GRT

Golden Research Thoughts

ISSN: 2231-5063 Impact Factor : 4.6052(UIF) Volume - 6 | Issue - 6 | December - 2016

ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF WILD EDIBLE MUSHROOM *Morchella conica* Pers. FROM NORTH WEST HIMALAYAN REGION

Dr. Renu Rana

Associate Professor, Department of Botany, RKMV, Shimla.

ABSTRACT:

The specimens of *Morchella conica* were collected from nature during 2011-2012. Collections were made from different parts of Distt. Shimla (H. P.)[1], India. The antimicrobial activity, of *Morchella conica,* at four concentrations (20, 40, 60 and 80%) of extracts prepared in different solvents (water, methanol and ethanol) were screened against five pathogens including three fungal pathogens (*P. infestans, A. alternata* and *F. sambucinum)* and two bacterial pathogens *(E. coli* and *S. aureus).* Maximum inhibition against all the five test pathogens including fungal as well as bacterial pathogens was observed in methanol extract at a concentration level of 80%, followed by ethanol and aqueous extracts at the same concentration. On comparing antifungal and antibacterial activity, it was observed that all extracts were having more antifungal property as compared to antibacterial property. In case of *P. infestans,* methanol and ethanol extracts in the concentration range of 40-80% completely checked the mycelial growth i.e. 100%. Whereas, growth inhibition of remaining four pathogens i.e. *A. alternata, F. sambucinum E. coli* and *S. aureus* increased with increase in concentration level of different solvent extracts.

Keywords: *mycelial growth, concentrations, antimicrobial, methanol, ethanol, extract, growth inhibition, Morchella conica .*

I.INTRODUCTION

Although, fungicides and antibiotics have been very effective in controlling the fungal and the bacterial diseases, respectively but the use of those chemicals leads to health and environment hazards. Despite the use of half a million tones fungicides and pesticides annually, one third of all crop production is still lost. Continued use of fungicides is threatening the environment and health and is responsible for some major problems. Firstly, some fungi have acquired resistance against fungicides particularly the systemic fungicides; secondly, some fungicides are not biodegradable and tend to persist for years in the environment. This leads to third problem, the detrimental effects of chemicals on organisms other than target fungi (Brady, 1984; Agarwal *et al.,* 2001). Similarly, society is facing serious public health dilemma over the emergence of infectious bacteria displaying resistance to many antibiotics (Kapil, 2005). Human infections, particularly those involving skin and mucosal surfaces constitutes serious problem, especially in tropical and subtropical countries (El-Mahmood and Amey, 2007). Methicillin Resistant

[1] Jakhoo,NSharog,Navbahar Glen, Kufri (Shimla).

Staphylococcus aureus (MRSA), *Escherichia coli, Pseudomonas aeruginosa, Candida albicans* were observed to be the most frequent skin pathogens (Obeidat, 2011). Therefore, human health and environmental safety are the two most important issues in the long term application of pesticides, fungicides and antibiotics (Lin, 1995).

Due to increasing awareness about the risk involved in use of chemicals much attention is being focused on alternative methods of pathogen control. Spiraling up the cost of synthetic fungicides, pesticides and antibiotics and pollution to soil, water, air by the accumulation of obnoxious chemical residues due to continuous use of these chemicals and development of resistant races to these chemicals are therefore now forcing the scientists to look for methods which are eco-friendly, safe, cost effective and specific for pathogen. The recurrent and indiscriminate use of fungicides have posed a serious threat to human health and existing human eco-geographical conditions as some of them have been proved to be either mutagenic, carcinogenic. Keeping in view the drawbacks of chemical management of animal and plant diseases and to increase world food production and feed the ever increasing population, agricultural production can be augmented with biological control instead of chemicals (Mukerji and Garg, 1993; Joseph *et al.,* 2008).

Recently, in different parts of the world, attention has been paid towards exploitation of bio-products as novel chemo-therapeutants in plant protection because of non-phytotoxicity, sytemecity, easy biodegradability and stimulatory nature of host metabolism, plant products are considered valuable for controlling plant, animal and human diseases (Mishra and Dubey, 1994; Siva *et al.,* 2008).

Higher and lower plants contain a wide spectrum of secondary metabolites such as phenols, flavonoids, quinines, tannins, essential oils, alkaloids, saponins, sterols, polysaccharides particularly beta-glucan, chalcones, yellow polyphenol pigments composed of styryl pyrone. Such plant metabolites may be exploited for their different biological properties (Tripathi et al., 2004). Terresterial plants produce a wide spectrum of natural products viz. terpenoids, phenolic, alkaloids, tannins and quinines. Many of these are thought to be serving an ecological function for the plants from herbivores and pathogens (Islam and Akhtar, 2007). Both higher and lower plants generally produce many secondary metabolites which constitute an important source of micro-biocides, pesticides, fungicides and pharmaceutical drugs (Ibrahim, 1997; Mahesh and Satish, 2008).

The use of plants for curing various ailments is figured in ancient manuscripts such as the Rigveda and the Samhita etc. in early ages, man used raw drugs isolated or obtained from the plants leading to information about the interrelationship between primitive man and plants. Many of the plant materials used in traditional medicines are readily available in rural areas at relatively cheaper cost than modern medicines (Mann *et al.,* 2008). Phyto-toxins which are safe and eco-friendly are considered a good alternative for the disease management (Kumar and Yadav, 2007).

Due to increasing awareness about micro-biocide and fungicides hazards, a need was felt to develop biological agents for the control of plant, animal and human diseases. In the field of biological control, mushrooms have attracted attention of scientists all over the world for a long time and yet studies in other areas of the world have shown that mushrooms contain many bioactive compounds with diverse biological activaties like higher plants.Since long, mushrooms have been cultivated world-wide for commercial purposes (Olila *et al.,* 2008). Scientific research in this field indicates that metabolites of mushrooms are the potential source for the production of nutritiorial, neutraceutical and antimicrobial compounds (Veluchamy *et al.,* 2012). Keeping into consideration the antimicrobial property of mushrooms extract, it is considered worthwhile to take up the studies with wild edible mushroom *Morchella conica* against fungal pathogens viz, .

Phytophthora infestans, Fusarium sambucinum, Alternaria *alternata* and bacterial strains viz. *Staphylococcus* aureus and *Escherichia coli* with the following objectives:

II MATERIALS AND METHODS

Test Pathogens

1 Procurement of test fungal and Bacterial pathogens

Fungal isolates of *Phytophthora infestans, Alternaria alternata* and *Fusarium sambucinum* were procured from the Department of Plant Pathology of Central Potato Research Institute Shimla. Pathogenic strains of *Escherichia coli* and S*taphylococcus aureus* were procured from Indira Gandhi Medical College, Department of Microbiology, Shimla.

Maintenance and preservation of pure culture

Pure cultures of all the fungal isolates of test pathogens were maintained on Potato Dextrose Agar (PDA) medium while, pure cultures of bacteria used as test pathogens were maintained on nutrient medium broth and were preserved in refrigerator at 4°C. Sub culturing was done at regular intervals in order to maintain the culture.

Each fungal /bacterial species of test pathogens was transferred from parent source to a fresh slant/fresh nutrient medium (broth) in order to maintain and preserve the parent culture, respectively.

Extraction procedure for preparation of methanol, ethanol and aqueous extracts from *Morchella conica* mushroom.

A fine powder (20 meshes) was obtained using a mill (Restch ultra centrifugal mill and sieving machine). Dried mushroom powder sample (20 g) of *Morchella conica* was extracted by stirring with 100ml of methanol (solvent) at 25°C at 150 rpm for 24h and filtering through Whatman No.4 filter paper. The residue was then extracted with two additional 100ml of methanol as described above. The combined methanol extracts were then rotary evaporated at 40°C to dryness, re-dissolved in methanol to a concentration of 50mg/ml (stock solution) and stored at 4°C in a refrigerator for further use. The whole procedure was repeated with ethanol and distilled water as solvents, to get ethanol and aqueous extracts, respectively. Preparation of extracts of mushrooms was based on procedures described by Barros *et al.,* (2008) with some modifications.

Methodology for screening *Morchella conica* for antifungal activity againstfungal pathogens i.e. *Alternaria alternata, Fusarium sambucinum and Phytophthora infestans.*

Screening of methanol, ethanol and aqueous extracts of *Morchella conica* against fungal plant pathogens viz .*Phytophthora infestans, Alternaria alternata* and *Fusarium sambucinum* was done using poisoned food technique (Grover and Moore, 1962; Perrucci *et al.,* 1994; Mishra and Dubey, 1994).

Potato Dextrose Agar (PDA) medium (Potato: 200 gm, Dextrose: 20 gm, Agar-Agar: 15 gm, Distilled water: 1 It.) was used for culturing *Alternaria alternata* and *Fusarium sambucinum* while Rye B medium was used for *Phytophthora infestans*.

The respective medium was autoclaved at 121.6°C for 30 minutes. After cooling the medium to 45°C, ten milligram of streptomycin was added to it and was mixed thoroughly to prevent bacterial contamination (Gupta and Banerjee, 1970; Srivastava, 2008).

In Poisoned food technique, each mushroom extract i.e. methanol, ethanol and aqueous extract was tested at 20%[2] (10ml/ml), 40%[3] (20mg/ml), 60%[4] (30 mg ml^{-1}) and

[2] **20%** = 200 l ss + **800 l of** dw
[3] **40%=** 400 l ss + 600 l of dw
[4] **60%** = 600 l ss + **400 l of** dw

80%[5] (40mg/ ml) concentration, prepared separately by dissolving requisite amount in PDA medium *in case of Alternaria alternata* and *Fusarium sambucinum* and in Rye B medium for *(Phytophthora infestans),* cooled to 45°C in a beaker and then streptomycin was added to it and mixed thoroughly so as to prevent bacterial contamination. 10 ml of each concentration was poured into sterilized pertri plates (9.0 cm diameter). Disc (8mm diameter) of test fungal pathogen cut from the periphery of seven days old culture (*A. alternata* and *F. sambucinum*) and 12 days old culture (*P. infestans*) with the help of sterilized cork borer and was inoculated aseptically in each of the treatment.

Control sets: The medium of control sets, contained requisite amount of corresponding solvent (methanol, ethanol or distilled water) in place of corresponding extract. Three replicates were maintained in each case. The petri plates were incubated at 25 ± .5°C for seven days (*A. alternata* and *F. sambucinum*) and at 18°C for 12 days *(P. infestans)* in an incubation chamber. Diameters of fungal mycelial colonies of treatment and control sets were measured in mutually perpendicular direction on seventh day *(A. alternata and F. sambucinum)* and twelfth day *(P. infestans)*. The percentage inhibition of radial growth of test fungus by different extracts was calculated following Pandey *et al.*, (1982) method as:

% inhibition of fungal colony= *dc-dt/dcx100*

Where *dc*= average diameter of fungal colony in control sets.

dt= average diameter of fungal colony in treatment sets.

Percentage inhibition of growth of all the test fungi by different extracts of mushrooms samples using poisoned food technique was calculated on seventh day *(A. alternata and F. sambucinum* and twelfth day *(P. infestans)* and the results are represented in tables 1-10.

Methedolology for screening *Morchella conica* for antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*

Antibacterial activity of methanol, ethanol and aqueous extracts of *Morchella conica* was determined by the agar well diffusion assay. All the microorganisms mentioned above were incubated separately at 37±0.1°C for 24 h by inoculation into nutrient broth (Beef extract 1gm, yeast extract **2gm,** Sodium Chloride 1gm, Peptone 5gm, distilled water 1 It). The culture suspensions were prepared and adjusted by comparing against 0.4-05 McFarland turbidity standard tubes. Nutrient Agar (NA) medium (Beef extract 1gm, yeast extract 2gm, Sodium Chloride 1gm, Peptone 5gm, Agar-Agar 20 gm, distilled water 1 It.) was used throughout the investigation for the growth of microorganisms. The medium was autoclaved at 121.6°C for 30 minutes. Nutrient Agar medium (10ml) was poured into each sterilized petri dish (9cm). The plates were left over night at room temperature to check for any contamination to appear. Bacterial lawns were prepared by distributing 100 l nutrient broth culture of each bacterium homogenously over the petri dish medium.

Agar-wells of 8mm diameter were prepared with the help of stainless steel cork borer. One well was prepared in each nutrient agar plate. For investigating bacterial activity, the well in each plate was loaded with 20, 40, 60 and 80% concentrations prepared separately by dissolving extracts in requisite amount of corresponding solvent (methanol, ethanol and distilled water in control sets), agar wells were filled with bare corresponding solvent only.

Plates inoculated with bacterial culture were incubated at 37±0.1°C for 24 h. All determinations were done in triplicates. At the end of incubation period, inhibition zones formed on medium were evaluated. For evaluation, diameter of bacterial colonies of treated and control sets were measured in mutually perpendicular direction on second day. Percentage inhibition of radial growth of bacteria was calculated after subtracting the value of treated/tested extracts from control as standard (Hemasphenpagam N and Selvaraj T, 2010).

[5] **80%** = 800 l ss + **200 l of** dw

$$\% \text{ Inhibition of bacterial colony} = \frac{dc - dt}{dc} \times 100$$

Where, dc= average diameter of bacterial colony in control sets.
dt = average diameter of bacterial colony in treatment sets.

Statistics

For extract (methanol, ethanol and water) from mushroom samples, three samples were prepared for assaying every antimicrobial attribute and component. The experimental data was subjected to an analysis of variance for a completely random design, as described by Stell, Torrie and Dickey (1997) to determine the significant difference.

III OBSERVATIONS

Antimicrobial activity of *Morchella conica*

To test antimicrobial activity, four different concentrations (20, 40, 60 and 80%) of three different solvent extracts (Aqueous, methanol and ethanol) of *Morchella conica* were screened against five pathogens including both fungal as well as bacterial pathogens i.e. *P. infestans, A. alternata, F. sambucinum, E. coli* and *S. aureus.* The circular growth in petriplates was recorded after ten days incubation at temperature of 25 ± .2^{0}C. The % growth inhibition ± standard deviation at different concentrations in different solvent extracts in presented in Table 1, Plate –VIII and IX (Petriplates 1.1-1.15).

Evidence from table 1; indicates that all the three extracts i.e. aqueous extract, methanolic extract and ethanolic extract effectively checked the growth of all the test pathogens. Methanolic and ethanolic extracts in the concentration range of 40-80% totally inhibited the growth of *P. infestans* i.e. 100% inhibition. Growth inhibition increased with increase in the concentration of all the three solvent extracts in case of remaining four pathogens i.e. *A. alternata, F. sambucinum, E. coli* and *S. aureus*. (Table 1) maximum inhibition against all the five tested pathogens including fungal and bacterial pathogens was observed in methanolic extract at 80% concentration of ethanolic extract followed by ethanolic and aqueous extract. Comparison between fungal and bacterial growth inhibition revealed that extracts more effectively checked the growth of three fungal pathogens than two bacterial pathogens. Whereas, in case of both bacteria, growth of *S. aureus* was more effectively inhibited by all the extracts of *Morchella conica* as compared to *E. coli* bacterium.

Hence, conclusion drawn from the results inferred that all the three solvent extracts of *Morchella conica* restricted the growth of all the tested pathogens to different extents. Whereas methanol extract at 80% concentration proved really very effective against all the pathogens investigated.

Table 1: Antimicrobial activity of *Morchella conica*

Extract	Concentration in %	%age growth inhibition of test pathogens by different extracts of *Morchella conica*				
		P. infestans	***A. aternata***	***F. sambucinum***	***E-coli***	***S. aureus***
Aqueous extract	20	16.11 ± .19	12.44 ± .77	8.91 ± .42	2.22 ± .19	8.22 ± .39
	40	30.33 ± .34	26.33 ± .34	22.15 ± .46	10.78 ± .19	15.67 ± .58
	60	40.45 ± .39	37.33 ± .34	37.99 ± .69	18.22 ± .39	24.44 ± .77
	80	47.33 ± .34	52.55 ± .69	52.34 ± .45	24.22 ± .89	31.33 ± .39
Methanolic extract	20	80.11 ± .19	26.33 ± .34	24.37 ± .39	15.11 ± .19	15.44 ± .51
	40	100.00 ± .00	52.55 ± .69	49.67 ± .88	23.78 ± .19	28.44 ± .77
	60	100.00 ± .00	66.67 ± .67	66.22 ± .39	34.22 ± .39	37.89 ± .84
	80	100.00 ±	86.33 ± .34	87.77 ± .39	40.11 ± .69	54.00 ±

		.00				.96
Ethanolic extract	20	64.22 ± .39	20.44 ±.51	16.24 ± .37	11.11 ± 1.35	12.00 ± .67
	40	100.00 ± .00	40.11 ± .19	36.08 ± .23	21.67 ± .58	23.89 ± .51
	60	100.00	60.00 ± .33	52.42 ± .67	29.89 ± .84	37.00 ± 1.00
	80	100.00	80.22 ± .39	82.22 ± .39	37.33 ± .58	48.44 ± .77

Each data represents the mean of 3 replicates ± S.D.

PLATE-VIII

Showing the fungitoxic activity of different solvent extracts of *Morchella conica.*

1.1 Petriplates showing growth inhibition of *Phytophthora infestans* by aqueous extract of *Morchella conica.*

1.2 Petriplates showing growth inhibition of *Phytophthora infestans* by methanolic extract of *Morchella conica.*

1.3 Petriplates showing growth inhibition of *Phytophthora infestans* by *ethanolic* extract of *Morchella conica.*

1.4 Petriplates showing growth inhibition of *Alternaria alternata* by aqueous extract of *Morchella conica.*

1.5 Petriplates showing growth inhibition of *Alternaria alternata* by methanolic extract of *Morchella conica.*

1.6 Petriplates showing growth inhibition of *Alternaria alternata* by *ethanolic* extract of *Morchella conica.*

1.7 Petriplates showing growth inhibition of *Fusarium sambucinum* by aqueous extract of *Morchella conica.*

1.8 Petriplates showing growth inhibition of *Fusarium sambucinum* by methanolic extract of *Morchella conica.*

1.9 Petriplates showing growth inhibition of *Fusarium sambucinum* by *ethanolic* extract of *Morchella conica.*

•

PLATE-IX

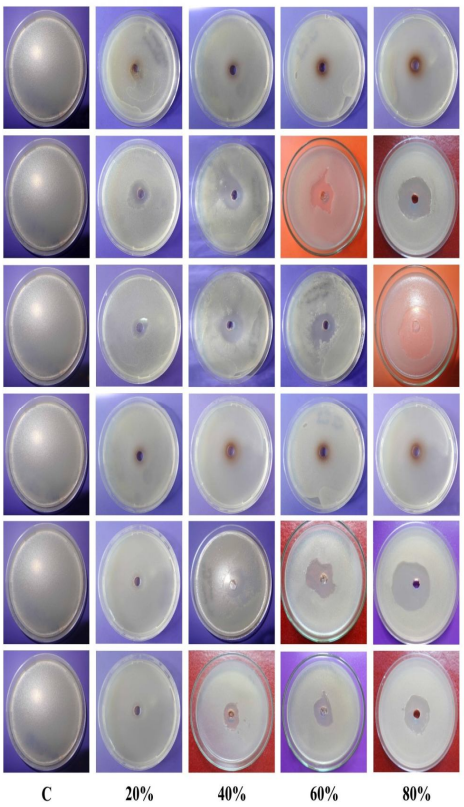

PLATE-IX

Showing bactericidal activity of different solvent extracts of *Morchella conica*.

1.10 Petriplates showing growth inhibition of *Escherichia coli* by aqueous extract of *Morchella conica*.

1.11 Petriplates showing growth inhibition of *Escherichia coli* by methanolic extract of *Morchella conica*.

1.12 Petriplates showing growth inhibition of *Escherichia coli* by *ethanolic* extract of *Morchella conica*.

1.13 Petriplates showing growth inhibition of *Staphylococcus aureus* by aqueous extract of *Morchella conica*.

1.14 Petriplates showing growth inhibition of *Staphylococcus aureus* by methanolic extract of *Morchella conica*.

1.15 Petriplates showing growth inhibition of *Staphylococcus aureus* by *ethanolic* extract of *Morchella conica*.

IV DISCUSSION

Although, there is a tremendous progress in human medicine; bacterial, fungal and viral diseases are still a threat to the public health especially in developing countries (Cos *et al.*, 2006). Relative unavailability of medicines and extensive drug resistance has a large impact on human health in these countries. Therefore, further research about investigation of new antimicrobial substances should be conducted. Natural products have potential of containing therapeutic agents against infectious diseases (Clardy *et al.*, 2004).

Natural products, either as pure compounds or as standardized plant extracts provide unlimited opportunities for new drugs but only a minute portion of the available diversity among fungi, marine flora and fauna, bacteria and plants has yet been explored for such purposes (Cos *et al.*, 2006). The use of plant extracts (including macro fungi**:** mushrooms) for antimicrobial activity is enjoying great popularity since 1990's when people realised that effective life span of antibiotics and other synthetic chemicals is limited and over dose and misuse of these chemicals is causing microbial resistance (Alam *et al.*, 2009). In this context, mushrooms are not only source of nutrients but also could be used to prevent diseases such as hypertension, hypercholesterolemia, cancer, skin-boils, fibrosis, diabetes, urinary and pulmonary infections in man (Wasser and Weis *et al.*, 1999a; Bobek *et al.*, 1995).

Fungal pathogens are also significant destroyers of food stuffs during storage, rendering them unfit for human consumption by retarding their nutritive value and sometimes by producing mycotoxins. Approximately, 20-40% of cereals world-wide are contaminated with mycotoxins produced by different fungi during storage (Kumar *et al.*,

2007). One third of the world's potential food supplies are estimated to be lost due to pre and post-harvest pathogens and diseases. According to FAO estimates, potential losses world-wide are 35% (Odhiambo, 1985).

Chemical control measures against plants have a long history. Already in nineteenth century and even earlier chemicals containing copper, sulphur or phenolic compounds were used to control various plant diseases (Backhaus, 2009). Fungicides are usually applied as effective, dependable and economical control measures to control fungal diseases. However, the indiscriminate use of chemical fungicides has resulted in several problems, such as toxic residues in food, water and soil and degradation of the ecosystem, leading to the fear that their regular use may harm the environment further. Hardly 0.1% of the agro-chemicals used in crop protection reach the target pathogen leaving the remaining 99.9% to enter environment to cause a hazard to non-target organisms including humans, animals and to environment (Pimentel and Levitan, 1986; Varma and Dubey, 1999). According to WHO estimates, approximately 0.75 million people are becoming ill every year with agrochemicals poisoning, further the resistance of pathogens to fungicides has rendered certain fungicides ineffective, giving rise to new physiological races of pathogens.

Basic research for over more than 40 years in biology and biochemistry has made it possible to envisage not only how new pesticides may be synthesized but also has generated a completely new approach to the production of plants using secondary plant products which may be toxic to a specific pathogen and harmless to humans and animals. Pesticide plants have been in nature for millions of years and their bioactive compounds are not having any ill or adverse effect on the ecosystem. They have distinct advantage in management of diseases caused by pathogens. Plants have natural potential to withstand the aggressiveness of pathogenic species. A wide spectrum of secondary substances is contained in higher as well as lower plants. The total number of plant chemicals may exceed 4000 and out of these 1000 are secondary bioactive metabolites. These bioactive metabolites act as a major defence mechanism for plants (Tripathi and Shukla, 2010).

The preservative nature of some plant extracts have been known for centuries and has been reported from higher as well as lower plants (especially mushrooms) (Datar, 1999). The antimicrobial metabolites are contained in the phytochemical constituents (alkaloids, saponins, tannins, chalcones, tocopherols, beta-glucans, flavonoids, polysaccharides and polyphenols etc.) of the plants (Edeoga and Mbaebie, 2005). Antimicrobial activities have been linked to the presence of bioactive compounds which sometimes serve to protect plants themselves against bacterial, viral and fungal infections as well as exhibiting their antimicrobial properties against these organisms (El-Mahmood and Amey, 2007). Therefore, these days plant extracts have assumed a special significance as an eco-friendly method for plant disease management. Further, mushrooms are proving most promising agents as the antimicrobial substances. Edible and non edible mushrooms have the potential to be developed into bio control agents for the control of plant as well as human and animal diseases (JinTong *et al.*, 2010).

Findings of the present investigation are in agreement with the work of (Hussain *et al.*, 2012). In this work methanol extract of *Morchella esculenta* exhibited maximum inhibitory activity against *V. cholerae* and also the ethanolic extract depicted higher bactericidal activity against *E. coli.*).

V CONCLUSION

Hence, in the present investigation, it was considered worthwhile to find out the antimicrobial (antibacterial and antifungal) properties of extracts of *Morchella conica* Since, mushrooms are perishable items, dried mushroom parts (fruiting bodies with stalk) have been used for their fungal toxicity and bactericidal property against test fungal and bacterial pathogens. In present study extracts of *Morchella conica* (dried powder) prepared in three different solvents separately i.e. methanol, ethanol and water were screened at

the concentrations 20%, 40%, 60% and 80% against three test fungal pathogens. P*hytophthora infestans, Alternaria* alternata and *Fusarium sambucinum* and two bacteria; *Staphylococcus aureus* (Gram-positive) and *Escherichia coli* (Gram-negative). It was observed that growth inhibition took place at every concentration studied i.e. 20, 40, 60 and 80% concentration in every extract with different solvents, except aqueous extract which remained totally ineffective against *E.Coli.* at all concentrations. Maximum growth inhibition was observed at 80% concentration in every extract with different solvents. Tables 1 clearly put forward that *Morchella conica* under investigation exhibited antimicrobial activity against every test pathogen Growth inhibition increased with increase in the concentration level. Therefore, maximum inhibition was observed at 80% concentration while minimum at 20% concentration.

Although, inhibition at 20% concentration was negligible but concentrations at 40%, 60%, and 80% levels significantly inhibited the growth of fungal pathogens;P*hytophthora infestans, Alternaria* alternata and *Fusarium sambucinum*. Moreover, on comparing the % inhibition growth of test pathogens, it was found that maximum growth inhibition was exhibited by methanol extract followed by ethanol and aqueous extracts. *Phytophthora infestans* was found most sensitive pathogen against methanol and ethanol extracts as its growth was totally inhibited even at 40% concentration. Aqueous extract was also fairly significant as it inhibited its growth up to 40% at 80% concentration. All the extracts proved very effective in controlling the growth of both tested bacteria. But growth inhibition was more pronounced against *Staphylococcus aureus* as compared to *Escherichia coli.*

The results of the present investigation are also in agreement with information available in the literature. Previous studies inferred that mushrooms have great potential to be used as source of nutritionally functional food and a source of biologically active, physiologically beneficial and nontoxic medicines (Wasser, 1999a). Many previous findings depicted the mushrooms as a source for the development of medicines and drugs due to their pharmacological effects against pathogenic microbes and drugs due to their pharmacological effects against pathogenic microbes (Jonathan and Fasidi, 2003; Gbolagade *et al.*, 2005; Gezer *et al.*, 2006). It is estimated that approximately 50% i.e. 5 million metric tons of cultivated edible mushrooms contains the functional therapeutic properties. The available literature confirms the anti-microbial property of macro fungi: *Tricholoma giganteum, Lentinula edodes, Lentinula boryana, Lactarius deliciosus, Lactarius indigo, Podaxis pistillaris, Russula paludosa*, *Russula delica*, *Pleurotus sajor-caju, Pleurotus eryngii, Pleurotus florida, Pleurotus pulmonarius, Pleurotus citirinopileatus, Pleurotus villosus, Oudmensiella mucida, Cantharellus cibarius, Ramaria botrytis, Ramaria cistidiophora, Agaricus bisporus, Agaricus bitorquis, Agaricus blazei, Hygrophorous chrysodon, Armillariella mellea, Ganoderma lucidum, Flammulina velutipes, Hypsizygus marmoreus, Volvariella volvacea, Armillariella tabescens* against fungal and bacterial pathogens.

REFERENCES

1. Agarwal, R., Singh, D.V., Srivastva and K.D. 2001. Plant Pathology. 1st Ed. pp. 51.
2. Alam, M.T., Karim, M.M. and Khan, S.N. 2009. Antibacterial activity of different organic extracts of *Achyranthes aspera* and *cassia alata*. *J. Sci. Res.* **1**: 393-398.
3. Backhaus, G.F. 2009. Regulatory aspects in chemical control of fungal diseases: Impact on efficient plant production. *Recent Development in Management of Plant Diseases.* **1**: 47-55.
4. Bobek, P., Ozdyn L and Kuniak L. 1995. The effect of oyster (*Pleurotus ostreatus)* its ethanolic extracts and extraction residues on cholesterol levels in cerum lipoproteins and liver of rat. *Nahrung.* **39**: 98-99.
5. Brady, N.C. 1984. The nature and properties of soils. MacMillan Publishing company, New York. pp. 528.

6. Centko, R.M., Raman-Garcia, S., Taylor, T., Patrick, B.O., Thompson, C.J., Miao, V.P. and Andersen, R.J. 2012. Ramariolides A-D, anti-mycobacterial, butenolides isolated from the mushroom *Ramaria Cystidiophora .Journal of Natural Products.* **75**: 2178-2182.
7. Chu, K.T., LiXin, X. and Ng, T.B. 2005. Pleurostrin, an antifingal peptide from the oyster mushroom. *Peptides.* **26**: 2098-2103.
8. Clardy J. and Walsh C. 2004. Lessons from natural molecules. *Nature.* **432**: 829-837.
9. Cos, P., Vlietinck A.J., Vanden Bergh, D. and Maes L. 2006. Anti-infective potential of natural products: How to develop a stronger in vitro 'proof-of-concept'. *Journal of Ethnopharmacology*. **106**: 290-302.
10. Datar, V.V. 1999. Bioefficacy of plant extracts against *Macrophomia phasiolina* (Transi) Goid. The incitant of charcoal rot of sorghum. *J. Mycol. Pl. Pathol.* **29**: 251-253.
11. Edeoga, H.O., Okwu, D.E., Mbaebie, B.O. 2005. Phytochemical constitutents of some Nigerian medicinal plants. *Afr. J. Biotechnol.* **4**: 685-688.
12. El-Mahmood. A.M., Amey, J.M. 2007. *In vitro* antibacterial activity of *Parkia biglobosa* (Jacq.) root bark extract against some microorganisms associated with urinary infections. *Afr. J. Biotechnol.* **6**: 1772-1275.
13. Gbolagade, J.S. 2005. Bacteria associated with cultures of Nigerian edible mushrooms, *Psathyrella antroumbonata* (Pegler) and *Schizophyllum commune* (Fr. Ex. Fr.). *Acta Phytopathologica et Entomologica Hungarica* **40**: 333-340.
14. Gbolagade, J.S., Fasidi, I.O., Ajayi, E.J. and Sobowale, A.A. 2006. Effect of physico-chemical factors and semi-synthetic media on vegetative growth of *Lentinus subnudus* (Berk.), and edible mushroom from Nigeria. *Food chemistry*. **99**: 742-747.
15. Gezer, K., Duru, M.E., Kivrak, I., Turkoglu, A., Mercan, N., Turkoglu, H. and Gulcan, S. 2006. Free radical scavenging capacity and antimicrobial activity of wild edible mushroom from Turkey. *African Journal of Biotechnology.* **5**: 1924-1928.
16. Gupta, S. 1970. A rapid method of screening antifungal antibiotics. *Ind. J. of Exp. Biol.* **8**: 148-149.
17. Hemeshenpagan, N. and Selvaraj, T. 2010. Antimicrobial potential of different extracts of *Solanum xanthocarpum.* Chand and Wendt. *Plant Archives.* **1**: 387-390.
18. Hussain, B., Qureshi, R.A., Khan, J. Ullah, F., Fahad, S., Ullah, F., Khan, A.M., Hussain, I. and Khan N. 2012. Pharmacological screening of *Morchella esculenta* (L.) Pers., *Calvatia gigantea* (Batsch ex Pers.) Llyod and *Astreatus hygrometricus* Pers., mushroom collected from South Waziristan (FATA). *Journal of Medicinal Plants Research.* **6**: 1853-1859.
19. Ibrahim, M.B. 1997. Antimicrobial effects of extract leaf stem and bark of *Angeissus leiocarpus* on *Staphylococcus aureus*, *Streptococcus pyogens*, *Escherichia coli* and *Protens vulgaris*. *J. Pharma. Devpt.* **2**: 20-30.
20. Iftekhar, A.F.M.H., Choudhry, Z.K., Khan, M.I. and Salah, A.A. 2011. Comparative study of antibacterial activity of wood-decay fungi and antibiotics. *Bangladesh Journal of Pharmacology.* **6**: 14-17.
21. Islam, M.B. and Akhtar, Y. 2007. Plant natural products as a source for developing environmentally acceptable insecticides: Insecticides design using advance of technologies (ed. Issac. Ishaaya, Ralf Nauen and A. Ramj Horowiz) Springer Verlang Berlin Heidelberg. pp. 235-248.
22. JinTong, C. and JennWen, H. 2010. Antimicrobial activity of edible mushroom culture filtrates on plant pathogens. *Plant pathology Bulletin.* **19**: 261-270.
23. Jonathan, S.G. and Fasidi, L.O. 2003. Antibacterial activities of Nigerian edible macro fungi – *Lycoperdon pusilum* (Bot. EX.) and *Lycoperdon giganteus* (Pers) *Afr. J. Bimed. Res.* **6**: 85-90.
24. Jones. F.A. 1996. Herbs – useful plants. Their role in history and today. *European Journal of Gastroenterology and Hepatology.* **8**: 1227-1231.
25. Joseph, B., Dar, M.A. and Kumar, V. 2008. Bioefficacy of plant extracts to control *F. solani F.* sp. Melogenae incitant of Brinjal wilt. *Global. J. Bichem* **3**: 56-59.
26. Kapil, A. 2005. The challenges of antibiotic resistance need to contemplate. *Ind. J. Med. Res.* **121**: 83-89.

27. Kumar, S. and yadav, B.P. 2007. Efficacy of fungicides and phyto extracts on *Colletotrichum* spp. *J. Mycol. Pl. Pathol.* 37: 363-364.
28. Lin, C.Y. 1995. Technology for sustainable agricultural development. *In:* APO-Japan Symposium on Prospective Agricultural Technologies. July 25- Aug 4. Tokyo Japan. pp. 1-26.
29. Mahesh, B. and Satish, S. 2008. Antimicrobial effects of some important medicinal plants and human pathogens. *World J. Agri. Sci.* **4**: 839-843.
30. Mann, A., Banso, A. and Clifford, L.C., 2008. An antifungal property of crude plant extracts from *Anogeissus leiocarpus and Terminalia avicennioides. Tanzania J. of Health Res.* **10**: 34-38.
31. Mishra, A.K., Dubey, N.K. 1994. Evaluation of some essential oils for their toxicity against fungi causing deterioation of stored food commodities. Appl. and *environ. Microbiol.* **60**: 101-105.
32. Mukerjii, K.G. and Garg, K.L. 1993. Bio control of plant diseases Vol. 1.Emkay Publications, New Delhi. pp. 38.
33. Obeidat. M. 2011. Antimicrobial activity of some medicinal plants against multidrug resistant skin pathogens. Journal of *Medicinal Plants research.* **5**: 3856-3860.
34. Odhiambo, T.R. 1985. Pest problems as constraints to agriculture in tropical Africa, pesticides management in East and Southern Africa: *Proceedings of a regional workshop, Nairobi.*
35. Olila, D., Kapaata, A., Kabasa, J.D., Kisovi, L. and Munishi, P.K.T. 2008. Antibacterial activity of nutritional composition of selected indigenous mushrooms of the Lake Victoria Basin. *Journal of Food Technology.* **6**: 1-4.
36. Parameswari, V. and Chinnaswamy, P. 2011. An *in vitro* studies of the inhibitory effect of *Pleurotus florida* a higher fungi on human pathogens. *Journal of Pharmacy Research.* **4**: 1948-1949.
37. Pimentel, D. and Levitan, L. 1986. Pesticides: amounts applied and amounts reaching pests. *Biol. Sci.* **36**: 86-91.
38. Silva, R.F., Pascholati, S.F. and Bedendo, I.P. 2008. Induction of resistance in eggplants by *Lentinula edodes* and *Agaricus blazei* against *Ralstonia solanacearum:* biochemical aspects and vegetative biomass. *Summa Phytopathologica.* **34**: 137-144.
39. Srivastva, B. 2008. Pesticidal activity of some higher plants in management of post harvest bio-deterioration of some food commodities. Ph.D. Thesis. Banaras Hindu University, Varanasi, India.
40. Steel, R.G.Torrie, J.H. and Dickey. D.A.1997. Principles and procedures of statistics: A biometrical approach. Singapore, McGraw-Hill.
41. Tripathi, P. and Dubey N.K. 2004. Exploitation of natural products as an alternative strategy to control post harvest rotting of fruits and vegetables. *Postharvest Biol. And Technol.* **32**: 235-245.
42. Tripathi, P. and Shukla, A.K. 2010. Exploitation of botanicals in the management of phyto-pathogenic and storage fungi. *In*: Management of fungal plant pathogens. Arya and perello (Eds.) CABI Publishers. pp. 36-49.
43. Varma, J. and Dubey, N.K. 1999. Prospective of botanical and microbial products as pesticides of tomorrow. *Current Science.* **76**: 172-179.
44. Veluchamy, R., Arivudainambi, U.S.E., Annamali, T., Chandran, K. and Ayyappan, R. 2012. Antibacterial activity of wild *Xylaria* sp. Strain R005 (Ascomycetes) against multidrug-resistant *Staphylococcus aureus* and *Pseudomonas aeruginosa. International Journal of Medicinal Mushrooms.* **14**: 47-53.
45. Wasser, S.P. and Weis. A.L. 1999a. Medicinal properties of substances occurring in higher Basidiomycetes mushrooms: Current perspective (review) *Internatinal Journal Medicinal Mushrooms.* **1**: 31-62.