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IDENTIFICATION AND CHARACTERIZATION OF FUSARIUM SPECIES ASSOCIATED WITH SOIL, SEED, AND FRUIT

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Abstract: Fusarium is a large genus of filamentous fungi and belongs to the class Ascomycetes and family Hypocreaceae. During the study total of 40 different species were isolated among them *Fusarium* genera have 14 species ware isolated from different sources like soil, seed, and fruit. The source of highest occurrence of *Fusarium* species from the soil source 10 *Fusarium* species was isolated these species were *Fusarium camptoceras, Fusarium chlamydosprum, Fusarium graminearum, Fusarium heterosporum, Fusarium moniliforme, Fusarium neoceras, Fusarium nivale, Fusarium goxysporum, Fusarium solani, Fusarium moniliforme var. minus*. Next, that seed was also a good source from 4 species *viz. F. equiseti, F. graminearum, F. redolens, F. oxysporum)* were isolated. The lowest occurrence observed from the fruit source of isolation is only 3 species *viz. F. avenaceum, F. langesthiae, F. oxysporum*.

Keywords: *Fusarium*, soil, seed, fruit

I. INTRODUCTION

The name of the *"fusarium"* comes from Latin *"fusus"*, it is means a spindle. *Fusarium* is a large genus of filamentous fungi belonging to the phylum- Ascomycota, class –Ascomycetes, order- Hypocreals. Since its establishment in 1809 by Link, the genus *fusarium* has received global attention in the literature. It was validated the genus in terms of the International Botanical Code where nearly 1000 species were described in the genus based on their hosts [1]. The differentiation of species and groups of the section has been dominated by the use of morphological characters. This genus is important as species of this genus causes plant diseases several economically important crops lead to huge economic losses, viz; crown, rots, head blights, scabs, vascular wilt, root rots, and canker. However, several other diseases caused by *Fusarium spp*. are of international importance, like the Panama disease of bananas and the wilts of *Cajanus cajan*. Similarly, other species like *F. moniliform, F. graminearum, F. avenaceum, and F. culmorum* are reported to be serious pathogens of the Graminaceous crop. Strains of *F. solani* are also worldwide occurrence as root rots, but may also cause cankers of hardwood trees. And they may occasionally cause opportunistic infection in animals. In addition, *Fusarium* species also causes a broad spectrum of infections in humans, including superficial keratitis and onychomycosis. Several species produce well-known mycotoxins, such as trichothecenes, fumonisin, naphthazarine pigment, fusaric acid, lycomarasmis, and fusicoccin, etc. Leads to mycotoxicosis in humans and animals. Some of its species are reportedly associated with cancer and other diseases in humans and domesticated animals.

Fusarium spp. is universal occurring fungi, which can cause diseases on a wide-range of host plants with significant economic impacts [2]. The morphological characteristics; the colony color and growth rate; the shape and size of macroconidia, microconidia, and chlamydospores, and the formation of conidiogenous cells have been the primary means for identifying *Fusarium spp.* [3]. For example, morphological characteristics such as the formation of conidiogenous cells are used to differentiate *F. commune* from *F. oxysporum* [4]. The genus Fusarium is complex, and different morphological characteristics at the species level may be subtle. Robust DNA-based analysis is needed for accurate, rapid identification and characterization of the species. Phylogenetic analysis based on DNA sequence data has made significant contributions to our understanding of the systematics of *Fusarium spp*. [5]. Various other plant pathogens, *F. oxysporum* has several specialized forms, known as formae specialize that infect a variety of hosts causing various diseases: banana *(Musa spp.) (F. oxysporum f.* sp. *cubense*), cabbage (*Brassica* spp.) (*F. oxysporum f.* sp. *conglutinans*), cotton (*Gossypium* spp.) (*F. oxysporum f.* sp. *vasinfectum*), flax (*Linum* spp.) (*F. oxysporum f.sp lini*), muskmelon (*Cucumis* spp.) (*F. oxysporum f.* sp. *melonis*), onion (*Allium* spp.) *(F. oxysporum f.* sp. *cepae*), pea (*Pisum* spp.) (*F. oxysporum f.* sp. *pisi*), tomato (*Lycopersicon* spp.) (*F. oxysporum f.* sp. *lycopersici*), watermelon (*Citrullus* spp.) *(F. oxysporum f.* sp. *niveum) China aster* (*Calistephus* spp.*) (F. oxysporum f.* sp. *callistephi),* carnation (*Dianthus* spp.) (*F. oxysporum f.* sp. *dianthi*), chrysanthemum (*Chrysanthemum* spp.) (*F. oxysporum f.* sp. *chrysanthemi),* gladioli (*Gladiolus* spp.) (*F. oxysporum f.* sp. *gladioli)* and tulip (*Tulipa* spp.)(*F. oxysporum f.* sp. *tulipae*) [6]. The form-genus *Fusarium* includes a large number of species and many forms inside species many of these are saprobic or saprophytic. Some are only mild facultative parasites. It is extensive. The hyphae are septate and

branched. They are both intercellular and intracellular. When young they may be a thing of pink, purple or yellow and become dark-colored at maturity. The dark mycelium produces thick bands which plug the vascular tissues and produce toxic secretions. The toxins are carried up in the xylem vessels. As a result, the plant wilts and dies. It takes place by the formation of three kinds of asexual spores, microconidia, macroconidia, and chlamydospores. The identification of *Fusarium* species is mainly based on distinctive characters of the shapes and sizes of macro-and microconidia, presence, and absence of chlamydospores as well as colony appearances, pigmentations, and growth rates on agar media [7]. The microconidia vary in form from round to oval. They are often held in small masses. At times they are elongated or crescent-shaped. Macroconidia are large multi-cellular usually two to four celled conidia. In form, they are elongated, sickle-shaped, or crescent-shaped. They are produced at the tips of simples or sparingly branched conidiophores which are assembled to form a sporodochium type of fructification. It has been reported that at least 80% of all cultivated plants are associated with at least one disease caused by a Fusarium species [7]. Crop losses in India due to biotic stresses, like pathogen and insect pests are huge. In India alone, 5000 plant diseases are prevalent [8]. Hence, better understandings of the biology of pathogens, diseases caused by them, and relationships with the hosts are essential to developing sustainable control for the management of diseases.

Among plant pathogenic fungi, *Fusarium* species has been considered the most diverse and widely dispersed and known to cause devastating diseases in a diverse range of economically important crops. The worldwide presence of *Fusarium* sp. attests to their potent pathogenic behaviors. *Fusarium* is common in nature and can be isolated from different plant organs, plant debris, and soil. While most species are common in the tropical and subtropical areas, some are reported to inhabit soils of cold climates also. This also shows their enormous adaptability to varying climatic conditions.

II. MATERIAL AND METHOD

2.1. **Isolation of fungi** Isolation of *Fusarium sp*. We are taken at. Department of botany Dr. Babasaheb Ambedkar Marathwada University, Aurangabad. Isolation is done with help of different sources like soil, seed, (pulses, oilseed, and cereal), and different fruits which were collected from the garden, different market places, shops, localities of Aurangabad at.2019.

2.2. Collection of the soil sample from different sources

2.2.1.**Soil;** Soil dilution was made by suspending 1g of the soil of each sample in 10ml of sterile distilled water. Dilution of 10-1, 10-2, and 10-3 were used to isolate fungi in fungal colonies. 1ml of suspension of each concentration was added to sterile Petri dishes, in triplicates of each dilution, containing sterile potato dextrose agar medium 1% streptomycin solution was added to the medium for preventing bacterial growth, before pouring into Petri plates, the plates were then incubated at 28 to 300 c for 4-7 days. Organisms were easily isolated because they formed surface colonies that were well dispersed particularly at higher dilution [9].

2.2.2.**Seed:** The collected different seed samples were isolation was taken by the method described by [10] by Agar plate method on media such as potato dextrose agar (PDA) and czapek dox agar (CZA) used as isolation technique.

2.2.3.**Fruit:** Fruit samples that were fresh as well as previously infected or rotten fruit and vegetables were collected in pre-sterilized polythene bags from the market to examine post-harvest fungi. The fruit samples cut out a small portion of infected parts using a sterile scalpel.

3.1. **Composition of media used in isolation of fungi:** Potato Dextrose Agar (PDA): Peeled Potato 200g, Dextrose 20g, Agar 20g Distilled Water 1000ml Streptomycin 0.2g pH was maintained 5.6. Along with the PDA Czapek Dox Agar (CZA) (Sucrose 30g, $NaNo_3$ 2.0g, K_2HPO_4 1.0g, $MgSo_4$ 0.5g, KCL 0.5g, $FeSo_4$ 0.01g, Agar 20g) pH was maintained around 5.6 to 6.0 in 1000ml of distilled water used for isolation of fungi from different samples like the seed, fruit, and soil.

4.1. **Slide Preparation:** On a clean slide, a drop of lactophenol cotton blue was taken & then the culture from the edge of the colony was placed over it followed by a coverslip. Examine the Fungal structure under a microscope.

5.1. **Microscopic Identification:** After the growth of fungus on Petri Plates, the macroscopic observations of colonies were external features, texture, colony color, growth rate & microscopic characteristics arrangement of spores. Macroscopic & microscopic features of fungi were helpful inaccurate identification of Fungi. The identification of fungi was done by using various research papers, monographs & other literature such as manual of soil Fungi [11], [12], Handbook of soil Fungi, [13], [14], "Fuskey Fusarium interactive key and *Fusarium* species: an illustrated manual for identification [15].

Table No.1. Isolation of Fusarium fungi from different sources

Sr. No.	Name of Fungi	Soil	Fruit	Seed
1	*Fusarium avenaceum*	-	+	-
2	*Fusarium camptoceras*	+	-	-
3	*Fusarium chlamydosprum*	+	-	-
4	*Fusarium equiseti*	-	-	+
5	*Fusarium graminearum*	+	-	+
6	*Fusarium heterosporum*	+	-	-
7	*Fusarium langsethiae*	-	+	-
8	*Fusarium moniliforme*	+	-	-
9	*Fusarium neoceras*	+	-	-
10	*Fusarium nivale*	+	-	-
11	*Fusarium oxysporum*	+	+	+
12	*Fusarium redolens*	-	-	+
13	*Fusarium solani*	+	-	-
14	*Fusarium moniliforme var. minus*	+	-	-

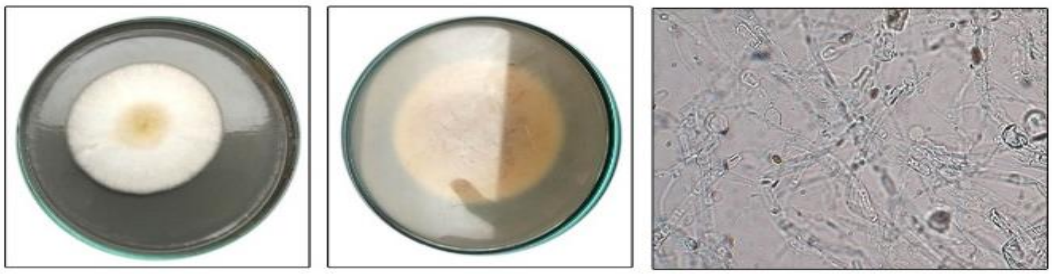
III. RESULT AND DISCUSSION:

During the investigation of fusarium isolation from different sources like seed, fruit, and soil. It is clear from table no. 1. Among total of 40 species the 14 *Fusarium* species was isolated. It is clear from the table. The highest occurrence of Fusarium species from the source of the soil from these source 10 *Fusarium* species was isolated these species are *Fusarium camptoceras, Fusarium chlamydosprum, Fusarium graminearum, Fusarium heterosporum, Fusarium moniliforme, Fusarium neoceras, Fusarium nivale, Fusarium oxysporum, Fusarium solani, Fusarium moniliforme var. minus*. Next to that 4 species (*F. equiseti, F. graminearum, F. redolens*, and *F. oxysporum*) were isolated from different seed sources. The lowest occurrence observed on the fruit is only 3 species (*F. avenaceum, F. langesthiae, F. oxysporum)*. The morphological studies were carried out and the result is mentioned in photo plate 1, photo plate 2, to Photo plate 7 were noted. Similar results were seen in the infection caused *by F. oxysporum* in banana and successfully isolated 13 isolates of *F. oxysporum* from Malaysia and two isolates from Indonesia [16]. The *F. musae* was responsible for the infection of banana fruits and the consumption of such infected fruits may cause fusariosis in human being reported [17]. From Dragon fruit (*Hylocereus polyrhizus*) plants were severely infected with *F. semitectum* leading to the economic loss of many cultivars. They isolated 79 isolates of *F. semitectum* from 3 different plant parts namely from the stem, fruit, and root of diseased *H. polyrhizus* from 9 states in Malaysia reported [18].

IV. CONCLUSION:

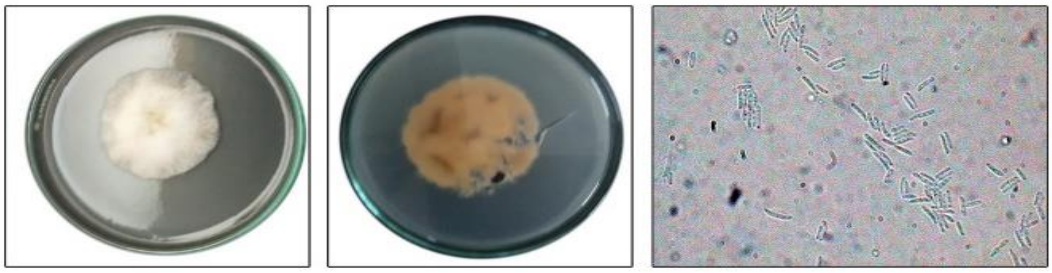
.During the investigation more occurrence were seen in the soil as compare to seed and fruit. Total 14 species were isolated from different sources *fusarium* genera is the more toxic genera towards the toxin production as well as pathogenic. It is need towards the further investigation on impact on seed, fruit and soil.

PLATE - 1



Fusarium avenaceum

Conidia seldom scattered in false heads or balls, as layer in sporochia and pinnotes, in gelatinous masses, orange, vermillion, scarelet, in resin like drops becoming darker in dry powdery condition lighter, becoming pink. Stroma yellow, ochre, carmine to brown red, µarial aknotty, roughened stromata and true glabose, single or gregarious sclerotia (60-80 µ in diameter) of adark blue or paler colour are very seldom found. Conidia long, awl- or thread like, proportionally circularaly or ellipsoidally curved or both ends especially at the tip. Conodia 3-5, seldom more or less septate. Conodiaphore simple or loosely to bushily branched with scattered or with two or four, seldom 5 membered whorls or side branches.



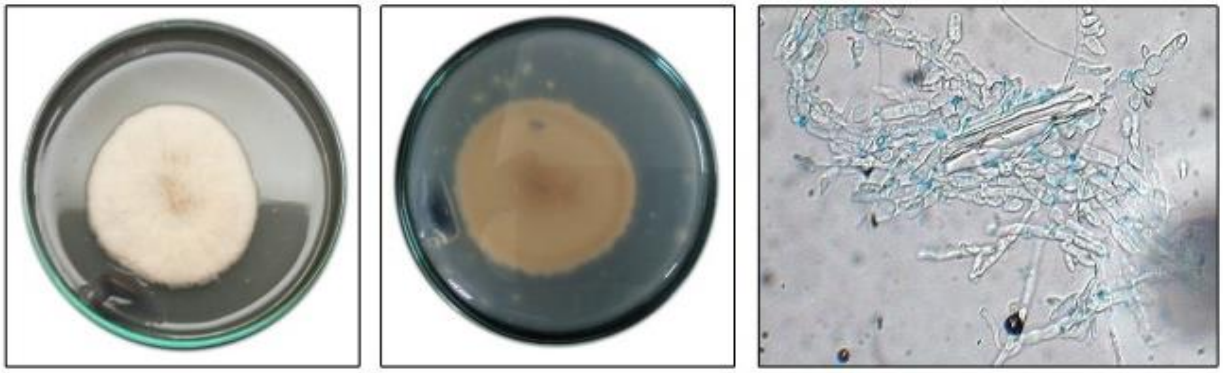
Fusarium camptoceras

Arial mycelium caramine- pink or isabellin, the lower part of the stroma leathery brown or rosy. Chlamydospres intercalary. Sporodochia lacking. Conidia scattered in arial mycelium, sickle-shaped, strongly curved, tapering at both ends, tip more or less constricted, basal cell rounded, conical pointed, not pedicellate, but at times with an attachement papilla, smaller conidia non to 2 septate, laeger 3 to 5 (6 or 7) septate.

PLATE - 2

Fusarium chlamydosporum

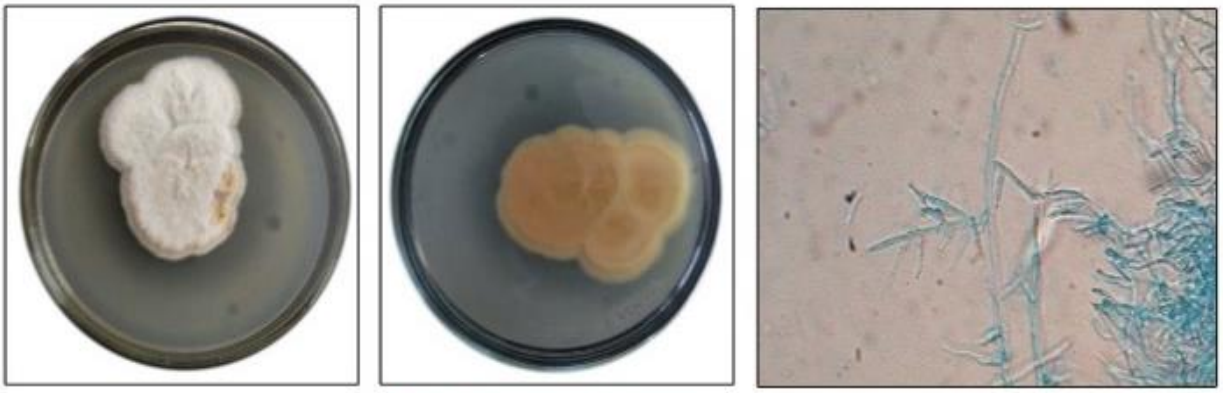
Fruiting layer, floccose,hyaline or pink, plectenchymatic below, at times forming somewhat warty, sclerotial knots, variously coloured, hyaline, carmine purple red, sulphur –yello, ochre or dark brown. The dark colour is caused by the occurrence in the mycelium of numerous, globose to pear – shaped smooth to rough spiny, intercalary or terminal single, paired, catnulate or muriform, chlamydospores 10 – 16 µ in diameter. Conidia small, spindle ellipsoid, not globose, lemon shaped, usually one celled, seldom, septate, three sepatate spore sickle shaped, weakly pedicellate.



Fusarium equiseti

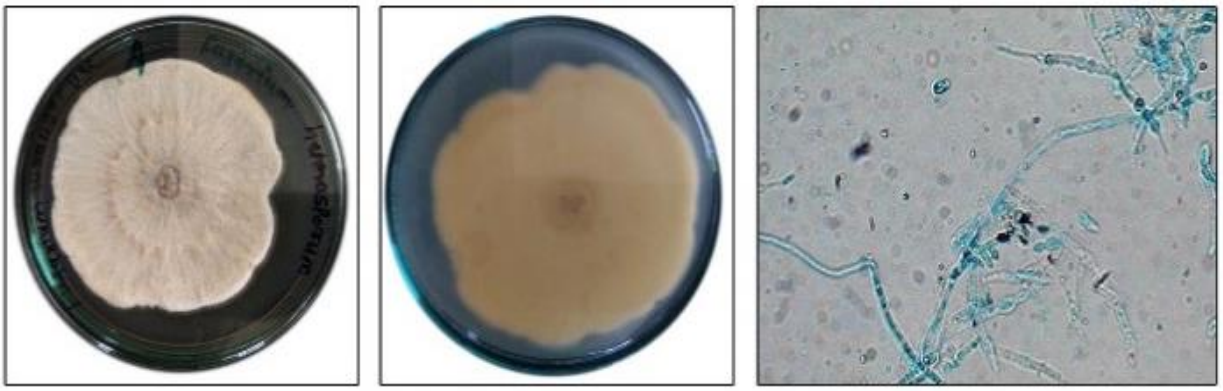
Conidia at first sparingly scattered in whitish to yellow to pink aerial mycelium, one celled or septate, oval or long to spindle sickle shaped, sometimes comma like sickle spores. Macroconidia in tubercular sporodochia on plectenchymatic pale brown, never caramine red stromata of various extent, or slimy, easily dissolving masses, also in balls. Conidiophore simple or branched. The lateral branches extended tree like, or compressed bush like. Conidiophore in 2 or 3, exceptionally more, many times multiplied whorls, and have at their ends small sterigma like papillae in ones, 2 or 3 also longer spore bearing members. Sterile and fertile hyphae are irregularly septate and 3 – 6 µ thick. Chlamydospores (6 - 14 µ in diameters) round, smooth or rough, on mycelium, old conidiophores as well as in conidia.

PLATE - 3



Fusarium graminearum

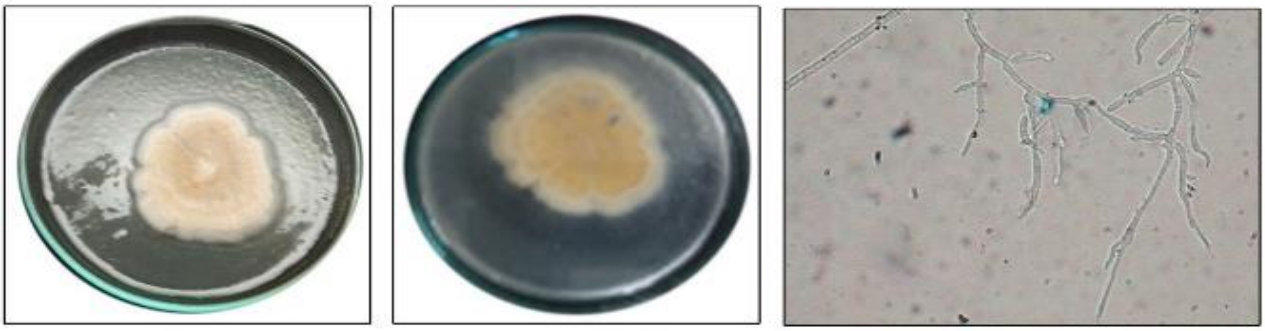
Fruiting layer variously colored, white-rosy, golden-yellow, ochre, (becoming blue-violet when treated with ammonia) or caramine-purple, sometimes plectenchymatic, extended, more or less covered with floccose arial mycelium, sometime limited, erumpent, sclerotial on which lies a conidial mass resembling pinnotes or sporodochia, ochre or bright orange red. Conidia at times compact as in F.culmorun, at times longer than in that variety, spindle-sickle shaped , strongly curved, tapering at both ends, with a rounded or constricted tip and pedicellate base, 3-5 septate, seldom 1 or 2 septate or 6-9 septate. Chlamydospores lacking or very rare, intercalary.



Fusarium heterosproum

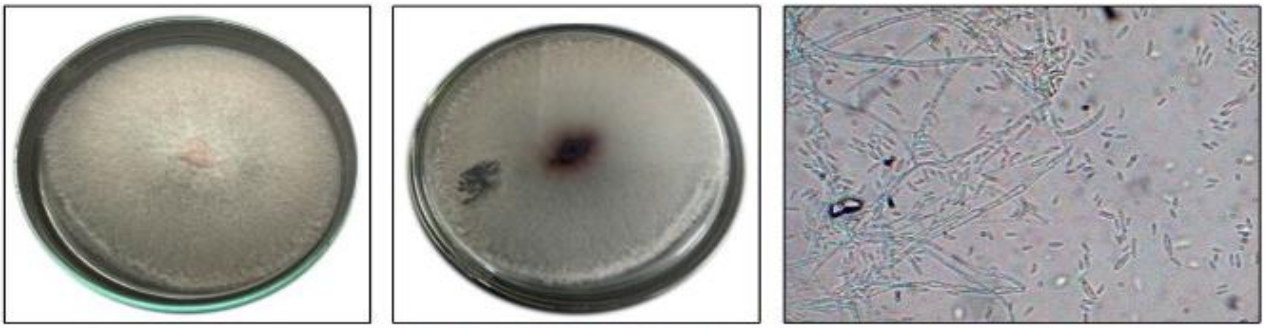
Microconidia are absent. Macroconidia are sickle-shaped, narrowed at both ends, and pedicellate Conidiophore. Unbranched and branched monophialides. Chlamydospores. They are present and are formed in chains. On PDA growth is rapid, with dense white to pink aerial mycelium. Orange sporodochia develop as the culture ages. The undersurface is light orange to tan in color The colony appearance on PDA and the bright orange sporodochia formed in fluffy white to pink aerial mycelium. along with the spore morphology on CLA.

PLATE - 4



Fusarium moniliforme

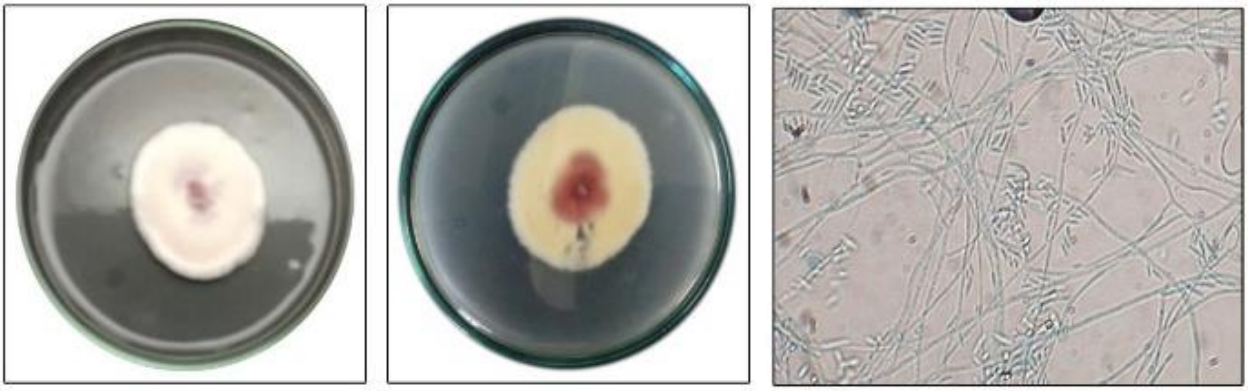
Microconidia produced in chains and remaining connected or held in false heads, later becoming scattered over the bright yellow to rosy-white aerial mucelium or a transparent shining powder , one or two – celled, spindle, egg-shaped. Macroconidia delicate, awl-shaped, slightly crescent-shaped or almost straight, tapering at both ends, often constricted at the tip, amd sometime shooked, at the base with a real or slight foot cell, scattered or gathered into sporodochia or pionnotes, bright in mass, isabellian orseldom 6 to 7 septate, chlamydospores lacking. Dark blue, spherical sclerotia 0.08 x 0.1 mm. may be present. Stroma more or less plectenchymatic, yellow, brown, violet, etc.



Fusarium langsethiae

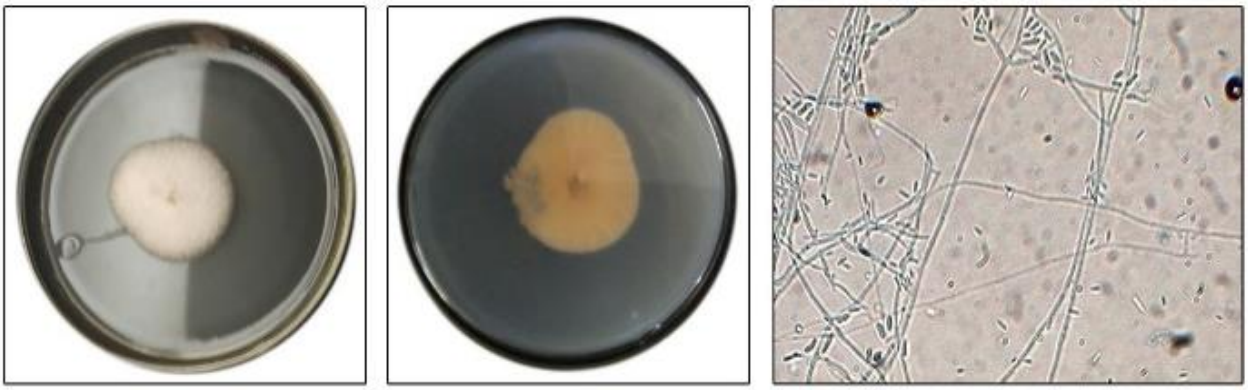
A new species of Fusarium, Fusarium langsethiae, is described, illustrated and discussed. This species is isolated from kernels of oats, wheat and barley in several European countries. Morphologically, the species resembles Fusarium poae. It is differentiated from F. poae by slower growth, less aerial mycelium and absence of odour; its napiform or globose conidia are borne in the aerial mycelium on the agar surface on often bent phialides which exhibit sometimes more than one opening, whereas those of F. poae are produced on straight monophialides mostly in the aerial mycelium. No sporodochial conidia are formed by F. langsethiae even under near-UV light (nUV). Based on morphological characters, the species is placed in the section Sporotrichiella.

PLATE - 5



Fusarium neoceras

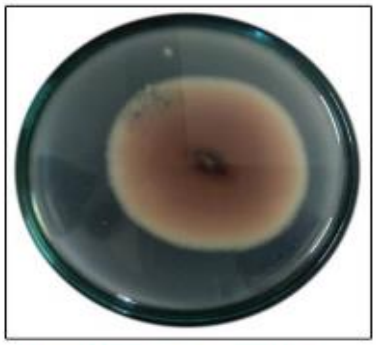
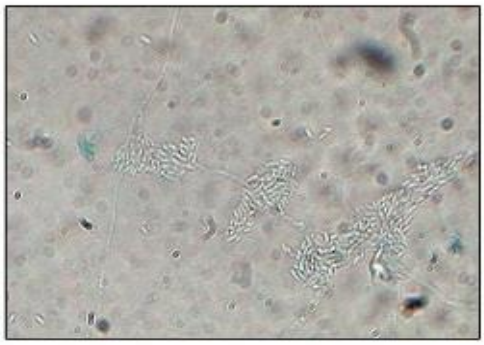
Microconidia single or in false heads, not in chains, one-called, oval-spindle-shaped, seldom two-celled, exceptionally three celled, later scattered as dust in mycelium. Microconidia in sporodochia and pionnotes, brownish-white cream to incarnate, at times becoming flecked with violet or blue tones and varying in concentric zones of the stroma and laid on it in rings, straight or weakly curved, tapering at both ends, slightly constricted at tip,with tenpin to slightly pedicellate base, 3 (3-5) very seldom 6-9 septate. Chlamydospores and sclerotia lacking.



Fusarium nivale

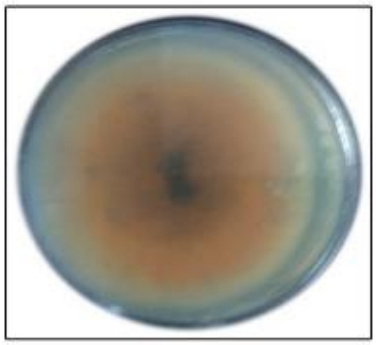
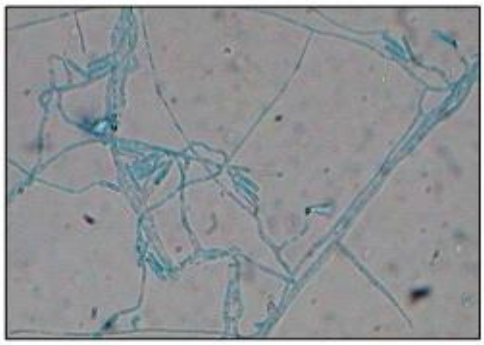
Conidia scattered on cobwebby, hyaline to rosy, fasciculate, loose or busy aerial mycelium, sometimes also in balls or wide – spread, slimy rosy, lack – orange masses which become darker, (cinnamon-browns) resinous on drying, paler (isabellin) in moist conditions, normally spindle-sickle-shaped, curved, tapered at the ends and tenpin-shaped to rounded, not pedicellate, seldom some what constricted at the base, 1-3 septate, occasionally 4-7 septate, mixed with scattered one celled forms. Chlamydospores and sclerotia lacking. Stroma delicate, thin, evanescent or plectenchymatic, wrinkled, hyalin, isabellin, rosy, orange to brick-red, later leather-brown.

PLATE - 6

Fusarium oxysporum

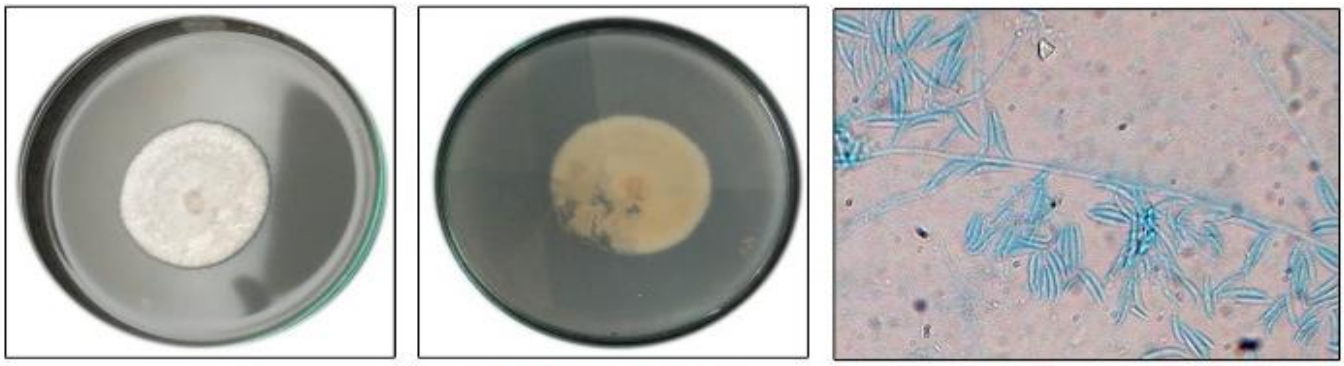
Microconidia are abundant. generally single-celled, oval to kidney-shaped, and produced only in false heads. Macroconidia are abundant, only slightly sickle-shaped, thin-walled, and delicate, with an attenuated apical cell and a foot-shaped basal cell. Conidiophores Unbranched and branched monophialides. Chlamydospores. They are present and are formed singly or in pairs. In most isolates they form readily and profusely in culture. On PDA. growth is rapid and the white aerial mycelium may become tinged with purple or be submerged by the blue color of the sclerotia when they are abundant, especially at the base of the slant, or by the cream to tan to orange sporodochia when these are abundant. Discrete, erumpent orange sporodochia are present in some strains.
The undersurface may be colorless to dark blue or dark purple, and these colors may be visible through the mycelium when viewed from above.

Fusarium redolens

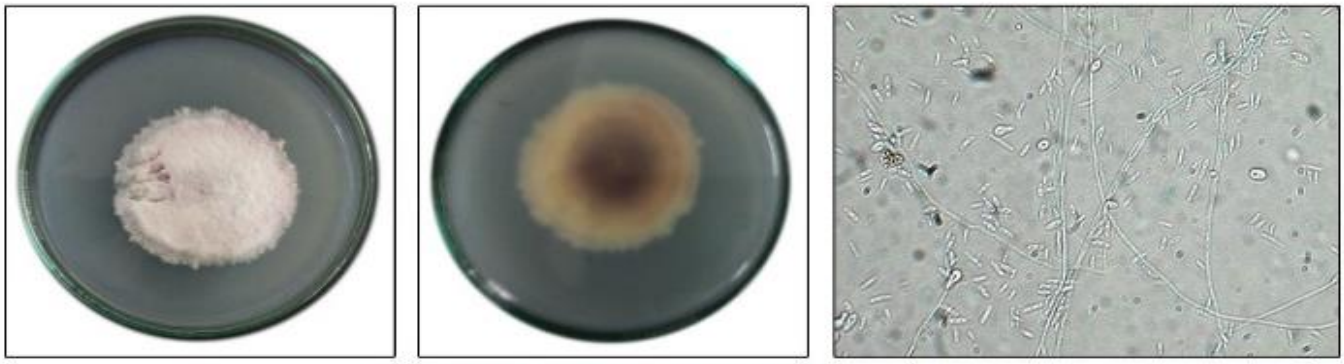
Microconidia one celled, 9 x 3 µ or sparingly septate, one septate, 16 x 4.5 µ. Macroconidia 3 seldom-4, exceptionally 5 septate, spindle-sickle- shaped, curved, sometimes resembling F.solani, but in theupper third thicker than the middle, generally becoming narrowed at the base to a pedicel or an attachement papilla, occurring in sporodochia or pionnotes, in massbrownish-white cream or bright incarnate, at first gelatiuos, then powdery, fading; 3-septate, 17-51 x 3-6.5 µ, 5-septate 31-61 x 3.5-6.5 µ. Chlamydospores terminal and intrcalary, one-celled, 8 µ or 3-12 µ, two celled, 11-24 x 5-14 µ, smooth or wrinkled, in conidia or mycelium. Blue sclerotia lacking. Plectenchymatic stromata extended, pale pink to lilac. The fungus is aromatic with an odor of lilac.

PLATE - 7



Fusarium solani

Microconidia are present, varying from sparse to abundant, generally single-celled, oval to kidney-shaped. The microconidia are similar in shape to those found in F. oxysporum, but they are larger and have thicker walls. Macroconidia are abundant, stout, thick-walled, and generally cylindrical, with the dorsal and ventral surfaces parallel for most of their length. The apical cell is blunt and rounded, and the basal cell is rounded or is distinctly foot-shaped or notched. Conidiophores Unbranched and branched monophialides. The monophialides bearing microconidia are long when compared to those in F. oxysporum. Chlamydospores They are present and are formed singly and in pairs. They are abundant in most clones. On PDA growth is rapid, often with abundant aerial mycelium. The surface is soon covered with confluent sporodochia that give the appearance of pionnotes and color the surface cream, blue-green, or blue, but never orange. Some clones may show a dark purple color on the upper surface. The undersurface is generally colorless, but some clones produce a dark violet pigment.



Fusarium moniliforme var.minus

Differs from the type by absence of pionnotal and sporodochial slime. Macroconidia in long chains or in false heads, then scattered in whitish-rosy aerial mycelium, oval-spindle form, one to two celled. Maceconidia few, sparingly scattered (to 1%) 3-5 septate, lanceform, seldom spindle-sickle-shaped, slightly curved, generally pointed at the tip, truncate at the base, tenpin-shaped, seldom with attachement papilla, not pedicellate. Dark blue globose sclerotia sometime present.

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