NOVATEUR PUBLICATIONS
JournalNX- A Multidisciplinary Peer Reviewed Journal
ISSN No: 2581 - 4230
VOLUME 7, ISSUE 12, Dec. -2021

SYNTHESIS OF METABOLITES OF THE GENUS FUNGUS FUSARIUM OXYSPORUM f.sp. VASINFECTUM

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ANNOTATION:

Fusarium oxysporum f. sp. vasinfestum W.S. Snyder & H.N. Hans isolated from soil during research. Studied the activity of the pathogens and the growth of fungi in a nutrient medium.

Isolation of pure cultures of the studied fungus and selection of the selected compounds in various nutrient media Fusarium oxysporum f. sp. vasinfestum W.S. Snyder & H.N. Hans. Studied the growth and development of fungi, the formation of micromacroconidia and the synthesis of metabolites.

Keywords: Nutrient medium, phytopathogens, Fusarium oxysporum f. sp. vasinfestum W.S. Snyder & H.N. Hans, CHapek nutrient medium, macroconidia and microconidia, suslo-agar.

I. INTRODUCTION:

Fusarium wilt is one of the most interesting but difficult to study crop diseases. One of the main reasons for the relatively weak study of Fusarium fungi in our country is the rapid variability of its morphological features and the difficulty of identifying species. They are common in soil, air, water, plant remains, and have the ability to infect more than 400 species of plants, including facultative phytoparasites or saprotrophs, depending on the method of feeding and their relationship with higher plants (16). .

Drying and root rot of plants due to diseases of the root system of plants is caused by a violation of the water balance due to damage to the connective tissue of plant organs. occurs as a result of obstruction.

Decay is the result of the breakdown of plant tissues by enzymes of the pathogen. Liver discoloration of damaged tissues is caused by the pigments of the pathogen. In this case, the breakdown of the cell wall and the substances between them by the enzymes of the pathogen and secondary metabolites causes the cells involved in the formation of tissues to separate from each other.

The development of the pathogen in cotton occurs through the roots in the soil. The first signs of the disease can be detected in the seedlings. Initially, the disease manifests itself in the form of necrotic spots, followed by necrosis of the leaves along the veins. Fusarium oxysporum Schl. f. vasinfestum W.S. Snyder & H.N. Sources of Hans infection are areas where soil is contaminated with plant debris contaminated with chlomidospores.

Plants are involved in a number of molecular and cellular processes to repair damaged tissue and prevent further damage (Abdusattarovich P. A., 2020. – T. 1. – №. 5.).

In the study of cotton diseases, a complex study of seeds, seedlings and vegetative and generative organs and fibers of the adult plant is of great practical importance (Abdusattorovich P. A. , 2021. – T. 2. – №. 04.). Seed diseases are treated with fungicides vitavax, montseren, panoktin. However, the effect of the recommended fungicide on local Fusarium species has not been studied and its effectiveness has not been established.

Humidity and temperature play a key role in the development of cotton plant diseases. Its normal temperature is 14-15^{o}C, humidity 80-85%. The fungi Fusarium, Pythium, Rhizostonia play a key role in seed molding. To reduce the risk of disease, it is recommended that the soil temperature at the time of sowing be 12-14^{o}C for 10 days (18), the seeds should be treated with fungicides, accompanied by biological control measures.

II. RESEARCH MATERIALS AND METHODS:

The research began with phytosanitary control of infected plant samples. In the study of the prevalence and damage of plants infected with Fusarium in the Republic, mycological analysis was carried out to determine the symptoms of infected plants in some samples. The date and area of the herbariums collected on the basis of the plants taken for the sample, and the location of the samples taken on the basis of the soil.

To isolate pure fungal cultures, the samples were placed in small bags and rinsed under running tap water for 90 minutes. The samples were then soaked in 0.0001% polysorbate-80 solution for 25 seconds, in 0.5% sodium hypochlorite (NaOCl) solution for 25 seconds, in 96% ethanol in 30% aqueous solution for 25 seconds, and in sterile distilled water for 2 to 2 minutes. .

II. 1. Methods of growing fungal strains:

The fungi were grown in a special climate chamber for 14 hours under light, with a temperature of 22-24^{o}C during the day and 18-22^{o}C at night for 3 to 15 days. We used Andijan-36 cotton seeds for sowing. Before sowing, the seeds were sterilized separately using a sterilization method: the seeds were first sterilized in 70% ethanol for 1 minute, in a 2.5% solution of sodium hypochlorite (NaOCl) for 15 minutes, and the seeds were thoroughly washed 4 times in distilled water (18).

II. 2. To study the growth of Fusarium fungi in nutrient media:

Detection of Fusarium fungi using natural nutrient media, including nutrient medium. The composition and preparation of the wort-agar medium: 1 liter of distilled water was mixed with 7 ^{o}C beer wort according to the hydrometer Balling, and 2% agar was heated. After preparation, it was poured into test tubes and sterilized at 1.0 atm for 30 min (18).

In this nutrient medium, Fusarium fungi can grow and develop well, as well as the color and appearance of the fungus.

In addition, Fusarium fungi macroconidia do not occur in some species, so the composition of the nutrient medium: water - 1 l; KNO_3-2 g; KH_2PO_4 - 1 g; KCl -0.5 g; $MgSO_4$ - 0.5 g; $FeSO_4$ - 1 drop; starch - 0.1 g; glucose - 0.1 g; sucrose - 0.1 g; is prepared at the expense of. The Zeitz bacteriological filter was used to separate the fruits and conidia of the Fusarium fungi. Fusarium fungi were grown in liquid artificial medium.

In the cultivation of Fusarium fungi, potato glucose agar (potato 200, glucose 100, agar-agar 20 g); potato sucrose agar (1000 ml potato extract, sucrose 40 g, agar-agar 20 g); potato destrose agar (potatoes 200 g, dextrose 20 g, agar-agar 20 g) natural nutrient media and CHapek nutrient medium were used: Preparation of CHapek nutrient medium agar-agar as follows; $FeSO_4$ - 0.001 g, Mg SO_4 - 0.5 g, K_2HPO_4 - 1.0 g, KSl - 0.5 g, KNO3 - 2 g, agar-agar 20 g, 1 liter of distilled water, sucrose - 20 g.

To study the toxicity properties of liquid CHapek-Dox medium was used: $CuSO_4$ - 0.001 g, $ZnSO_4$ - 0.01 g, $FeSO_4$ x $7H_2O$ - 0.01 g, $MgSO_4$ x $7H_2O$ - 0.5 g, K_2HPO_4 - 1.0 g , $NaNO_3$ - 3.0 g, KCl - 0.5 g, sucrose - 30.0 g, 1 liter of distilled water, agar-agar any, 20 g.

Fusarium fungi were used to monitor macroconidia (Carnation listovoy agar-GLA) (1 liter of distilled water, KCl 6 g, agar-agar 20 g, carnation leaves 5-6 pieces, 5-10 mm).

In the study of the morphological structure of the mycelium of Fusarium fungi SNA (SNA Nirenberg) from the culture medium: KNO_3 - 1, KH_2PO_4 - 1, $MgSO_4x7H_2O$ - 0.5, KCl - 0.5, sucrose - 0.2, glucose - 0.2 , agar-agar 20 g, was used at the rate of 1 liter of distilled water. Pieces of sterile filter paper (1-2 cm) were placed in Petri dishes to facilitate the formation of spores on the surface of the sterile medium.

The laminar box was sterilized in bactericidal lamps for 30 minutes to ensure the purity of the cultivated Fusarium species by creating a sterile environment for the cultivation of the species intended to study the growth and morphological characteristics of the Fusarium species.

1. The purity of the food medium and containers was ensured during the purity of the research in the laboratory. The glassware used in the experiments was 1 atm in an autoclave. was sterilized for 60 min under pressure.

II. 3 To study the morphological and cultural characteristics of Fusarium fungi:

The structure of macroconidia and microconidia was observed by binocular microscopy in the study of cultures of species of Fusarium fungi isolated by mycological examinations.

1. In these studies, chlamydospores, septa, cells, mycelium, conidia were observed under the microscope of the organs of Fusarium fungi and the images were of good quality. In order to determine the colors formed by Fusarium fungi, drugs were prepared using the Kiray scale and Lugol's solutions, methyl blue, dyes, and the shapes of macroconidia and microconidia, cells and mycelium were photographed under a binocular microscope.

2. Fusarium fungi Macroconidia and microconidia were measured using a micrometer-mounted micrometer. The length and width of the isolate were measured by measuring 15 macroconidia and 15 microconidia in the isolates. This condition was performed in the laboratory in 4 repetitions.

After planting the mycelium of the Fusarium fungi, the morphological characteristics of the cultures were studied under the microscope (Nf / Seissberk Iyena) in situ and drop methods. Identification of Fusarium fungi was carried out by measuring with an eyepiece-micrometer.

III.1. RESULTS:

III. 1. GX-MS spectrum of metabolites formed in Chapek nutrient medium:

Metabolites - These substances are involved in various biological energy exchanges that are necessary for cell growth and the maintenance of vital functions.

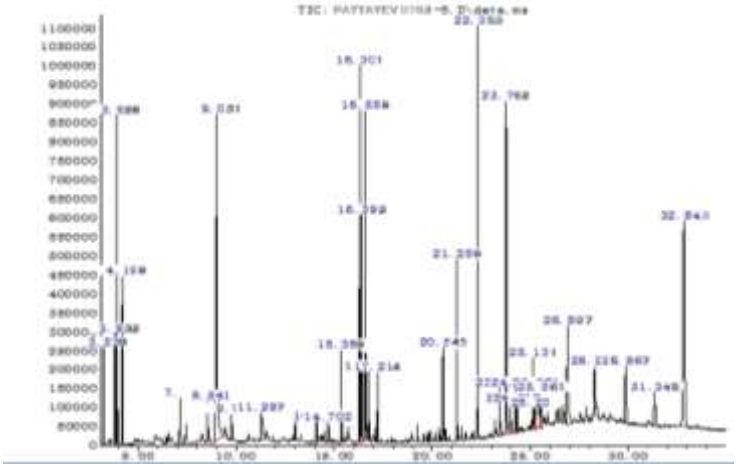

Figure III.1.1. Synthesis of metabolites in Chapek nutrient medium with fungal strain yeast extract.

Figure 3.1.1 shows the synthesis of several different metabolites in the Chapek nutrient medium mixed with yeast culture yeast extract. During this observation, the metabolism of fungi is more active and the metabolism is more extensive. The metabolic processes and fungi of fungi can also be attributed to the development of specific habitats in nature. The addition of yeast extract to the nutrient medium as a source of nitrogen allows the fungus to fully enhance its genetic process.

Table III.1.1. Metabolites of fungal cultures identified using GX-MS

№	RT	m/z	Modda nomi	Muvofiqligi ch, %
1.	3.239	106.078	Benzene, 1,3-dimethyl-	94
2.	3.832	106.078	o-Xylene	97
3.	3.928	106.078	p-Xylene	95
4.	4.199	116.084	Hexanois asid	78
5.	7.127	117.058	Benzyl nitrile	97
6.	9.031	136.052	Benzeneasetis asid	94
7.	11.287	187.121	5-Amino-1-pentanol, N,O-diasetyl-	59
8.	14.703	198.137	3,6-Diisopropylpiperazin-2,5-dione	59
9.	15.369	154.099	5-Isopropylidene-3,3-dimethyl-dihydrofuran-2-one	53
10.	16.392	423.371	l-Leusine, N-syslopropylsarbonyl-, hexadesyl ester	50
11.	16.589	210.137	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-	94
12.	17.214	278.152	Dibutyl phthalate	96
13.	20.545	244.121	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(phenylmethyl)-	97
14.	21.259	338.391	Tetrasosane	97
15.	22.353	212.214	2-Tetradesanone	64
16.	25.563	146.073	1(2H)-Naphthalenone, 3,4-dihydro-	25
17.	26.897	198.116	Propanedinitrile, trisyslo[3.3.1.1(3,7)]desylidene-	72
18.	28.225	190.121	Dimethyl(trimethylsilylmethyl)ethoxysilane	22
19.	29.867	320.189	Asetamide, 2-(adamantan-1-yl)-N-(quinolin-6-yl)-	15
20.	31.345	304.121	4-Hydroxy-3-(.alpha.-iminobenzyl)-1-methyl-6-phenylpyridin-2(1H)-one	27
21.	32.843	404.174	1,8-Diphthalimidoostane	25

The names of the substances are given in English as in the GX-MS database

A total of 21 metabolites were reported under fungal cell culture (nutrient medium with 5 g / l yeast extract) (Table 3.1.1). As shown in Figure 3.1.1, each cell produces its own metabolite. Metabolite analysis revealed that the fungal cell synthesized 21 metabolites. This proves that the conditions under which the fungus grows are affected by the growth of the cells.

In our experiments, nutrient media were characterized by the effect of compounds on the growth and development of phytopathogenic fungal cells.

CONCLUSION:

The experiments examined the growth characteristics of previously studied strains of phytopathogenic micromycetes, including Fusarium oxysporum f.sp.vasinfestum, which infect cotton plants (Gossypium hirsutum L.) in various nutrient media. The metabolites of the isolated fungal cultures, the composition and spectra of the substances they synthesize were analyzed.

Metabolite analysis revealed that the fungal cell synthesized 21 metabolites. Evidence suggests that fungal cells affect cell growth under growing conditions.

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