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CHARACTERIZATION OF *FUSARIUM SOLANI* KUSF204 AS A POTENTIAL METAL TOLERANT PLANT GROWTH PROMOTING FUNGUS

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ABSTRACT

The work depicts characterization and plant growth promoting features of a fungal isolate, designated as KUSF204 which was recovered from a soil sample of Lochanpur agricultural field located in Raninagar block 2 of Murshidabad district in West Bengal. The isolate was identified as Fusarium solani on the basis of morphological characters and rDNA sequence analysis. The growth characteristics of the isolate was studied in four different culture media viz., potato dextrose agar (PDA), potato carrot agar (PCA), Czapek's Dox agar (CDA) and synthetic nutrient agar (SNA) medium. Maltose and glycine were found to be the best utilized carbon and nitrogen, respectively by the isolate. The isolate was able to produce indole acetic acid both in absence (50 µg/ml) and presence (350 µg/ml) of tryptophan (2000 ppm). It also produced gibberellin (1460 µg/ml) and siderophore (500 nmole/ml) under in vitro condition. It can tolerate heavy metals such as cadmium, copper, iron, nickel and zinc and exhibited by high tolerance index (TI) value. Minimum inhibitory concentrations (MIC) of the different heavy metals showed that the soil isolate was most tolerant to the iron and least tolerance was found in case of cadmium. Dual culture assay against two soil borne phytopathogens (Rhizoctonia solani and Fusarium oxysporum) also revealed the potential of the Fusarium isolate as an antagonist. The culture filtrate of the particular Fusarium isolate did not show any inhibitory effect on germination of gram, cucumber and rice seeds. Moreover, growth stimulatory effects of the fungal culture filtrate on the three plants were evidenced by the increase in percentage of seed germination and vigour index. Therefore, this soil isolate has tremendous potential to be exploited in the agricultural fields to increase crop yield.

KEY WORDS

Fusarium, culture media, phytohormones, siderophore, heavy metals, antagonist, plant growth promotion.

INTRODUCTION

Plant growth promoting fungi (PGPF) are non-pathogenic soil-inhabiting saprotrophs or symbionts or plant associated endophytes reported to improve growth upon root colonization in plants and to suppress soil borne diseases, similar to the plant growth promoting rhizobacteria [1]. They might be potentially applied in agriculture as biofertilizer since they stimulate plant growth through nutrient mineralization and/or as biostimulator for their growth promotive effect due to synthesis of phytohormones or transport in plants [2]. Indole acetic acid (IAA) and gibberellin (GA) are the major phytohormone that can be synthesized by diverse soil microorganisms including bacteria [3-4], fungi [5-6] and algae [7-8]. PGPF met their iron requirement by secreting siderophore that chelates iron and also supply iron to the plant. Siderophore is also helpful in suppression of disease caused by plant pathogens with inefficient iron uptake system. Some PGPF are reported to produce enzyme, 1-amino cyclopropane-1-carboxylate deaminase for lowering the action of ethylene in the plants [9]. Indirect mechanisms of plant

growth promotion involve their antagonistic activity to reduce the deleterious effects of plant pathogens on crop yield by producing antibiotics, cell wall degrading enzymes such as chitinase and the ability to induce systemic resistance. Competition between pathogenic and biocontrol strains for space and/or nutrients on the rhizospheric region is also important for biocontrol activity.

Heavy metal pollution in soil affects the activities of microorganisms and alters their population structure. Many filamentous fungi were reported to possess metal tolerance properties and become dominant organisms in some polluted habitats [10]. Fungi are furthermore known to accumulate high amounts of metals [11]. Due to this property, fungi have great importance for bioremediation of metals from natural environments [12].

Fusarium species are widely distributed in soil as well as in organic substrates and can utilize on wide range of substances [13]. They survive as free-living saprophytes in soils, and as pathogens and endophytes in plants. The non-pathogenic isolates with potential plant growth promoting attributes can be explored to improve crop productivity. Furthermore, metal tolerance property of the PGPF is of great advantage for their application in metal contaminated agricultural field.

To view this objective, the present study dealt with isolation of a *Fusarium* sp., characterization of its metal tolerance property and exploration on its ability to promote plant growth by way of producing phytohormones and metabolites like siderophore which are potentially involved in pathogen suppression in agricultural fields. The study was also conducted to formulate an optimal basal medium for growth of the fungus for its commercial exploitation at a later stage. This work also pertains to the study of optimum physiological requirements (carbon and nitrogen sources) of the *Fusarium* isolate.

MATERIALS AND METHODS

Collection of soil sample: A soil sample was collected from the rhizospheric region of the rice plant located in Lochanpur village of Raninagar block 2 in Murshidabad district, West Bengal, India. The agricultural field was placed in close vicinity of Padma river near Indo-Bangladesh boarder region and cultivated for several crops throughout the year where no *Fusarium* diseases were reported previously.

Isolation and identification of fungus: The soil was screened for isolation of the fungi by dilution plate technique on selective peptone PCNB agar medium [composition (g/l): peptone 15, KH_2PO_4 1.0, $MgSO_4$, $7H_2O$ 0.5, PCNB 1.0, agar 20, pH 6] supplemented with streptomycin sulphate 1.0 g/l and neomycin sulphate 0.12 g/l. The plates were incubated at 28°C for 5-7 days until visible sign of colony growth occurred. Fungal isolates were identified by observing their colony morphology, sporulation and pigmentation on Czapek's Dox agar (CDA) medium [14]. One promising isolate was further identified based on rDNA gene analysis. For this, genomic DNA was extracted and used as template for amplification of the rDNA region using the primer pair ITS1 (TCCGTAGGTGAACCTGCGG) and ITS2 (GCTGCGTTCTTCATCGATGC). The amplified product was sequenced, and the sequence was analysed using Nucleotide BLAST function at NCBI [15] to find similarity of the sequence with nucleotide database. Phylogenetic tree was constructed using the software MEGA6 [16]. Multiple sequences alignment was carried using CLUSTALW and the evolutionary history was inferred using the Neighbour-joining method [17].

Study on effect of culture media on growth and sporulation: The fungal isolate was grown separately on four culture media viz., potato dextrose agar (PDA), potato carrot agar (PCA), Czapek's Dox agar (CDA) and synthetic nutrient agar (SNA) medium at 28°C for 7 days to find out the most suitable one for growth and sporulation of the isolate. The composition of potato dextrose agar (PDA) medium was (g/l) [grated potato 200, dextrose 20, agar 20, pH 6]. The composition of potato carrot agar (PCA) medium was [composition (g/l): grated potato 20, grated carrot 20, agar 15, pH 6]. The composition of CDA medium was (g/l) K_2HPO_4 1, $MgSO_4$, $7H_2O$ 0.5, KCl 0.5, $NaNO_3$ 2, sucrose 30, agar 20. The composition of SNA medium was (g/l) KH_2PO_4 1, $MgSO_4$, $7H_2O$ 0.5, KCl 0.5, $NaNO_3$ 1, glucose 0.2, sucrose 0.2, agar 20. Reproductive structures of the isolate were studied through microscopic observation following cotton blue staining.

Study on utilization of carbon and nitrogen sources: Carbon source utilization was studied by using the carbohydrate free Czapek's Dox broth supplemented with 2% of the respective carbon sources viz., dextrose, maltose, lactose, sucrose, mannitol, sorbitol, cellulose and starch with respect to a control having no carbon source. Likewise, nitrogen source utilization was studied

using nitrogen free Czapek's Dox broth supplemented with 0.2% of the various nitrogen sources viz., sodium nitrate, potassium nitrate, sodium nitrite, ammonium nitrate, glycine, tryptophan, asparagine and glutamine. Two control sets one without carbohydrate and another without nitrogen source were also prepared. Mycelial disc of 5 mm was inoculated in each of the flask and incubated at 28ºC for 14 days. The data of mycelial dry weight was taken to determine the growth of the isolate.

Metal tolerance study: Tolerance against heavy metals viz., Cd, Cu, Fe, Ni and Zn was studied by determination of tolerance index (TI) and minimum inhibitory concentration (MIC). The fungal isolate was grown in potato dextrose (PD) broth supplemented with increasing concentration of each metal at 50 ppm interval. A control set without any metal supplementation was also inoculated. Incubation was conducted at 28°C for two weeks. Growth of the fungal isolate was monitored by measuring the mycelial dry weight. TI was calculated as the percentage value of the ratio of the dry weight of treated culture to that of untreated culture. Three concentrations (50, 100 and 150 ppm) of each of the heavy metals were used for evaluation of TI. MIC was determined as the lowest concentration of metal that was found to inhibit visible growth of the isolate. The fungal isolate was also tested for its ability to tolerate all the heavy metals present together in PD broth at three different concentrations (5, 15 and 25 ppm) with respect to control. Tolerance indices of the soil isolate were calculated accordingly.

IAA estimation: The fungal isolate was grown in PD broth supplemented with three different concentrations of tryptophan viz, 1000 ppm, 1500 ppm and 2000 ppm at 28ºC and in a control set having no tryptophan. After 14 days of incubation, concentration of IAA in the culture filtrate was estimated using Salkowski reagent. One ml of the supernatant was mixed with 2 ml of Salkowski reagent (2 ml of 0.5 M $FeCl_3$ + 98 ml 35% $HClO_4$) and the intensity of red colour developed after 30 minutes was measured at 530 nm. The concentration was determined using standard solution of IAA.

Assay of GA production: Efficacy of gibberellin production by the fungal soil isolate was assayed by growing it in Czapek's Dox broth at 28ºC for 30 days and the amount of gibberellin in the culture supernatant was determined by spectrophotometric method using phosphomolybdic acid reagent [85 % molybdic acid 35 g, sodium tungstate 5 g, 10 % sodium hydroxide 200 ml, 85 % concentrated phosphoric acid 125 ml, distilled water upto 500 ml]. One ml of the supernatant sample was taken, mixed with 15 ml of phosphomolybdic acid reagent and placed in a boiling water bath for 1 hour. After that, the temperature of the flask was reduced to room temperature and then final volume was made to 25 ml with distilled water. The absorbance of blue color developed was measured at 780 nm using distilled water as blank and the concentration was determined using a standard curve prepared from the standard solution of gibberellin [18].

Bioassay of plant growth promotion: Fungal isolate was grown in CD broth for 30 days and culture supernatant was taken to study its effect on plants. Seeds of paddy, gram and cucumber were surface sterilized with 0.1 % mercuric chloride and then washed three times with sterile distilled water. After washing, the seeds were suspended in the culture supernatant and kept in dark at 4ºC for 24 hours. A control set was prepared using uninoculated CD broth. On the next day, seeds were removed from culture filtrate, washed with sterile distilled water, and 10 seeds were placed on each of the pre-soaked blotting paper in separate petridishes. The sets were kept for 7 days in well-illuminated place. Germination percentage, root length, shoot length and vigor index (VI) [= (Length of shoot + root) X Germination %] of the seeds were calculated.

Siderophore production test: Fungal isolate was grown in Czapek's Dox broth (without addition of $FeSO_4$) at 28°C for 30 days and the culture filtrate was assayed for detection siderophore using CAS reagent [19] with desferal as standard. Chemical nature of the siderophore was determined by the method of Czaky [20] and Arnow [21] for hydroxamate and catecholate type, respectively.

Study on antagonism of the fungal isolate against phytopathogenic fungi: The fungal isolate was tested for its antagonistic property against two soil borne fungal pathogens viz., *Fusarium oxysporum* and *Rhizctonia solani* by dual culture technique [22]. Mycelial discs (5 mm) from each pathogen and the antagonist were placed 3 cm apart on fresh PDA plates and the plates were kept at 28°C for 7 days. The antagonism effect was determined by observing inhibition of mycelial growth of the pathogen and percentage of inhibition (I) was measured using the formula: I = (R –

r)/R X 100; where r = radius of the pathogen colony opposite the antagonist colony and R = maximum radius of the pathogen colony in untreated (control) plate.

RESULTS AND DISCUSSION

Isolation and identification of the fungus: On selective peptone PCNB agar medium the several fungal colonies with similar morphology had appeared. One fungal isolate, designated as KUSF204 was selected for further study. On CDA medium the fungal isolate showed slow growth, white, circular and compact mycelial colony having no pigmentation (Fig. 1). It produced curved macroconidia (16.25-26.25 µm X 3.12-3.75 µm) having 3-5 septa with tapering apical cell and foot shaped basal cell (Fig. 1). Microconidia were lacking. Chlamydospores were abundant, globose in shape (10-12.5 µm in diameter) intercalary or terminal in position (Fig. 1). They were found singly, in pairs, clusters as well as in chains. Molecular identification of the fungal isolate KUSF204 was performed based on rDNA sequence analysis. ~ 500 bp amplicon of rDNA region of the isolate was observed on agarose gel and a stretch of 229 bp had been sequenced. Search for sequence homology through nucleotide BLAST function in NCBI database was performed and maximum identity (99%) was found with the rDNA sequence of *Fusarium solani*. When a phylogenetic tree was constructed by Neighbour-joining method based on rDNA sequence of KUSF204 and other similar sequences obtained from BLAST search and one outgroup as *Alternaria* sp., relatedness of KUSF204 with related fungal species was observed and it belonged to the same evolutionary branch with *Fusarium solani* (Fig. 2). Based on all these key specifics, the fungal isolate was identified as *Fusarium solani.* The partial rDNA region of the fungal isolate KUSF204 had been submitted to the genebank under the accession no. KY593923.

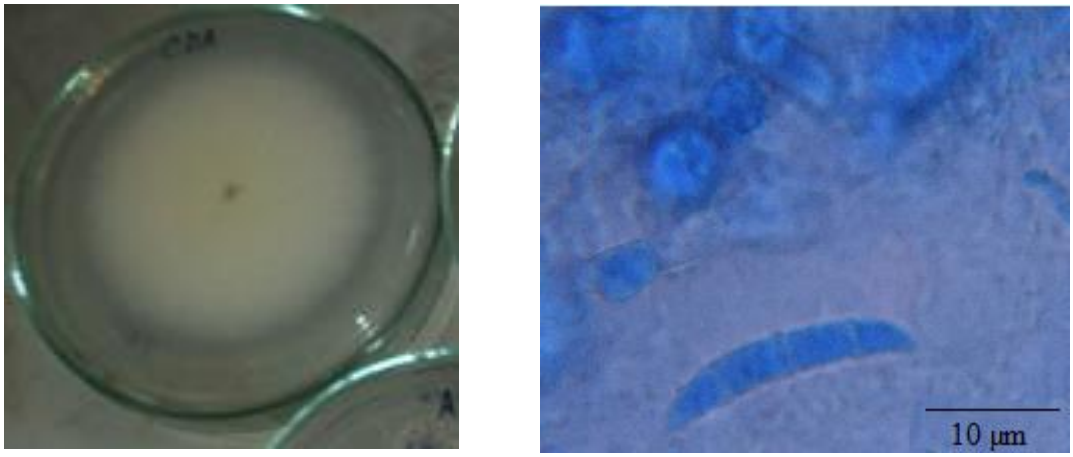

Fig. 1: Growth of the *Fusarium* isolate on CDA medium (left); cotton blue stained slide showing macroconidia and chlamydospore (right) of *Fusarium* isolate

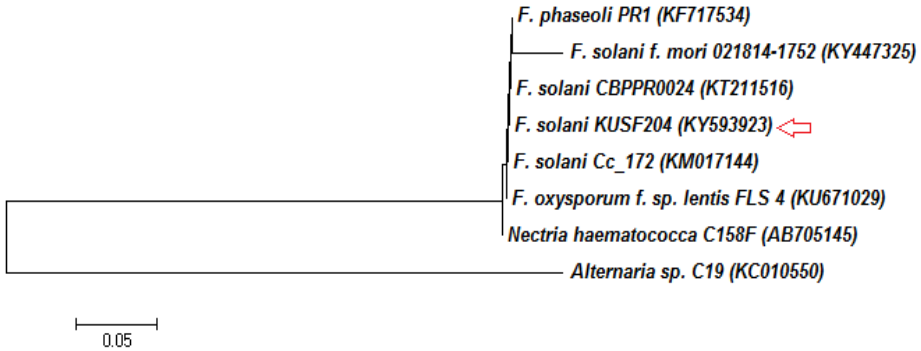

Fig. 2: Phylogenetic tree of *Fusarium solani* KUSF204 showing relatedness with other fungal species based on rDNA sequence comparison, where *Alternaria* sp. was used as an outgroup. The topology of the tree was estimated by bootstraps based on 1000 replications. The numbers at the branch points are the percentage support by bootstraps. Bar represents 1% sequence divergence. Except for the sequence determined in this study, all rDNA sequences were retrieved from GenBank. GenBank accession numbers are included in parentheses.

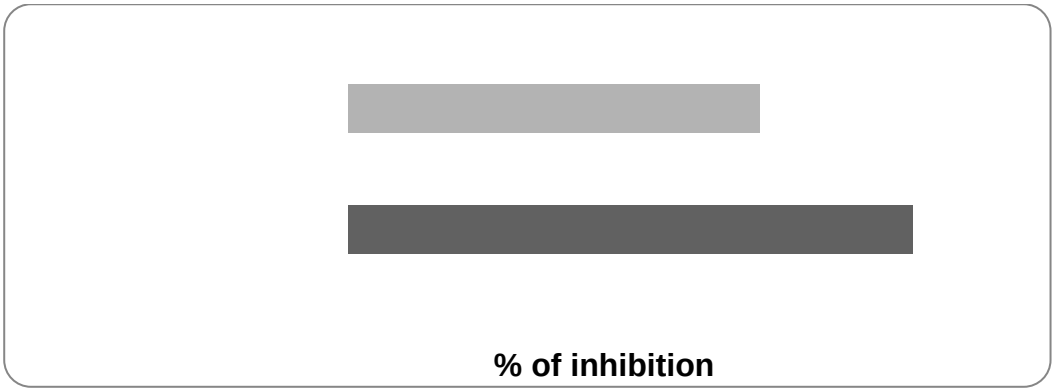

Fig 3: Percentage of growth inhibition of soil borne fungal pathogens by *F. solani* KUSF204

Effect of culture media: Among the four-culture media tested, potato carrot agar (PCA) medium was found to be most conducive in terms of both growth (colony diameter 73 mm after 7 days of incubation) and sporulation. SNA medium was also equally found to be favourable for the growth of the *Fusarium* isolate. Surprisingly, the *Fusarium* species did not grow well on either of the PDA and CDA medium. The sporulation was extremely sparse on PDA medium. The fungal isolate did not produce any pigment on any of the culture media tested (Table 1). Our results conform to the findings of Yonggang [23] where PDA and CDA media were found to influence mycelial growth of *Fusarium semitectum* moderately. Earlier, it was reported that PDA and Richard's agar media supported best mycelial growth of *F. udum* [24, 25]. Maximum growth and sporulation of *F. oxysporum* f. sp. *vanillae* were on PDA, Richard's agar and Czapek's Dox agar [26].

Table 1: Growth and sporulation of *F. solani* KUSF204 on different culture media

Culture media	Colony diameter (mm)	Pigmentation	Sporulation
PDA	21	nil	sparse
PCA	73	nil	abundant
CDA	42	nil	moderate
SNA	71	nil	abundant

Table 2: Utilization of carbon and nitrogen sources by *F. solani* KUSF204

Carbon sources	Mycelial dry wt. (g)	Nitrogen sources	Mycelial dry wt. (g)
Maltose	0.201	Sodium nitrate	0.127
Dextrose	0.130	Potassium nitrate	0.097
Lactose	0.153	Sodium nitrite	0.073
Sucrose	0.143	Glycine	0.267
Mannitol	0.130	Ammonium nitrate	0.087
Sorbitol	0.096	Tryptophan	0.179
Starch	0.046	Glutamine	0.064
Cellulose	0.047	Asparagine	0.139
Control	0.018	Control	0.010

Table 3: Metal tolerance of *F. solani* KUSF204

Heavy Metals																						
Cd				**Cu**				**Fe**				**Ni**				**Zn**				All the five metals present together		
Tolerance index				**Tolerance index**				**Tolerance index**				**Tolerance index**				**Tolerance index**				**Tolerance index**		
050 ppm	100 ppm	150 ppm	**MIC (ppm)**	050 ppm	100 ppm	150 ppm	**MIC (ppm)**	50 ppm	100 ppm	150 ppm	**MIC (ppm)**	050 ppm	100 ppm	150 ppm	**MIC (ppm)**	050 ppm	100 ppm	150 ppm	**MIC (ppm)**	05 ppm each	15 ppm each	25 ppm each
73.7	80.8	28.3	200.0	54.1	59.6	66.1	300.0	111.1	106.1	75.8	300.0	69.0	85.3	64.7	250.0	81.7	95.0	96.7	425.0	75.3	97.5	3.7

Table 4: Phytohormones and siderophore production by *F. solani* KUSF204

IAA production (µg/ml)				**GA production (µg/ml)**	**Siderophore production (nmole/ml)**
Tryptophan concentration					
0 ppm (Control)	1000 ppm	1500 ppm	2000 ppm		
50	175	325	350	1460	500

Table 5: Growth promoting effects of the *F. solani* KUSF204 on different seeds

	Gram				**Cucumber**				**Paddy**			
Experi-mental sets	% of germination	Root length (mm)	Shoot length (mm)	Vigor index	% of germination	Root length (mm)	Shoot length (mm)	Vigor index	% of germination	Root length (mm)	Shoot length (mm)	Vigor index
With organism	100	56	10	46.0	80	101	110	168.8	100	20.8	3.2	24.0
Without organism	80	23	6	23.2	70	91	80	119.7	80	19.4	0.9	16.2

Utilization of carbon and nitrogen sources: Utilization of each of the eight-different carbon and nitrogen sources by the isolate were recorded and presented in the Table 2. The isolate was found to be capable of utilizing all the carbon and nitrogen sources to a varied extent. Maltose, lactose, sucrose and dextrose were mostly preferred by the *Fusarium* soil isolate (Table 2). Significantly, the tested *Fusarium* soil fungus could also utilize sugar alcohol viz., mannitol and sorbitol to a considerable extent. But polysaccharides were least preferred by the soil isolate. Both the homopolysaccharide (cellulose) and complex heteropolysaccharide (starch) were utilized to the same little extent. In control, where the carbon source was lacking, the growth of the isolate ceased. These results were similar to the findings where sucrose was best utilized followed by maltose, starch, glucose, fructose and cellulose by *F. oxysporum* causing cotton wilt [27]. The fungus may possess enzymatic mechinary for conversion of complex carbon compounds to simple forms, which may be metabolized readily [28]. It was also reported that sucrose was the best carbon source for the growth of *Fusarium solani* followed by lactose, maltose, dextrose and fructose [29].

Out of eight different forms of nitrogen sources utilized, glycine was found to be the best nitrogen source for the growth and development of the *Fusarium* species (Table 2). The soil isolate also preferred the other amino acid tryptophan as a potential nitrogen source. The nitrate ($NaNO_3$, KNO_3 and NH_4NO_3) and nitrite ($NaNO_2$) sources were also moderately utilized by the *Fusarium* isolate. The results are in agreement regarding use of

ammonium nitrate by *F. oxysporum* causing cotton wilt. Out of the 10 nitrogeneous compounds tested against *F. oxysporum* f.sp. *elaeidis,* good growth and sporulation were recorded in presence of nitrates of sodium, ammonium and potassium, peptone and DL-leucine [30]. While the fungus grew moderatly in ammonium sulphate, calcium nitrate, L-asparagine and DL- aspartic acid, sporulation was sparse in these compounds and chlamydospore formation induced in presence of ammonium sulphate [27]. Among the two-amide nitrogen, asparagine was found to be favorable for mycelial growth of the fungal isolate. The results were also in agreement with of *F. oxysporum* f.sp. *aurentifoliae* which showed good growth on D-leucine and aspargine [31]. It was also reported that aspargine supported maximum mycelial growth of *F. solani* [32]. However, ammonium nitrate and glutamine had minimum influence on the growth of the isolate. This was in conformity with the results reported by Agarwal [33]. Similar results were reported where the best utilized carbon for *F. semitectum* was glucose, and the nitrogen sources were glycine and NH_4NO_3 [22].

Metal tolerance of the *Fusarium* sp:

Tolerance index and minimum inhibitory concentrations of different heavy metals against the *Fusarium* sp. are presented in Table 3. *Fusarium* sp. was found to tolerate Zn and Fe mostly as was evident by increased tolerance index values. Zn is an essential element required for physiological function and growth of organisms, although at higher concentrations it can be toxic**.** Iron is highly toxic for biological substrates due to its high oxidative potential with its ability to generate reactive oxygen species (ROS). Fungi have evolved special mechanism for selective uptake of iron by way of producing iron chelator called siderophore. The fungal isolate could also tolerate the presence of Ni and Cu moderately but in presence of Cd at 150 ppm concentration, the growth of the fungus declined sharply. Cadmium is highly toxic to organisms even at very low concentration and can cause cellular damage. It inhibits DNA replication and makes it more susceptible to nucleolytic attack [34]. Similar results were obtained when the influence of lead, copper, zinc and cadmium was studied on the growth of *F. oxysporum*, and the greatest influence on the inhibition of growth was exhibited by cadmium, whereas the least influence was exhibited by zinc [35-36]. High metal ion concentration causes a reduction in growth and increased the length of the lag phase compared to the control. In contrast to these results, the influence of cadmium on the mycelium growth of *F. oxysporum* showed that it was tolerant to the presence of cadmium [37].

Interestingly, the mycelial dry weight of the soil isolate increased with increasing concentrations of Zn and Cu. Copper tolerance in fungi ascribed to diverse mechanisms involving trapping of the metal by cell wall components, chelation or precipitation by extracellular metabolites, and intracellular complexing by metallothioneins and phytochelatins [38]. In case of Ni, Fe and Cd the dry weight increased only up to 100 ppm concentration and later declined at 150 ppm concentration. Ni (II) is more toxic against all living systems. Ni negatively influenced soil microbial communities [39]. Highest MIC value as found in case of Zn. Expectedly, the minimum inhibitory concentrations of Cu and Fe were also in the higher range (300 ppm in each). The *Fusarium* isolate could even tolerate all the five heavy metals in mixture each at a concentration of 5 ppm as well as 15 ppm, but the mixture of the five heavy metals each at a concentration of 25 ppm showed inhibitory effect (Table 3).

IAA production by the *Fusarium* sp.: The *Fusarium* soil isolate was found to possess IAA producing ability. The fungal species produced IAA both in presence and absence of the precursor molecule. Significantly, with the increase in tryptophan concentrations, IAA production was also increased significantly in the *Fusarium* isolate (Table 4). At 2000 ppm tryptophan concentration, IAA production was maximum (350 µg/ml). *Fusarium oxysporum* isolated from root regions of *Corchorus olitorius*, *Sesamum indicum* and *Glycine max* also produced IAA (100-140 µg/ml) [6] . Since the *Fusarium solani* KUSF204 produced IAA both in absence and presence of tryptophan, tryptophan-independant as well as tryptophan-dependent pathways of IAA biosynthesis may exist in this species as in *Azospirillum* [40] and *Anabaena* [8].

GA production by the *Fusarium* sp.: Gibberellin production by the *Fusarium solani* KUSF204 has been estimated and depicted in Table 4. In our findings, spectrophotometric analysis showed presence of GA in the culture filtrate of the *Fusarium* species. The soil isolate was found to be a moderate GA producer (1460 µg/ml). Our results did conform to the findings where *Fusarium fujikuroi* isolate SG2 produced gibberellins

(1175 µg/ml) in modified medium having low concentration of nitrogen and carbon [41]. Microbial synthesis of gibberellin by using various *Fusarium* strains has been studied. Among these, *F. moniliforme* was found most impressive fungus for the production of gibberellin. GA_3 was also reported to be produced by *F. oxysporum* [42], *F. avenaceum* [43], *F. graminearum, F. solani* and *F. semitectum* [44] , and *F sacchari* [45].

Effect of *Fusarium* culture filtrate on seed germination and seedling growth: The effect of *Fusarium* culture filtrate on the growth attributes of *Pisum sativum, Cucumis sativa* and *Oryza sativa* have been depicted in Table 5. The culture filtrate of the *Fusarium* soil isolate was found to be stimulatory on the germination of all the three different treated seeds. The fungal isolate was also assayed on the seeds for their growth promoting capacity in terms of increasing shoot and root lengths of the seedling plant. Gram, cucumber and paddy seeds were chosen for the bio-assay experiment as they lack seed dormancy with high germination rate and easily availability. In addition, paddy seeds have multiseriate aleurone layer surrounding the endosperm which is the principle site for gibberellin activated α-amylase synthesis. This hydrolytic enzyme is involved in solubilization of endosperm reserves and thus helps in seed germination.

Seed germination in all the inoculated treatments increased conspicuously as compared to the control. There was also early onset of seed germination in the inoculated seeds as well. Cent percent seed germination was noticed in the treated seeds of gram and paddy. There were also marked differences in the root and shoot lengths of the treated seeds as compared to the untreated seeds. This was clearly evident by the increased vigor index value in treated seeds (Table 5). Comparison of the vigor index in different treatments with control yet again proved that the culture filtrate of *Fusarium solani* KUSF204 significantly promoted the seedling growth possibly with the aid of phytohormones (IAA and GA) produced substantially in the culture filtrates. Current results also confirm previous reports of shoot length promotion through microbial culture filtrate treatment [46]. It was also reported that the culture filtrate obtained from *F. oxysporum udum* isolated from pigeon pea *(Cajanus cajan*) var. BDN-708 increased seed germination (90%) and also root-shoot length as compared to other varieties [47]. The results may help to explain the beneficial effects of fungi to host plants and the role of microbial synthesis of phytohormones to improve crop productivity as well as soil fertility as reported earlier [48-49].

Siderophore production by the *Fusarium* sp.: The fungi present in the rhizospheric regions are more potent siderophore producer. The average amount of siderophore produced by fungi isolated from rhizosphere soil was found 1.2 fold higher than others [50]. Fungal siderophores are generally of the hydroxamate in nature which might be either of three types, ferrichrome, coprogen, fusarinine. *Fusarium roseum* strain ATCC 12822 produced malonichrome, a ferrichrome siderophore [51], *F. oxysporum* strain FGSC 9935 reported to produce three different ferrichrome type siderophores: ferricrocin, ferrichrome C, and malonichrome [52]. In this study *Fusarium solani* KUSF204 was found positive for siderophore production (Table 4). The *Fusarium* species produced siderophore 500 nmole/ml. This is also in conformity with the results where it was reported that the rhizosphere fungi viz., *Penicillium* sp*., Trichoderma* sp. and *Beauveria bassiana,* showed higher production of siderophore of 90.89%, 92.33%, and 89.44%, respectively [47]. The type of siderophore produced by the *Fusarium* species was solely hydroxamate as has been revealed by Czaky assay. Hydroxamate siderophore production was also reported in a number of *Aspergillus* spp. and *Penicillium chrysogenum* [53].

Antagonism of *Fusarium* sp.: Fungal antagonists suppress growth of plant pathogens through antibiosis, competition for nutrients and hyperparasitism [54]. In dual culture assay, *Fusarium solani* KUSF204 was found to inhibit the growth of the two-soil borne phytopathogens viz., *Rhizoctonia solani* and *Fusarium oxysporum* to a reasonable extent with percentage of inhibition (I) estimated as 85% and 62%, respectively (Fig 3). Maximum antagonism was observed against *Rhizoctonia solani* by the soil isolate inhibiting both mycelial growth and sclerotia formation. Although the antagonism mechanism of the *Fusarium* isolate was not identified in this study, it might be suggested that their antagonism must have involved the production of siderophore as well as other antifungal metabolites. Siderophore directly stimulates the biosynthesis of other antibiotic compounds by increasing the bioavailability of iron and other minerals which would suppress the growth of pathogenic microorganisms [55]. After the additional incubation period, the pathogenic

mycelia did not cover the surface of the tested soil isolate, which indicates that the antagonism was very strong. It was reported that *Fusarium* isolate JDF12 produced siderophore and exhibited antifungal activity against a number of phytopathogens [56]. It was suggested that microbial siderophore stimulate plant growth directly by increasing the availability of iron in the rhizospheric region or indirectly by competitively inhibiting the growth of plant pathogens with less efficient iron uptake system.

CONCLUSION

From our study, it can be concluded that *Fusarium solani* KUSF204 is a nonpathogenic saprophytic soil fungus which could tolerate different concentrations of a wide variety of heavy metals and also has the potential to produce phytohormones adequately. The *Fusarium* species is also peculiar in the sense that it produced an iron chelator called siderophore which negatively influences the microbial populations.

Our findings clearly demonstrated the relative efficacy of the heavy metal tolerant *Fusarium solani* KUSF204 in production of phytohormones viz., IAA, gibberellin and metabolites like siderophore which were directly or indirectly involved in growth stimulation and pathogen suppression. Thus, the present study served as a good indication of the *Fusarium* species to be exploited in agriculture as plant growth promoting fungus (PGPF) and also as a potential biocontrol agent.

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REFERENCES

[1]. Hyakumachi M. Plant growth promoting fungi from turfgrass rhizosphere with potential for disease suppression. Soil Microorganisms, 1994; 44, 53–68

[2]. Yadav J, Verma JP, Tiwari KN. Plant growth promoting activities of fungi and their effect on chickpea plant growth, Asian journal of Biological sciences. 2011; 4(3): 291-299.

[3]. Idris EE, Iglesias DJ, Talon M, Borriss R. Tryptophan-Dependent Production of Indole-3-Acetic Acid (IAA) Affects Level of Plant Growth Promotion by *Bacillus amyloliquefaciens* FZB4220. Molecular Plant-Microbe Interactions. 2007; 6: 619–626.

[4]. Shahab S, Ahmed N, Khan NS. Indole acetic acid production and enhanced plant growth promotion by indigenous PSBs. African Journal of Agricultural Research. 2009; 4 (11): 1312-1316.

[5]. Stein A, Fortin JA, Vallee G. Enhanced rooting of *Picea mariana* cuttings by ectomycorrhizal fungi. Can. J. Bot. 1985; 68: 492-498.

[6]. Hasan HAH. Gibberellin and auxin production by plant root fungi and their biosynthesis under salinity-calcium interaction. Rostlinna Vyroba. 2002; 48(3): 101-106.

[7]. Finnie JF, Van Staden J. Effect of seed weed concentrate and applied hormones on in vitro cultured tomato roots. J. Plant Physiol. 1985; 120: 215-222.

[8]. Prasanna R, Joshi M, Rana A, Nain L. Modulation of IAA production in Cyanobacteria by Tryptophan and light. Polish journal of Microbiology. 2010; 59(2): 99-105.

[9]. Viterbo A, Landau U, Kim S, Chernin L, Chet I. Characterization of ACCdeaminase fromthe biocontrol and plant growth-promoting agent *Trichoderma asperellum* T203 FEMS Microbiol Lett .2010; 305, 42–48

[10]. Martino E, Turnau K, Girlanda M, Bonfate P, Perroto S. Ericoid mycorrhizal fungi from heavy metal polluted soils: their identification and growth in the presence of zinc ions.Mycological Research. 2000; 184:338-44

[11]. Morley GF, Sayer JA, Wilkinson S.C., Gharieb M.M. and Gadd G. M. Fungal sequestration, mobilization and transformation of metals and metalloids. Cambridge University Press, Cambridge, 1995.

[12]. Gadd GM, White C, de Rome L. Heavy metal and radio-nuclide uptake by fungi and yeasts. In Bio Hydro Metal-lurgy: Proceedings of the International Symposium; Norris, P. R., Kelly, D. P., Eds.; Sci. Tech. Letters: Kew, Surrey, U.K., 1988; pp 421-436.

[13]. Tudzynski B, Sharon A. Biosynthesis, biological role and application of fungal hormones. In: Osiewacz, H. D. (ed.). The Mycota X: Industrial Applications. Berlin-Heidelberg: Springer-Verlag. 2002; p. 183-195.

[14]. Leslie JF, Summerell BA. *The Fusarium Laboratory manual.* Blackwell Publishing: Iowa, USA. 2006

[15]. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. *Journal of molecular biology*, 1990; 215(3), 403-410

[16]. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: molecular evolutionary genetics analysis version 6.0. Molecular biology and evolution, 2013; 30(12), 2725-2729

[17]. Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular biology and evolution*, 1987; *4*(4), 406-425

[18]. Graham HD, Henderson JHM. Reaction of gibberellic acid & gibberellins with Folin-Wu phosphomolybdic acid reagent & its use for quantitative assay. Plant physiology, 1961; 36(4), 405

[19]. Schwyn B, Neilands JB. Universal chemical assay for the detection and determination of siderophores. *Anal. Biochem*. 1987; 160, 47-56

[20]. Czaky TZ. On the estimation of bound hydroxylamine in biological materials. *Acta Chemica Scandinavia,* 1948; 2, 450-454

[21]. Arnow LE. Colorimetric determination of the components of 3-4 dihydroxyphenylalaninetyrosine mixtures. The Journal of Biological Chemistry. (1937); 118, 531-537

[22]. Idris HA, Labuschagne N, Korsten L. Screening rhizobacteria for biological control of fusarium root and crown rot of sorghum in Ethiopia. *Biological Control,* 2007; 40, 97–106

[23]. Yonggang Li. Biology characteristic determination of *Fusarium semitectum* in soybeans. International Conference on Agricultural and Natural Resources Engineering. Advances in Biomedical Engineering, 2011; Vol.3-5, 76-80

[24]. Ingole M.N. Estimation of losses, variability among isolates and management of pigeonpea wilt caused by Fusarium udum Butler. M.Sc. (Ag.) Thesis, Dr. PDKV, Akola, 1995; pp.146.

[25]. Anjaneyareddy B. Variability of Fusarium udum and evaluation of Pigeonpea (Cajanus cajan (L). Mills) genotypes. M.Sc (agri) Thesis, Univ. Agril. Sci. Bangalore, 2002; pp.115.

[26]. Jamaria SL., Nutritional requirement of Fusarium oxysporum f.sp. niveum. Indian Phytopath, 1972; 25: 29-32.

[27]. Naim MS, Sharoubeem HH. Carbon and nitrogen requirements of *Fusarium oxysporum* causing cotton wilt. Mycopathologia, 1963; 22: 59-64

[28]. Bais BS, Singh SB, Singh DV. Effect of different carbon and nitrogen sources on the growth and sporulation of *Curvularia pallescens*. Indian Phytopathology, 1970; 23: 511–7

[29]. Ramteke PK, Kamble SS. Physiological studies in *Fusarium solani* causing rhizome rot of ginger (*zingiber officinale* rosc.) The Bioscan, 2011; 6(2) : 195-197

[30]. Oritsejafor JJ. Carbon and nitrogen nutrition in relation to growth and sporulation of *Fusarium oxysporum f.sp. elaeidis*. Transactions of the British Mycological Society, 1986; 87: 519-524

[31]. Bhatnagar CC, Prasad N, Mathur RL. Nitrogen requirement of *Fusarium oxysporum f.sp. aurentifoliae* Bhat and Prasad. Indian Phytopathology, 1968; 21: 237 – 239.

[32]. Patel ST. Studies on some aspects of wilt of chickpea. Ph.D. Thesis Univ. Agric. Sci., Dharwad, 1991; pp. 205.

[33]. Agarwal GP. Utilization of different sources for *Fusarium udum* Butler. Phyton., 1958; 11: 143-151.

[34]. Volesky B, Holan ZR. Biosorption of Heavy Metals, Biotechnol. Prog. 1995; 11: 235-250.

[35]. Mittra B, Gnosh P, Henry SL, Mishra J, Das TK, Gnosh S, Babu CR, Mohanty P. Novel mode of resistance to *Fusarium* infection by a mild dose pre-exposure of cadmium in wheat, Plant Physiology and Biochemistry, 2004; 42 (10): 781–787.

[36]. Rai B, Chandra A, Singh SK. Effects of heavy metals on growth of some pathogenic and non-pathogenic soil microflora. Indian Botanical Society, Madras, 1995; 74 (1–4): 35–39.

[37]. Babich H, Stotzky G. Effect of Cadmium on Fungi and on Interactions Between Fungi and Bacteria in Soil ∴ Influence of Clay Minerals and pH. Applied and Environmental Microbiology, 1977; 33 (5) ∴1059–1066.

[38]. Cervantes C, Gutierrez-Corana F. Copper resistance mechanism in bacteria and fungi.FEMS Microbiol Rev, 1994; 14:121-137.

[39]. Baath E, Diaz-Ravina M, Frostegord A, Effect of metal-rich sludge amendments on the soil microbial communities. Appl. Environ. Microbiol. 1998; 64: 238-245.

[40]. Costacurta A, Vanderleyden J, Accumulation of phytohormones by plant-associated bacteria Critical Review of Microbiology, (1995); 21: 1-18.

[41]. Uthandi S, Karthikeyan S, Sabarinathan KG, Gibberellic acid production by *Fusarium fujikuroi* SG2 Journal of Scientific and Industrial Research. 2010; Vol. 69: pp. 211-214

[42]. Malcolm HD. Fahy PC. Abnormal growth of lettuce associated with a gibberellin producing strains of *F. oxysporum*. 1971; Search 2: 141-147.

[43]. Manaka M. Auxin and gibberellin like substance synthesis by *Fusarium* isolates pathogenic to corn seedlings. Acta Microbiol. Pol. 1980; 29: 365-373

[44]. Graham HD. Henderson JHM. Reaction of gibberellic acid & gibberellins with Folin-Wu phosphomolybdic acid reagent & its use for quantitative assay. Plant physiology, 1961; 36(4),405

[45]. Troncoso et al. Gibberellin biosynthesis and gibberellin oxidase activities in *Fusarium sacchari, Fusarium konzum* and *Fusarium subglutinans* strains, Phytochemistry. 2010; 71, 1322–1331

[46]. Choi WY, Rim SO, Lee JH Isolation of gibberellins producing fungi from the root of several *Sesamum indicum* plants. Journal of Microbial Biotechnology, 2005; 151:22 28

[47]. Jalander V, Gachonde BD Effect of cutture filtrates of *Fusarium oxysporum* on seed germination and seedling growth of ptgeonpea (*Cajanus cajan*) varieties. Bioscience Discovery. 2011; 02 (2):185-188.

[48]. Asghar HN, Zahir ZA, Arshad M, Khaliq A. Relationship between in vitro production of auxins by rhizobacteria and their growth promoting activities in *Brassica juncea L*, Biol. Fert. Soils, 2002; 35:231-237.

[49]. Patten CL, Glick BR. Role of *Pseudomonas Putida* Indole acetic Acid in Development of the Host Plant Root System. Appl Environ Microbiol. 2002; 68(8): 3795–3801.

[50]. Hussein KA, Joo JH. Comparison between Siderophores Production by Fungi Isolated from Heavy Metals Polluted

and Rhizosphere Soils. Korean J. Soil Sci. Fert. 2012; 45(5), 798-804.

[51]. Emery T. Malonichrome, a new iron chelates from *Fusarium roseum*. *Biochim. Biophys*. *Acta,* 1980; 629, 382–390

[52]. Lopez-Berges MS, Capilla J, Turra D, Schafferer L, Matthijs S, Jochl C. HapX-mediated iron homeostasis is essential for rhizosphere competence and virulence of the soil borne pathogen *Fusarium oxysporum*. *Plant Cell,* 2012; 24, 3805–3822

[53]. Mahmoud ALE, Abdalla MH. Siderophore production by some microorganisms and their effect on Bradirhizobium-Mung bean symbiosis. Int. J. Agri. Bio. 2001; Vol 3, No.2, 157-162

[54]. Woos L, Scala F, Ruocco M, Lorito M. the molecular biology of interactions between *Trichoderma* sp., phytopathogenic fungi and plants. Phytopathology. 2006; 96: 181–185

[55]. Hass H, Defago G. Biological control of soil borne pathogens by fluorescent *Pseudomonas,* 2005; Nature, 3. 307-319.

[56]. Dalal JM, Kulkarni NS, Bodhankar MG. Antagonistic and plant growth promoting potentials of indigenous endophytic fungi of soybean (Glycine max (L) Merril) Indian Journal of Advances in Plant Research (IJAPR), 2014. Vol.1 (7): -9-16.

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