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Evaluation of Antifungal Activity 3-(1-Methoxy Napthalen-2-Yl)-5-Phenylisoxazole.

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Abstract:*Isoxazole derivatives have been prepared bycondensing alpha-naphtholwith acetic unhydrideres pectively. While compounds have been synthesized by the reaction of3-(1-methoxy napthalen-2-yl)-5-phenylisoxazole.All the compounds were screened for their antifungalactivities. The structures of newly synthesized compounds were established on thebasis of elemental analyses, IR. The newly synthesized heterocyles were characterized basedon their chemical properties and spectroscopic data, and were found to inhibitFusariumoxysporum.*

Keywords:*Isoxazole derivatives,Fusariumoxysporum,anti-fungal activity.*

I. Introduction

Fungi

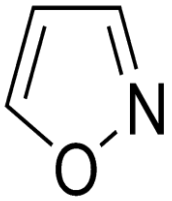
The branch of microbiology that deals with the study of fungi (yeasts and molds) is called mycology[1]. The different groups of fungi have different levels of cellular organization. Some groups consist of single-celled organisms that have a single nucleus per cell. (A nucleus is a membrane-enclosed structure within a cell that contains the cell's genetic material and controls its growth and reproduction.) Other groups consist of single-celled organisms in which each cell has hundreds or thousands of nuclei.[2]

Fungi, the word for more than one fungus, can be found on different parts of the body. Here are some common types of fungal infections:

- **Tinea** (say: **tih**-nee-uh) is a type of fungal infection of the hair, skin, or nails. When it's on the skin, tinea usually begins as a small red area the size of a pea. As it grows, it spreads out in a circle or ring. Tinea is often called ringworm because it may look like tiny worms are under theskin. Because the fungi that cause tinea (ringworm) live on different parts of the body, they are named for the part of the body they infect. Scalp ringworm is found on the head, and body ringworm affects any other skin areas.
- **Athlete's foot** is another type of fungal infection that usually appears between the toes but can also affect toenails and the bottom or sides of the feet.
- **Jock itch** is a fungal infection of the groin and upper thighs. You might think only men and boys get it, but girls and women can get it, too.
- **Candida** (say: **kan**-duh-duh) is a yeast, similar to a fungus. It most often affects the skin around the nails or the soft, moist areas around body openings. Diaper rash in babies can be from one type of candidal infection, as can thrush (white patches often found in the mouths of babies.) Older girls and women may develop another form of candidal infection in and around the vagina. This is called a yeast infection.

Fusarium spp. infects neutropenic patients to cause pneumonia, fungemia, and disseminated infection with cutaneous lesions.[3]It is a common vascular wilt fungal disease, exhibiting symptoms similar toVerticillium wilt. The pathogen that causes Fusarium wilt is *Fusariumoxysporum* (*F. oxysporum*). The fungal pathogen *Fusariumoxysporum* affects a wide variety of hosts of any age. Tomato, tobacco, legumes, cucurbits, sweet potatoes and banana are a few of the most susceptible plants, but it will also infect other herbaceous plants.[4]*Fusariumoxysporum* generally produces symptoms such as wilting, chlorosis, necrosis, premature leaf drop, browning of the vascular system, stunting, and damping-off. [5] Fusarium wilt starts out looking like vein clearing on the younger leaves and drooping of the older lower leaves, followed by stunting of the plant, yellowing of the lower leaves, defoliation, marginal necrosis and death of the plant. *F. oxysporum* f. sp. *batatas* affects sweet potato.*F. oxysporum* f. sp. *cubense* causes Panama disease on banana. It is found everywhere bananas are grown in Africa, Asia, Central and South America.The disease starts out as yellowing and drooping on one side of the plant.[6]*F. oxysporum* f. sp. *melonis* attacks muskmelon and cantaloupe.[5] These remarkably diverse and adaptable fungi have been found in soils ranging from the Sonoran Desert, to tropical and temperate forests, grasslands and soils of the tundra.[7] *F. oxysporum* strains are ubiquitous soil inhabitants that have the ability to exist as saprophytes, and degrade lignin [8]and complex carbohydrates[9,6]associated with soil debris. Isoxazole is a five membered heterocyclic compound having two hetero atoms: oxygen at position 1

and nitrogen at position 2. Claisen first reported an isoxazole (I) for a product from the reaction of 1,3diketone with hydroxylamine hydrochloride.Subsequently a solid foundation for the chemistry of isoxazole was laid down by Claisen and his students. It was shown to possess typical properties of an aromatic system but under certain reaction conditions. Particularly in reducing or basic media, it becomes very highly labile. [10]



The next important contribution to the chemistry of isoxazoles was made by Quelicoin 1945, when he begane to study the formation of isoxazoles from nitrile N-oxide and unsaturated compounds.

Synthetic Aspect: Isoxazoles can be prepared by various methods; some of them are described as under.

1. A variety of 3,5-disubstituted 4-bromoisoxazoles(II) are readily prepared in good to excellent yields under mild reaction conditions.
2. Tayade V. B. et al.have synthesized some new 3,5-diarylisoxazoles from the reaction of 2-aryl acetophenones with hydroxyl amine hydrochloride in presence of alkali.[11]
3. Dawood Kamal etal.have prepared isoxazole derivatives from enamino nitriles[12].
4. Mark Lautens and Ame´lie Royhave constructed isoxazoles (III), wereachieved in good yields in a rapid and simple way by using *N*-acetoacetyl derivatives
5. Solid phase synthesis of isoxazole derivatives based on aminoacids was reported by Lidia De Luca and co-workersin the presence of basic catalyst and dichloromethane used as a solvent. One pot synthesis of polyfunctionalizedisoxazoleshave been synthesized by the reaction of dipyrrolidinium 3,3-dimethylpentanedinitrile-2,4-dinitronate and acetyl chloride in benzene.[13]
6. Keisuke Suzuki et al.have synthesized functionalized isoxazole derivatives (IV) by cyclocondensation of C-chlorooximes with cyclic 1,3-diketones. [14]
7. Crawley L. S. and Fan Shawe W. Jhave prepared isoxazole (V) from α,β-unsaturated carbonyl compounds, hydroxyl amine hydrochloride and KOH in methanol.[15]
8. R. Kalirajan*etal*.have synthesized and check antimicrobial screening against

various gram positive and Gram negative bacteria and anti-fungal activity against various fungal stains compared with standard drug (Ampicillin and Ketoconazole) using solvent control.[16]

Therapeutic Importance

Isoxazole derivatives exhibit various biological activities such as,

1. Antibacterial [14]
3. Anticholestermic[17]
4. Anticancer [18]
5. Anthelmintics[19]
6.Anticonvulsant[20]

Table 1.IR Interpretation of 3-(1-methoxy napthalen-2-yl)-5-phenylisoxazole.

Sr.No.	Wave Number (cm^{-1})	Remark
1	1678	C=N
2	3066	Ar C—H
3	1593	Ar C=C
4	682	C-Cl

II. Material & Methods

Antifungal Activity

Materials:

Culture media: Potato dextrose agar (PDA) medium , Nutrient Broth.

Collection of Fungal strains:

Pure cultures of F. Oxysporum (FO) were obtained from the Government institute of science, Department of botany, Aurangabad.The collected fungi were cultured on potato dextrose agar (PDA) as the growth medium for all test fungi on petridishes and Incubated at room temperature.

Assay for antifungal activity:
Preparation of fungal inoculums
For fungal inoculums, Potato dextrose agar (PDA) pours plates were prepared. At the center of these plates 5 days old test fungiwere transferred and incubated at (25±2)0C. After 5 days of
Incubation they were ready to use.

Procedure :

The poisoned food technique was used to assay antifungal Activity. From this, required concentration of test sample was taken by sterilized pipette in a sterilized petriplate andThen 15 ml medium was poured into the petriplate and mixed wellAnd allowed to solidify. Inoculation was done at the center of eachPlate with 6 mm mycelium block for each fungus. The myceliumblock was prepared with the help of cork borer from the growingarea of a 5 days old culture of the test fungi on PDA. The blockswere placed at the center of each petriplate in an inverted positionto get greater contact of the mycelium with the culture medium. The inoculated plates were incubated at(25+_ 2)0C.The experiment was repeated for three times.Proper control (PDAwithout extract) was also maintained. After 7 days of incubation the diameter of fungalcolonies were measured. The average of three measurements wastaken as colony diameter of the fungus in mm.[21]Inhibition effect of 3-(1-methoxynapthalen-2-yl)-5-phenylisoxazole on *Fusarium oxysporum*By using poisoned food technique in potato dextrose agar medium(PDA).
Formula:
The percentage inhibition of mycelial growth of the test fungus was calculated by
the following formula: I= (C-T)/C X 100,
Where, I=Percentage of Inhibition,
C=Diameter of the fungal colony in control,
T=Diameter of the fungal colony in treatment

III. Results and Discussion

The targeted compounds synthesized were screened for the antifungal potential against *Fusariumoxysporum*was found to be sensitive to Itraconazole, Fluconazole, Ketoconazole and Clotrimazole but developed resistance against common antifungal antibiotics such as Nystatin and Amphoterecin-B. For convenience the synthesized compounds 3-(1-methoxynapthalen-2-yl)-5-phenylisoxazole. The test compounds with the final treatment concentrations of 50- 1000 µg/mL was prepared. The fungistatic assay was carried out using potato dextrose agar(PDA)medium.

The synthesized compound were evaluated for their antifungal activity against *Fusariumoxysporum.*The relative broad spectrum of activity of the azoles against common fungal pathogens, ease of administration and limited toxicity are highly attractive features. Fluconzole and itraconazole are better tolerated and more effective thaketoconazole.These agents have several drawbacks and limitations also. One potential limitation of the azole antifungal agent is the frequency of their interaction with co administered drugs, which results in adverse consequences. A second limitation of the azoles is the emergence of resistance of fungal organisms, especially *fusiariumoxysporum,* to fluconazole. These limitations of the azoles will become more problematic if fluconazole and other azoles continue to be used injudiciously.In poisoned food technique showed percentage inhibition of mycelial growth of the test fungus fusiariumoxysporum.

Observation Table:
Table.2. In vitro antifungal activity of 3-(1-methoxy napthalen-2-yl)-5-phenylisoxazole against the strains of F. oxysporum.

Concentration/ days	**50ug/ ml**	**100ug/ ml**	**200ug/ml**	**300ug/ ml**	**400ug/ml**	**500ug/ml**	**Std-10ug/ml**	**Control DMF**
1st day	9mm	8mm	8mm	7mm	6mm	Growth inhibition	Growth inhibition	14mm
2nd day	11mm	11mm	10mm	9mm	8mm	Growth inhibition	Growth inhibition	21mm
3rd day	13mm	12mm	11mm	10mm	9mm	Growth inhibition	Growth inhibition	36mm
4th day	16mm	15mm	12mm	11mm	9mm	Growth inhibition	Growth inhibition	48mm
5th day	20mm	17mm	12mm	11mm	9mm	Growth inhibition	Growth inhibition	62mm
6th day	20mm	17mm	12mm	11mm	9mm	Growth inhibition	Growth inhibition	79mm
7th day	20mm	17mm	12mm	11mm	9mm	Growth inhibition	Growth inhibition	90mm

The different test concentration 50- 500 µg/mL was prepared.
50ug/ml % of growth inhibition **[77.77%]**,
100ug/ml % of growth inhibition **[81.11%],**
200ug/ml% of growth inhibition **[86.66%],**
300ug/ml % of growth inhibition**[87.77%]**,
400ug/ml % of growth inhibition **[90.00%],**
500ug/ml % of growth inhibition [**100%],**
Std-10ug/ml % of growth inhibition [**100%].**

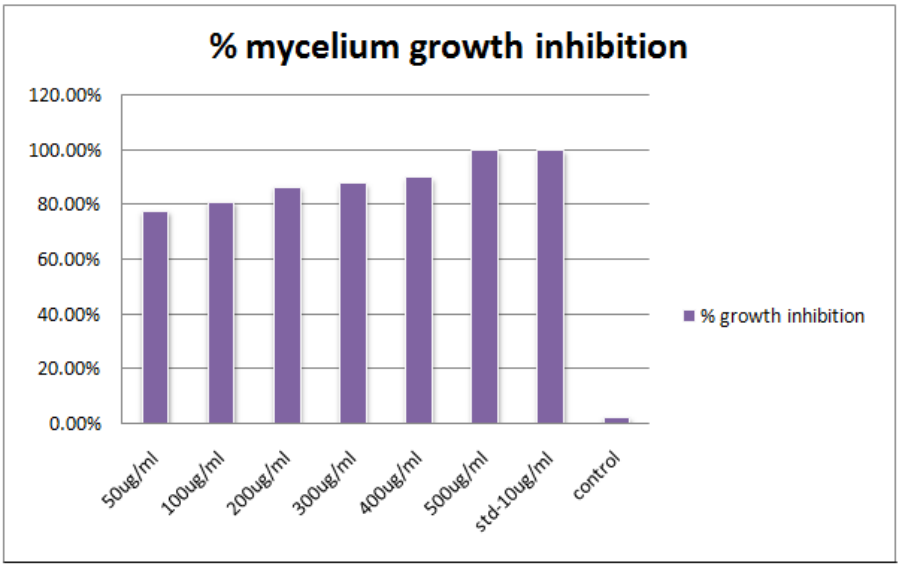


Figure 2. Inhibition effect of 3-(1-methoxynapthalen-2-yl)-5-phenylisoxazole on Fusariumoxysporum.

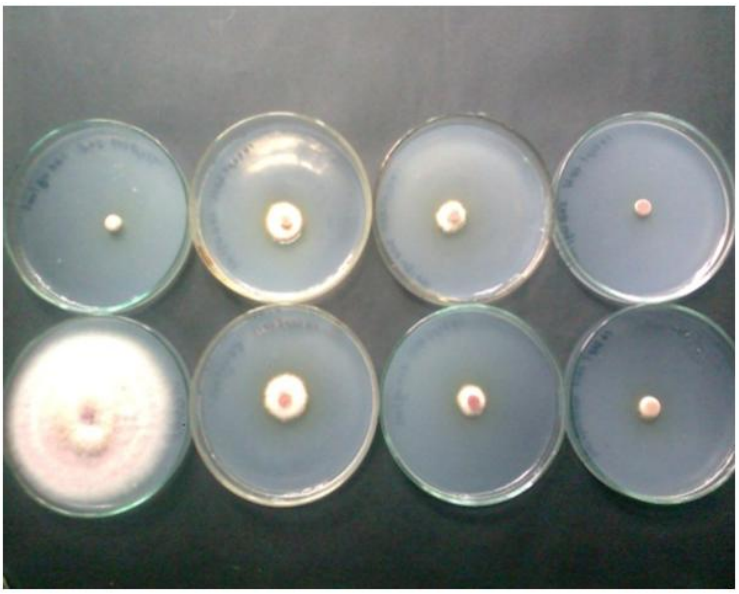


Figure 1: Inhibition effect of 3-(1-methoxy napthalen-2-yl)-5-phenylisoxazole on *F. oxysporum.*

IV. Conclusion

The aim of present study is to design the new synthetic compound which is more potential against *Fusariumoxysporum* (growth of inhibition in 500ug/ml).The relative broad spectrum of activity of the azoles against common fungal pathogens, ease of administration and limited toxicity are highly attractive features. Fluconzole and itraconazole are better tolerated and more effective thaketoconazole.These agents have several drawbacks and limitations also. One potential limitation of the azole antifungal agent is the frequency of their interaction with co administered drugs, which results in adverse consequences. A second limitation of the azoles is the emergence of resistance of fungal organisms, especially *fusiariumoxysporum* to fluconazole.

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