ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com
Journal DOI: 10.21474/IJAR01

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

FUNGICIDAL PROPERTIES OF HIGH ALTITUDE MEDICINAL PLANT: AN ANTIFUNGAL SUBSTANCE FROM THE EXTRACTS OF SELECTED MEDICINAL PLANTS.

[*]Suman Tiga, Prabhu Prasad Sarangi, Narendra Singh, Bhuvnesh Kumar and Om Prakash Chaurasia
Defence Institute of High Altitude Research, C/o 56 APO, Leh, Jammu and Kashmir -194101.

Manuscript Info

Manuscript History:

Received: 12 March 2016
Final Accepted: 22 May 2016
Published Online: May 2016

Key words:
Antifungal properties, Inhibition, Medicinal plant, Phytopathogenic fungi.

Corresponding Author

Suman Tiga.

Abstract

Different concentration i.e. 1000ppm, 3000ppm, 5000ppm of eighteen different methanolic extracts from fifteen different medicinal plant species were tested for their efficacy against *Aspergillus niger* and *Aspergillus flavus.* The plants selected were *Codonopsis clematidea, Hippophae rhamnoides, Artimesia dracunculus, Galium aparine, Mentha longifolia, Foeniculum vulgare, Rubia coradifolia, Saussurea lappa, Inula racemosa, Rheum webbianum, Arnebia euchroma, Rhodiala heterodanta, Rhodiala imbericate, Achelia millefolium and Hypericum perforantum*. The plants were selected on the basis of their reported ethnobotanical uses and locally available. The percentage inhibition was carried out using food poison technique. Results revealed that all the concentrations of plant extracts except *Rhodiala heterodanta, Rhodiala imbericate, Achelia millefolium and Hypericum perforantum* brought about inhibition in the growth of fungi *Aspergillus niger* and *Aspergillus flavus*. The result revealed that highly significant inhibition percent (79.43%) of mycelial growth against *A.flavus* was observed in PDA media amended with the 5000ppm concentration of *Inula racemosa* extract. Similar results were observed against *A.niger;* percent inhibition (79.86%) was found in *Inula racemosa* at concentration 5000ppm. The present investigation is an important step in developing plant based fungicide.

Introduction:-

Plant pathogens cause great losses to agricultural crops and thus threaten food resources all over the world [1]. A significant portion of the agricultural produce in the country and the world over become unfit for human consumption due to mycotoxins contamination produced by species of *Aspergillus* [2], [3], [4]. *Aspergillius niger* as a strong pathogen can cause the rotting of numerous fruits and vegetable [5], [6], [7], [8] such as black rot of onion and rot of tomato [9], [10]. *Aspergillus flavus* is a weak opportunistic and a mycotoxigenic fungus and infects several agricultural crops [11]. These pathogenic fungi not only involve in food spoilage by retarding its nutritive value [12], [13] but also involve in many fungal infections. The toxin produced by them shows the effect of carcinogenicity, genotoxicity, teratogenicity, nephrotoxicity, hepatotoxicity, reproductive disorders [14], [15], [16] on consumption of spoiled food. The disease in crop caused by phytopathogenic fungi is mainly controlled by synthetic fungicides. However, the application of this agrochemical is increasingly restricted due to the harmful effects of pesticides on human health and the environment [17]. In many countries the use of synthetic fungicide has been banned due to the non-biodegradability, residual toxicity, pollutative nature. In order to have safe methods for plant disease control in sustainable agriculture there is a need for reducing the use of synthetics chemicals fungicides there by replacing it with biocides with plant origin. Plants are known to produce a variety of compounds to protect themselves against a variety of pathogens. The plants are rich sources of numerous bioactive secondary metabolites

such as alkaloid, flavonoids, terpenoids, saponins, tannins and phenolic compounds which are the important sources of microbiocides, pesticides, antifungal activity and many pharmaceutical compound [18], [19]. Thus plant extracts which is a source of natural pesticides can be developed into new biocidal pesticides [20], [21]. Therefore the present study investigate; the efficacy of various high altitude medicinal plant extracts from *Codonopsis clematidea, Hippophae rhamnoides, Artimesia dracunculus, Galium aparine, Mentha longifolia, Foeniculum vulgare, Rubia coradifolia, Saussurea lappa, Inula racemosa, Rheum webbianum, Arnebia euchroma, Rhodiala heterodanta, Rhodiala imbericate, Achelia millefolium and Hypericum perforantum*, for the thirst of antifungal property against the two *Aspergillus spp.*, through *in vitro* analysis. Recent efforts have focused on development of environmentally safe, long lasting and effective biocontrol methods for the management of plant diseases.

Material and methods:-

Plant material:-

Fifteen different plant species, known for their medicinal value in traditional medicine were collected from the medicinal garden of Defence Institute of High Altitude Research (DIHAR), Leh, Ladakh. The selected plant parts of different medicinal plants were cut into small pieces and shade dried at room temperature for fifteen days and finely powdered. The taxonomic characters and important medicinal uses of these plant and their parts used which showed antifungal activity are presented in Table 1.

Table 1:- Taxonomic characters and important medicinal uses of the plants selected for this investigation.

Species(family)	Common name	Popular uses	Part used
C.clematidea (Campanulaceae)	Asian Bell flower	Antitumor, antioxidant, anti-aging, anti-ulter and antibacterial properties.	Leaf Root
H.rhamnoides (Elaeagnaceae)	Seabuckthorn	Antimicrobial, anticancer, antifungal, antiulcerogenic etc.	Leaf
A.dracunculus (Asteraceae)	Dragon wort	Antiscorbutic, hypnotic, odontalgic, appetizer, febrifuge, and vermifuge.	Leaf
G.aparine (Rubiaceae)	Cleavers	Anti-inflammatory, antiphlogistic, astringent etc.	Leaf
M.longifolia (lamiaceae)	Horsemint	Antimicrobial, stimulant, antispasmodic, antiseptic, antiasthmatic etc.	Leaf
F.vulgare (umbelifers)	Sweet Fennel	Diaphoretic, febrifuge, anesthetic, disinfectant and vermifuge.	Leaf
R.coradifolia (Rubiaceae)	Madder wort	Antibacterial, astringent, antidysenteric, antiseptic, antipyretic etc.	Root
S.lappa (Asteraceae)	Costus	Anti-ulcer, hepatoprotective, anti-viral activities, antiseptic etc.	Root
I.racemosa (Asteraceae)	Indian elecampane	Thermogenic, cardiotonic, anodyne, anti-inflammatory etc.	Root
R.webbianum (Polygonaceae)	Rhubarb	Antifungal, antitumor, antibacterial and anti-inflammatory.	Root
A.euchroma (Boraginaceae)	Pink Arnebia	Antifungal, antitumour, antipyretic and antibacterial.	Root

The above plants are chosen to be studied because they come in abundant sources, easily available and some of them are already being utilized in traditional medicine.

Preparation of solvent extracts:-

75gms of finely powdered plant materials were successively extracted with organic solvent; methanol using soxhlet apparatus for 24 hrs. The raw extracts were filtered using whatman No.1 filter paper. The different filtered extracts obtained were subsequently concentrated under reduced pressure using rota vapour to get their corresponding residues. All the extracts were stored in sterile glass bottles at -20^{o}C until screened.

Sterilization of plant extract and medium:-
25mg, 75mg and 125mg of extract was added to 25ml of PDA medium separately. The two were mixed thoroughly to make its concentration 1000ppm, 3000ppm and 5000ppm respectively. The medium was sterilizes at 15psi at 121^{o}C for 15 minutes. 25ml of the medium containing extract was poured in sterilized petri dishes and was solidified at room temperature for further use.

Isolation and identification of fungi:-
Two fungal species selected for this study, were *A.niger and A.flavus.* The tested fungal species were isolated from the leaves of onion and tomato in a potato dextrose agar. The fungi isolated were identified by Indian agricultural research institute (IARI), New Delhi.

Antifungal activity assay:-
Determination of mycelia inhibition by poisoned food technique:-
Inhibition of mycelia growth was assayed using the food poison method [22] with slightly modification. The sterilized solidified PDA medium with different concentration of extract were inoculated by placing 3.0mm diameter mycelium agar disc of seven days old culture of toxigenic fungus in the center of each petri plate. All plates were incubated at 28 ± 2°C for 4 days. Fungal growth was measured as colony diameter. The petri dishes of PDA without extract served as control. Percentage mycelia inhibition by the extract was calculated using formula: Growth Inhibition (%) = [(*Dc –Dt)/Dc]** 100
Where, *Dc*: Diameter of colony in the control (cm) and *Dt*: Diameter of colony in the treatment (cm)

Statistical analysis:-
The data were analyzed by a one-way analysis of variance (ANOVA), and comparisons were carried out for each pair with HSD Tukey test using SPSS statistical software (SPSS Inc., Chicago, IL, USA). All treatments were carried out in triplicate, and the values are given as means ± standard errors. Differences were considered to be significant when the *P* value was less than or equal to 0.05.

Results:-
Different concentration i.e. 1000ppm, 3000ppm, 5000ppm of eighteen different methanolic extract from fifteen different medicinal plant species was tested for their efficacy against *A.niger* and *A. flavus.*

Table 2:- Inhibition % of *A. flavus* by solvent extract of high altitude medicinal plant.

Percent mycelia inhibition							
Concentration (ppm)							
	1000ppm		3000ppm		5000ppm		
S. No.	Colony Diameter	% Inhibition	Colony Diameter	% Inhibition	Colony Diameter	% Inhibition	F value
1	3.6±0	23.4±1.0a	2.96±0.03	36.87±0.7b	2.6±0.05	44.67±1.22c	173.39
2	2.5±0.03	46.09±0.7a	1.3±0.057	72.33±1.22b	1.16±0.05	75.18±0.71b	307.49
3	2.6±0.08	43.26±1.87a	1.53±0.03	67.34±0.69b	1.5±0.05	68.08±1.22b	108.77
4	3.43±0.06	26.95±1.42a	3.2±0.03	31.20±0.7a	2.76±0.03	41.13±0.7b	52.66
5	3.2±0.05	31.91±1.22a	1.96±0.03	58.15±0.71b	1.63±0.03	65.24±0.7c	367.08
6	3.2±0.03	31.2±0.7a	3.06±0.06	34.75±1.42a	2.5±0.05	46.81±1.22b	49.84
7	3.06±0.03	34.75±0.71a	2.66±0.03	43.26±0.71b	2.1±0.05	55.32±1.22c	127.15
8	2.36±0.03	49.64±0.7a	1.43±.033	69.5±0.7b	1.1±0.05	76.59±1.22c	233.81
9	3±0.05	36.16±1.22a	2.2±0.05	53.19±1.22b	2.1±0.05	55.32±1.22b	72.98
10	1.5±0.05	68.08±1.22a	1.03±0.06	78.01±1.41b	0.96±0.06	79.43±1.41b	20.76
11	3.9±0	17.02±0a	3.7±0.06	20.56±1.4a	3.13±0.06	33.33±1.4b	54.86
12	3.46±0.03	26.24±.0.7a	3.3±0.05	29.78±1.11a	2.3±0	51.06±0b	269.06

The value means of three replicates ± standard error. The values followed by different alphabets differ significantly when subjected to Tukey HSD at 0.5 subset; 1: *C.clematidea*(L); 2: *C.clematidea*(R); 3:*H. rhamnoides*(L); 4: *A.dracunculus*(L); 5: *G.aparine*(L); 6: *M.longifolia*(L); 7: *F.vulgare*(L); 8: *R. coradifolia*(R); 9: *S.lappa*(R); 10: *I.racemosa*(R); 11: *R.webbianum*(R); 12: *A.euchroma*(R); R: Root; L: Leaf.

Antifungal activity of tested plant extract was investigated at different concentration (Table 2) against *A.flavus*. The result revealed that highly significant percent inhibition (79.43%) of mycelial growth of *A.flavus* was observed in

PDA media amended with the 5000ppm concentration of extract of *Inula racemosa,* followed by *Rubia coradifolia* (76.59%), *Codonopsis clematidea*(R) (75.18%), *Hippophae rhamnoides* (68.08%), *Galium aparine* (65.24%) respectively.

Moderate or low activity was observed in the extract of *Saussurea lappa* (55.32%), *Foeniculum vulgare* (55.32%), *Arnebia euchroma* (51.06%), *Mentha longifolia* (46.81%), *Codonopsis clematidea*(L) (44.67%), *Artimesia dracunculus* (41.13%) *and Rheum webbianum* (33.33%) showed least activity. The percentage inhibition showed significant at $p<0.05$. The antifungal activity was observed to be dose- dependent i.e. with increase in concentration of plant extract percentage inhibition of mycelium growth increases.

Antifungal activity of 12 different plant extracts showed significant activity when compared with the leaf/root extract against *A.flavus.* Root extract of *Inula racemosa* (79.43%), *Rubia coradifolia* (76.59%), *Codonopsis clematidea*(R) (75.18%) exhibit highest activity and least activity was observed in *Rheum webbianum* (33.33%) against *A.flavus* when compared with leaf extract. However leaf extract also had significant level of antifungal activity in the extract of *Hippophae rhamnoides* which has potential to inhibit mycelia growth of *A.flavus* upto (68.08%) followed by *Galium aparin* (65.24%), *Foeniculum vulgare* (55.32%), and *Mentha longifolia* (46.81%). Least activity was observed in *Artimesia dracunculus* (41.13%).

Similarly the antifungal activity of tested plant extracts was investigated at different concentration (Table 3) against *A.niger*. The result revealed that highly significant percent inhibition (79.86%) of mycelial growth of *A.niger* was observed in PDA media amended with the 5000ppm concentration of extract of *Inula racemosa, followed by* of *Rubia coradifolia* (77.08%), *Codonopsis clematidea(R)* (75.69%), *Hippophae rhamnoides*(68.75%), *Galium aparine* (64.68%). Moderate or low activity was observed in the extract of *Saussurea lappa* (59.72%), *Foeniculum vulgare* (56.25%), *Arnebia euchroma* (52.77%), *Mentha longifolia* (47.91%), *Codonopsis clematidea*(L) (45.83%), *Artimesia dracunculus* (40.97%) *and Rheum webbianum* (36.11%) showed least activity. The percentage inhibition showed significant at $p<0.05$. It showed, the antifungal activity was dose-dependent manner i.e. with increase in concentration of plant extract percentage inhibition of mycelium growth increases.

Table 3:- Inhibition of *A.niger* by solvent extract of high altitude medicinal plant.

Percent mycelia inhibition							
Concentration (ppm)							
	1000ppm		3000ppm		5000ppm		
S. No.	Colony Diameter	% Inhibition	Colony Diameter	% Inhibition	Colony Diameter	% Inhibition	F value
1	3.43±0.06	28.47±1.39a	2.96±0.03	38.19±0.69b	2.6±0.057	45.83±1.2b	58.83
2	1.4±0.05	70.83±1.2a	1.3±0.05	72.91±1.2ab	1.16±0.03	75.69±0.69b	5.27
3	3.63±0.03	24.30±0.69a	1.8±0.05	62.46±1.29b	1.5±0.05	68.75±1.2c	514
4	3.63±0.08	24.30±1.83a	3.23±0.03	32.63±0.69b	2.83±0.08	40.97±1.83c	28.8
5	2.26±0.08	52.77±1.83a	1.86±0.08	61.11±1.83b	1.7±0.05	64.68±1.2b	13.47
6	3.06±0.06	36.83±1.39a	2.6±0.05	45.83±1.2b	2.5±0.05	47.91±1.2b	24.69
7	3.06±0.03	36.10±0.69a	2.5±0.05	47.91±1.2b	2.1±0.05	56.25±1.2c	91.01
8	2.46±0.03	48.61±0.69a	1.43±0.03	70.13±0.69b	1.1±0.05	77.08±1.2c	274.22
9	2.36±0.06	50.74±1.41a	2.06±0.03	56.94±0.69b	1.93±0.06	59.72±1.39b	14.3
10	1.3±0.05	72.91±1.2a	1.03±0.06	78.47±1.4ab	0.96±0.06	79.86±1.39c	7.62
11	3.9±0	18.75±0a	3.46±0.03	27.77±0.69b	3.06±0.11	36.11±1.39c	93.54
12	3.46±0.08	27.77±1.83a	3.3±0.05	31.25±1.2a	2.26±0.03	52.77±0.69b	103.8

The value means of three replicates ± standard error. The values followed by different alphabets differ significantly when subjected to Tukey HSD at 0.5 subset; 1: *C.clematidea*(L); 2: *C.clematidea*(R); 3-*H. rhamnoides*(L); 4: *A.dracunculus*(L); 5: *G.aparine*(L); 6: *M.longifolia*(L); 7: *F. vulgare*(L); 8: *R. coradifolia*(R); 9: *S.lappa*(R); 10: *I.racemosa*(R); 11: *R.webbianum*(R); 12: *A.euchroma*(R); R: Root; L : Leaf.

Antifungal activity of tested plant extracts showed significant activity when compared with the leaf/root extract against *A.niger*. Root extract of *Inula racemosa* (79.86%), *Rubia coradifolia* (77.08%), *Codonopsis clematidea* (75.69%) exhibit highest activity and least activity was observed in *Rheum webbianum* (36.11%) against *A.niger* when compared with leaf extract. However leaf extract also showed significant level of antifungal activity in the extract of *Hippophae rhamnoides* which has potential to inhibit mycelia growth of *A.niger* up to (68.75%) followed

by *Galium aparin* (64.68%), *Foeniculum vulgare* (56.25%) and *Mentha longifolia* (47.91%). Least activity was observed in *Artimesia dracunculus* (40.97%).

Discussion:-

Bio-deterioration and spoilage of vegetables, fruits and agricultural product due to infestation by insects and microorganism can cause losses up to 100%. Several phytopathogenic fungi including species of *Aspergillus* causes significantly decrease in nutritional value [23]. *Aspergillus spp*. has been recorded as dominant fungus in fruit and vegetables. Use of many agriculturally important synthetic fungicide against this fungus have been banned by World health organization (WHO) in several countries due to its toxicity effect against non target organisms including human [24]. Thus, there is an urgent need to search for alternative method for prevention of bio deterioration of vegetable without any toxicity to the consumer. Plants are rich sources important organic compounds, pharmaceuticals and pesticidal compounds. Many reports on the antiviral, antibacterial, antifungal, anthelmintic, antimolluscal and anti-inflammatory properties of plants [19], [25], [26], [27], [28], [29], [30], [31] have reviewed. Plant derived secondary metabolites possessing antifungal activity. There are plenty of biologically active constituent which can be used as potential sources of commercially valuable pesticides which still remain to be discovered [32]. This may be due to lack of information on the screening/evaluation of diverse plants for their antifungal potential. Biologically active plant derived pesticides are expected to play an increasingly significant role in crop protection strategies. Considering this as the first step in the present investigation; solvent extract of fifteen plants species were screened for *in vitro* antifungal activity against important plant borne phytopathogenic *Aspergillus* species. These plants were selected based on traditional medicine knowledge and random choosing from the local flora. All the extract except *Rhodiala heterodanta, Rhodiala imbericate, Achelia millefolium and Hypericum perforantum* were found to inhibit the growth of selected fungi. Among the 18 extracts evaluated, 3 extract showed mycelia inhibition above 75%, 5 showed moderate effect of 50 -58% and 4 showed least 46%- 33%. Remaining six extract from root and leaf extract of *Rhodiala heterodanta,* root and leaf extract of *Rhodiala imbericate,* leaf extract of *Achelia millefolium,* and leaf extract of *Hypericum perforantum;* showed no antifungal activity against the two tested fungi. This may be due to lack of antifungal compound in the above mentioned six extracts. The percentage growth inhibition of *A.flavus* and *A.niger* was found maximum with *Inula racemosa.* The plant *Inula racemosa, Rubia coradifolia, Codonopsis clematidea*(R), *Hippophae rhamnoides, Galium aparine, Saussurea lappa, Foeniculum vulgare and Arnebia euchroma* which inhibit the mycelia growth above 50% would probably can be an important candidates plants for prevention of biodeterioration of fruits and vegetable against *A.flavus* and *A.niger*. The finding of the present investigation is an important step towards crop protection strategies for antifungal activity against important phytopathogen *Aspergillus*.

Conclusion:-

The results obtained from the present investigation clear that the antifungal activity vary with the species of the plants, plant material and the concentration. In general it revealed that the root extract have more antifungal activity than leaf extract this may be due to the presence of more antifungal compound in root. It also concluded that; with increasing concentration of plant extract, the mycelial growth of the *Aspergillus niger* and *Aspergillus flavus* decrease. The study ascertains the value of plants used in traditional medicine thereby concluding that *Inula racemosa, Rubia coradifolia, Codonopsis clematidea(R)* and *Hippophae rhamnoides* extracts can be emerged as safe alternatives to replace chemical fungicides against *Aspergillus niger* and *Aspergillus flavus*. Further investigation will carried out to determine the biologically active ingredient present in extracts which are responsible for antifungal activity as well as its mode of action; for the development of commercial formulation by field trail and toxicological experiment. The finding will help in managing eco-friendly way to combat against selected phytopathogenic fungi.

Reference:-

1. Baniasadi F, Bonjar GHS, Karimi NA, Jorjandi M, Aghighi S, Farokhi PR (2009): Biological control of Sclerotinia sclerotiorum, causal agent of sunflower head and stem rot disease, by use of soil borne actinomycetes isolates. AJABS 4: 146–151
2. Janardhana, G.R., Raveesha, K.A. and Shetty, H.S. (1999). Mycotoxin contamination of maize grains grown in Karnataka (India). Food Chemical Toxicology 37: 863 – 868
3. Chandra, R. and Sarbhoy, A.K. (1997) Production of Aflatoxins and Zearalenone by the toxigenic fungal isolates obtained from stored food grains of commercial crops. Indian Phytopathology 50: 458-68
4. Devi, K.T., Mayo, M.A., Reddy, G., Emmanuel, K.E., Larondelle, Y. and Reddy, D.V.R. (2001). Occurrence of Ochratoxin A in black pepper, coriander, ginger and turmeric in India. Food Additives Contamination 18: 830 – 835.
5. Leong S, Hocking AD, Pitt JI (2004): Occurrence of fruit rot fungi (Aspergillus sectionNigri)on some drying varieties of irrigated grapes.Austral J Grape Wine Res 10: 83–88.
6. Diedhiou, P.M., Mbaye. N., Dramél A., Samb.P.I., (2007): Alteration of post harvest diseases of mango Mangifera indica through production practices and climatic factors. Afr J Biotechnol 6: 1087–1094
7. Fatima N, Batool H, Sultana V, Ara J, Ehteshamul- Haque S (2009): Prevalence of post-harvest rot of vegetables and fruits in Karachi, Pakistan. Pakist J Bot 41: 3185–3190.
8. Mathew S (2010): The prevalence of fungi on the post-harvested guava Psidiumguajava. Int J Pharmac Sci Res 1: 145–149.
9. Narayana, K.J.P., M. Srikanth, M. Vijayalakshmiand N. Lakshmi, 2007. Toxic spectrum of Aspergillus niger causing black mold rot of onion.Res.J.Microbiol., 2:881-884
10. Sinha P and SK Saxena, 1987. Effect of treating tomatoes with leaf extract of Lantana camara on development of fruit rot caused by A. niger in presence of Drosophila busckii. Indian Journal of Experimental Biology **25** 143-144.
11. Bankole, S.A., B.M. Ogunsanwo, O.O. Mabekoje, 2004. Natural occurrence of moulds and aflatoxin B1 in melon seeds from markets in Nigeria. Food and chemical toxicology 42(8):1309-14.
12. Marin. S., Homedes, V., Sanchis, V., Ramos, A.J. and Magan, N. (1999).Impact of Fusarium moniliforme and F. proliferatum colonisation of maize on calorific losses and fumonisin production under different environmental conditions. Journal of Stored ProductResearch 35: 15 – 26.
13. Janardhana, G.R., Raveesha, K.A. and Shetty, H.S. (1998). Modified atmosphere storage to prevent mould-induced nutritional loss in maize. Journal of Science Food and Agriculture 76: 573 – 578.
14. Lacey, J. (1988) The microbiology of cereal grains from areas of Iran with a high incidence of oesophageal cancer. Journal of Stored Product Research 24: 39-50.
15. Desjardins, A.E., Manandhar, G., Plattner, R.D., Maragos, C.M., Shrestha, K. and McCormick, S.P. (2000) Occurrence of Fusariumspecies and mycotoxins in Nepalese Maize and Wheat and the effect traditional processing method on mycotoxin levels. Journal of Agricultural and Food Chemistry 48: 1377-1383
16. Satish S, Mohana DC, Raghavendra MP, Raveesha KA. Antifungal activity of some plant extracts against important seed borne pathogens of Aspergillus sp. J of Agric Tech, 2007; 3(1): 109-119.
17. Harris,C.A., M.J.Renfrew, and M.W.Woolridge. 2001. Assessing the risk of pesticide residues to consumers: Recent and future developments. Food Additives and Contamination 18:1124-1129.)
18. Mahesh B. and Satish S. (2008). Antimicrobial activity of some important medicinal plant against plant and human pathogens.World Journal of Agricultural Sciences, **4:** 839-843.
19. Arif T, Bhosale JD, Kumar N, Mandal TK, Bendre RS, Lavekar GS, Dabur R. Natural products- antifungal agents derived from plants. J Asian Natural Products Res, 2009; 11(7): 621-63820.
20. Gangadevi, V., Yogeswari, S., Kamalraj, S., Rani, G and Muthumary, J. (2008).The antibacterial activity of Acalypha indica L. Indian journal of Science and Technology.**1**(6).
21. Brindha, V., Saravanan, A and Manimekalai, R. (2009). Drug designing for ring finger protein 110 involved in adenocarcinoma (human breast cancer) using casuarinin extracted from Terminaliaarjuna. Indian Journal of Science andTechnology, **2**(2)**:** 22-26
22. Grover, R.K. and J.D. Moore. 1962. Toximetric studies of fungicides against brown rot organism. Sclerotinafruticola.Phytopathology 52:876-880
23. Koirala, P., Kumar, S., Yadar, B.K. and Premarajan, K.C. (2005) Occurrence of Aflatoxin in some of the food and feed in Nepal. Indian Journal of Medical Sciences 59: 331-336
24. Barnard, C., Padgitt, M. and Uri, N. D. (1997). Pesticide use and its measurement. International Pest Control 39**:**161-164

25. Samy, R.P. and S. Ignacimuthu, 2000. Antibacterial activity of some folklore medicinal plants used by tribals in Western Ghats in India. J. Ethnopharmacol., 69: 63-71.
26. Palombo, E.A. and S.J. Semple, 2001. Antibacterial activity of traditional medicinal plants.Ethnopharmacol., 77: 151-157.
27. Kumaraswamy, Y., P.J. Cox, M. Jaspars, L. Nahar and S.D. Sarker, 2002. Screening seeds of Scottish plants for antibacterial activity. J. Ethnopharmacol., 83:73-77
28. Stepanovic, S., N. Antic, I. Dakic and M. Svabic- vlahovic, 2003. In vitro antimicrobial activity of propilis and antimicrobial drugs.Microbiol. Res.,158: 353-357.
29. Bylka, W., M. Szaufer-Hajdrych, I. Matalawskan and O. Goslinka, 2004. Antimicrobial activity of isocytisoside and extracts of Aquilegia vulgaris L.Lett. Appl. Microbiol., 39: 93-97.
30. Behera, S.K. and M.K. Misra, 2005. Indigenous phytotherapy for genito-urinary diseases used by the Kandha tribe of Orissa, India. J. Ethnopharmacol., 102: 319-325.
31. Govindarajan, R., M. Vijayakumar, M. Singh, C.H.V. Rao, A. Shirwaikar, A.K.S. Rawat and P. Pushpangadan, 2006. Antiulcer and antimicrobial activity of Anogeissus latifolia. J. Ethnopharmacol., 106: 57-61.
32. Balandrin, M.F., Klocke, J.A., Wurtele, E.S. and Bollinger, W.H. (1985). Natural plant chemicals: Sources of Industrial and Medicinal materials. Science 228: 1154-1160.