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RESEARCH ARTICLE

INTERACTIONS BETWEEN MUSHROOMS AND FUNGI IN DUAL-CULTURE EXPERIMENTS.

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Abstract

The current study was undertaken to measure the interaction between five wild mushrooms collected from natural habitat and mycoparasitic fungi such as Fusarium sp. Pythium sp. and Aspergillus sp. Study showed that replacement was 53.32% and deadlock (40.00%). Therefore in 53.32% of pairings the mushrooms replace effectively the pathogenic fungi. Complete replacement and partial growth were take place in equal frequency (6.66%, 6.66%). The results revealed that 93.32% of pairings led to prevention and limited the fungal growth. Identification of wild mushroom strain based on DNA Sequencing by ITS1and ITS4. Through molecular taxonomy sample identified as 8/12 (Clitopilus scyphoides), 33/12 (Agrocybe pediades), 45/12 (Agaricaceae sp.), 48/12 (Trametes ochracea) and (112/12) Irpex lacteus.

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Introduction:-

Only limited information is available on competition of mushroom against the pathogenic fungus. Fusarium, Pythium, Aspergillus sp. is commonly cause plant pathogen. Plant pathogens, Fusarium species cause various diseases on plants [1]. As well as in animals[2]. Fusarium species cause a various infectious diseases in humans such as septic arthritis, endophthalmitis, osteomyelitis, cystitis and brain abscess[3,] fusariosis depends largely on the immune status of the host and the portal of entry[4] and largely concerned with allergic diseases such as sinusitis and mycotoxicosis in individuals[5]. Pythium is pathogen that causes diseases in animal as well as in human[6] , several cases of human pythiosis have been reported[7] and arteritic infection[8-9]. Aspergillus is a group of saprophytic moulds and cause illness in humans and animals. Commonly disease caused by Aspergillus is apergillosis and it takes several forms like Invasive aspergillosis that affects patients with neutropenia[10]. Infections of the larynx, trachea, and bronchus have been reported in immunocompromised hosts[11]. Aspergillus species have been also investigated that were responsible for both native and prosthetic valve endocarditis[13], The A. fumigatus and A. nidulans most commonly causative agents of invasive aspergillosis (IA) in patients with chronic granulomatous disease (CGD**)**[14]**.** As we know the ratio of well investigated mushroom for antagonistic is low. Therefore, in this study we focused on the awareness for wild mushrooms as biologically active with medicinal potential. Therefore in the antagonistic study we have used five unknown wild strains of mushroom were collected from Himachal Pradesh from different places, against the three pathogenic strains (Fusarium, Pythium and Aspergillus sp.).

And in further research we have subjected these five samples of mushroom for isolation of bioactive compounds.

Materials and methods:-

Collection and isolation of mushroom sample:-

The wild strain of fleshy fungi was collected from forest of district Solan (Kasauli) from Himachal Pradesh in INDIA. Sample was placed into plastic bag and cut, cleaned with disinfectants. After that dried at 50 to 60°C and then put in refrigerator for further use. The fruiting body of the mushroom was collected at a young stage with the aid of sterile forceps, wrapped with sterile foil paper and transported to the laboratory, washed thoroughly with

several changes of sterile distilled water and 0.001% Mercuric Chloride and aseptically break lengthwise exposing the inner tissue (trama) with the aid of a sterile blade. A small piece of 2 x 2mm of the sterile tissue will aseptically transfer onto plates of (2%) malt extract agar and the plates were incubated at 25°C to 30°C temperature for 7 days. Sub sculturing for pure tissue mycelial production was prepared by transferring a small square of 5 x 5mm from the mother plate onto a fresh solid media plates.

Antagonistic activity of wild mushrooms against pathogenic fungi:-

Testing antagonistic activity:-

The pathogenic fungus tested were collected from molecular biology lab from Shoolini university, India. Competitive interaction between wild mushrooms and soil born plant pathogenic microorganisms was evaluated by dual culture experiments on Petri dishes (90 mm diameter containing 20 ml PDA. In each Petri dish 2 mm diameter mycelial disks, one each from mushroom and fungal colonies was placed on the agar surface 30 mm apart. Immediately after inoculation, the plates were sealed with plastic film and incubated in darkness at 24°C for 10 days. Colony growth and type of interaction was examined regularly. The presence of dense zones of mycelium, aggregated structures such as mycelial cords, pigmented hyphae, exudate droplets and dark pseudo sclerotial lines. The antagonistic activity of the various mushroom cultures was tested by the method described by [15].

Antagonistic rating scale:-

The antagonistic ability of each mushroom was determined by using antagonistic rating scale given by [16] with slight modifications.

The following score was assigned to each type or sub-type of reaction: A=1; B=2; C=3; CA1=3.5; CB1=4; CA2=4.5; CB2=5, as shown in **(Table 1).**

Table 1:- The antagonism index (AI)

Subtypes	Interaction category	Score
A	deadlock with mycelial contact	**1**
B	deadlock at a distance	**2**
C	overgrowth without initial deadlock	**3**
CA1	partial replacement after initial deadlock with contact	**3.5**
CA2	complete replacement after initial deadlock with contact	**4.5**
CB1	partial replacement after initial deadlock at a distance	**4**
CB2	complete replacement after initial deadlock at a distance; * overgrowth of the mushroom by the	**5**

The antagonism index (AI) was then calculated for each fungal species using the following formula: AI=A(n1)+B(n2)+C(n3)+CA1(n3.5)+CB1(n4)+CA2(n4.5)+CB2(n5) where n= frequency of each type or sub-type of reaction.

Molecular Identification of mushroom:-

Molecular identification was done to identify the unknown samples of mushroom. DNA isolated and PCR amplification: Genomic DNA isolated from HIMEDIA kit. Then PCR amplification was performed using a pair of universal primers ITS-1(5'- TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'TCCTCCGCTTATTGATATGC-3') for the region containing ITS1, ITS4 and 5.8S rDNA. The reaction mixture was followed by the kit (HIMEDIA X-PERT). Amplifications will be performed in 50-µl reactions of PCR buffer and PCR reactions will be consists of an initial denaturation at 94°C for 1 min 25 s, 35 cycles of amplification, and a final extension at 72°C for 10 min; each cycle of amplification will be consisted of denaturation at 95°C for 35 s, annealing for 55 s (at 55°C for reactions with ITS1 and ITS4 and at 60°C for reactions with ITS1 and ITS4), and extension at 72°C for 1 min. Amplification products was electrophoreses in 0.8% agarose gel, stained with ethidium bromide and amplicans will be observed under UV light [17].

Sequencing:-

The PCR product was subjected for sequencing and the DNA sequences was used for. The DNA sequences were used in NCBI (National Center for Biotechnology Information) BLAST (Basic Local Alignment Search Tool) search for bioinformatics analysis, to look for homologus nucleotide sequences. On the basis of this information, species status was assigned.

Results:-

The frequency of each type and sub-type of reaction is shown in **(Table 2, 3)**. Study revealed that replacement was more frequent (53.32% + 6.6%) than deadlock (40.00%). In 53.32% of pairings the mushrooms replace effectively the pathogenic fungi. Complete replacement and partial growth were take place in equal frequency (6.66%, 6.66%) except overgrowth (40.00%) as shown in (**Figure 1, 2 and 3)**. Overall 93.32% of pairings led to prevention and limited the fungal growth. This clearly indicated that the mushrooms were competetive against the pathogenic fungi.

Table 2:- Antagonism index values (in brackets) and competitive reactions between mycelium mushrooms and pathogenic fungi in pairings on potato dextrose agar medium.

Mushroom samples	Pathogenic fungus			
	Phythium sp.	**Aspergillus sp.**	**Fusarium sp.**	**Total antagonism index**
8/12	A	C	A	5
33/12	C*	C	C	6
45/12	C	C	C_{A1}	9.5
48/12	C	A	A	4
112/12	A	C_{B1}	A	6.5

A, deadlock with mycelial contact; **B**, deadlock at a distance; **C**, overgrowth without initial deadlock; $\mathbf{C_{A1}}$, partial replacement after initial deadlock with contact; $\mathbf{C_{A2}}$, complete replacement after initial deadlock with contact; $\mathbf{C_{B1}}$, partial replacement after initial deadlock at a distance; $\mathbf{C_{B2}}$, complete replacement after initial deadlock at a distance; (* overgrowth of the mushroom by the phytopathogenic fungus).

Table 3:- Frequency of type and subtype of interactions between mycelium of xylotrophic mushrooms and pathogenic fungi of cereals in dual culture experiments on potato dextrose agar medium, expressed as a percentage of the total number (31) of pairings tested.

Deadlock		**Replacement of pathogenic fungi by mushrooms**		**Replacement of by mushrooms by pathogenic fungus**	
Subtype	**%**	**Subtype**	**%**	**Subtype**	**%**
A	40.00	C	40.00	C*	6.6
B	0	C_{A1}	6.66	C_{A1*}	0
		C_{A2}	0	C_{A2*}	0
		C_{B1}	6.66	C_{B1*}	0
		C_{B2}	0	C_{B2*}	0
Total	40.00	Total	53.32	Total	6.6

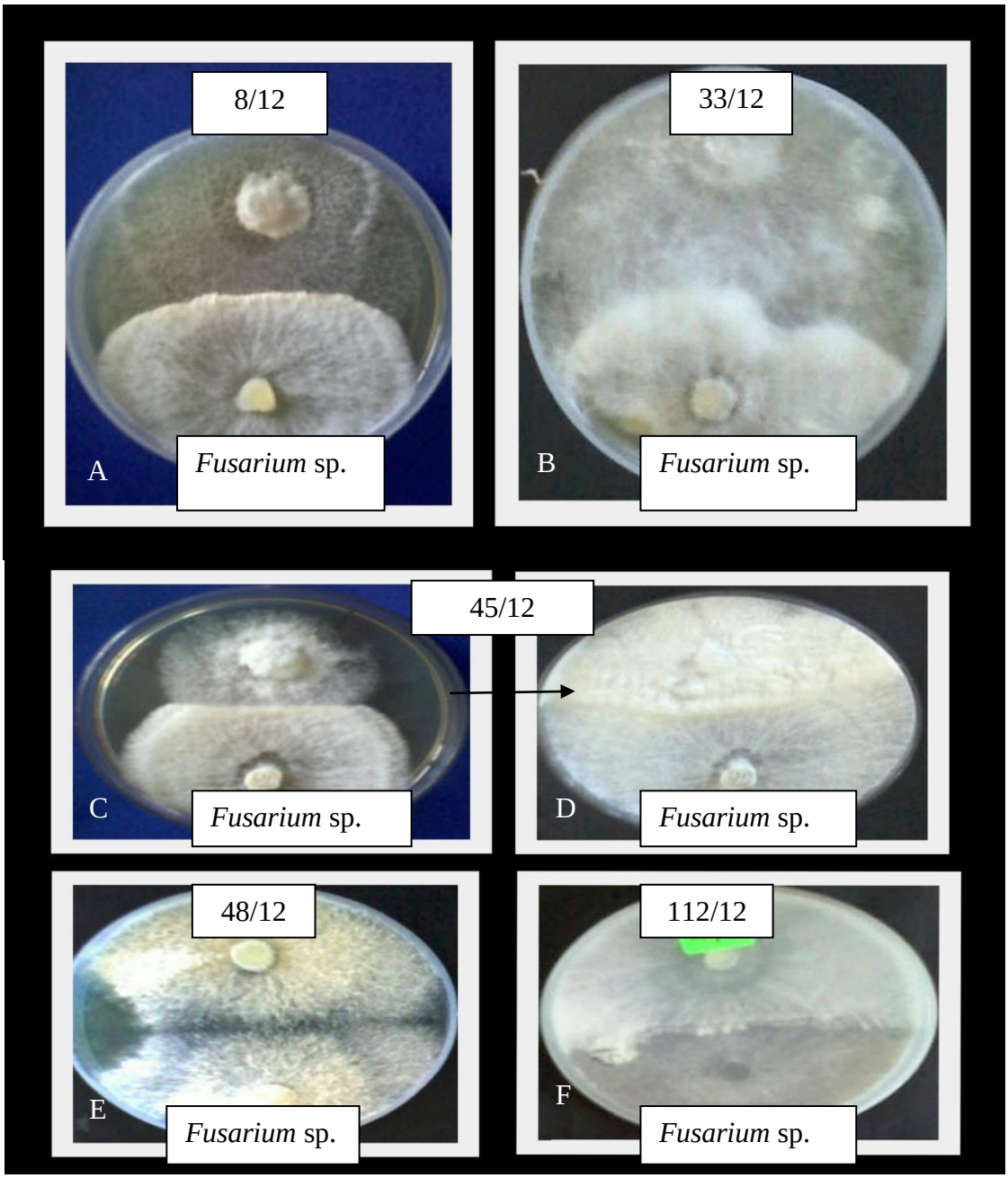

Figure 1: (A) Deadlock with mycelial contact between 8/12 and Fusarium sp. 10 days after inoculation. **(B)** Over growth by 33/12 wihout initial contact in Fusarium sp. within 25 days after inoculation . In **(C) and (D)** after dead lock in 8 and 10 days then followed by complete replacement of 45/12 by Fusarium in 14 days and 17 days **(E)** Deadlock with mycelial contact between 48/12 and Fusarium 7 days after inoculation **(F)** Deadlock with mycelial contact between 112/12 and Fusarium 10 days after inoculation.

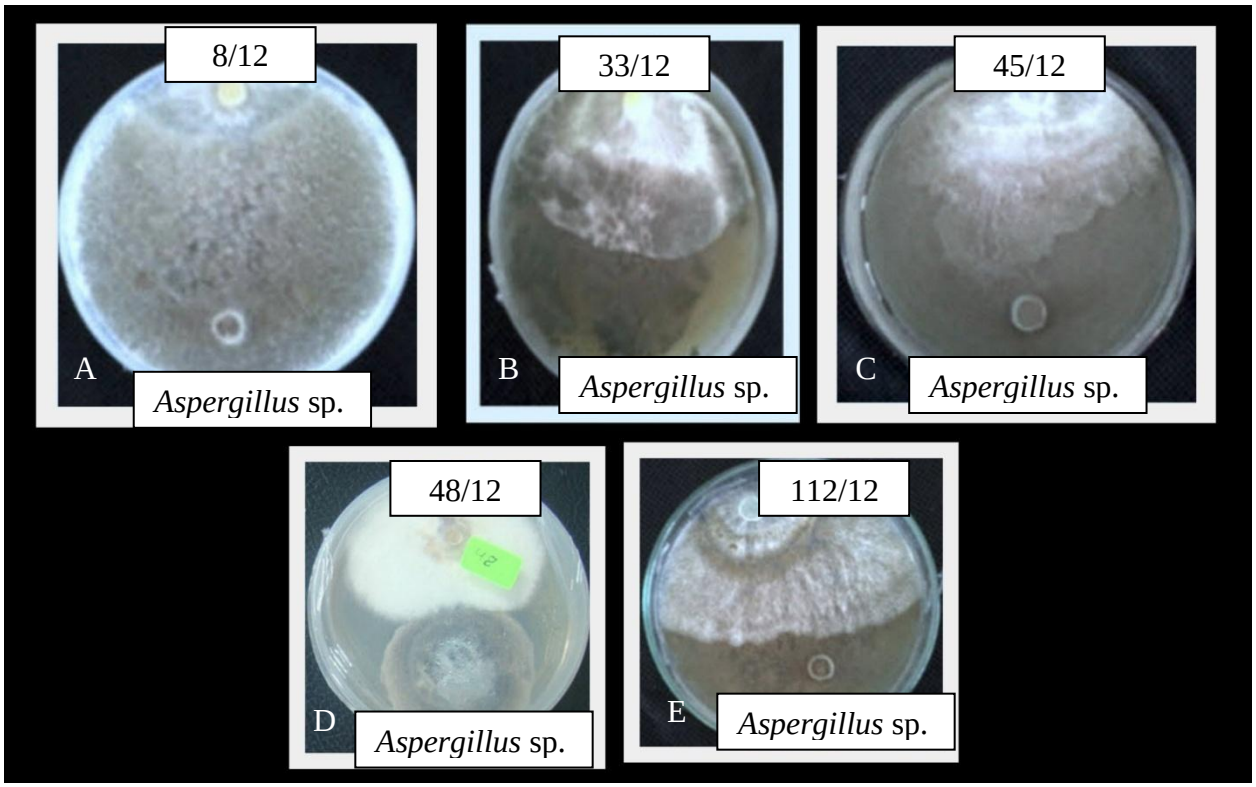

Figure 2: (A) Replacement of the pathogenic fungus by dense mycelial cords of 8 after 20 days of inoculation. **(B)** Initial deadlock between 33 and pathogenic fungus 7 days after inoculation then followed by replacement of Aspergillus after 14 days of inoculation. **(C)** Partial replacement of pathogenic fungus by 45 in 10 days after inoculation. **(D)** Deadlock with mycelial contact between 45/12 pathogenic fungus 20 days after inoculation. **(E)** Partial replacement of the pathogenic fungus by mycelial waves of 112 without dead lock.

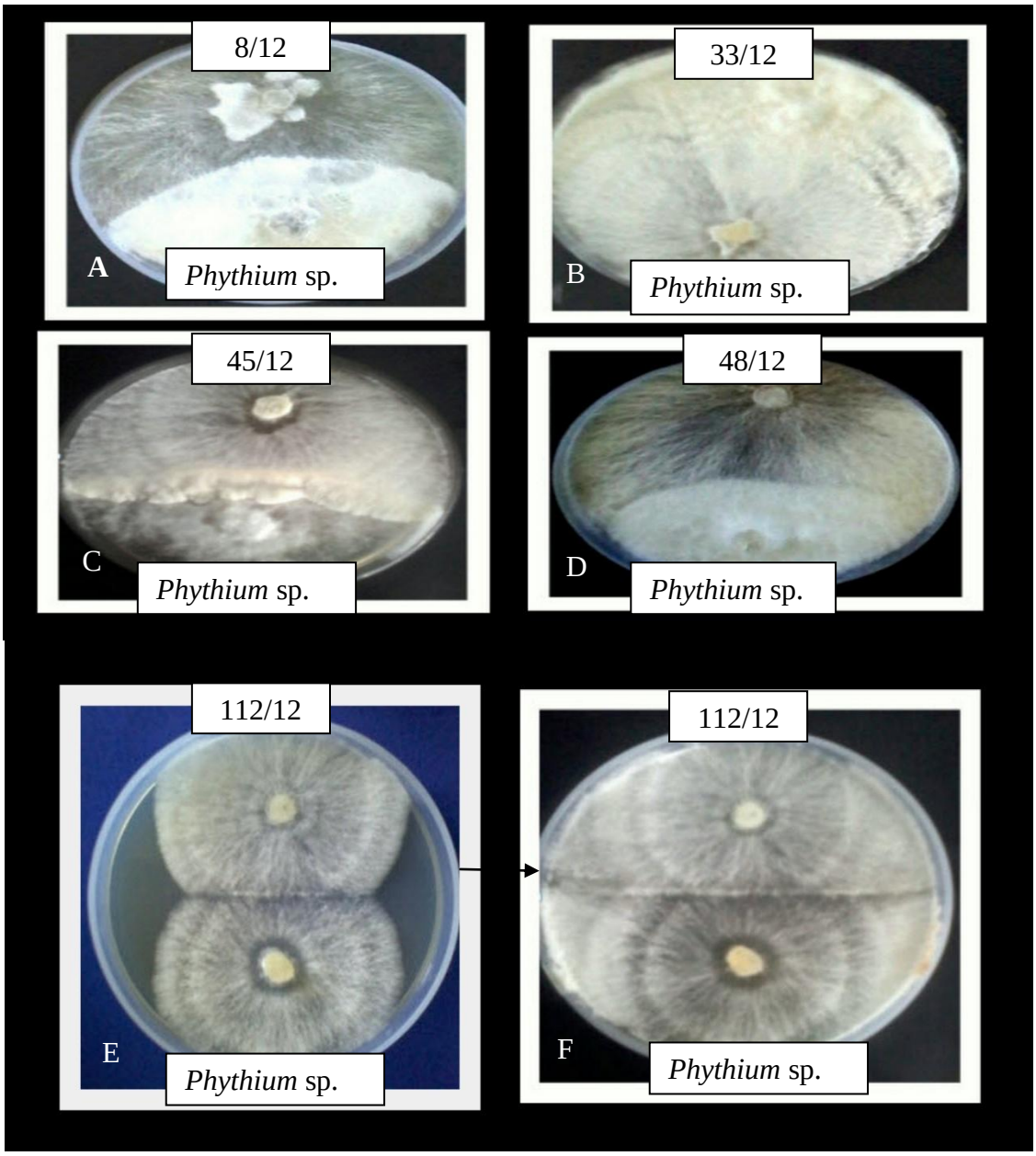

Figure 3: Combative interactions between mushrooms and phytopathogenic fungi in dual cultures on potato dextrose agar. **(A)** Deadlock with mycelial contact between 8/12 and Pythium sp. 20 days after inoculation. **(B)** Complete replacement of 33/12 by phythium sp. in 10 days after inoculation. **(C)** Partial replacement of Pathogenic fungus by dense mycelial waves of 45 after 20 days. **(D)** Over growth by 48 over the pathogenic fungus wihout initial contact in 21 days after inoculation. (**E) and (F)** Deadlock with mycelial contact between 112 and Pythium sp. 7 days after inoculation.

Therefore five new species were subjected to identify on the basis of molecular indentification. The size of the DNA of five mushroom samples was around 600bp in **(Figure 4)**. The rDNA-ITS (Ribosomal DNA Internal Transcribed Spacers) fragments of the genomic DNA were amplified using ITS1 and ITS4 primers. The PCR amplification products (**Figure 5)** showed that 8/12 gave around (500 bp) amplified band, while 33/12 and 112/12 bands around 490 bp, 45/12 and 48/12 showed bands at 650 bp. These PCR products were gel purified, run in 1% agarose gel, and processed for nucleotide sequencing. The nucleotide sequences of five samples were obtained and then analyzed for Basic Local Alignment Search Tool (BLAST). The BLAST result were presented in (**Table 4)**. Identification was based on % highest coverage and followed by the Accession no. The mushrooms may be identified as follows: 8-

Clitopilus scyphoides (99% KC176282.1), 33-Agrocybe pediades (99% FJ8 10133.1), 112- Irpex lacteus (100% JX290579.1), Trametes ochracea (99% KC492579.1) as shown in (**Table 5)**. Tested 112/12 collections are potential producers of antifungal metabolites which significantly suppressed the growth of test phytopathogenic fungi. Among tested species the highest activity was detected in 112/12.

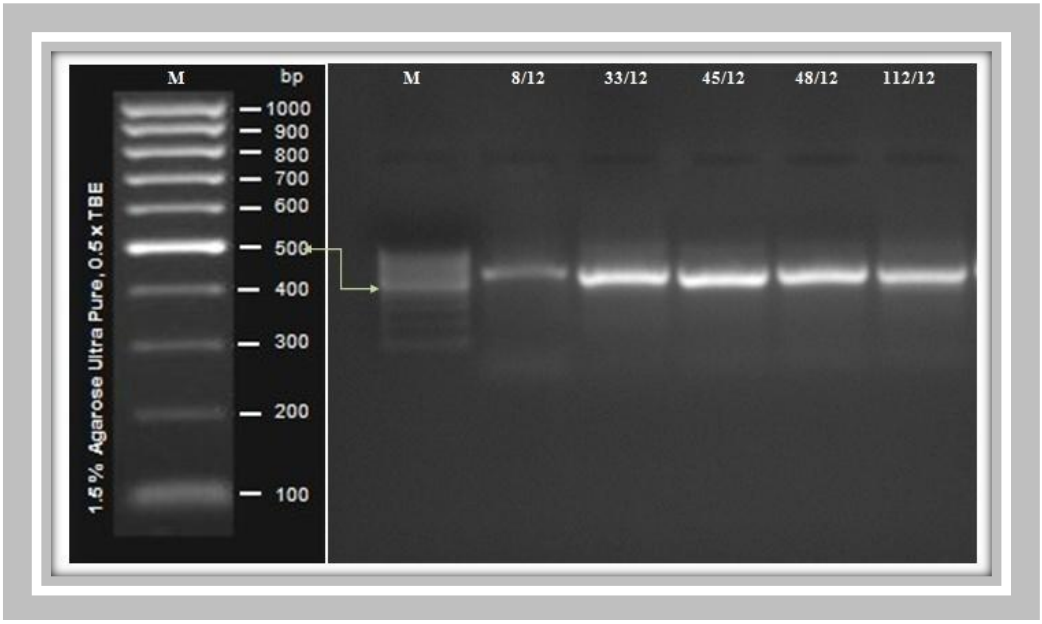

Figure 4: Gel purified DNA product of the isolated five mushroom samples, mentioned above and marker M (marker lane), 100bp plus ladder.

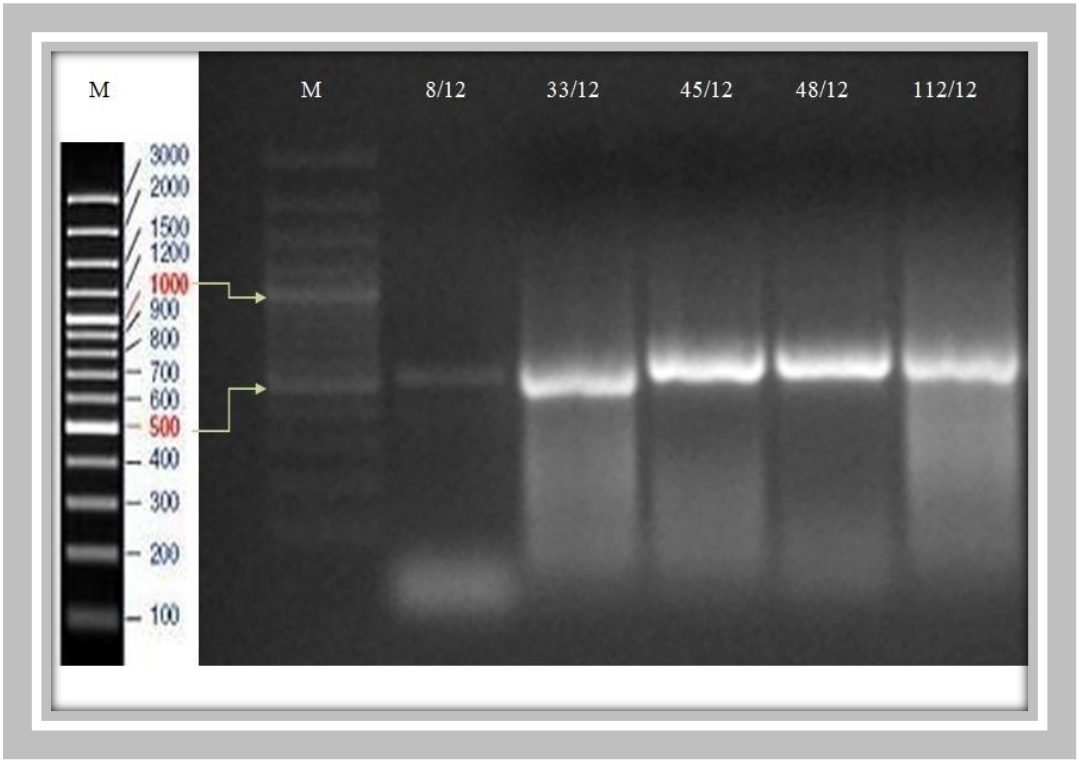

Figure 4: Gel purified PCR product of the isolated wild mushroom samples M (marker lane), 100 bp plus ladder).

Table 4:- Nucleotide sequences of the field isolated mushroom samples

Sample No.	Sequences
8/12	>8 TGCGGAAGGATCATTATTGAATAAACTTGGTCAAGCTGTTGCTGGTCCTTCGGG GCATGTGCACGCTTGCCACCAATTTTAACCACCTGTGCACCTTTTGTAGACTAGA AACGTTTCTCGAGGCAACTCGGATTGAGAACTGCTGCGCGAAAGCCAGCTTCTT GTGTTTCTCAGTCTATGTTTTTACATACCCCGAATGAATGTATCAGAATGTATTG CTTGGCCTTAGTGCCTTTAAATCAAATACAACTTTCAACAACGGATCTCTTGGCT CTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATGCGAATT CAGTGAATCATCGAATCTTTGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAG CATGCCTGTTTGAGTGTCATTAAATTCTCAACTATACAAGTTTTTATTAACATGT ATAGCTTGGATCATGGGATTTGCGGGCTTTCACAAGTCGGCTATCCTCAAATGC ATTAGCAGAGCTTTTGCCGCTAATCTCTGGTGTGATAATTATCTACGCCATTGAG AAGTGACATATTGAGGCTTCGCTTCTAATCGTCTTTACCGACAATTTTTGACAAT CTGACCTCAAATCAGGTAGGATTACCCGCTGAACTTAAGCATACAATA
33/12	>33 CTTTCCGTAGGGGGAACCTGCGGAAGGATCATTATTGAATAAACCTGGCGTGGT TGTAGCTGGTCCTCTCGGGGACATGTGCTCGCCCGTCATCTTTATATCTCCACCT GTGCACCCTTTGTAGACCTGGACGATAACTTTCCGAGGCAACTCGGTCGGGAGG ACTGCTGGCTTTCACGAGTCGGCTTTCCTTGTATTATCCAGGCCTATGTCTTACA CATACCCCAAAGAATGTAACAGAATGTATTGTATATGGCCTAGTGCCTATAAAC TATATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCA GCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTT GAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATT AAATTCTCAACCTTATTAGCTTTTGCTGATAATGGCTTGGACTTGGGGGTCTTTT TGCTGGCTTTCATTAGTCTGCTCCCCTTAAATGTATTAGCCGGTGCCCCCCAGTG GAACCGTCTATTGGTGTGATAATTATCTACGCCGTGGACGTCTGCTATAATGGGT TTGCGCTGCTTCTAACCGTCTCTCGGGACAACACAAATGACAATTTGACCTCAA ATCAGGTAGGACTACCCGCTGAACTTAAGCATATCAATAA
45/12	>45 TCTTCCTGCATTTCAGATTGAATAACGCAGCGAAATGCGATAAGTAATGTGAAT TGCAGAATTCATTGAATCATCGAATCTTTGAACGCACCTTGCGCCCTTTGGTATT CCGAGGGGCATGCCTGTTGAGTGTCATTAAATACCATCAACCCTCTTTTGACTTC GGTCTCGAGAGTGGCTTGGAAGTGGAGGTCTGCTGGAGCCTAACGGAGCCAGCT CCTCTTAAATGTATTAGCGGATTTCCCTTGCGGGATCGCGTCTCCGATGTGATAA TTTCTACGTCGTTGACCATCTCGGGGCTGACCTAGTCAGTTTCAATAGGAGTCTG CTTCTAACCGTCTCTTGACCGAGACTAGCGACTTGTGCGCTAACTTTTGACTTGA CCTCAAATCAGGTAGGACTACCCGCTGAACTTAAGCATATCAATAAGCGGAGGA A
48/12	>48 TTCCTCCGCTTATTGATATGCTTAAGTTCAGCGGGTAGTCCTACCTGATTTGAGG TCAGATGTTAAAAAGTTGTCCTAATGGACGGTTAGAAGCTCGCCAAAACACTTC ACGGTCACAGCGTAGACAATTATCACACTGAGAGCCGATCCGTACGGAATCGA GCTAATGCATTCAAGAGGAGCCGACCAACGAGGGCCAGCAAGCCTCCAAGTCC AAGCTTATAGATCACAAGGATTTATAAGTTGAGAATTCCATGACACTCAAACAG GCATGCTCCTCGGAATACCAAGGAGCGCAAGGTGCGTTCAAAGATTCGATGATT CACTGAATTCTGCAATTCACATTACTTATCGCATTTCGCTGCGTTCTTCATCGAT GCGAGAGCCAAGAGATCCGTTGCTAAAAGTTGTATTATAGATGCGTTAGACGCG TTTACATTCTGATACTTTAAAGTGTTTGTAGTATACATAGGCCGGCAGAATGCTC CCGCAAAGGAGCCCACGCCAACCTACAGTAAGTGCACAGGGGTAGAGTGGATG

	AGCAGAGC
112/12	>112 AACCTGCGGAAGGATCATTATCGAGTTTTGAACGGGTTGTAGCTGGCCTCTCAC GAGGCATGTGCACGCCTGGCTCATCCACTCTTAACCTCTGTGCACTTTATGTAAG AGAAAAAAATGGTGGAAGCTTCCAGGATCTCGCGAGAGGTCTTCGGTTGAACA AGCCGTTTTTCTTTCTTATGTTTTACTACAAACGCTTCAGTTATAGAATGTCAACT GTGTATAACACATTTATATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATC GATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGA ATCATCGAATCTTTGAACGCACCTTGCACTCCTTGGTATTCCGAGGAGTATGCCT GTTTGAGTCTCATGGTATTCTCAACCCCTAAATTTTTGTAATGAAGGTTTAGCGG GCTTGGACTTGGAGGTTGTGTCGGCCCTCGCTGGTCGACTCCTCTGAAATGCATT AGCGTGAATCTTACGGATCGCCTTCAGTGTGATAATTATCTGCGCTGTGGTGTTG AAGTATTTATGGTGTTCATGCTTCGAACCGTCTCCTTGCCGAGACAATCATTTGA CAATCTGAGCTCAAATCAGGTAGGACTACCCGCTGAACTTAAGCATATC

Table 5: Alignment view and distance matrix **Table** with sample mushroom sequence taken as reference sequence.

Samples	Sequence length blasted (bp)	Highest coverage	% identity (Accession no.)	Identified name of samples
8/12	647	100%	99% KT272409	Clitopilus scyphoides
33/12	694	99%	99% KT272410	Agrocybe pediades
45/12	438	99%	99% KT272413	Agaricaceae sp
48/12	548	100%	99% KT272412	Trametes ochracea
112/12	650	100%	100% KT2724	Irpex lacteus

Discussion:-

Antagonistic activity of mushrooms against fungi has been reported. Changes in colony colour were due to diffusion of metabolites of two species and this pigmentation occurred by mycelial phenoloxidase or peroxidase activity [18,19]. The mushroom produce metabolites with antifungal properties. The most active mushrooms were 45 and 112. This study provides a simple strategy that used to answer relevant questions how mushroom can overpowered the growth of pathogenic fungi. The human getting infected indirectly through foods of plant origin, animal origin through fungal toxins [20]. As discussed by [21] that Fusarium spp. produce mycotoxins Deoxynivalenol (DON) affects animal and human health causing acute temporary nausea, vomiting, diarrhoea, abdominal pain, headache, dizziness, and fever. Therefore against Fusarium all five sample gave good results but 33/12 and 45/2 showed better results and had more tendency to suppress the Fusarium sp. Fungal pathogens cause disease in plant and animal hosts [22] . The [23] determined that mushrooms had more species with antifungal, (Pleurotus ostreatus, Pleurotaceae) has shown effectiveness against Aspergillus niger causing aspergillosis lung disease, a malady that can pose a serious threat to persons with compromised immune systems. Hericium erinaceus has also shown anti-fungal activity against the mold A. niger. L. edodes represents a promising medicinal mushroom to control the infection by A. parasiticus and the aflatoxin production.[24] cyanthus sp. inhibition of human pathogenic fungus (Aspergillus fumigatus) [25].

The analysis of genomic DNA using PCR-based methods has proven to be a fast and reliable method to determined genetic relationships among basidiomycetes[26]. Nuclear rDNA, and particularly the internal transcribed spacer (ITS) regions are good targets for the Phylogenetic analysis in fungi because the ITS regions are often highly variable between isolates of the same species [27, 28]. The genomic DNA of the mushroom was extracted, the rDNA - ITS fragment of the genomic DNA was amplified using ITS1 and ITS4 primers and subjected to nucleotide sequence determination.

Conclusion:-

Therefore Studies aiming at the isolation and identification of basidiomycetes because with increasing demand for edible and medicinal mushroom, it becomes a necessity to unravel the rich biodiversity of basidiomycetes. Identification of those high-quality fungal species is not only necessary but has great economic significance as it will help in detecting fraudulent products being marketed. The best of our knowledge, this study is the first to investigate the potential of basidiomycetes against the pathogenic fungi and can serve to stimulate the investigation.

References:-

1. **Nelson, B.C**.; Patrícia, M.A.; Lis Ribeiro, M.C.; Luzia, D.P.M.; Giani, A.L.(2008). Temperature and pH conditions for mycelial growth of Agaricus brasiliensis on axenic cultivation. Agri. Scie. Lon., 307-312.
2. **Evans, J.**; Levesque, A.; de Lahunta, H.; Jensen, E. (2004). Intracranial Fusariosis: A Novel Cause of Fungal Meningoencephalitis in a Dog. Vet. Patho. On., 41: 5-510.
3. **3.Gupta, A.K.**, Baran, R., Summerbell, R.C. (2000). Fusarium infections of the skin. Curr. Opin. Infect. Dis., 13(2): 121-128.
4. **Marcio, N.**; Elias, A. (2007). Fusarium Infections in Immunocompromised Patients. Clin. Microbiol. Rev., 20(4): 695–704.
5. **Wickern, G.M**. (1993). Fusarium allergic fungal sinusitis. J. Allergy Clin. Immunol., 92**:** 624-625.
6. **Nongnuch, V.**; Jitwadee, S.; Chantana, K.; Jutarut, P.; Sophit, T.; Narawudt, P.; Worawit, Louthrenoo, S.; Chiewchanvit.; Napaporn, T. (2004). Identification of Emerging Human Pathogenic Pythium insidiosum by Serological and Molecular Assay-Based Methods. J. Clin. Microbiol., 42(9): 3970–3974.
7. **Imwidthaya, P**. (1994). Human pythiosis in Thailand. Postgrad. J. Med., 70**:** 558-560.
8. **Thitithayanont, A.**; Mendoza, L., Chuansumrit, A.R. Pracharktam, J. Laothamatas, B. Sathapatayavongs, S. Lolekha, and L. Ajello. (1998). Use of an immunotherapeutic vaccine to treat a life-threatening arteritic infection caused by Pythium insidiosum. Clin. Infect. Dis., 27: 1394-1400.
9. **Wanachiwanawin, W.**, M. Thianprasit, S. Fucharoen, A. Chaiprasert, N. Sudasna, N. Ayudhya, N. Sirithanaratkul, and A. Piankijagum. (1993). Fatal arteritis due to Pythium insidiosum infection in patients with thalassaemia. Trans. R. Soc. Trop. Med. Hyg., 87: 296-298.
10. **Gerson, S.L.**; Talbot, G.H.; Hurwitz, S. (1984). Prolonged granulocytopenia: the major risk factor for invasive pulmonary aspergillosis in patients with acute leukemia. Ann. Intern. Med., 100: 345–351.
11. **Kemper, C.A.**, Hostetler, J.S., Follansbee, S.E., Ruane, P., Covington, D., Leong, S.S. (1993). Ulcerative and plaque-like tracheobronchitis due to infection with Aspergillus in patients with AIDS. Clin. Infect. Dis., 17: 344-52.
12. **Biggs, V.J.**, Dummer, S., Holsinger, FC., Loyd, JE., Christman, BW., Frist, WH. (1994). Successful treatment of invasive bronchial aspergillosis after single-lung transplantation. Clin. Infect. Dis., 18, 123-4.
13. **Barst, R.J.**, Prince, A.S., Neu, H. (**1981).** Aspergillus endocarditis in children: case report and review of the literature. Pedia., 68: 73-8.
14. **Sugui, J.A.**, Peterson, S.W., Lily, P., Nardone, C.G., Folio, L., Riedlinger, G., Zerbe, C.S., Shea, Y., Henderson, C.M., Zelazny, A.M., Holland, S.M., Kwon-Chung, K.J. **(2012)**. Aspergillus tanneri sp. nov., a New Pathogen That Causes Invasive Disease Refractory to Antifungal Therapy. J. Clin. Microbiol.**,** 50(10): 3309–3317.
15. **Susanna, M.B.**, Gloria, I., Marine, G., Garibyan, L. (2002). Antagonistic activity of xylotrophic mushrooms against pathogenic fungi of cereals in dual culture. Phytopatho. Med., 41, 200-225.
16. **Badalyan, S.M.**, Innocenti, G., Garibyan, NG. (2002). Antagonistic activity of xylotrophic mushrooms against pathogenic fungi of cereals in dual culture. Phytopatho. Med. 41(3): 220–225.
17. **White T.J**, Bruns T.D, Lee S.B, Taylor J.W. (1990). Analysis of phylogenetic relationships by amplification and direct sequencing of ribosomal genes," PCR protocol. In: Innis MA, Gelfand DH, Sninsky JJ and White TJ, editors. New York: Academic Press, 315-322 pp.
18. **Li, C.Y.** (1981). Phenoloxidase and peroxidase activities in zone line of Phellinus weirii. Myco., 73: 811–821.
19. **Li, C.Y.** (1983). Melanin-like pigment in zone lines of Phellinus weirii - colonized wood. Myco., 75: 562–566.
20. Hussein, H.S., Brasel, J.M. (2001). Toxicity, metabolism, and impact of mycotoxins on humans and animals. Toxicol., 67: 101–134.
21. **Pavlina, S., Vojtech, A**., **Anna, V.**, **Miroslava, B.**, **Ladislav, Z., Rene, Kizek**. (2010). Deoxynivalenol and its toxicity. Interdiscip. Toxicol., 3(3): 94–99.

22. Montserrat, O**.**, Josep, G., Marta, P., Madrid, Zaira C., Isabel, G., Roncero., Emilio, M., Antonio, Di P. (2004). Fusarium oxysporum as a Multihost Model for the Genetic Dissection of Fungal Virulence in Plants and Mammals. Infect. Immun., 72 (3), 1760-1766.
23. **Suay, I.**, Arenal, F., Asenio, F., Basilio, A., Cabello, M., Diez, MT.(2000). Screening of basidiomycetes for antimicrobial activities. Antonie van Leeuwenhoek., 78: 129-39.
24. **Reverberi, M.**, Fabbri, A. A., Zjalic, S., Ricelli, A. Punelli, F., Fanelli, C. (2005). Antioxidant enzymes stimulation in Aspergillus parasiticus by Lentinula edodes inhibits aflatoxin production. Appl. Microbio. Biotechnol., 69: 207-215.
25. **Liu, Y.J.**, Zhang, K.Q. (2004). Antimicrobial activities of selected Cyathus species. Mycopath.**,** 157(2), 185-9.
26. **Singh, S.K.**, Yadav, M.C., Upadhyay, R.C., Rai, RD., Tewari, R.P. (2003). Mush. Res., 12: 67-68.
27. **Bruns, T.D.**, White, T.J., Taylor, J.W. **(1991)**. Fungal molecular systematics. Annual Review of Ecology, Evolution, and Systematics., 22: 525–564.
28. **Salazar, O.**, Schneider, J.H.M., Julian, M.C., Keijer, J., Rubio, V. (1999). Phylogenetic sub grouping of Rhizoctonia solani AG2 isolates based on ribosomal ITS sequences. Myco., 91 (3): 459–467.