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Isolation and evaluation of chickpea rhizospheric microflora against wilt disease in chickpea (*Cicer arietinum* L.) under *in vitro* condition

T Priyanka, Dr. M Prameela, Dr. B Vidyasagar and Dr. S Triveni

Abstract
The six isolates of *Fusarium oxysporum f. sp. ciceris* FOC 1, FOC 2, FOC 3, FOC 4, FOC 5, FOC 6 are isolated from Kamareddy, Gadwal, Sangareddy, Nizamabad, Nirmal, Adilabad districts respectively. A total of 40 bacterial isolates and 10 actinomycetes isolates and 18 fungal isolates are isolated from 18 rhizosphere soil samples. These isolates were further tested in dual culture for antagonism against *Fusarium oxysporum* f. sp. *ciceris*. Among 18 fungal isolates f-17 showed maximum inhibition with 86.66 per cent inhibition. Among 40 bacterial isolates GS1B21 showed maximum inhibition with 86.66 per cent inhibition followed by NS1B1, AS2B9, GS2B24 with 83.33 per cent inhibition. Among 10 actinomycetes isolates A-8 showed maximum inhibition with 84.00 per cent inhibition. These isolates have high efficacy against *Fusarium oxysporum* f. sp. *ciceris* under *in vitro* conditions.

Keywords: *Fusarium oxysporum f. sp. ciceris*, districts, per cent inhibition, efficacy, *in vitro*

Introduction
Chickpea (*Cicer arietinum* L.) is third most important pulse crop and extensively grown as major winter crop in more than 50 tropical and subtropical countries in the world (Gaur *et al*., 2014; Patil *et al*., 2017). India is the largest producer contributing to around 70 per cent of the world production; where during *Rabi* (2019-20) about 107.21 lakh ha area coverage was reported (*www.nipgr.ac.in*). Chickpea productivity is adversely affected by various abiotic and biotic factors around the globe (Tarafdar *et al*., 2018 and Cabollo *et al*., 2019) [26, 7] where Fusarium wilt has been found a devastating fungal disease posing adverse effects on chickpea productivity among the biotic factors and become a major threat for chickpea productivity and the yield losses range from 10-19% depending upon the severity of disease (Haware *et al*., 1989) [10].

The most efficient method for the management of disease is using resistant cultivars although new races of the pathogen appear to overcome resistant genes. In addition, chemical control is not satisfactory, therefore biological control is an alternative to chemical control of the disease (Anjajah *et al*., 2003; Landa *et al*., 2004) [4, 14]. Use of biological control agents, such as plant growth promoting rhizobacteria (PGPR), can be a suitable approach in control of disease (Schmidt *et al*., 2004) [23]. Bio control methods utilizing antagonistic microorganisms associated with the plant rhizosphere have great potential for control of soil borne plant pathogens (Prashar *et al*., 2013) [19].

Methods and Materials
Survey for the occurrence of Fusarium wilt in different Bengal gram growing areas of Telangana
A roving survey was conducted in major chickpea growing regions of Telangana covering 6 districts in which 3 places are surveyed in each district during Rabi 2020-21 for the collection of infected plant samples and rhizosphere soil samples. Infected plant samples were collected for isolation of Fusarium wilt pathogen *Fusarium oxysporum* f. sp. *Ciceris*. Rhizosphere soil samples adhering to the roots of chickpea plants were collected for isolation of rhizosphere micro flora. Five plots measuring 1 m x 1 m were selected such that one plot was in the centre of the field and the rest were randomly placed on the four corners leaving 1 m from the border. Total number of plants and number of wilt infected plants were counted in each plot and per cent disease incidence was calculated by the following formula:

Per cent disease incidence (PDI) = Number of infected plants / Total number of plants × 100

Isolation and characterization of test pathogen

Isolation of pathogen

The pathogen *Fusarium oxysporum* f. sp. *Ciceris* was isolated from infected chickpea plants by tissue segment method (Rangaswami and Mahadevan, 1999) [20]. The infected Bengal gram plant roots were washed under running tap water to remove excess soil adhered to the root zone and dried on blotter paper before isolation to avoid contamination. These roots were then cut into small pieces of size 2-3 mm with sterilized blade. These bits were then surface sterilized with 1 per cent sodium hypochlorite solution for one minute and rinsed with sterilized water at three intervals to remove traces of sodium hypochlorite on the root. Then each bit was dried on a blotter paper and four bits of each were placed on the Potato Dextrose Agar (PDA) medium poured plates and were incubated at 28±2 °C for seven days in an incubator.

Purification and Identification of wilt pathogen

Spore suspension of the isolated pathogen (*F. oxysporum* f. sp. *ciceris*) was prepared by dissolving spores in sterile distilled water. One ml of spore suspension was taken and spread uniformly on two per cent water agar plates and drained the excess suspension. The plates were incubated at 28±2 °C and observed for spore germination under the microscope. Hyphae coming out from the single spore was observed and marked with the marker on the reverse side of the Petri plates. The tip of hypha was cut and transferred on to PDA plates and incubated at temperature of 28±2 °C for 10 days. Later pure culture of the fungus was transferred to slants and were used for further studies. The purified isolates of *Fusarium* were identified on the basis of cultural and morphological characteristics such as colony colour, mycelial growth, pigmentation and sporulation (macro conidia, micro conidia and chlamydospores) by using monographs of *Fusarium* described by Booth (1971) [6]. The morphology of the conidia were observed under low power magnification (40X) of stereo binocular microscope and data were recorded. Based on the above cultural and morphological characteristics the isolates of *Fusarium* were identified.

Mass multiplication of *F. oxysporum* f. sp. *ciceris*

Purified cultures of six isolates of *F. oxysporum* f. sp. *ciceris* were mass multiplied on sorghum grains. The sorghum grains were soaked in water for overnight and excess water was drained out from it. Sorghum grains were filled in 500 ml conical flask and tightly closed with non-absorbent cotton and were autoclaved at 121 °C 15 lbs pressure for 30 min for 2 days. Then the flasks were allowed to cool and inoculated with 5 mm mycelial disc of *F. oxysporum* f. sp. *ciceris* and incubated at 28±2 °C for 15 days in BOD incubator. The flasks were manually shaken on daily basis for a few minutes to avoid clumping in order to get early growth with uniform colonization of seeds. After 15 days, of inoculation fungal cultures are ready for further use which is fully multiplied.

Pathogenicity test

Pathogenicity test was carried out in earthen pots of (12×10 cm) diameter filled with three kg steam sterilized soil. The mass multiplied fungus was added to these pots @ 30 g / kg soil and mixed thoroughly for uniform distribution. The pots were watered lightly and was incubated for 4 days. Surface sterilized seeds of Bengal gram cultivar JG-11 were sown in these pots (5 seeds / pot) and three replications was maintained. The pots were regularly watered to maintain sufficient moisture needed by the plants. Wilt symptoms developed were observed for 25 days after sowing and the per cent disease incidence was calculated (Jamil and Ashraf, 2020) [11].

PDI = Number of wilted plants per pot / Total number of plants per pot ×100

Isolation and characterization of chickpea rhizosphere micro flora

Isolation of rhizosphere micro flora

Serial dilution and plate count method by Timonin (1940) [27] was used for the isolation of fungi, bacteria, and actinomycetes from chickpea rhizosphere soil. Ten grams of rhizosphere soil sample was suspended in 90 ml of sterile distilled water blank and kept in a rotary shaker at 160 rpm for 30-45 minutes. One ml of 10^{-1} dilution thus obtained was transferred to a test tube containing 9 ml of sterile distilled water blank using a 1 ml micropipette which gives 10^{-2} dilution. This step was repeated to obtain concentrations 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} respectively.

Dilutions of 10-3 and 10-4 were used for isolation of fungus and actinomycetes while dilutions of 10-5 and 10-6 were used for isolation of bacteria. Hundred µl of respective dilutions were spread onto potato dextrose agar medium (PDA) and Martin‘s Rose Bengal agar medium (RBA) for isolation of fungus, Nutrient agar medium (NA) 32 and King‘s B medium (KB) for isolation of bacteria, and actinomycetes isolation agar medium (AIA) for isolation actinomycetes present in rhizosphere soil. These media plates were incubated at 25±2 °C for 3-4 days, 28±2 °C for 24-48 hours 28±2 °C for 10-15 days for isolation of fungus, bacteria and actinomycetes respectively.

After completion of incubation, the number of similar colonies was counted and sub culturing was done to obtain pure cultures. Pure cultures of fungal isolates were obtained by single spore and single hyphal tip method. While bacterial and actinomycetes pure cultures were obtained by picking a single colony with sterilized inoculation loop and streaking in fresh sterile media plates. Fungal, bacterial, and actinomycetes isolates were maintained by sub culturing onto potato dextrose agar medium (PDA), nutrient agar medium (NA) and starch M protein agar medium (SMPA) respectively.

Screening of rhizosphere fungal isolates against *F. oxysporum* f. sp. *ciceris in vitro*

Five mm mycelial discs of 5 day old culture of *F. oxysporum* f. sp. *ciceris* and the test fungal isolate were placed at opposite ends of the Petri dish 1 cm away from the periphery and incubated at 25±2 ºC. A control plate with only *F. oxysporum* f. sp. *ciceris* was also maintained. When full growth was achieved in the control plate, the mycelial growth of the pathogen was measured in each Petri dish separately and expressed in mm. Per cent inhibition of mycelial growth of the pathogen by different test fungal isolates was calculated using the formula given by Vincent (1947) [28].

Screening of bacterial and actinomycetes isolates against *F. oxysporum* f. sp. *ciceris in vitro*

Primary screening of bacterial and actinomycetes isolates was done for their inhibitory effect on the growth of *F. oxysporum* f. sp. *ciceris*. Selected bacterial and actinomycetes isolates

were tested for their antagonism against *F. oxysporum* f. sp. *ciceris* by following the dual culture technique. Loopful of 24 hour old pure cultures of test isolate was streaked 1 cm away from the periphery of PDA plates and a 5 mm mycelial disc of 5 days old culture of *F. oxysporum* f. sp. *ciceris* was placed at the opposite end and incubated at 25±2 ºC. A control plate with only *F. oxysporum* f. sp. *ciceris* was also maintained. When full growth was achieved in the control plate, the mycelial growth of the pathogen was measured in each Petri dish separately and expressed in mm. Per cent inhibition of the mycelial growth of the pathogen by different test isolates was calculated using the formula given by Vincent (1947) [28].

Results

A roving survey was conducted in chickpea major growing areas of Telangana state during Rabi 2020-21 for collection of rhizosphere soil samples and wilt infected plant samples. The survey covered all major growing districts *viz*., Adilabad, Kamareddy, Nizamabad, Gadwal, Nirmal, Sangareddy where 18 rhizosphere soil samples were collected and infected plant samples were collected from six districts. Per cent disease incidence of wilt in the survey fields ranged between 6.06 and 30.30 per cent with a mean of 18.18 per cent. The field in Shanthinagar village, Wadepally mandal, Gadwal district of recorded the highest wilt incidence with 30.30 per cent followed by Dilawarpur village, Dilawarpur mandal, Nirmal district with disease incidence of 27.27 per cent while the lowest disease incidence of 6.06 per cent was observed in Brahmanpally village of Jakranpalle mandal, Nizamabad district (Table 1). Nikam *et al*., 2011 [17] revealed average wilt complex to the tune of 12.26% by survey and surveillance of chickpea wilt in the Latur district where maximum wilt incidence in Tashil Ausa (15.4%) followed by Jalkot (14.8%) and Renapur (14.0%). Shahzaman *et al*., 2015 [24] collected rhizosphere soil from different areas of Rawalpindi division of Pakistan of chickpea fields. Mathur and Mathurt, 2021 [16] collected soil from rhizosphere of chickpea fields of different areas of Rawalpindi division of Pakistan.

The test pathogen *Fusarium oxysporum* f. sp. *ciceris* was isolated on potato dextrose agar media by following the tissue isolation method from infected chickpea plants collected from six districts during sample collection (Fig.1.) The six isolates of F. oxysporum f. sp. *ciceris* FOC 1, FOC 2, FOC 3, FOC 4, FOC 5, FOC 6 are from Kamareddy, Gadwal, Sangareddy, Nizamabad, Nirmal, Adilabad districts respectively (Fig.2). The cultural and morphological variations like colony character, pigmentation, colony growth and sporulation were recorded. The cultural characteristics of six *Fusarium* isolates differed in colony type, pigmentation, sporulation and growth (Table.2). The maximum radial growth was recorded by isolate FOC5 (86.5 mm) which showed a fast growth followed by isolate FOC3 (83 mm), FOC1 (80mm), FOC2 (80mm), FOC4 (78mm) which showed a moderate growth whereas isolate FOC6 (75mm) showed a slow growth (Fig.3). The isolate FOC 1 and FOC4 showed cottony white mycelium and FOC2 showed dense cottony white mycelium, FOC3 showed pinkish white mycelium with concentric rings, FOC5 showed fluffy cottony white mycelium and FOC6 showed yellowish white mycelium. The Fusarium wilt pathogen bears two types of asexual spores i.e., macro conidia and micro conidia. The size of the macro conidia ranged from (16.631×2.091 to 24.725×3.616 µm) and size of micro conidia ranged from (3.570×1.596 to 5.420×2.327). The septation is varying with the isolate, most of the isolates showed more than 2 septa in macro conidia. In micro conidia most of the isolates showed single septum. The shape of macro conidia was sickle shaped of isolates FOC1, FOC2, FOC3, FOC5 while isolates FOC 4 and FOC 6 showed blunt type of macro conidia. The micro conidia of the isolates were oval shaped. The isolate FOC 1, FOC 2 and FOC 3, FOC5 showed profuse sporulation. The isolate FOC 4 has shown moderate sporulation, whereas isolate FOC5 shown slightly moderate. All six isolates of *F. oxysporum* f. sp. *ciceris* produced chlamydospores in which size varies within the isolates. The maximum (10.65 µm) size was seen in isolate FOC 5 and least (5.48 µm) size was seen in isolate FOC6 (Fig. 4) (Table 2). Similar findings were found by Andrabi *et al*., 2011 [3] isolated *Fusarium oxysporum* f. sp. ciceriss from the wilted chickpea (*Cicer arietinum*) plants. Sankar *et al*., 2018 [22] studied the cultural variability of fifteen isolates have pale yellowish to dark pinkish (Foc4) in pigmentation with aerial compact mycelial growth within 7- 9 DAI. The morphological characterization all the isolates produced micro, macro-conidia and chlamydospores within 20 DAI and the size of the spores varied from (micro conidia) 5.6 x 2.5 µm (Foc2) to 12.7 x 3.1 µm (Foc14) and the isolate (Foc4) maximum size of macro conidia in 29.1x 4.9 µm.

Pathogenicity test was carried out by inoculating *F. oxysporum* f. sp. *ciceris* culture in pots by sick pot soil method (Fig.5) All the six isolates of *Fusarium* (FOC 1, FOC 2, FOC 3, FOC 4, FOC 5, and FOC 6) were inoculated in soil pots individually, and Bengal gram seeds were sown in the pots after incubating the cultures for 5 days. Seeds inoculated with fungus showed wilting symptoms within 30 days after inoculation. Based on per cent disease incidence the most virulent isolate was taken up for further studies. The isolate FOC5 was identified as the most virulent isolate among the six isolates of FOC. The symptoms developed were yellowing of leaves, later turn brown or straw colour, finally wilting and death of the plants was noticed. Plants which were not inoculated with the fungal cultures served as control and they did not show any wilt symptoms. The results obtained are in agreement with findings of Nikam *et al*., 2011 [17] who proved the pathogenicity of *F. oxysporum* f. sp. *ciceris*. Sankar *et al*., 2018 [22] isolated fifteen isolates in which Foc4 (Gomangalampudur) is highly pathogenic when compared to other and causing early wilt in JAKI-9218 where Foc4, Foc5, Foc6, Foc8, Foc10, Foc11, Foc12, Foc13 and Foc14 are highly pathogenic nature and other isolates were strongly pathogenic.

The rhizosphere soil samples which were collected from chickpea fields in all major growing districts of Telangana were assessed for their microbial population by serial dilution and plate count method on three different media *viz*., nutrient agar medium (NA), actinomycetes isolation agar medium (AIA), and Martin‘s Rose Bengal agar medium (RBA) (Fig.6). A total of 18 fungal isolates (Fig.7) were isolated from RBA and 40 isolates were isolated from NA (Fig.9) and 10 isolates from AIA (Fig.10) and they were maintained as pure cultures for further studies. The isolates were designated using district and rhizosphere soil sample number followed Colony number (1, 2, 3, 4, n). E.g. NS1B1– First bacterial isolate isolated from nizamabad district and rhizosphere soil sample S1 (Table 4). The fungal colonies formed in Martin‘s Rose Bengal agar medium were isolated and pure cultures were obtained. Suthar *et al*., 2017 [25] obtained fourteen

potential antagonistic bacterial isolates from healthy chickpea rhizospheric soil samples. Kumari *et al*., 2013 [13] isolated 200 isolates from chickpea rhizospheric soils and Anusha *et al*., 2019 [5] isolated 40 bacterial isolates Rangeshwaran *et al*., 2000 collected three hundred rhizospheric bacteria isolates from different regions of Karnataka. Kumari *et al*., 2013 [13] isolated forty two rhizobacterial isolates from chickpea rhizosphere soils collected from twelve different locations of the Punjab. Verma and Yadav, 2018 isolated five indigenous soil microbes from chickpea rhizosphere soils of different location of Jaunpur, Mirzapur, Varanasi and Azamgarh district of eastern Uttar Pradesh. Amini *et al*., 2016 [2] isolated about 112 isolates of Streptomyces from chickpea rhizospheric soils. Zaim *et al*., 2016 [29] isolated five rhizobacteria Rb29, Rb6, Rb12, Rb4, and Rb15 from rhizosphere soils of healthy chickpea plants. Joseph *et al*., 2007 isolated a total of 150 bacterial isolates from different rhizospheric soil of chick pea in the vicinity of Allahabad. Gopalakrishnan *et al*., 2015 [9] isolated five strains of Streptomyces sp. (CAI-24, CAI-121, CAI-127, KAI-32, and KAI-90.

The fungal, bacterial, actinomycetes isolates were studied for their morphological and cultural characteristics. (Table 3) (Table 4) (Table 6). The fungal isolates from Martin's Rose Bengal agar medium were culturally and morphologically identified Biochemical characterization of the bacterial and actinomycetes isolates were done by conducting tests including indole production test, methyl red test, Voges Proskauer's test, oxidase test, catalase test and KOH test along with gram staining (Fig.11) (Table 5).

The fungal isolates from the chickpea rhizosphere were observed for their cultural and microscopic characteristics (Barnett and Hunter, 1972) [30] and the results are presented in (Table3). Photomicrographs of the major fungal isolates are presented in (Fig.8). It was observed that Aspergillus spp. was predominant Mycoflora. Apart from Aspergillus, other genera like *Penicillium*, Trichoderma, *Chaetomium*, Colletotrichum, *Fusarium*, Rhizoctonia spp. were also isolated from the chickpea rhizosphere soil under study.

Different colonies selected during isolation were studied for colony morphology characteristics like shape, margin, elevation, size, texture, appearance, pigmentation and optical property. The results suggested there is considerable variation in colony morphology both in type and number among isolates isolated from different rhizosphere soil samples suggesting the presence of diverse organisms in chickpea rhizosphere soil (Table 4).

The biochemical tests including indole production test, methyl red test, Voges Proskauer test, oxidase test, catalase test and KOH test along with gram staining were conducted for each isolates. Among the 40 rhizosphere isolates evaluated for indole production, 7 isolates tested positive while 33 isolates tested negative. A total of 10 isolates tested positive and 30 tested negative for the methyl red test. In the Voges Proskauer test, 14 tested positive while 26 tested negative. All the isolates tested positive for the catalase test. In the oxidase test, 18 isolates tested positive and 22 tested negative. Of the total 40 isolates tested, 12 isolates were KOH test positive and had a negative Gram reaction while 28 tested negative for the KOH test and gave a positive Gram reaction. The results suggested that the majority of chickpea rhizosphere isolates studied were indole production negative, methyl red negative, Voges Proskauer positive, catalase positive and oxidase negative. Gram-positive rods dominate the isolates studied indicating most of the isolates present in the chickpea rhizosphere soil come under the Bacillus genus followed by others. Suthar *et al*., 2017 [25] diversified fourteen potential antagonistic bacterial isolates from each other by colony and morphological characterization. Kumari *et al*., 2013 [13] characterized forty two rhizobacterial isolates biochemically and found belonging to genera Pseudomonas (19), Bacillus (22) and Serratia (1). Anusha *et al*., 2019 [5] found 10 strong antagonistic potential to be Streptomyces spp. (five isolates) and Bacillus spp. (five isolates) in the morphological and biochemical characterisation. Abed *et al*., 2016 [] found out bacterial isolates were varied in production of Protease, Gelatinase, Amylase, Cellulase, Acid Indole acetic, Lipase, Catalase and Cyanid Hydrogen.

Antagonistic activity of rhizosphere isolates against *Fusarium oxysporum* f. sp. *ciceris*

These isolates were further tested in dual culture for antagonism against *Fusarium oxysporum* f. sp. *ciceris*. Observations were taken on the day when the radial growth of *Fusarium oxysporum* f. sp. *ciceris* in the control plate was full. Among 18 fungal isolates f-17 showed maximum inhibition with 86.66 per cent inhibition followed by f-8, f-16 and f-18 with 83.33 per cent inhibition and least inhibition by f-6 with 50 per cent inhibition (Table 7) (Fig.12). Among 40 bacterial isolates GS1B21 showed maximum inhibition with 86.66 per cent inhibition followed by NS1B1, AS2B9, GS2B24 with 83.33 per cent inhibition and least inhibition by NMS2B30 with 40 per cent inhibition (Table 8) (Fig.13). Among 10 actinomycetes isolates A-8 showed maximum inhibition with 84.00 per cent inhibition followed by A-5 with 73.33 per cent inhibition and least inhibition by A-2 and A-6 with 6.66 per cent inhibition (Table 9) (Fig.14).

Anusha *et al*., 2019 [5] tested antagonistic activity for 40 bacterial isolates against FOC out of which 10 were found to have strong antagonistic potential. Maitlo *et al*., 2019 [15] tested different antagonistic fungi *viz*., *Trichoderma pseudo koningii*, *T. polysporum*, *T. harzianum*, *Paecilomyces lilacinus*, *P. variotii* and *Gliocladium virens* against *Fusarium oxysporum* f. sp. *ciceris* under *in vitro* where all the antagonistic fungi inhibited the growth of test pathogen in dual assay where *P. lilacinus* produced the least inhibition of test pathogen while, *G. virens* produced maximum inhibition. Mathur and Mathurt, 2021 [16] determined antagonistic activity against *Fusarium oxysporum* f. sp. *ciceris* for 40 chickpea rhizobacteria in which twenty eight isolates showed antagonistic activity against test fungus ranging from 18.2 to 41.8%.

Conclusions

Chickpea rhizosphere soil samples and infected plant samples were collected from eighteen different fields covering three villages in each district of Telangana state during Rabi 2020-21. The mean per cent wilt incidence in all the chickpea fields surveyed was 15.15 per cent and it ranged between 6.06 and 30.30 per cent. Rhizosphere isolates and test pathogen *Fusarium oxysporum* f. sp. *ciceris* were isolated from collected samples. Six isolates of test pathogen *Fusarium oxysporum* f. sp. *ciceris* were isolated. A total of 40 isolates isolated from nutrient agar medium (NA) and 10 isolates isolated from actinomycetes isolation agar medium (AIA) and 18 fungal isolates isolated from Martin's Rose Bengal agar

medium (RBA) were maintained as pure cultures for further studies. Six isolates of test pathogen *Fusarium oxysporum* f. sp. *Ciceris* were studied for cultural characteristics. Further, virulence variability among the isolates were also tested by conducting pathogenicity tests on chickpea varietyJG-11. Though all the isolates recorded cent per cent disease incidence, *Fusarium oxysporum* f. sp. *ciceris* isolate FOCNm recorded the lowest mean values for incubation period (4.25 days) and days to permanent wilting point (12.25 days) and hence was selected for further studies. Among the fungal isolates, the Aspergillus genus majorly A. Niger and *A. flavus* dominated the groundnut rhizosphere Mycoflora and were present in 15 out of 18 rhizosphere soil samples used for isolation. Other Aspergillus species identified include *A. ochraceus*, *A. terreus* and *A. fumigatus*. Apart from Aspergillus, *Penicillium*, Trichoderma, *Chaetomium*, Colletotrichum, *Fusarium* were the other genera present in the chickpea rhizosphere soil under study. Further, these isolates were tested for antagonistic activity against *Fusarium oxysporum* f. sp. *ciceris*. Among 18 fungal isolates F-17 showed maximum inhibition with 86.66 per cent inhibition. Among 40 bacterial isolates GS1B21 showed maximum inhibition with 86.66 per cent inhibition followed by NS1B1, AS2B9, GS2B24 with 83.33 per cent inhibition. Among 10 actinomycetes isolates A-8 showed maximum inhibition with 84.00 per cent inhibition.

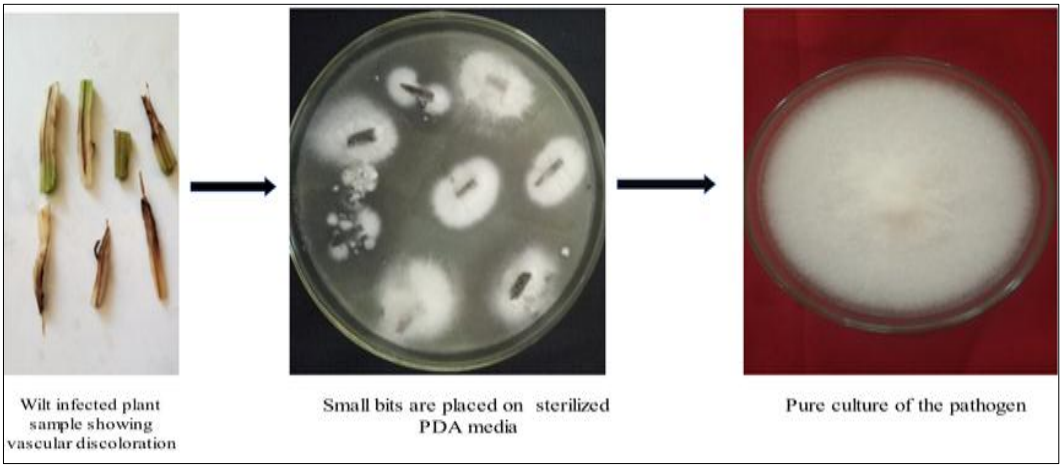

Fig 1: Isolation of pathogen by tissue segment method

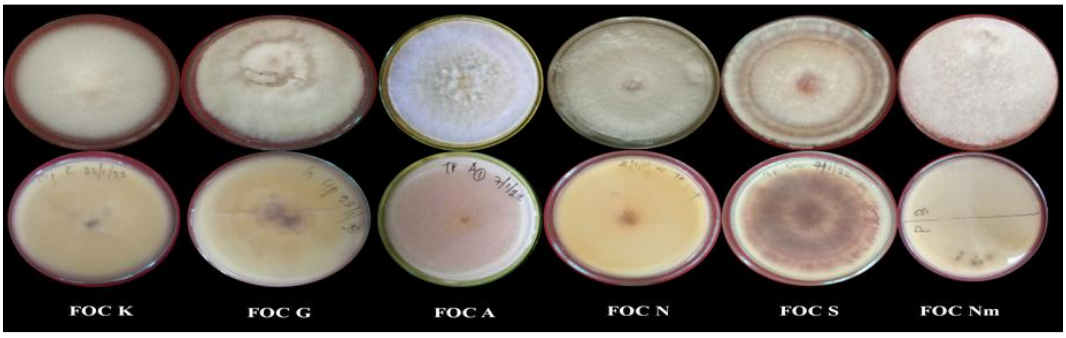

Fig 2: Pure cultures of *Fusarium oxysporum* f. sp. *Ciceris* isolated from different Bengal gram growing areas of Telangana

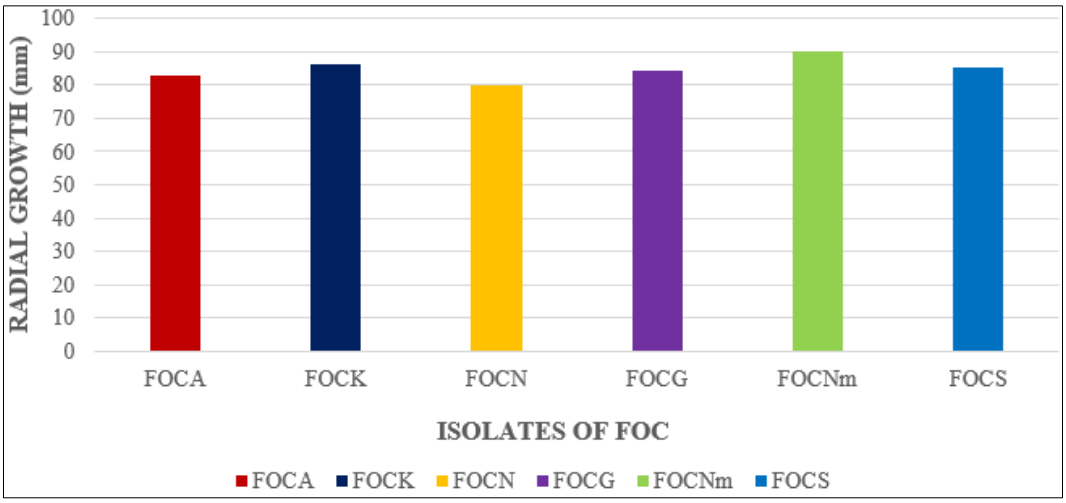

Fig 3: Variability in mycelial growth rate of different isolates of *F. oxysporum* f. sp. *ciceris* of chickpea wilt pathogen isolated from major growing areas of Telangana

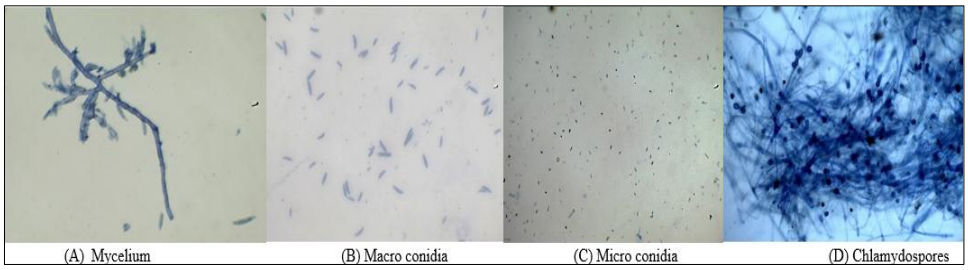

Fig 4: Photomicrographs of conidia of *Fusarium oxysporum* f. sp. *ciceris* observed under (40X)

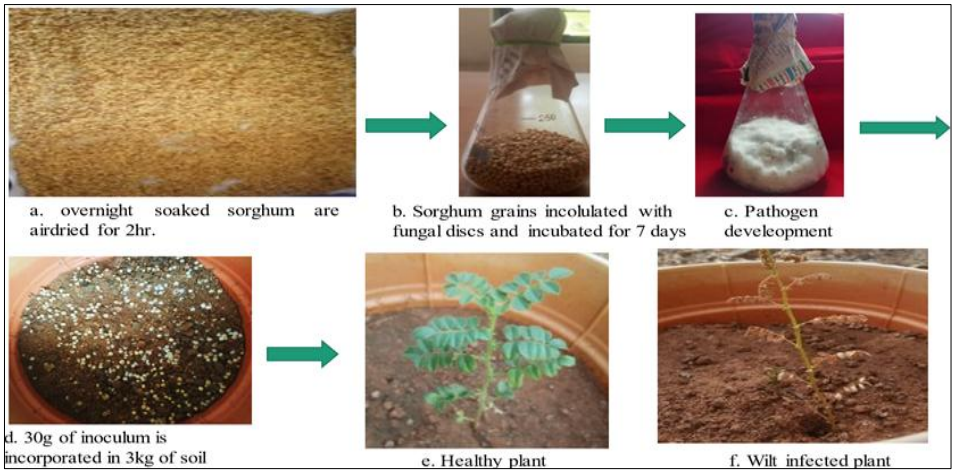

Fig 5: Pathogenicity test of *F. oxysporum* f. sp. *ciceris* on Bengal gram plants

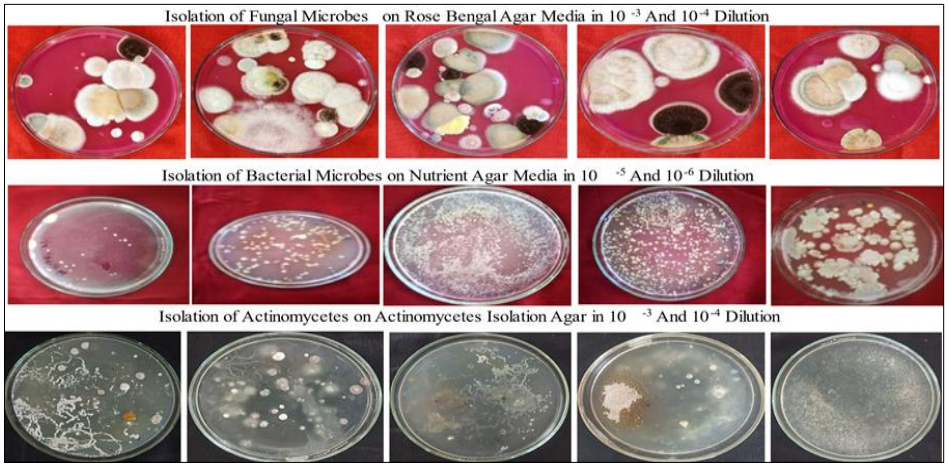

Fig 6: Isolation of micro flora from chickpea rhizosphere soil samples on different media

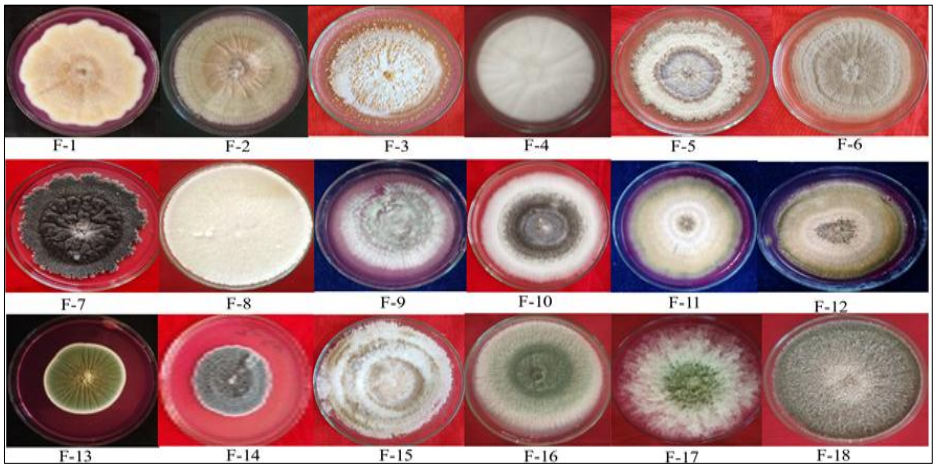

Fig 7: Pure cultures of major fungi isolated from chickpea rhizosphere soil samples

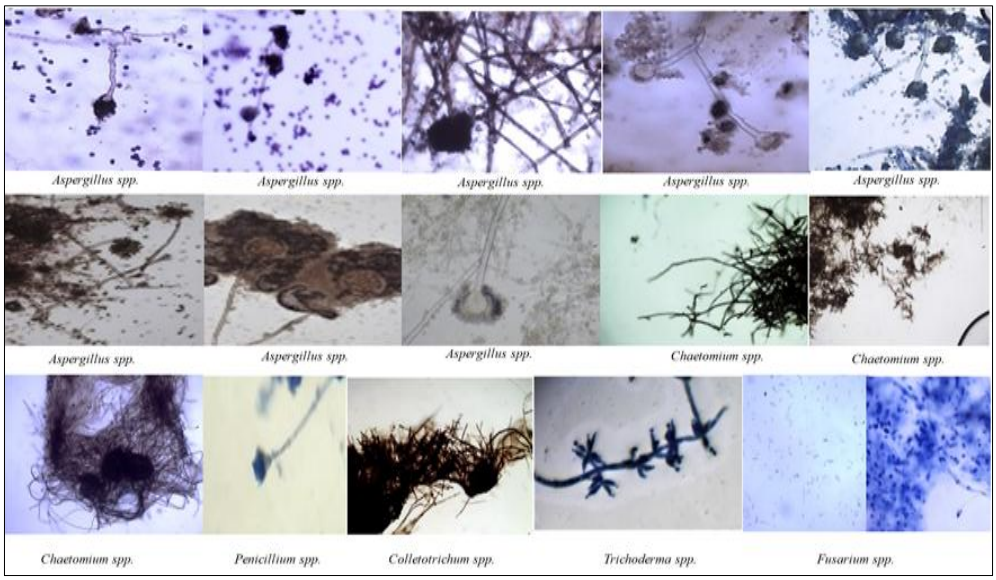

Fig 8: Photomicrographs of major fungal isolates observed at 20X magnification

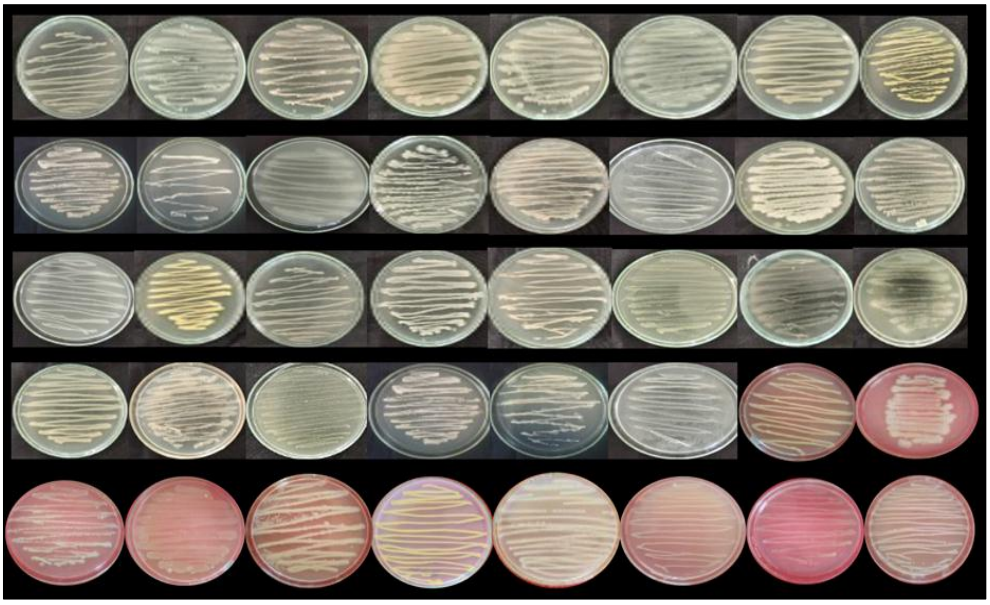

Fig 9: Rhizosphere isolates isolated on nutrient agar media

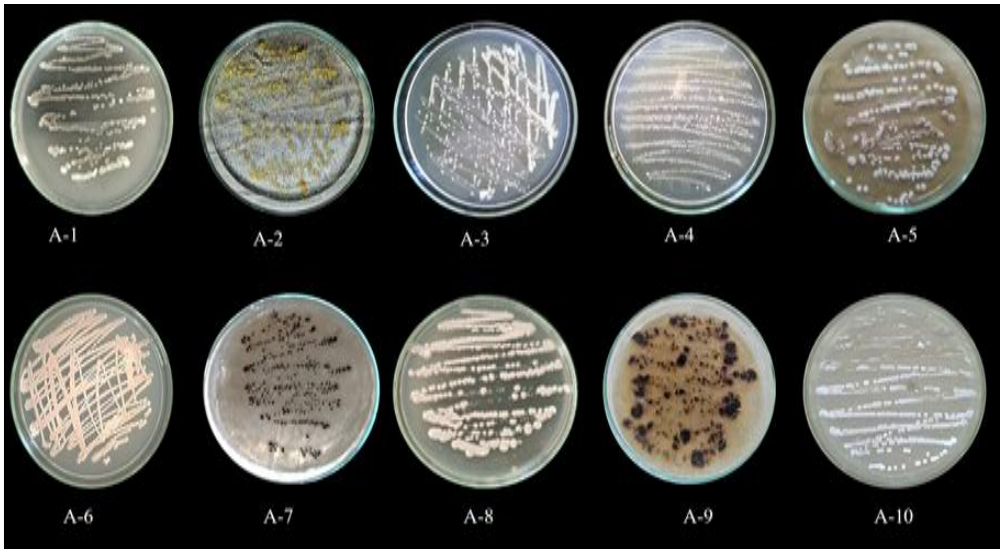

Fig 10: Rhizosphere isolates isolated on actinomycetes isolation agar media

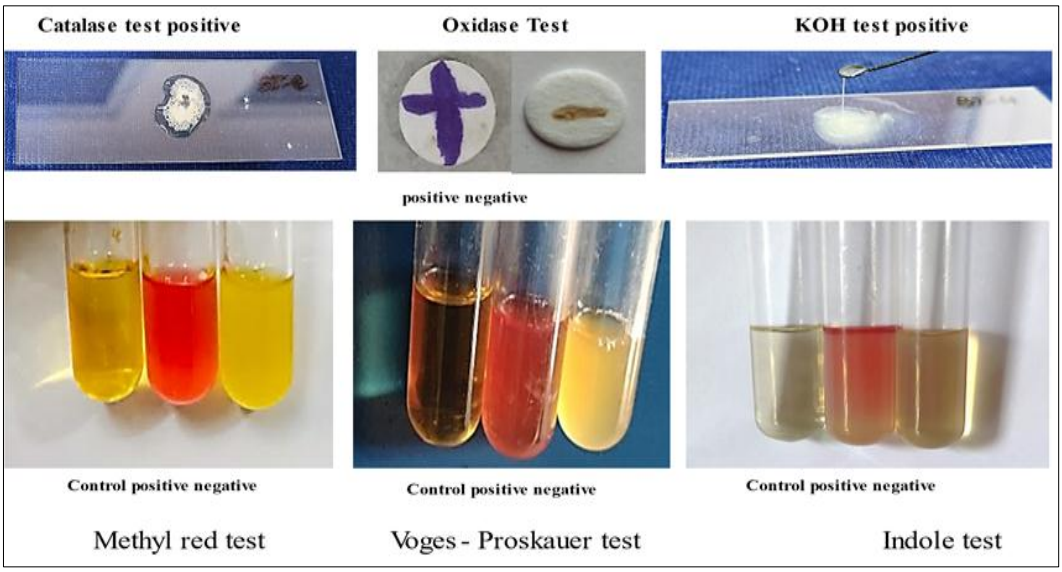

Fig 11: Biochemical characterization of rhizosphere isolates isolated on nutrient agar and actinomycetes isolation agar media

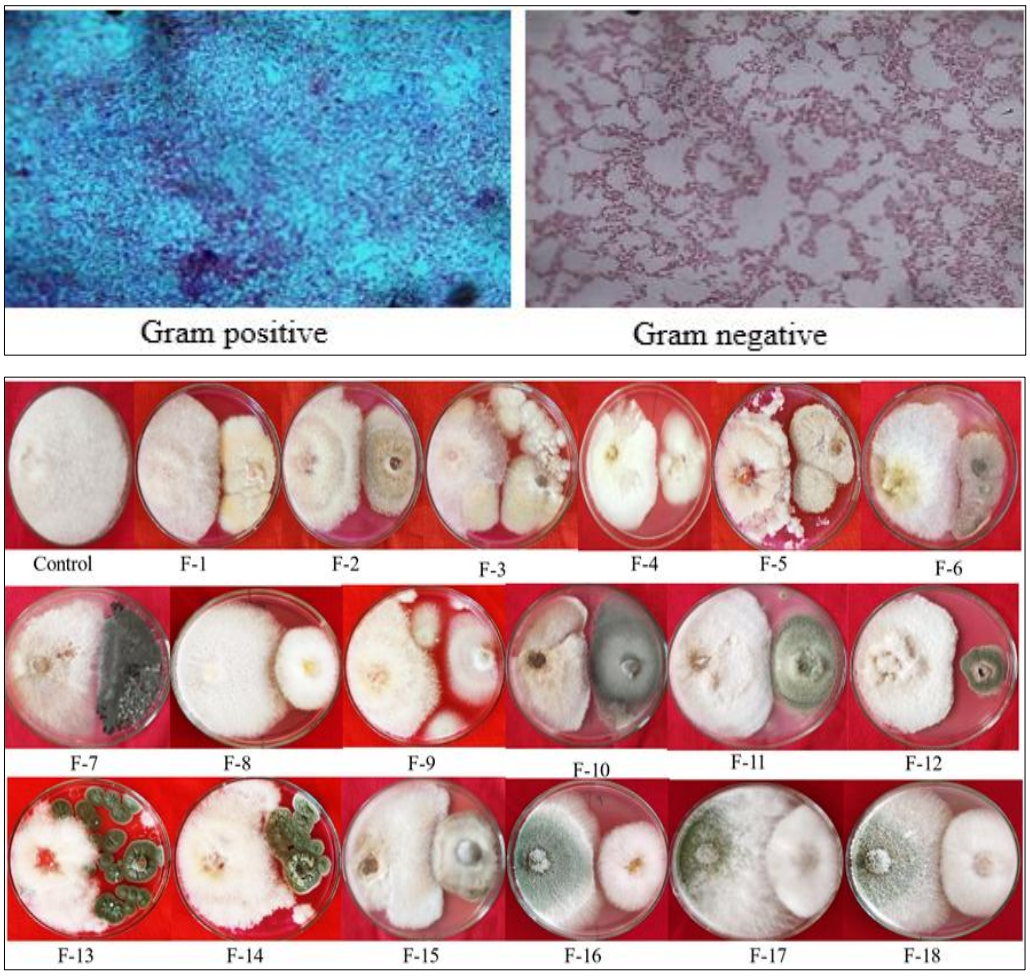

Fig 12: Antagonistic activity of fungal isolates from chickpea rhizosphere against *Fusarium oxysporum* f. sp. *Ciceris*

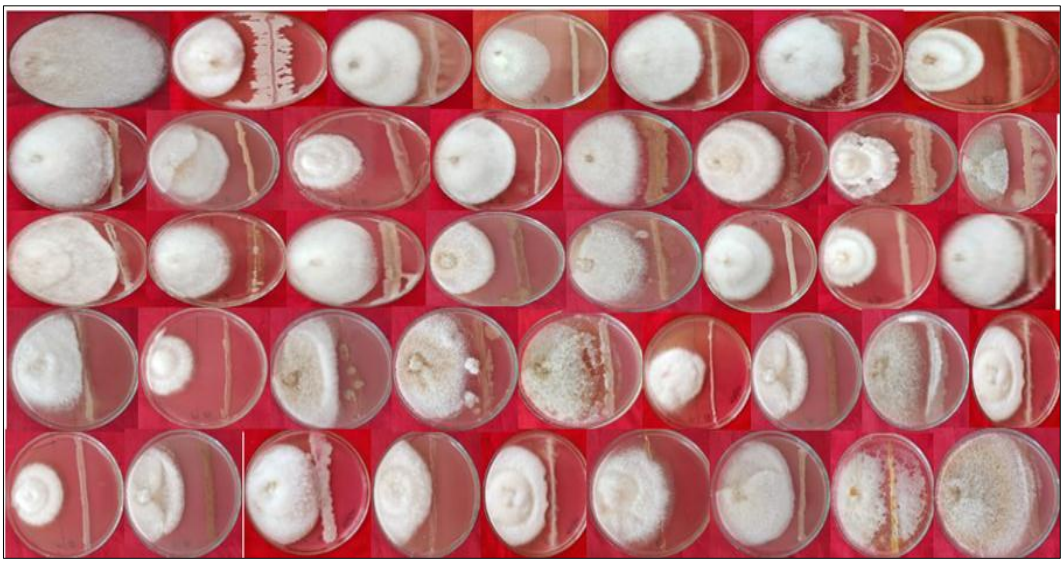

Fig 13: Antagonistic activity of rhizosphere isolates from nutrient agar medium against *Fusarium oxysporum* f. sp. *Ciceris*

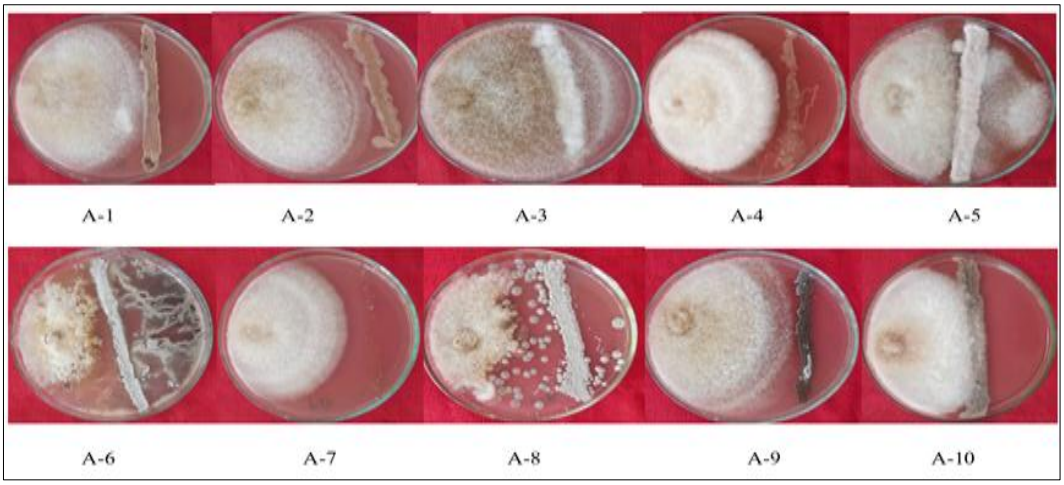

Fig 14: Antagonistic activity of rhizosphere isolates from actinomycetes isolation agar against *Fusarium oxysporum* f. sp. *ciceris*

Table 1: List of rhizosphere soil samples collected from chickpea major growing areas of Telangana state

Sample ID	Location					Soil type	Previous crop grown	Irrigation Method	Variety	Other agronomic practices	PDI (%)
	District	Mandal	Village	Latitude	Longitude						
S1	Nizamabad	Armoor	Govindpet	18.7848	78.2951	Black	Soybean	Flood	JG-11	Complex fertilizer application (12-32-16)	15.15
S2			Suyyapet	18.7653	78.3287	Red	Soyabean	Flood	JG-11	Weeding and fertilizer application	12.12
S3		Jakranpalle	Brahmanpally	18.7031	77.9572	Red	Soyabean	Flood	JG-11	-	6.06
S4	Adilabad	Indervelly	Mutnoor	19.5133	78.6299	Black	Soyabean	Rain fed	JG-11	Weeding and fertilizer application	21.21
S5			Dodanda	19.5190	78.7419	Black	Soyabean	Rain fed	JG-11	-	24.24
S6		Narnoor	Umri	19.5208	78.7080	Red	Soyabean	Rain fed	JG-11	-	18.18
S7	Kamareddy	Madnoor	Madnoor	18.4924	77.6511	Black	Maize	Rain fed	JG-11	Weeding and fertilizer application	9.09
S8			Limboor	18.3151	77.4123	Black	Maize	Rain fed	JG-11	-	15.15
S9		Jukkal	Jukkal	18.3603	77.6028	Red	Maize	Rain fed	JG-11	-	18.18
S10	Gadwal	Wadepally	Wadepally	15.9358	77.8417	Black	Tobacco	Rain fed	JG-11	Weeding and fertilizer application	21.21
S11			Shanthinagar	15.9485	77.8470	Black	Tobacco	Rain fed	JG-11	-	30.30
S12		Rajoli	Rajoli	15.8925	77.8259	Red	Tobacco	Rain fed	JG-11	Weeding and fertilizer application	18.18
S13	Nirmal	Dilawarpur	Dilawarpur	19.0919	78.2275	Black	Soyabean	Rain fed	JG-11	-	27.27
S14			Sirgapur	19.0889	78.2819	Black	Soyabean	Rain fed	JG-11	-	24.24
S15		Kaddam	Kaddam	19.1126	78.4727	Black	Soyabean	Rain fed	JG-11	-	18.18
S16	Sangareddy	Munipally	Munipally	17.7776	77.8624	Red	Maize	Flood	JG-11	-	12.12

S17			Chilepalle	18.7566	78.2376	Red	Maize	Flood	JG-11	-	12.12
S18		Kandi	Kandi	17.5826	78.1089	Black	Maize	Flood	JG-11	Weeding and fertilizer application	9.09

Table 2: Cultural and morphological characteristics of isolates of *F. oxysporum* f. sp. *ciceris* isolated from different Bengal gram growing areas of Telangana

Isolate Name	Location	Mycelial arrangement and colour	Pigmentation of substrate	Radial growth (mm)	Type of growth	Sporulation	Chlamydospore diameter (µm)	Macro conidia			Micro conidia		
								Size (µm)	Sepatation	Shape	Size (µm)	Sepatation	Shape
FOC1	Kamareddy	cottony white mycelium	Light yellow	80 mm	moderate	++	9.77	21.895×2.927	2-3	Sickle	4.823×1.767	0-1	Oval
FOC2	Gadwal	dense cottony white mycelium	Light yellow	80 mm	moderate	++	8.56	20.331×3.213	2-3	Sickle	4.544×1.514	0-1	Oval
FOC3	Sangareddy	pinkish white mycelium with concentric rings	Purple	83 mm	moderate	++	10.10	22.690×2.450	3-4	Sickle	5.198×2.327	0-1	Oval
FOC4	Nizamabad	cottony white mycelium	Light yellow	78 mm	moderate	+	6.83	18.645×2.039	2-3	Blunt	4.291×1.596	-	Oval
FOC5	Nirmal	Fluffy cottony white mycelium	Pale yellow	86.5 mm	fast	+++	10.65	24.725×3.616	3-4	Sickle	5.420×2.327	0-1	Oval
FOC6	Adilabad	creamy white mycelium	Creamy white	75 mm	slow	+	5.48	16.631×2.091	2	Blunt	3.570×1.596	-	Oval

Table 3: Cultural and morphological characteristics of major fungal isolates found in chickpea Rhizosphere

ID	Organism	Cultural characters	Morphological characteristics
F-1	*Aspergillus spp.*	Cream colour culture	Conidia spores produced on globose conidiophores
F-2	*Aspergillus spp.*	Cream brown coloured appressed culture	Conidia chains directly attached to globose vesicles
F-3	*Aspergillus spp.*	white colony colour with yellow sporulation	Dark brown conidia spores, conidiophore becomes dark at the apex and terminating in a globose vesicle
F-4	*Fusarium spp.*	Dull white appressed culture	Oval Conidia with no septa with chlamydospores formation
F-5	*Aspergillus spp.*	Cream colour culture with violet sporulation	Conidia spores produced on globose conidiophores
F-6	*Aspergillus spp.*	dull brown culture	Conidia chains directly attached to globose vesicles
F-7	*Chaetomium spp.*	Black colour appressed culture with irregular margins	Perithecia with ascospores
F-8	*Trichoderma spp.*	White colour culture	Globose conidia produced on bottle shaped conidiophores
F-9	*Chaetomium spp.*	Initially white later turning into dull green colour	Perithecia with ascospores
F-10	*Chaetomium spp.*	Initially white later turning into black colour	Perithecia with ascospores
F-11	*Aspergillus spp.*	Initially white later turning into light green colour	Conidia globose-shaped
F-12	*Aspergillus spp.*	Dirty Green colour culture	Conidia globose-shaped
F-13	*Penicillium spp.*	Dark green appressed culture	Conidiophores arising from the mycelium branched near the apex, penicillate, ending in a group of phial ides
F-14	*Penicillium spp.*	Shiny green culture	Conidiophores arising from the mycelium branched near the apex, penicillate, ending in a group of phial ides
F-15	*Aspergillus spp.*	Profuse cottony white culture with brown sporulation	Conidia spores produced on globose conidiophores
F-16	*Trichoderma spp.*	White-coloured culture turns to green	Globose conidia produced on bottle shaped conidiophores
F-17	*Trichoderma spp.*	White-coloured culture turns to green	Globose conidia produced on bottle shaped conidiophores
F-18	*Trichoderma spp.*	White-coloured culture turns to green	Globose conidia produced on bottle shaped conidiophores

Table 4: Cultural and morphological characteristics of rhizosphere isolates isolated on nutrient agar medium

Bacterial isolates ID	Shape	Margin	Elevation	Size	Texture	Appearance	Pigmentation	Optical property	Gram staining	shape
NS1B1	Irregular	Wavy	Raised	Pinpoint	Moist	Veined	Dull white	Opaque	+	Rod
NS1B2	Circular	Entire	Raised	Moderate	Mucoid	Smooth	Creamy white	Opaque	+	Coccus
NS2B3	Circular	Wavy	Slightly raised	Large	Butyrous	Smooth	peach	Opaque	+	Coccus
NS3B4	Irregular	Entire	Raised	Pinpoint	Mucoid	Smooth	Dull white	Opaque	+	Coccus
NS3B5	Irregular	Entire	Flat	Moderate	Mucoid	Smooth	white	Opaque	+	Coccus
AS1B6	Circular	Entire	Flat	Moderate	Dry	Smooth	white	Opaque	+	Coccus
AS2B7	Circular	Wavy	Raised	Moderate	Moist	Smooth	Dull yellow	Opaque	+	rod
AS2B8	Irregular	Wavy	Slightly raised	Small	Butyrous	Shiny	yellow	Opaque	+	Rod
AS2B9	Irregular	Wavy	Raised	Moderate	Moist	Veined	Dull white	Opaque	+	rod
AS3B10	Circular	Entire	Raised	Moderate	Mucoid	Smooth	white	Opaque	+	rod
AS4B11	Circular	Entire	Flat	small	Mucoid	Smooth	Dull white	Opaque	+	Coccus
KS1B12	Circular	Entire	Raised	Moderate	Mucoid	Smooth	white	Opaque	+	Coccus
KS1B13	Irregular	Wavy	Slightly raised	Large	Butyrous	Wrinkled	Dirty white	Opaque	+	Coccus
KS1B14	Circular	Entire	Raised	Moderate	Mucoid	Smooth	white	Opaque	+	Coccus
KS2B15	Circular	Wavy	Flat	Moderate	Mucoid	Smooth	white	Opaque	+	Coccus

KS2B16	Irregular	Wavy	Flat	Moderate	Mucoid	Smooth	Dull white	Opaque	-	Coccus
KS2B17	Irregular	Entire	Raised	Moderate	Mucoid	Brittle Rough	white	Opaque	-	Coccus
KS3B18	Circular	Entire	Slightly raised	Small	Butyrous	Shiny	yellow	Opaque	-	Rod
KS3B19	Circular	Wavy	Flat	Moderate	Mucoid	Smooth	Dull white	Opaque	-	Rod
KS3B20	Circular	Entire	Raised	Moderate	Mucoid	Smooth	white	Opaque	-	Rod
GS1B21	Irregular	Wavy	Slightly raised	Large	Butyrous	Veined	Dull white	Opaque	+	Rod
GS1B22	Circular	Entire	Raised	Moderate	Mucoid	Smooth	peach	Opaque	+	Rod
GS2B23	Circular	Entire	Raised	pinpoint	Mucoid	Smooth	Dull white	Opaque	+	Coccus
GS2B24	Irregular	Wavy	Raised	Moderate	Mucoid	Veined	white	Opaque	+	rod
GS2B25	Circular	Entire	Raised	Large	Mucoid	Smooth	Dull yellow	Opaque	+	Coccus
GS3B26	Circular	Entire	Flat	Moderate	Mucoid	Smooth	Dull white	Opaque	+	Coccus
GS3B27	Circular	Entire	Flat	small	Mucoid	Smooth	white	Opaque	+	Coccus
NMS1B28	Circular	Entire	Raised	Moderate	Mucoid	Brittle Rough	white	Opaque	+	Coccus
NMS1B29	Circular	Wavy	Raised	small	Mucoid	Smooth	white	Opaque	+	Rod
NMS2B30	Irregular	Wavy	Flat	Moderate	Mucoid	Smooth	white	Opaque	+	
NMS2B31	Circular	Entire	Slightly raised	Small	Butyrous	Shiny	yellow	Opaque	+	Coccus
NMS3B32	Irregular	Entire	Raised	Moderate	Mucoid	Smooth	white	Opaque	+	Coccus
NMS3B33	Circular	Entire	Raised	Moderate	Mucoid	Smooth	Dirty white	Opaque	+	Coccus
NMS3B34	Circular	Wavy	Flat	Large	Moist	Smooth	Dull yellow	Opaque	+	Coccus
SS1B35	Irregular	Entire	Raised	Moderate	Mucoid	Brittle Rough	Dull yellow	Opaque	+	Coccus
SS1B36	Circular	Entire	Slightly raised	Small	Butyrous	Shiny	yellow	Opaque	+	Rod
SS2B37	Circular	Entire	Raised	Moderate	Mucoid	Veined	white	Opaque	+	Rod
SS2B38	Circular	Wavy	Flat	pinpoint	Mucoid	Smooth	white	Opaque	+	Rod
SS2B39	Irregular	Entire	Flat	small	Mucoid	Smooth	white	Opaque	+	Rod
SS3B40	Circular	Entire	Raised	Moderate	Mucoid	Smooth	white	Opaque	-	Coccus

Table 5: Biochemical characterization of rhizosphere isolates isolated on nutrient agar medium and actinomycetes isolation agar medium

Bacterial isolates ID	Indole production test	Methyl red test	Voges Proskaeur test	Oxidase test	Catalase test	KOH test
NS1B1	-	-	+	+	+	+
NS1B2	-	-	+	+	+	+
NS2B3	-	-	+	+	+	+
NS3B4	-	+	+	+	+	+
NS3B5	-	+	+	+	+	+
AS1B6	-	+	+	+	+	+
AS2B7	+	+	+	+	+	+
AS2B8	+	+	-	+	+	+
AS2B9	+	+	-	+	+	-
AS3B10	-	-	-	+	+	-
AS4B11	-	-	+	-	-	-
KS1B12	+	-	+	+	+	+
KS1B13	+	-	+	+	+	+
KS1B14	-	-	+	+	+	+
KS2B15	-	-	+	+	+	+
KS2B16	-	-	+	+	+	-
KS2B17	-	-	+	-	-	-
KS3B18	-	-	-	+	-	-
KS3B19	-	-	-	+	+	-
KS3B20	-	-	-	+	+	-
GS1B21	-	-	-	+	+	-
GS1B22	-	-	-	+	+	+
GS2B23	-	-	+	+	+	+
GS2B24	-	-	+	+	+	+
GS2B25	-	-	+	+	+	+
GS3B26	-	-	+	+	+	+
GS3B27	-	-	+	+	+	+
NMS1B28	+	-	+	+	+	+
NMS1B29	-	-	+	+	+	+
NMS2B30	-	-	+	+	+	+
NMS2B31	-	-	+	+	+	+
NMS3B32	-	-	+	+	+	+
NMS3B33	-	-	-	+	+	+
NMS3B34	-	-	-	-	+	+
SS1B35	-	-	-	-	+	+
SS1B36	-	-	-	-	+	+
SS2B37	+	-	-	-	+	+
SS2B38	-	-	-	-	+	+

SS2B39	-	-	-	-	+	+
SS3B40	-	-	-	+	+	+
AI-1	-	-	-	+	+	+
AI-2	-	-	-	+	+	+
AI-3	-	-	-	+	+	+
AI-4	-	-	-	+	+	+
AI-5	-	-	-	+	+	+
AI-6	-	-	-	+	+	+
AI-7	-	-	-	+	+	+
AI-8	-	-	-	+	+	+
AI-9	-	-	-	+	+	+
AI-10	-	-	-	+	+	+

Table 6: Cultural and morphological characteristics of rhizosphere isolates isolated on actinomycetes isolation agar medium

S. No.	isolate id	Shape	Margin	Elevation	Size	Texture	consistency	Pigmentation	Optical property	Gram staining	shape
1	AI-1	irregular	irregular	raised	large	powdery	tough	whitish grey	opaque	positive	Rod
2	AI-2	irregular	regular	flat	small	mucoid	tough	yellow	opaque	positive	Rod
3	AI-3	irregular	irregular	flat	small	mucoid	tough	dull white	opaque	positive	Rod
4	AI-4	irregular	irregular	flat	small	mucoid	tough	dull white	opaque	positive	Rod
5	AI-5	circular	circular	raised	large	powdery	dry	chalk white	opaque	positive	Coccus
6	AI-6	irregular	regular	flat	large	powdery	dry	dull pink	opaque	positive	Rod
7	AI-7	irregular	circular	flat	small	mucoid	tough	black	opaque	positive	Coccus
8	AI-8	irregular	irregular	convex	large	powdery	dry	greyish pink	opaque	positive	Coccus
9	AI-9	irregular	irregular	flat	small	mucoid	tough	black	opaque	positive	Rod
10	AI-10	regular	irregular	flat	large	powdery	dry	greyish	opaque	positive	Rod

Table 7: Antagonistic activity of fungal isolates from chickpea rhizosphere on radial growth of *Fusarium oxysporum* f. sp. *Ciceris* by dual culture technique

Isolate	Radial Growth(cm)	Percent inhibition (%)
F-1	2.4	60.00(50.40)*
F-2	2.5	58.33(49.82)*
F-3	2.0	66.66(53.92)*
F-4	2.0	66.66(54.90)*
F-5	2.5	58.33(50.04)*
F-6	3.0	50.00(44.46)*
F-7	2.5	58.33(49.18)*
F-8	1.0	83.33(65.00)*
F-9	2.0	66.66(53.91)*
F-10	2.4	60.00(50.42)*
F-11	2.8	53.33(46.94)*
F-12	3.0	50.00(45.12)*
F-13	2.0	66.66(54.57)*
F-14	2.5	58.33(49.50)*
F-15	2.0	66.66(53.99)*
F-16	1.0	83.33(64.60)*
F-17	0.8	86.66(67.99)*
F-18	1.0	83.33(65.38)*
control	6.0	0.00(0.00)*
C.D.		4.35(2.63)*
S.E.(m)		1.51(0.91)*
C.V.		4.27(3.10)*

Table 8: Antagonistic activity of bacterial isolates from chickpea rhizosphere on radial growth of *Fusarium oxysporum* f. sp. *ciceris* by dual culture technique

Bacterial Isolates ID	Radial growth (cm)	Per cent inhibition (%)
NS1B1	1.5	80.00(62.17)*
NS1B2	3.5	53.33(46.63)*
NS2B3	2.5	66.66(53.64)*
NS3B4	2.5	66.66(54.44)*
NS3B5	2.5	66.66(53.91)*
AS1B6	2.5	66.66(54.44)*
AS2B7	3.0	60.00(50.23)*
AS2B8	2.8	62.66(51.79)*
AS2B9	1.5	80.00(63.09)*
AS3B10	3.7	50.66(45.10)*

AS4B11	2.3	69.33(55.25)*
KS1B12	3.0	60.00(51.27)*
KS1B13	3.8	49.33(44.09)*
KS1B14	3.5	53.33(46.63)*
KS2B15	2.5	66.66(53.91)*
KS2B16	2.0	73.33(58.03)*
KS2B17	2.3	69.33(55.53)*
KS3B18	2.5	66.66(54.17)*
KS3B19	4.0	46.66(42.56)*
KS3B20	3.0	60.00(51.27)*
GS1B21	1.0	86.66(67.83)*
GS1B22	2.0	73.33(58.31)*
GS2B23	3.0	60.00(51.27)*
GS2B24	1.5	80.00(62.48)*
GS2B25	2.5	66.66(53.64)*
GS3B26	2.8	62.66(51.79)*
GS3B27	2.3	69.33(55.53)*
NMS1B28	2.4	68.00(54.98)*
NMS1B29	4.3	42.66(41.79)*
NMS2B30	4.5	40.00(38.95)*
NMS2B31	2.7	64.00(51.01)*
NMS3B32	2.5	66.66(53.64)*
NMS3B33	3.5	53.33(44.85)*
NMS3B34	2.5	66.66(53.64)*
SS1B35	4.3	42.66(41.79)*
SS1B36	3.0	60.00(48.69)*
SS2B37	3.7	50.66(43.83)*
SS2B38	3.5	53.33(45.87)*
SS2B39	4.3	42.66(41.79)*
SS3B40	3.5	53.33(44.85)*
Control	7.5	0.00(0.00)*
C.D.		3.06(1.83)*
S.E.(m)		1.08(0.65)*
C.V.		3.18(2.25)*

Table 9: Antagonistic activity of actinomycetes isolates from chickpea rhizosphere on radial growth of *Fusarium oxysporum* f. sp. *ciceris* by dual culture technique

ID	Radial growth(cm)	Percent inhibition (%)
A-1	3.5	53.33(48.17)*
A-2	7.0	6.66(4.98)*
A-3	4.5	40.00(36.57)*
A-4	3.5	53.33(44.85)*
A-5	2.0	73.33(57.20)*
A-6	7.0	6.66(14.95)*
A-7	6.8	9.33(18.04)*
A-8	1.2	84.00(65.40)*
A-9	3.0	60.00(51.27)*
A-10	6.0	20.00(25.90)*
Control	7.5	0.00(0.00)*
C.D.		4.86(5.36)*
S.E.(m)		1.64(1.81)*
C.V.		7.98(9.41)*

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