nitrogen fixation (Han *et al.*, 2005), solubilization of nutrients (Rodriguez and Fraga 1999), and indirectly by antagonizing pathogenic fungi by the production of siderophores, -1,3-glucanase, antibiotics, fluorescent pigments and cyanide (Pal *et al.*, 2001). Agricultural crops are exposed to approximately 70,000 species of pests, but of these only 10% are considered serious pests (Pimentel, 1997). Classes of fungi that commonly cause diseases in agricultural crops are Plasmodiophoromycetes (root disease of cereals, and powdery scab of potato), Oomycetes (late blight and white rust disease), Zygomycetes (cause soft rot of fruit), Ascomycetes and Deuteromycetes (cause leaf spots, blights, cankers, fruit spots, fruit rots, stem rots, root rots, vascular wilts, soft rot), and Basidiomycetes (cause rust and smut diseases (Agrios, 2005).

The fungi referred to as *Fusarium* spp. are the asexual state and are mostly known by their anamorph name and placed in the class Deuteromycotina (syn. Fungi imperfecti) (Nath, et al.1970; Smith, 1981).The genus *Fusarium* includes plant pathogenic spp. such as *F. avenaceum*, *F. culmorum*, *F. equiseti*, *F. graminearum*, *F. oxysporum*, *F. sporotrichoides*, *F. verticillioides* (syn. *F. moniliforme*). When acting as pathogens they mainly attack immature host plants and causes seedling blight, root-, crown- and foot-rot (Pettitt et al.1996); can penetrate only in damage tissues e.g. snow mould, leaf and stem infections (Parry et al. 1990); or senescent, e.g. node infection, or as in the case with scab (syn. head blight, ear blight) of wheat (*Triticum aestivum*) infection takes place at the stage of anthesis. At the time of grain formation the plant are tender and relatively unprotected (Parry et al.1995). Disease caused by *Fusarium* are popularly referred to as fusarioses. The objective of this present study was to isolate and identify the diversity of bacteria from the rhizospheric soil of agrobased area of bank of Arpa river Bilaspur and to screen its ability to antagonize phytopathogenic fungi.



**Figure01.** *bacterial colony*

## **2. MATERIAL AND METHOD**

### **2.1. Culture Media**

The media used in the present study were potato dextrose agar (PDA) for *fusarium* cultivation and nutrient agar medium for maintaining bacterial strains. The potato agar media consisted of a liter of potato extract prepared in laboratory from 200 g peeled potato boiled in water and 20 g agar for The reason behind choosing this media for the culture of *Fusarium* strains relies in the findings of ( Jiao et al.2008) according to whom the expression of important trichothecene biosynthesis genes (*tri4* and *tri5*) were up-regulated in a high sucrose containing media but not in glucose. Also, Vujanovic and Mansour (2011) reported that 15-ADON chemotype of *Fusarium graminearum* produced DON and ZEA mycotoxins only in the presence of high sucrose concentrations (20% and above).

### **2.2. Soil Samples**

Soil samples were collected in the month of February 2015 from agro based areas of bank of Arpa river in Bilaspur city and brought to the laboratory. The method used was composite sampling (Walworth 2006). Samples were collected to a depth of about 6 to 8 inches from rhizospheric area of crops. 5-8 sub-samples were taken depending on field size and uniformity. The sub-samples were thoroughly mixed to obtain a representative sample and analysis of this sample gave average values for the entire area. Soil samples were immediately air-dried at room temperature for two to three days. The desired sample amount was then removed (200gm), for microbiological analysis and the remainder was discarded.

### **2.3. Analysis of Soil Samples for Microbial Content**

Analysis of each soil samples was done separately, bacterial-flora were isolated by serial dilution and plating on suitable growth media (Pandey *et al.*, 2006). Upon incubation each cell gave rise to a colony on the agar surface (Steubing, 1993). Nutrient agar medium used was from Hi Media, Bombay, India.

### **2.4. Characterization Of Bacterial Culture**

Isolated bacterial cultures were characterized by studying cultural characteristics of individual isolates on nutrient agar and morphological characteristics of bacterial cultures were studied microscopically. (Aneja, 2003).

### **3. BIOCHEMICAL CHARACTERIZATION**

For determination of the biochemical activity of antagonistic bacteria many biochemical tests were carried out for both epiphytic and soil isolates. cellular characteristics such as gram stain reaction, cell size, morphology and arrangement were observed and identified. Bacterial isolates were inoculated with nutrient agar media of unknown (14) and incubated at 37 degrees Celsius for 48 hours. After the incubation period, a small sample of a bacterial colony was placed on a slide, heat fixed and gram stained. The gram stained slide was then observed under the microscope at 1000x magnification. Both Purple and pink (coccus/bacillus) shaped bacteria were observed. The bacteria appeared to be arranged in grape-like clusters. Based on the observations, it was determined that unknown bacterium (14) are gram(+ve & -ve) bacillus, coccus. In addition, a catalase test was also done in order to determine the presence of the enzyme catalase. A small sample of a live bacterial colony of unknown (14) from the NAM media was placed on a slide. Two small drops of 3% hydrogen peroxide H<sub>2</sub>O<sub>2</sub> were added. Gas bubbles were observed, resulting in a catalase positive test result. Unknown (14) contains the enzyme catalase and is able to convert into water and Oxygen. Another test was for the screening of starch hydrolysis in culture streaked in starch agar plate with starch only as carbon sources. After incubation individual plates were flooded with gram's iodine to produce a deep blue colour and in zone of degradation no blue colour forms which is the basis of detection and screening of amylolytic strain. Urea hydrolysis is a liquid medium used to test for the presence of the urea hydrolyzing enzyme, Urease. The enzyme hydrolyses urea to ammonia and carbon dioxide. It differentiates rapid urease-positive bacteria from slow urease-positive bacteria and Urease-negative bacteria. Color change to shocking pink indicates a positive test, but sometimes the incubation period might take up to eight days to see this result.

### **4. ISOLATION OF FUNGI**

The conventional method of isolating well separated spores streaked on the agar medium under a stereoscopic microscope was used by Tuite (1969). fungal cultures stored in a high nutrient medium, such as potato dextrose agar [PDA] may lose their ability to produce enzymes or metabolites, or perform other functions and care is therefore needed in selecting a storage medium (Smith and Onions, 1994). Some other sophisticated single spore isolation technique using micromanipulators and a hand maid glass needle were described by Matsushima (1975), Teik-Khiang (1999). Also preparing *Fusarium* cultures for identification was held by the single spore technique, devised by Hansen (1926) and Toussoun and Nelson (1976). In fact fungal isolates grown from single spores demonstrate species characteristics and uniform growth more clear than those of mass transfer of inoculums (Leslie and Summerell, 2006).

### **5. ANTIMICROBIAL ACTIVITY BY DUAL CULTURE METHOD**

The antagonistic activity of all bacteria isolates was studied on *Fusarium* by dual culture technique (Francisco et al., 2011). On Petri dishes with PDA placing equidistantly a 5 mm-disk of the bacteria and on the other side of the Petri dish, a disk of mycelium of the same diameter of *Fusarium*. The plates inoculated were incubated at 25 ± 2°C until the growth of control treatment, covered the Petri dish. The effect of bacteria on plant pathogens was determined by mycelia growth inhibition (Saeideh et al., 2008).

### **6. POINT INOCULATION METHOD**

Each of the bacterial isolates was point inoculated at sides 3cm from the center of the plate and after that six day old culture of *F. oxysporum* was point inoculated in the center of the plate. Control plate was only point inoculated with *F. oxysporum* (G.Shobha, BS.Kumudini). The plates were sealed with parafilm and incubated at 28±2°C for 4-5 days (Kumar et al. 2002). Antagonistic activity was investigated for four to seven days after incubation at room temperature (28±2°C).

After 5 days incubations the percent inhibition in growth of pathogen was calculated by the formula:

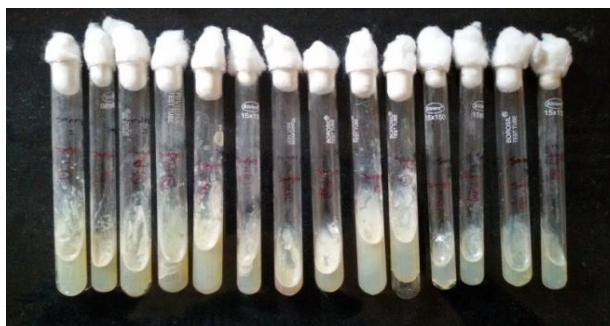
$$\% \text{ inhibition} = \frac{R1 - R2}{R1} \times 100$$

$$r1 = \frac{\text{radial growth of } Fusarium \text{ in control}}{\text{control}}$$

**r2= radial growth of *fusarium* in dual  
innoculation**

**7. RESULT AND DISCUSSION**

In the present study 3 composite soil samples (A, B and C ) were obtained as representative sample from agro based area of bank of Arpa river Bilaspur by composite sampling method. A total of 14 bacterial isolates were differentiated and maintained as pure cultures on nutrient agar. From soil sample (A) pure culture of five bacteria were isolated, from soil sample (B) 4 bacterial culture were isolated and from sample (C)5 bacterial isolates were obtained.

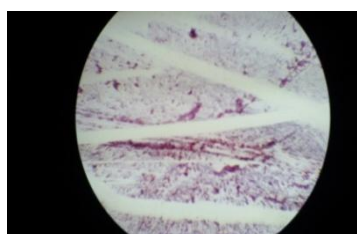


**Figure02. bacterial isolates**

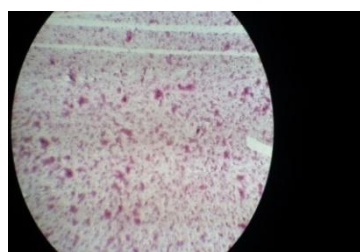
**TABLE 01 COPOSITE SOIL SAMPLE**

| S.NO | FIELD | CROP       | SOIL SAMPLE | COMPOSITE SAMPLE |
|------|-------|------------|-------------|------------------|
| 01   | A     | SPINACH    | SA1 (200gm) | 200gm            |
|      |       |            | SA2 (200gm) |                  |
|      |       |            | SA3 (200gm) |                  |
|      |       |            | SA4 (200gm) |                  |
|      |       |            | SA5 (200gm) |                  |
| 02   | B     | METHI      | SB1 (200gm) |                  |
|      |       |            | SB2 (200gm) |                  |
|      |       |            | SB3 (200gm) |                  |
|      |       |            | SB4 (200gm) |                  |
|      |       |            | SB5 (200gm) |                  |
| 03   | C     | CORRIANDER | SC1 (200gm) |                  |
|      |       |            | SC2 (200gm) |                  |
|      |       |            | SC3 (200gm) |                  |
|      |       |            | SC4 (200gm) |                  |
|      |       |            | SC5 (200gm) |                  |
|      |       |            |             |                  |

Cultural and morphological characteristics of all 14 bacterial isolates are depicted in table- (02) Both gram positive and negative bacilli along with gram negative cocci were isolated in the present investigation. A total of 06 Gram positive bacilli,04 Gram negative bacilli, 04 Gram positive cocci were isolated of the total samples used in the current study.



**Gram positive**



**Gram negative**

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Bacteria are the most abundant and predominant organisms found in the soil habitat. The bacterial abundance is 2–20 times higher in the rhizosphere than in the bulk soil (Morgan et al. 2005).Kloeppe et al., (1989); Okon and Labandera-Gonzalez, (1994) ;Glick, (1995) reported that large number of Gram positive and Gram negative bacteria are common inhabitants of rhizospheric soil including species

of *Pseudomonas, Azospirillum, Azotobacter, Klebsiella, Enterobacter, Alcaligenes, Arthobacter, Burkholderia, Bacillus & Serratia.*

TABLE-02: CULTURAL AND MORPHOLOGICAL CHARACTERISTICS OF BACTERIAL ISOLATES.

| S. NO | FIELD | BACTERIAL SAMPLE | MORPHOLOGY CHARACTER | GRAM STAIN | COLONY COLOUR | COLONY FORM | COLONY MARGIN | ELEVATION | OPTICAL |
|-------|-------|------------------|----------------------|------------|---------------|-------------|---------------|-----------|---------|
| 1     | A     | BU-1             | COCCUS               | GRAM -VE   | WHITE         | IRREGULAR   | LOBATE        | RAISED    | OPAQUE  |
|       |       | BU-2             | ROD SHAPE            | GRAM +VE   | WHITE         | REGULAR     | LOBATE        | RAISED    | OPAQUE  |
|       |       | BU-3             | COCCUS               | GRAM -VE   | WHITE         | REGULAR     | UNDOLATED     | RAISED    | OPAQUE  |
|       |       | BU-4             | COCCUS               | GRAM -VE   | WHITE         | IRREGULAR   | UNDOLATED     | FLAT      | OPAQUE  |
|       |       | BU-5             | ROD SHAPE            | GRAM +VE   | WHITE         | IRREGULAR   | UNDOLATED     | FLAT      | OPAQUE  |
| 2     | B     | BU-6             | ROD SHAPE            | GRAM +VE   | LIGHT YELLOW  | IRREGULAR   | UNDOLATED     | RAISED    | OPAQUE  |
|       |       | BU-7             | ROD SHAPE            | GRAM +VE   | LIGHT YELLOW  | IRREGULAR   | UNDOLATED     | FLAT      | OPAQUE  |
|       |       | BU-8             | ROD SHAPE            | GRAM +VE   | WHITE         | IRREGULAR   | UNDOLATED     | FLAT      | OPAQUE  |
|       |       | BU-9             | ROD SHAPE            | GRAM +VE   | YELLOW        | IRREGULAR   | UNDOLATED     | FLAT      | OPAQUE  |
|       | C     | BU-10            | ROD SHAPE            | GRAM -VE   | WHITE         | REGULAR     | LOBATE        | FLAT      | OPAQUE  |
|       |       | BU-11            | ROD SHAPE            | GRAM -VE   | WHITE         | REGULAR     | LOBATE        | FLAT      | OPAQUE  |
|       |       | BU-12            | ROD SHAPE            | GRAM -VE   | WHITE         | REGULAR     | LOBATE        | FLAT      | OPAQUE  |
|       |       | BU-13            | ROD SHAPE            | GRAM -VE   | YELLOW        | IRREGULAR   | UNDOLATED     | FLAT      | OPAQUE  |
|       |       | BU-14            | COCCUS               | GRAM -VE   | WHITE         | IRREGULAR   | UNDOLATED     | FLAT      | OPAQUE  |

NOTE: BU- BACTERIAL SAMPLE

Antagonists BU2, BU5, BU6, BU7, BU8, BU9, were aerobic Gram-positive rod-shaped bacteria, motile and able to grow at high temperature. Catalase and starch hydrolysis tests showed variable reaction. These biochemical tests and physical properties show that both antagonists are *Bacillus* sp. (Fahy and Hayward, 1983; Cappuccino and Sherman, 1996).

BU1, BU3, BU4, BU10, BU11, BU12, BU13, BU14, were short Gram-negative rods and coccus. Catalase were positive. starch hydrolysis testes were positive in negative. Hence, these antagonists were placed in the family Enterobacteriaceae (Fahy and Hayward, 1983; Cappuccino and Sherman, 1996).

Table (03) depicts biochemical characterization of 14 bacterial isolates. Screening of Amylase Producing Bacteria was done on starch agar medium. Among 14 bacterial strains 10 bacterial strains showed amylase activity while the rest 4 bacterial isolates showed negative test for amylase activity.

BU1, BU2, BU3, BU6, BU7, BU9, BU10, BU11, BU12, BU13, BU14 bacterial isolate were able to produce catalase enzyme while the BU4, BU5, BU8 shows negative catalase activity. High catalase activity were observed in BU3, BU6, BU10, BU13, BU14 isolates. Yellow indicated in all the 14 bacterial sample the organism only has low urease activity the phosphate buffer is not neutralise and no production of NH<sub>3</sub> take place. Thus urease test by all the bacterial sample is negative. In the similar studies made by Fahy and Hayward, (1983); Cappuccino and Sherman, (1996), bacterial antagonists isolated by them were aerobic Gram-positive rod-shaped bacteria, motile and able to grow at high.

TABLE 03. BIOCHEMICAL CHARACTERISATION FOR BACTERIAL ISOLATES

| BACTERIAL ISOLATES | GRAM STAINING | CATALASE TEST | STARCH TEST | UREASE TEST |
|--------------------|---------------|---------------|-------------|-------------|
| BU SAMPLE-1        | GRAM -VE      | POSITIVE(+)   | POSITIVE(+) | NEGATIVE(-) |
| BU SAMPLE-2        | GRAM +VE      | POSITIVE(+)   | POSITIVE(+) | NEGATIVE(-) |
| BU SAMPLE-3        | GRAM -VE      | POSITIVE(++)  | POSITIVE(+) | NEGATIVE(-) |
| BU SAMPLE-4        | GRAM -VE      | NEGATIVE(-)   | NEGATIVE(-) | NEGATIVE(-) |
| BU SAMPLE-5        | GRAM +VE      | NEGATIVE(-)   | POSITIVE(+) | NEGATIVE(-) |
| BU SAMPLE-6        | GRAM +VE      | POSITIVE(++)  | POSITIVE(+) | NEGATIVE(-) |
| BU SAMPLE-7        | GRAM +VE      | POSITIVE(+)   | POSITIVE(+) | NEGATIVE(-) |
| BU SAMPLE-8        | GRAM +VE      | NEGATIVE(-)   | NEGATIVE(-) | NEGATIVE(-) |
| BU SAMPLE-9        | GRAM +VE      | POSITIVE(+)   | NEGATIVE(-) | NEGATIVE(-) |
| BU SAMPLE-10       | GRAM -VE      | POSITIVE(+)   | POSITIVE(+) | NEGATIVE(-) |
| BU SAMPLE-11       | GRAM -VE      | POSITIVE(++)  | NEGATIVE(-) | NEGATIVE(-) |
| BU SAMPLE-12       | GRAM -VE      | POSITIVE(+)   | POSITIVE(+) | NEGATIVE(-) |
| BU SAMPLE-13       | GRAM -VE      | POSITIVE(++)  | POSITIVE(+) | NEGATIVE(-) |
| BU SAMPLE-14       | GRAM -VE      | POSITIVE(++)  | POSITIVE(+) | NEGATIVE(-) |

### 8. ISOLATION AND SCREENING OF POTENTIAL ANTAGONISTS

Screening for antagonism between the microorganisms isolated from the rhizosphere and fusarium species were done by dual culture technique. The potato dextrose agar (PDA) medium in culture plates was simultaneously seeded with actively growing 3mm mycelial blocks of fusarium and the antagonist isolates. Four days old *Fusarium* point inoculated in one side of the pda plate near centre. where as each individuals antagonists were seeded at 2cm equidistant point near the periphery from the centre and incubated at 28± 1oC. Isolations yielded 14 different colonies of bacteria and among which 9 colonies Of bacteria showed potential as antagonists for inhibiting *fusarium species*(Table04 ). Among these, bacterial isolates ‘4’ and ‘5’ displayed a high ability to suppress *fusarium* growth, with inhibition zone over 10 mm. out of 14 bacterial isolates 9 isolates samples were effective in tested methods (Table04 ).



Figure03. microscopic view of identified fusarium species

TABLE 04. IN VITRO SCREENING OF MICROORGANISMS FOR ANTAGONISTIC ACTIVITY TOWARDS FUSARIUM.

| S.NO | BACTERIAL ISOLATES | PATHOGEN      | ANTAGONISTIC ACTIVITY |
|------|--------------------|---------------|-----------------------|
| 1    | BU-1               | FUSARIUM SPP. | POSITIVE(+)           |
| 2    | BU-2               | FUSARIUM SPP. | POSITIVE(+)           |
| 3    | BU-3               | FUSARIUM SPP. | POSITIVE(+)           |
| 4    | BU-4               | FUSARIUM SPP. | POSITIVE(+)           |
| 5    | BU-5               | FUSARIUM SPP. | POSITIVE(+)           |
| 6    | BU-6               | FUSARIUM SPP. | NEGATIVE(-)           |
| 7    | BU-7               | FUSARIUM SPP. | NEGATIVE(-)           |
| 8    | BU-8               | FUSARIUM SPP. | NEGATIVE(-)           |
| 9    | BU-9               | FUSARIUM SPP. | POSITIVE(+)           |
| 10   | BU-10              | FUSARIUM SPP. | NEGATIVE(-)           |
| 11   | BU-11              | FUSARIUM SPP. | POSITIVE(+)           |
| 12   | BU-12              | FUSARIUM SPP. | NEGATIVE(-)           |
| 13   | BU-13              | FUSARIUM SPP. | POSITIVE(+)           |
| 14   | BU-14              | FUSARIUM SPP. | POSITIVE(+)           |

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TABLE NO.4 depicts invitro antagonism effect of 14 bacterial isolates against test pathogen fusarium species.the result of invitro experiment reveals that isolates number BU1,BU2,BU3,BU4,BU5,BU9,BU11,BU12,BU14 these are positive for antagonistic activity against the test pathogen.

TABLE 05. BIOLOGICAL CONTROL OF FUSARIUM SPP. BY ANTAGONIST BACTERIA AND INHIBITION (%)

| S.NO | BACTERIAL ISOLATES | INHIBITION % |
|------|--------------------|--------------|
| 1    | BU-1               | 56%          |
| 2    | BU-2               | 66%          |
| 3    | BU-3               | 66%          |
| 4    | BU-4               | 73%          |
| 5    | BU-5               | 70%          |
| 6    | BU-9               | 66%          |
| 7    | BU-11              | 66%          |
| 8    | BU-12              | 41%          |
| 9    | BU-14              | 33%          |
| 10.  | control            | 00%          |

result depicts in table no.5 indicates the potentiality of bacterial antagonist against fusarium spp. the highest antagonist effect showed by isolates (BU4) with (73%) inhibition followed by isolates (BU5) with (70%)inhibition and isolates (BU2) with (66%) inhibition.

## 9. DISCUSSION

Rajput et al., 2013 in their Investigation on leaf spot disease (*Alternaria alternata* (FR.) Keissler) of Brinjal (*Solanum melongena* L.) under south Gujarat condition find out suitable management strategies to control the pathogen alternative to chemical fungicides by using bioagents to control the pathogen. They evaluated eight known bioagents by dual culture, pathogen at periphery and pathogen at the centre technique to monitor antagonistic effect. Their results revealed that out of all the eight bioagents used, three bioagents viz., *Trichoderma viride* (IARI isolate) (74.77%, 69.04% and 79.45%), *Trichoderma viride* (Navsari isolate) (74.14%, 66.08%, and 76.99%) maximum growth inhibition in dual culture, pathogen at periphery and pathogen at the centre methods respectively), *T. harzianum* (Junagadh isolate) (71.25%, 59.96% and 74.78%) maximum growth inhibition in dual culture, pathogen at periphery and pathogen at the centre methods respectively, showed strong antagonistic effect to inhibit the mycelia growth of the pathogen significantly.

Thirty isolates of *Pseudomonas fluorescens* obtained from citrus rhizosphere were tested for antifungal activity against *Phytophthora* spp. *P. fluorescens* isolate Pf20 was found efficient in inhibiting the mycelial growth upto 38.88%. The antifungal compounds were extracted were found inhibitory to the growth of *Rhizoctonia solani* (42.79%), *Phytophthora parasitica* (28.57%), *P. palmivora* (25.98%) and *Fusarium solani* (20.45%) (Koche et al., 2013). In another investigation carried out by Dewangan et al., 2014 to screen different media for growth and colony formation of test bacterium *Pseudomonas fluorescens* and to check its antagonistic activity on fungal plant pathogens by in vitro dual culture techniques. Out of the seven different media tested, King's 'B' media was best with an OD value of 2.50 and  $6 \times 10^8$  cfu among the different media evaluated. The growth was fast in King's 'B' medium, fair in Sim agar medium and slow in remaining media tested. In dual culture method, *P.fluorescens* on co-inoculation with fungal pathogens decreased their growth rate. Maximum inhibition was observed in *Sclerotium rolfsii* (63.15%) followed by *Fusarium oxysporum* (61.85%) *Rhizoctonia bataticola* (55.56%) and

*R.solani* (53.15 %). Also Bacteria and *Fusarium oxysporum* from the rhizoplane soil and surrounding soil of healthy and diseased tomato plants of district regions of Karnataka were collected. The best antagonist bacterial strains against *Fusarium oxysporum* isolate, were identified as BS1, BS5 and BS18. All bacterial isolates resulted effective for the in vitro control of growth of *Fusarium oxysporum*. Their results suggested that *Pseudomonas fluorescens* isolates had an excellent potential to be used as biocontrol agents of *Fusarium oxysporum* in tomato greenhouses at the field level. (ASHA et al., 2011). The work of Mezeal, (2014) reveals the antagonistic effect of microorganism

against tomato disease caused by *Rhizoctonia solani* and *Fusarium oxysporum*. For which four bacterial strains of, *Bacillus subtilis* and five strain of *Pseudomonas fluorescens* were isolated from tomato field soil. The antagonistic microorganisms against the pathogens were observed by Dual Culture Technique. *P. fluorescens* 5 isolate was found to show 81.3% and 77.4 of growth inhibition against *R.solani* and *F. oxysporum* respectively while *B. subtilis* 177.4% and 73.2 of growth inhibition against test pathogens respectively. Their report suggested that *P. fluorescens* 5 isolate were more effective than *Bacillus subtilis* 1 might be used potential biological control agents of tomato plants in Iraqi soil.

All the above observations reviewed supported our finding that soil rhizospheric bacteria are potential enough to antagonize plant pathogenic fungi *Fusarium* spp. and these finding will help in future endeavor on biocontrol of fungal phytopathogens in Bilaspur.

## **10. CONCLUSION**

The results of this study proved that properly selected bacterial isolates have the potential to significantly suppress the growth of *Fusarium* spp. The efficiency of the inhibition could be influenced by the specific interactions between bacterial strains (competition, antagonism) and by the particularities of target fungal pathogens species.

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## **REFERENCES**

- [1]. K.R.Aneja,(2003).Experiment In Plant Pathology ,Microbiology and Biotechnology (pp. 74-275). New Age International (p) Ltd., Publishers, New Delhi.
- [2]. Dal Bello, G.M., C.I. Mónaco, M.R. Simón. 2002. Al-Mughrabi, K.I. 2010. Biological control of Fusarium dry rot and other potato tuber diseases using *Pseudomonas fluorescens* and *Enterobacter cloacae*. *Biological Control*. 53. 280-284.
- [3]. AK Patel, KM Nampoothiri, S Ramachandran, G Szakacs, A Pandey, *Indian J. Biotechnol*, **2005**, 4: 336–342.
- [4]. Alabouvette, C., Edel, E., Lemanceau, P., Olivain, C., Recorbet, G., Steinberg, C. (2001) Diversity and interactions among strains of *Fusarium oxysporum*: Application to biological control. In: Jeger MJ, Spence NJ, eds. *Biotic interactions in plant–pathogen associations*. Wallingford, UK: CAB International, 131–158.
- [5]. Mina D. Koche, R. M. Gade1 And A.G. Deshmukh2.antifungal activity of secondary metabolites produced by *pseudomonas fluorescens*.
- [6]. Elad et al., 1980,Baker and Cook,1974, Blakeman&Fokkema,1982,Campbell,1989).An isolate of *Trichoderma harzianum* capable of lysing mycelia of *Sclerotium rolfsii* and *Rhizoctonia solani* was isolated from a soil naturally infested with those pathogens.
- [7]. Biological control of seedling blight of wheat caused by *Fusarium graminearum* with beneficial rhizosphere microorganisms. *World Journal of Microbiology and Biotechnology*. 18. 627-636.
- [8]. T.J Beveridge, J.A. Davies (1983) Cellular responses of *Bacillus subtilis* and *Escherichia coli* to the Gram stain. *J Bacteriol*. 1983 Nov;156(2):846-58.
- [9]. G.Shobha, BS.Kumudini \* Department of Biotechnology, Center for Post Graduate Studies, Jain University, Bangalore Antagonistic effect of the newly isolated PGPR *Bacillus* spp. on *Fusarium oxysporum*(2006)
- [10].G.Shobha, BS.Kumudini \* Department of Biotechnology, Center for Post Graduate Studies, Jain University, Bangalore Antagonistic effect of the newly isolated PGPR *Bacillus* spp. on *Fusarium oxysporum*(2006)
- [11]. Beveridge, T.J. 1990. Mechanism of Gram Variability in Select Bacteria *J. Bacteriol*. 172(3):1609- 1620.
- [12].B. Asha, S. Chandra Nayaka1, A. C. Udaya Shankar1, C. Srinivas\* And S. R. Niranjana Selection Of Effective Bio -Antagonistic Bacteria For Biological Control Of Tomato Wilt Caused By *Fusarium Oxysporum* F.Sp. *Lycopersici* B.



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- [13]. Dewangan.P.k.\*,Koma.B.,BaghelS., Khare N & H.K.Singh. Characterization *Pseudomonas Fluorescens* In Different Media And Its Antagonistic Effect On Phytopathogenic Fungi.
- [14]. Dewangan Et Al., 2014,9(1): 317-321, 2014 (Supplement On Plant Pathology) Www.Thebioscan.In 317
- [15]. D. Koche, R. M. Gade<sup>1</sup> And A. G. Deshmukh<sup>2</sup>. Antifungal Activity Of Secondary Metabolites Produced By *Pseudomonas Fluorescens*mina.
- [16]. J. M. Whipps and M. P. Mc Quilken (1993). Aspects of biocontrol of plant pathogens. In: Exploitation of Microorganisms (Ed. D.G. Jones), pp. 45-68. Chapman and Hall, London.
- [17]. J. M. Whipps and M. P. Mc Quilken (1993). Aspects of biocontrol of plant pathogens. In: Exploitation of Microorganisms (Ed. D.G. Jones), pp. 45-68. Chapman and Hall, London.
- [18]. K. K. Pandey and J. P. Upadhyay (2000), Microbial population from Rhizosphere and Non-Rhizosphere soil of pigeonpea : Screening for Resident Antagonist and Mode of Mycoparasitism J. Mycol. Pl. Pathol. 30(1): 7 -10.
- [19]. K. K. Pandey, P. k. Pandey and J. P. Upadhyay (2005). Mycoparasitism of *Trichoderma spp.* on *Fusarium* and *Rhizoctonia*. J. Mycol. Pl. 35 (1) : 174 – 176.
- [20]. KK Pal, KVB Tilak, AK Saxena, R Dey, S Singh (2001) Suppression of maize root disease caused by *Macrophomina phaseolina*, *Fusarium moniliforme* and *Fusarium graminearum* by plant growth-promoting rhizobacteria.
- [21]. R. B. Rajput\*, K.U.Solanky, V.P.Prajapati, D.M.Pawar and S.R.Suradkar. effect of fungal and bacterial bioagents against *alternaria alternata* (fr.) keissler in vitro condition