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Effect of selected botanical extracts against *Curvularia Sp*. and *Meloidogyne incognita* (J_2)

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Abstract
Antifungal activity of aqueous extract of leaves and flowers of *Plumeria rubra, Chromolaena odorata, Rauwolfia serpentina, Ocimum tenuiflorum, Ziziphus mauritiana, Tectona grandis, Catharanthus roseus, Lantana camara, Peltophorum pterocarpum* was tested *in vitro* and it showed antifungal activity against the plant pathogen *Curvularia sp*. and *Meloidogyne incognita*. As compared to the control, the extract of *Chromolaena odorata* was found to be most effective to inhibit the growth of the *Curvularia* sp. and also showed the highest emergence of juveniles (J_2) of *Meloidogyne incognita* released from the egg masses. The best inhibitory effect which showed 40.6% reduction as compared to the control was *Chromolaena odorata* against *Curvularia* sp. whereas *Chromolaena odorata* at 99.63% also showed the highest significant reduction released from the egg masses of *Meloidogyne incognita* of J_2. The experiment was laid out in randomized complete block design with three replications and nine treatments.

Keywords: *Curvularia sp*, antifungal activity, botanicals, *Meloidogyne incognita*

Introduction
Antimicrobial agents such as medicinal plants represent a rich source. Plants with possible antimicrobial activity should be tested against some microbes to confirm the activity (Shinwari *et al*., 2009) [21]. The activity of plant extracts on bacteria and fungi has been studied by a very large number of researchers in different parts of the world (Vuuren and Naido, 2010; Bhengraj *et al*., 2008; Walter *et al*., 2011) [27, 3, 28]. Medicinal plants represent a rich source of antimicrobial agents (Mahesh and Satish, 2008; Adnan *et al*., 2010) [11, 1]. Aqueous extracts of some plants are known to have toxic properties, roots, leaves and other parts of plants contain chemicals which when present in sufficient concentration exerts toxic effect on the plant pathogens.

Curvularia is a dematiaceous hyphomycetes which is wooly, pale brown or black in color with cylindrical or slightly curved conidia and is a facultative pathogen of many plant species and common in soil and mostly found in tropical regions, though a few are found in temperate zones. The central cell generally larger than the other cells which gives a specific feature of identification of the genus *Curvularia*. Conidia is solitary, simple, often curved, clavate, ellipsoidal, fusiform, obovoid or pyriform with 3 or more transverse septa (Ellis, 1971) [7]. The disease produces small necrotic or chlorotic spot with a light colored halo and lesions are about 0.5 cm per spot when fully developed.

Root knot nematode as *Meloidogyne incognita* are root parasitic worms which belongs to family Meloidogynidae and from the genus *Meloidogyne*, which are widely distributed geographically. They are among the most damaging agricultural pests attacking a wide range of vegetable crops (Sahebani and Hadavi, 2008) [18] causing dramatic yield losses. Hyperplasia of surrounding root cells forms the gall in which the developing juvenile is placed, which is a recognizable symptom of plant infection by root knot nematode.

Medicinal uses of selected botanicals
Plumeria rubra (Frangipani) is a deciduous and a semi-succulent shrub. The juice of the bark is also used to treat amoebic dysentery. The milky juice is used to treat boils and rheumatic pain. It is also applied to remove worms or germs from wounds. The chemical classes of compounds present in the flowers oil were aliphatic compounds (25.1%), sesquiterpene hydrocarbons (22.9%), fatty acids (22.0%) and monoterpene hydrocarbons (14.9%).

Chromolaena odorata (Siam weed) infusion of the leaves is taken to cleanse the blood. The young leaves are crushed, and the resulting liquid can be used to treat skin wounds. The leaves are used to treat eye pains. The seed contains alkaloids. The leaves contain cerylic alcohol, sitosterol, isosakuranetine and odoratine. An essential oil in the plant contains sesquiterpenic acid, eupatol and anisic acid. The whole plant contains triterpenic alcohols.

Catharanthus roseus (Periwinkle) is an important medicinal plant and used for the treatment of mouth ulcer. The extract gives comfort during the depression, headache, nausea and fatigue. *Catharanthus roseus* contains significant amounts of volatile and phenolic compounds including caffeoylquinic acids and flavonal glycosides which are known to antioxidant activity.

Rauwolfia serpentina (Sarpagandha) is alkaloids in the plants reduce blood pressure, depress activity of the central nervous system and act as hypnotics. It contains a number of bioactive chemicals, including ajmaline, aricine, corynanthine, deserpidine, lankanescine, rauwolscine, rescinnamine, reserpine, reserpiline, isoreserpine, isoreserpiline, serpentinine, and yohimbine.

Lantana camara (Wild Sage) is a perennial flowering plants and the decoction of dried roots are used for gonorrhea, cough, mumps, malaria and influenza. The decoction of dried flowers is used for hemoptysis and pulmonary tuberculosis. Monoterpenes, triterpenes, flavones coumarin, steroids, iridoid glycosides, are reported from Lantana camara. Leaf extracts of lantana exhibit antimicrobial, fungicidal, insecticidal and nematicidal properties.

Peltophorum pterocarpum (Copper Pod) is used for intestinal disorders, sprains, bruises and swellings and as a postpartum remedy. It is also used as a constituent of gargles, tooth-powders and lotions for sores and muscular pains. Chemical constituents from this plant species include aliphatic alcohol, fatty acids, amino acids, terpenoids, phenolics, flavonoids, alkaloids, steroids.

Tectona grandis (Teak) is a laxative, for piles, leucoderma, dysentery, headache, burning pain over the region of the liver and removes itchiness of the skin. The compounds present are alkaloids, glycosides, saponins, steroids, flavonoids, proteins and carbohydrates and secondary metabolites such as tectoquinone, 5-hydroxylapachol, tectol, betulinic acid, betulinic aldehyde, squalene, lapachol.

Ziziphus mauritiana Lam. (Ber) leaves are applied as poultices and are helpful in liver troubles, asthma and fever and, together with catechu, are administered when an astringent is needed, as on wounds. The bitter, astringent bark decoction is taken to halt diarrhea and dysentery and relieve gingivitis. The bark paste is applied on sores. The compounds present are Tannins, Saponins, Alkaloids, Flavonoides, Glycosides, Phenol.

Ocimum tenuiflorum (Tulsi) plant extract has been proved to possess various propertries including anti-diabetic, antioxidant and antimicrobial as well as wound healing properties. It is used to treat a wide variety of skin conditions, fevers, coughs and internal ailments. It is also used in treating asthma, arthritis and heart problems. Some of the phytochemical constituents of *tulsi* are oleanolic acid, ursolic acid, rosmarinic acid, eugenol, carvacrol, linalool, β-caryophyllene (about 8%).

Materials and Methods

Collection and isolation of *Curvularia* sp: Infected fresh plant samples of *Populus deltoides* were collected from the campus of SHUATS as shown in Plate 1 and also six important medicinal planst viz: *Plumeria rubra, Chromolaena odorata, Rauwolfia serpentina, Ocimum tenuiflorum, Ziziphus mauritiana, Tectona grandis* were also collected. They were collected and washed with tap water as well as surface sterilized with 1% sodium hypochloride and washed with distilled water. Leaf material were weighted at 10 gram and chopped adding 20 ml water and the leaves were grinded using mortar and pestel, the leaf solution were then filtered out using muslin cloth and centrifuged at 4000rpm for 10 minutes. To isolate the pathogen infected portions of leaf samples were surface sterilized (70% ethanol and 0.1% sodium hypochlorite) and cultured at the centre of Potato Dextrose Agar (PDA) slants. Poison food technique of plant extract against *Curvularia* sp. was determined in lab condition under laminar flow (Plate 2). Preparation of plant extracts was prepared with the help of a cork borer and 10 ml of plant extract were added with 90 ml of distilled and water sterilized at 15lb pressure to get 10% concentration and were poured into sterilized conical flask. After proper sterilization the PDA was poured into sterilized plates and kept for 10 minutes to let it get solidify. Using 5mm cork borer, the freshly grown *Curvularia* sp. was introduced in the middle of the plates. And three plates of PDA without extract was maintained as a control and a total of 7 treatments with 3 replicate each were maintained and incubated at 28±2 ^{0}C. Radial growth of mycelium were measured every 24 hours for 4 days and compared with the control which was without plant extract. The following formula of per cent inhibition growth of the botanicals was calculated by using the formula suggested by (Vincent, 1947) [26].

$$I = \frac{C - T}{C} \times 100$$

Where,
I = Per cent inhibition
C = Mycelium growth in control
T = Mycelium growth in treatment

Plate 1: Chlorosis symptom in Poplar leaves caused by *Curvularia* sp.

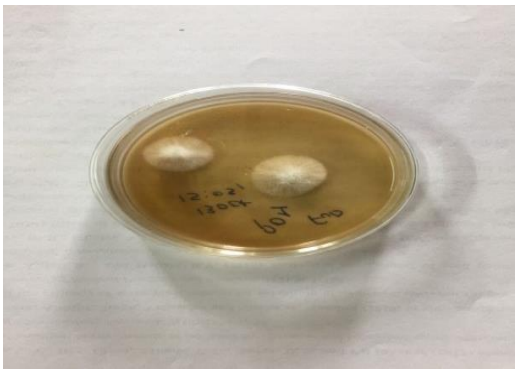


Plate 2: Pure culture of *Curvularia* sp.

Collection and screening of *Meloidogyne incognita*: Nine plant extracts were used for this experiment viz; *Catharanthus roseus, Lantana camara, Peltophorum pterocarpum, Tectona*

grandis, Plumeria rubra, Rauwolfia serpentina, Ziziphus mauritiana, Chromolaena odorata and Ocimum tenuiflorum. The root-knot nematode (*Meloidogyne incognita)* infested in the roots of *Vigna unguiculata* were uprooted gently and these were found to have 11-30 galls (Plate 2). The infected roots were washed with gentle care to remove the soil and it was soaked in fresh water for 24 hours in normal temperature. For the experiment root –knot nematode was prepared by blending 0.4 cm of chopped roots in 20 ml of distilled water. Extracts were collected and weighted for 10 gm each and washed with tap water as well as surface sterilised. These measured extracts were grinded to form powdery substance and 30 ml of distilled water was used while crushing the plant materials. Using muslin cloth the extract was filtered and the botanical extracts was taken and Centrifuged for about 10 minutes. The galls were collected and washed properly for the collection of egg masses of *Meloidogyne incognita* placed in a glass cavity slide with 50% concentration of different plant extracts. And 10 milliliter of egg suspension and 50 ml of each extract was transferred to nematode counting disc. They were then kept at room temperature allowing the eggs to hatch at 24, 48 and 72 hours while the juvenile released in distilled water was served as control (Alam, 1985) [2]. The number of J_2 was counted using a stereoscopic microscope as shown in plate 4.

Plate 3: Root knot symptoms caused by *Meloidogyne incognita*.

Plate 4: Egg masses of *Meloidogyne incognita* (J_2) under microscope.

Result and Discussion

Fungus (*Curvularia* sp.)

Scientific proof for antifungal activities of plants usually stagnates with the studies of respective plant parts against diverse group of fungal organisms (Gayatri and Ramesh, 2013) [9]. The literature survey concerns to antimicrobial activities of plants indicated that an aqueous as well as organic solvent extract of various parts of the plants have been used against the plant-pathogenic fungi to inhibit their activities involving degradation and deterioration of substrates (Swami and Alane, 2013; Nanthakumar *et al*., 2014; Vimal and Das, 2015) [24, 15, 25].

Antifungal activity of some plant extracts was studied by Disc diffusion method. The cultures were allowed to grow and get incubated in the BOD incubator. The results shows that the plant extracts were effective in significantly reducing the growth of mycelia as compared with control plate. However, all plants show antifungal activity against *Curvularia* sp. The data presented in the table 1 and depicted in figure 1 indicates that all the plant extracts inhibit the mycelial growth of the fungal organisms on culture medium. Maximum inhibition percentage was recorded in leaf extract of *Ziziphus mauritiana (T_5) at 24 hours* and it was significantly reduced from Control (T_0), *Ocimum tenuiflorum (*T_4*), Tectona grandis (*T_6*), Plumeria rubra (*T_1) while *Chormolaena odorata (*T_2*), Rauwolfia serpentina (*T_3*) and Ziziphus mauritiana (*T_5*)* were non significant from each other. At 48, 72 and 96 hours, T_2 was significantly reduced from T_0, T_5, T_6, T_3 and T_4 and T_1. Therefore, among the six plant extracts Chromolaena *odorata (*T_2*) and Plumeria rubra* (T_1) were observed to be more effective in reducing the growth of *Curvularia* sp.

All six plants showed better antifungal activities against *Curvularia* sp. and some of the plant extracts has suppressed the mycelia growth. Considering the need for an alternative eco-friendly approach to control the phyto-pathogens, it was believed to be worthwhile to screen the antifungal effects of locally available flora (Bhardwaj, 2012) [4]. Plant extracts of many higher plants have been reported to exhibit antibacterial, antifungal and insecticidal properties under laboratory trails (Okigbo and Ogbonnaya, 2006; Shariff *et al*., 2006; Bouamama *et al*., 2006; Ergene *et al*., 2006; Kiran and Raveesha, 2006; Mohana and Raveesha, 2006) [16, 20, 5, 8, 10, 13]. This finding can be investigated further to reach concrete conclusions and on the possible use of the plant extracts against the dreaded plant pathogen in agriculture and forest.

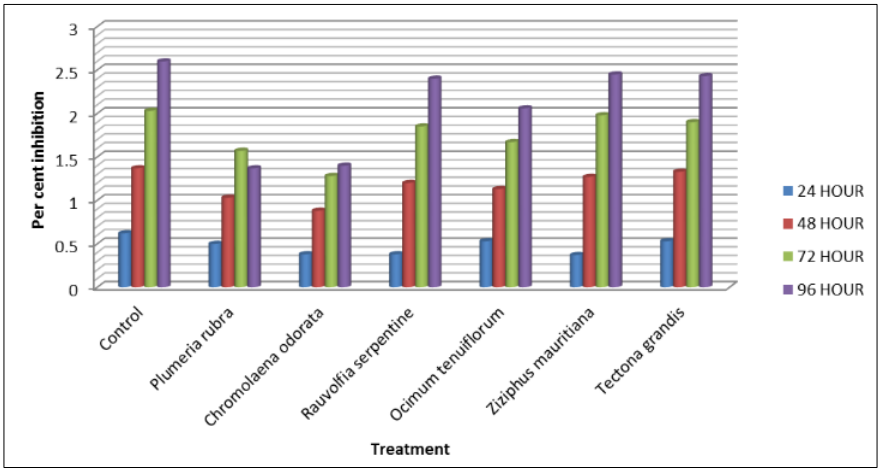


Fig 1: Per cent inhibition of *Curvularia* sp. as affected by the selected botanical extracts.

Table 1: Effect of the botanicals on the radial growth (mm) of *Curvularia* sp.

Botanicals	Part used	Extract concentration	Radial Mycelial growth of *Curvularia* sp. (colony diameter in mm)				Per cent inhibition
			24 hrs	48 hrs	72 hrs	96 hrs	
Control (T_0)		10%	0.62	1.37	2.03	2.6	0
Plumeria rubra (T_1)	Flower	10%	0.50	1.03	1.57	2.4	16.9
Chromolaena odorata (T_2)	Leaf	10%	0.38	0.88	1.28	1.40	40.6
Rauwolfia serpentina (T_3)	Leaf	10%	0.38	1.20	1.85	2.40	12.12
Ocimum tenuiflorum (T_4)	Leaf	10%	0.53	1.13	1.67	2.06	18.78
Ziziphus mauritiana (T_5)	Leaf	10%	0.37	1.27	1.98	2.45	8.48
Tectona grandis (T_6)	Leaf	10%	0.53	1.33	1.90	2.43	6.66
CD at 5%			0.134	N.S.	0.222	0.288	

Root knot nematode *(Meloidogyne incognita)*

When exposed to the botanicals the result as shown in table 2 and depicted in figure 2 that all the tested plant extracts caused a significant increase in the mortality rate of hatching of J_2. Extracts of all the nine plants tested shows significant in inhibiting the hatching of *Meloidogyne incognita* from egg masses over the control. However there was variation among the botanicals in reducing the hatching of the juveniles. All the nine plant extracts caused an increase in immobility of M. incognita juveniles after 24 hours of exposure but *Ocimum tenuiflorum* (T_9) was observed to be significantly reduced from Control (T_0), *Plumeria rubra* (T_5), *Catharanthus roseus* (T_1), *Lantana camara* (T_2), *Peltophorum pterocarpum* (T_3), *Tectona grandis* (T_4) and *Rauwolfia serpentina* (T_6) while *Ziziphus mauritiana* (T_7), *Chromolaena odorata* (T_8) and *Ocimum tenuiflorum* (T_9) were non significant from each other. From 48 to 72 hours, it was observed that T8 was significantly reduced from T_0, T_5, T_1, T_2, T_3 and T_4 but T_6, T_9, T_7 and T_8 were non-significant from each other. Among the tested botanicals, the leaves of *Chromolaena odorata* (T_8) and *Ziziphus mauritiana* (T_7) extracts were effective in reducing *Meloidogyne incognita* for egg hatching. These studies have clearly enlightened that the tested botanical extracts inhibit hatching of juveniles of M. incognita. Medicinal plants represent a rich source of antimicrobial as well as antifungal agents (Mahesh and Satish 2008) [11]. Chitwood (2002) [6] suggested that the nematicidal properties of plants species vary considerably with plant species and cultivar, the plant tissue used, plant growth stage, application method and the nematode species tested. The result of this study indicates the different activities of plant extracts on the mycelium growth and significant inhibition against *Curvularia* sp. as well as on *Meloidogyne incognita*. *Chromolaena odorata* showed to have more potential to suppress the hatching of the egg masses.

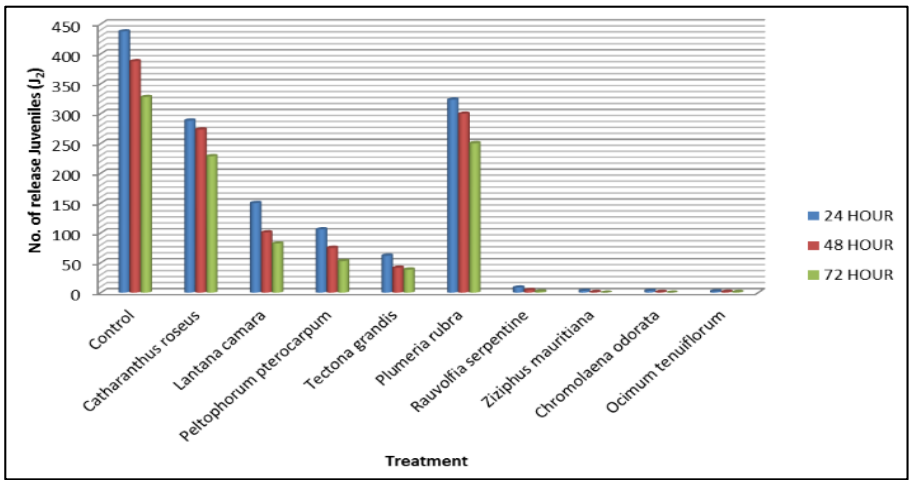


Fig 2: Per cent inhibition of second stage juveniles of *Meloidogyne incognita* as affected by the selected botanical extracts

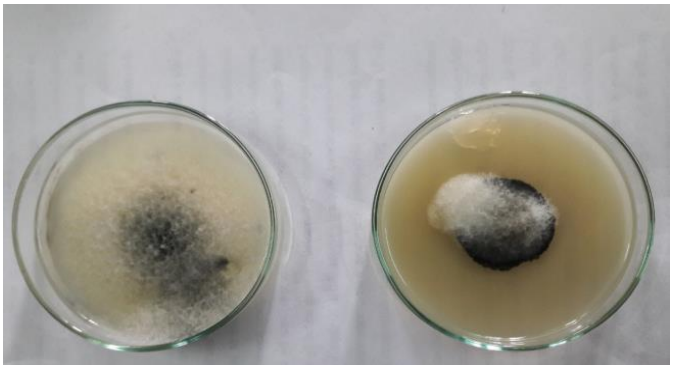
Table 2: Effect of the botanicals on emergence of *Meloidogyne incognita* of second stage juveniles from eggs.

Botanicals	Extract concentration	Number of eggs	Release of Juveniles (J_2) of *Meloidogyne incognita* after exposing the eggs to the botanicals. (Mean of the three replications)			Per cent inhibition
		0 day	24h	48h	72h	
Control (T_0)	50%	623	137.6	187.7	227.67	0
Catharanthus roseus (T_1)	50%	510	288.3	273.3	228.7	31.43
Lantana camara (T_2)	50%	390	150.3	101	82.33	71.05
Peltophorum pterocarpum (T_3)	50%	450	106	75	53	79.69
Tectona grandis (T_4)	50%	593	62	41.67	38.67	87.66
Plumeria rubra (T_5)	50%	550	323.3	299.7	250.3	24.23
Rauwolfia serpentina (T_6)	50%	534	8.67	4.33	2	98.69
Ziziphus mauritiana (T_7)	50%	497	3.33	1	0	99.63
Chromolaena odorata (T_8)	50%	400	3.33	1	0	99.63
Ocimum tenuiflorum (T_9)	50%	590	2.33	1.33	1	99.60
CD at 5%			4.015	4.406	4.054	

Conclusion

The study has shown that plants namely *Chromolaena odorata* (T_2), *Plumeria rubra* (T_1) *and Ocimum tenuiflorum* (T_4), *Rauwolfia serpentina* (T_3), *Tectona grandis* (T_6), *Ziziphus mauritiana* (T_5) are suitable for inhibiting the mycelial growth of *Curvularia sp*. These plants could be utilized to field trials to access their effectiveness in field condition. It will help in the formulation of eco-friendly control measures, environmentally safe, which is cheap and can be recommended for the farmers as reported by Sultana *et al*. (2011) [23] and Saqib *et al*. (2011) [19]. The control of fungal diseases in trees is an area of in-depth research and integrated approaches (Mehrotra BS (1992) [12]; Singh Y, Verma RK, Jamaluddin 2002) [22]. It is concluded that *Chromolaena odorata* (T_2) have antifungal activities against pathogenic fungi as it was found to be aggressively inhibiting the growth of *Curvularia sp.* against the control as shown in plate 5.

The result from this study also revealed that the botanicals of *Catharanthus roseus* (T_1), *Lantana camara* (T_2), *Peltophorum pterocarpum* (T_3), *Tectona grandis* (T_4), *Plumeria rubra* (T_5), *Rauwolfia serpentina* (T_6), *Ziziphus mauritiana* (T_7), *Chromolaena odorata* (T_8) *and Ocimum tenuiflorum* (T_9) leaves and flowers were significantly effective as shown in plate 5 and the leaves of *Chromolaena odorata*(T_8) showed the highest emergence on the hatching of juveniles (J_2) of *Meloidogyne incognita* against the control.



T_0 T_2

Plate 5: Growth of *Curvularia* sp. in the control (T_0) and *Chromolaena odorata* (T_2) treated in PDA (96 hours after inoculation)

References

1. Adnan, Hussain MJ, Shah MT, Ullah F, Shinwari ZK, Bahadar A, *et al*. Proximate and nutrient composition of Medicinal Plants of Humid and Subhumid regions in Northwest Pakistan Journal of Medicinal Plant Research. 2010; 4:339-345.
2. Alam MM. A simple method for *in vitro* screening of chemicals for nema- toxicity. International Nematology Network Newsletter. 1985; 2:6.
3. Bhengraj AR, Dar SA, Talwar GP, Mittal A. Potential of a novel polyherbal formulation BASANT for prevention of Chlamydia trachomatis infection. International Journal of Antimicrobial Agents. 2008; 32:84-88.
4. Bhardwaj SK. Evaluation of plant extracts as antifungal agents against *Fusarium solani* (Mart.) Sacc. World Journal of Agricultural Sciences. 2012; 8:385-388.
5. Bouamama H, Noel T, Villard J, Benharref A, Jana M. Antimicrobial activities of the leaf extracts of two Moroccan *Cistus* L. species. Journal of Ethnopharmacology. 2006; 104:104-107.
6. Chitwood DJ. Phytochemical based strategies for nematode control. Annual Review of Phytopathology. 2002; 40:221-249.
7. Ellis MB. *Dematiaceous Hyphomycetes* Commonwealth Mycological Institute, Kew Surrey, England, 1971, 608.
8. Ergene A, Guler P, Tan S, Mirici S, Hamzaoglu E, Duran A. Antibacterial and antifungal activity of *Heracleum sphondylium* subsp. *artvinense*. African Journal of Biotechnology. 2006; 5:1087-1089.
9. Gayathri A, Ramesh V. Antifungal activity of *Euphorbia hirta* L. inflorescence extract against *Aspergillus flavus.* A mode of action study. International Journal of Current Microbiology and Applied Sciences. 2013; 2(4):31-37.
10. Kiran B, Raveesha KA. Antifungal activity of seed extracts of *Psoralea corylifolia* L. Plant Disease Research. 2006; 20:213-215.

11. Mahesh B, Satish S. Antimicrobial activity of some important medicinal plants against plant and human pathogens. World Journal of Agricultural Sciences. 2008; 4:839-843.
12. Mehrotra BS. The Fungi: An introduction, form Genus *Fusarium* Link. Today and Tomorrows Printers and Publishers, New Delhi, India, 1992, 421-422.
13. Mohana DC, Raveesha KA. Anti-bacterial activity of *Caesalpinia coriaria* (Jacq.) Willd. Against plant pathogenic *Xanthomonas* pathovars: an eco-friendly approach. Journal of Agricultural Technology. 2006; 2:317-327.
14. Mohana DCS, Ranhavendra MP, Raveesha KA. Antifungal activity of some plant extracts against important seed borne pathogens of *Aspergillus* sp. Journal of Agricultural Technology. 2007; 3:109-119.
15. Nanthakumar R, Udhayasankar MR, Ashadevi V, Arumugasamy K, Shalimol A. *In vitro* antimicrobial activity of aqueous and extracts of *Rhinacanthus nasutus*-a medicinal plant. International Journal of Pharmaceutical Chemical and Biological Sciences. 2014; 4(1):164-166.
16. Okigbo RN, Ogbonnaya UO. Antifungal effects of two tropical plant leaf extracts (*Ocimum gratissimum* and *Aframomum melegueta*) on post-harvest yam (*Dioscorea* spp.) rot. African Journal of Biotechnology. 2006; 5:727-731.
17. Mohana DCS, Ranhavendra MP, Raveesha KA. Antifungal activity of some plant extracts against important seed borne pathogens of *Aspergillus* sp. Journal of Agriculture Technology. 2007; 3:109-119.
18. Sahebani N, Hadavi N. Biological control of the root knot nematode Meloidogyne javanica by Trichoderma harzianum. Soil Biology and Biochemistry. 2008; 40(8):2016-2020.
19. Saqib Z, Malik RN, Shinwari MI, Shinwari ZK. Species richness, ethnobotanical species richness and human settlements along a Himalayan altitudinal gradient: Prioritizing plant conservation in Palas Valley, Pakistan. Pakistan Journal of Botany. 2011; 43(SI):129-133.
20. Shariff N, Sudarshana MS, Umesha S, Hariprasad P. Antimicrobial activity of *Rauvolfia tetraphylla* and *Physalis minima* leaf and callus extracts. African Journal of Biotechnology. 2006; 5:946-950.
21. Shinwari ZK, Khan I, Naz S, Hussain A. Assessment of antibacterial activity of three plants used in Pakistan to cure respiratory diseases. African Journal of Biotechnology. 2009; 8(24):7082-7086
22. Singh Y, Verma RK, Jamaluddin. An integrated approach to control *Fusarium wilt* of Dalbergia sissoo. Indian Forester. 2002; 128(4):432-438.
23. Sultana S, Khan MA, Ahmad M, Bano A, Zafar M, Shinwari ZK. Authentication of herbal medicine neem (*Azadirachta indica* A. Juss.) by using taxonomic and pharmacognostic techniques. Pakistan Journal of Botany. 2011; 43(SI):141-150.
24. Swami CS, Alane SK. Efficacy of some Botanicals against Seed - borne fungi Of Green Gram (*Phaseolus Aureus* Roxb.) Bioscience Discovery. 2013; 4(1):107-110.
25. Vimal JB, Das SM. Antifungal activity of *Euphorbia antiquorum* L. late an *in vitro* study. International Journal of Applied Research. 2015; 1(3):25-28.
26. Vincent JM. Distortion of fungal hyphae in the presence of certain inhibitors. Nature. 1947; 159:850.
27. Vuuren SFV, Naidoo D. An antimicrobial investigation of plants used traditionally in southern Africa to treat sexually transmitted infections. Journal of Ethnopharmacol. 2010; 130:552-558.
28. Walter C, Shinwari ZK, Afzal I, Malik RN. Antibacterial activity in herbal products used in Pakistan. Pakistan Journal of Botany. 2011; 43(SI):155-162