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ANTIFUNGAL ABILITY OF *NERIUM OLEANDER* AGAINST *FUSARIUM OXYSPORUM, SCLEROTIUM ROLFSII* AND *MACROPHOMINA PHASEOLINA*

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ABSTRACT

Nerium oleander L. belonging to Apocynaceae family was screened for antifungal activity against three economically important fungi *Macrophomina phaseolina, Sclerotium rolfsii* and *Fusarium oxysporum,*.Leaves stem and root extracts in aqueous, methanol, ethanol, choloroform and acetone were prepared and were evaluated for their in vitro antifungal ability. All the parts of the plant displayed variable results for the three fungi. In case of *M. phaseolina* roots have proved to be the most effective one in reducing the growth of the fungus. Chloroform root extract has reduced the growth of the fungi to its maximum, 2.03 followed by acetone root extract respectively. Leaves chloroform extract displayed the best antifungal activity thus giving a colony diameter of 1.43 in *S.rolfsii* followed by methanol, acetone, and ethanol leaves extracts. In *F. oxysporum* trend has entirely changed, shoot induced the maximum effect as the diameter of the fungal colony was 0.37 in acetone; this trend was followed by chloroform and ethanol shoot extracts respectively. The consequences of using the *N.oleander* extracts in controlling *F. oxysporum, S.rolfsii* and *M. phaseolina* are discussed.

Key words: *Nerium oleander, Macrophominaphaseolina, Sclerotiumrolfsii, Fusarium oxysporum,* Antifungal ability, Fungal biomass.

Abbreviations: W: Water, E:Ethanol, M:Methanol, A:Acetone, Chl: Chloroform, Mp: *Macrophomina phaseolina,* Sr: *Sclerotium rolfsii,* Fo: *Fusarium oxysporum*

INTRODUCTION

Plants are under constant strain of soil borne micro-organisms. Among hundreds of soil-borne micr-organisms, fungal pathogens, *Fusarium oxysporum, Sclerotium rolfsii* and *Macrophomina phaseolina* are more common which cause diseases in a large number of plant species. Fusarium wilt is the disease of tomatoes (*Lycopersicum esculentum*) caused by *F.oxysporum* f. sp. *lycopersici.* It is one of the most destructive diseases because it is responsible for a significant economic loss in tomato yield (Alam *et al*., 2011; Ojha *et al*., 2012). In this disease, seedlings become stunted and leaves become yellow with abscission. Eventually the infected plant dies (Jones *et al*., 1991). *S. rolfsii* causes serious diseases of a wide variety of plants including field crops, vegetables, fruit and ornamental crops. The fungus infects the lower stem near the soil surface and for some plants, the roots may be infected (Mullen, 2001).Similarly stem and root rot disease of tomatoes caused by *S.rolfsii,* are serious diseases as they greatly reduce the yield of the crop (Monaim, 2010). *M.phaseolina* (Tassi) Goid causes a charcoal rot. The fungus infects the root and lower stem of 500 plant species (Smith and Wyllie, 1999). The fungus causes diseases on soybean, peanut and corn. In peanut it causes seed and seedling rots, wilt, root and stem rots, leaf spot and rotting of developing pods and seed. Charcoal rot on soybeans leads to early maturation, chlorosis and incomplete pod filling. While in corn the fungus causes a stalk rot of corn during hot, dry conditions, which is one of the most prevalent and destructive disease of corn (Khokhar *et al*., 2014).

The effective management of plant pathogenic fungi can be successfully done with fungicides but these chemicals have both short term or long term adverse effect on the environment (Bhandari, 2014).The inappropriate use of fungicides can put the life at risk as they can be carcinogenic (Stranger and Scott, 2005).In addition there are also reports that plant pathogenic fungi can adapt to fungicide treatments by mutations leading to resistance and loss of fungicide efficacy (Dissanayake, and Jayasinghe, 2013; Hahn, 2014). Recently some pesticides have been banned in Europe due to their toxicity, residues of the chemical persisting in the soil or long degradation period and destroying the useful animals as well (Komarek *et al*., 2010;Gatto *et al*., 2011).

It has necessitated an alternative approach to reduce the dependency on the synthetic fungicides. Plant extracts have proved to be complementary control means as they displayed good antimicrobial ability (Javaid and Iqbal, 2014; Javaid and Rauf, 2015). Their non toxic behavior and biodegradability have led to a new door of safety (Talibi *et al*., 2012; Ibrahim and Al-Ebady, 2014).Many secondary metabolites which plants produce show antifungal ability, include flavonoids, phenols and phenolic glycosides, unsaturated lactones, sulphur

compounds, saponins, cyanogenic glycosides glucosinolates and tannins (Dissanayake, and Jayasinghe, 2013;Vinale *et al*.,2014).

Literature survey has highlighted the medicinal properties of *Nerium oleander* L (Family: Apocynaceae) like anti-inflammatory, antibacterial, anticancer, antinociceptive, insecticidal and CNS depressant activity (Ali *et al*., 2008; Zibbu and Batra, 2010).

Previous studies have revealed the presence of carbohydrates, proteins, phenols, oleandrin and its aglycone oleandrigenin, triterpenoids, a resin, tannins, glucose, a paraffin, ursolic acid, vitamin C and an essential oil in different parts of this plant (Zibbu and Batra, 2010; Zibbu and Batra, 2012). *N.oleander*, roots and leaves have shown antimicrobial activity against *Bacillus pumilus, B.subtilis, Staphylocoocus aureus, Escherichia coli* and *Aspergillus niger* (Zibbu and Batra, 2010). Methanolic extract of *N.oleander* showed antifungal ability against *Fusarium oxysporum* f. sp. *albedinis* (Boulenouar, 2009). In another study ethanolic extract of *N.* oleander was highly effective in inhibiting the growth of *Staphylococcus aureus* and Klebsiella spp (Aboud, 2015).

The presence of bioactive compounds in different parts of the plant can be exploited against plant pathogenic fungi. The present project has been designed to test antifungal activity of various parts of *N.oleander* viz root, stem bark and leaves, against commercially important three fungi namely *F.oxysporum f. sp. Lycopersici, M. phaseolina and S. rolfsii* using different extracting solvents. Hence for the first time the above mentioned fungi have been exploited by the organic extracts of root, stem and leaves of *N.oleander* .

MATERIALS AND METHODS

Isolation and Identification of Fungi: Infected part of tomatoes and corn were cut into small pieces and were surface sterilized with 1% sodium hypochlorite solution for 2 minutes, for the isolation of the pathogens. Sterilized water was used for thorough washing, and pieces were placed on potato dextrose agar (PDA) medium in 9-cm diameter Petri plates and incubated at 25±2 °C for one week. The organisms were identified from the First Culture Bank of Pakistan as *Fusarium oxysporum f. sp. Lycopersici, Macrophomin aphaseolina and Sclerotiumrolfsii* respectively .Pure cultures of the fungi were maintained by placing in the refrigerator at 4 °C.

Aqueous and Organic extracts of Medicinal Plant: The aqueous and organic extracts of roots, stem and leaves of *N. oleander* were prepared by cutting 10 g of sun dried plant material and macerating it in the blender. The powdered plant material produced was blended with 100 ml of distilled water or organic solvents (ethanol, methanol, chloroform and acetone), (1:10 w/v). Extraction of the plant material in the solvents was done under cold conditions for 24 h. Filtration of the resultant extract was done through a Whatman filter paper and then rinsed with a small quantity (about 30 ml) of 96% ethyl alcohol. Evaporation of the extract solutions was done under reduced pressure at 40 °C. Consequently, distilled water diluted the resultant extracts and hence were stored in the deep freezer at -10 °C.

Antifungal screening: Agar well diffusion method described by Holder and Boyce (1994) was applied to screen the antifungal ability of the above mentioned extracts. Water was used as the negative control. Inoculums of 10^6 of the respective test fungi were evenly spread with a sterile glass spreader on to the PDA agar plates. A sterile borer of 7mm in diameter was used to make wells in the media. The wells were filled with 100µl of plant extracts. Plates in triplicates were incubated at 37°C. Diameter of zone of inhibition was the basis to check the antimicrobial activity after 7 days of incubation.

Statistical analysis: Two factor Completely Randomized Design (CRD) was applied. All the data were analyzed by analysis of variance (ANOVA). The comparisons among means were worked out using Tukey HSD test at 5% level of significance (Tukey, 1977).

RESULTS

Analysis of variance indicated that there was a significant ($P \leq 0.01$) effect of fungi (F), parts of *N. oleander* assayed (P) and organic solvent extracts (E) for fungal biomass. Likewise, all the interactive effects viz. F×P, F×E, P×E and F×P×E were also significant for fungal growth (Table 1).

Variability in the effect of extracts of the three parts (leaves, stem and roots) of *N. oleander* with organic extracts (methanol, ethanol, acetone and chloroform) was observed with reference to growth of *M. phaseolina*, *S. rolfsii* and *F. oxysporum*. Aqueous extract of any of the three parts of plant did not show antifungal activity. In order of effectiveness, chloroform and acetone root extracts showed the best antifungal activity followed by leaves and stem extracts in the same solvents against *M. phaseolina*. Methanol and ethanol showed variable results with reference to the parts of the plant assayed.

Chloroform root extract inhibited the colony growth up to 2.03±0.03 cm, which was the best effect, followed by acetone root extract (2.10±0.06 cm). The effect of Chloroform leaf and stem extracts was moderate in reducing the fungal growth (2.30±0.06 cm). Similar trend was recorded due to acetone extract of leaves (3.20±0.06) and stem (3.60±0.06 cm), respectively.

The effect of methanol and ethanol extracts of roots were not pronounced (5.10±0.06 and 5.20±0.10

cm). However, methanol and ethanol leaf extract reduced growth of the fungus up to 4.430±0.15 cm and 4.27±0.15 cm. Conversely, ethanol stem extract displayed a moderate antifungal activity (3.23±0.09 cm). Likewise, methanol extract of stem reduced the fungal growth to 4.60±0.06, which was not convincing (Table.2).

In case of *S. rolfsii*, colony growth was greatly reduced by the leaf extract of *N. oleander* followed by stem and root extracts in all the four organic extracts. Chloroform extract was found to be the most effective among all the organic extracts as it greatly reduced the growth of the fungus, thus the diameter of the colony was 1.43±0.23, with leaf extracts followed by stem (2.30±0.06) and root (2.73±0.07) respectively. Acetone extract followed the same trend i.e. leaves extracts reduced the colony growth up to 3.20±0.06 followed by stem and root extracts (3.60±0.06 and 4.13±0.09). Ethanol extracts of leaves and stem reduced the growth of the fungus up to 3.87±0.09 and 4.17±0.15 respectively, but ethanol extract of root showed the lowest (4.40±0.12 cm) antifungal activity among all the parts and extracts (Table 2).

Stem extract of *N. oleander* proved to be the most effective in inhibiting the growth of *F. oxysporum*. Whereas leaves and root were less effective as compared to stem. All the extracts showed antifungal activity but the acetone extract proved the best in suppressing the fungal growth. Acetone extract of shoot decreased the fungal growth to 0.37±0.09 cm which was the maximum effect followed by chloroform (0.87±0.03 cm) and ethanol (1.83±0.03 cm). Methanol extract of root did not show any promising result (5.13±0.20). Acetone extract of leaves and root showed a good decrease in the colony diameter (0.80±0.06 cm and 0.87±0.03 cm). After acetone, chloroform extracts of leaves and roots followed the same trend (1.30±0.06 cm and 1.40±0.06 cm), respectively. Ethanol extract of leaves and root displayed the colony diameter as 2.30±0.06 cm and 3.97±0.12 cm, respectively. Contrary to the trend methanol extract of root incurred colony growth up to 4.33±0.12 followed by methanol, extract of leaves (4.97±0.17)(Table 2).

Table 1. Analysis of variance for the effect of extracts of different parts of *Nerium*on growth of three phytopathogenic fungi

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F-value
Fungus (F)	2	39.2	19.59	602*
Part (P)	3	16.3	5.43	167*
Extract (E)	4	256.5	64.13	1970*
F × P	6	6.2	1.04	32*
F × E	8	77.1	9.64	296*
P × E	12	16.8	1.40	43*
F × P × E	24	23.7	0.99	30*
Error	120	3.9	0.03	
Total	179	439.7		

* = Significant at P≤0.01.

Table 2. Fungus x part interaction mean±SE

Fungus x Extract	Colony Diameter				Mean
	Control	Leave	Shoot	Root	(Fungus x Extract)
Mp x W	5.40 ± 0.06ab	5.40 ± 0.15ab	5.40 ± 0.10ab	5.40 ± 0.06ab	5.40 ± 0.04A
Mp x E	5.23 ± 0.07ab	4.27 ± 0.15fgh	3.23 ± 0.09lmn	5.20 ± 0.10abc	4.48 ± 0.25C
Mp x M	5.03 ± 0.09a-d	4.43 ± 0.15d-g	4.60 ± 0.06c-f	5.10 ± 0.06abc	4.79 ± 0.09B
Mp x A	4.20 ± 0.06f-i	3.23 ± 0.15lmn	3.23 ± 0.09lmn	2.10 ± 0.06p-s	3.22 ± 0.22E
Mp x Chl	3.50 ± 0.06klm	2.3 0 ± 0.06qrs	2.30 ± 0.06pqr	2.03 ± 0.03q-t	2.48 ± 0.18F
Sr x W	5.60 ± 0.12a	5.60 ± 0.12a	5.60 ± 0.12a	5.60 ± 0.12a	5.60 ± 0.05A
Sr x E	5.30 ± 0.15ab	3.87 ± 0.09g-k	4.17 ± 0.15f-j	4.40 ± 0.12efg	4.43 ± 0.17C
Sr x M	3.73 ± 0.09h-l	2.17 ± 0.09p-s	2.60 ± 0.06opq	3.57 ± 0.09j-m	3.02 ± 0.20E
Sr x A	4.37 ± 0.19efg	3.20 ± 0.06l-o	3.60 ± 0.06i-m	4.13 ± 0.09f-j	3.83 ± 0.15D
Sr x Chl	3.50 ± 0.06klm	1.43 ± 0.23t-x	2.30 ± 0.06pqr	2.73 ± 0.07nop	2.49 ± 0.23F
Fo x W	5.57 ± 0.15ab	5.57 ± 0.12ab	5.57 ± 0.15ab	5.57 ± 0.15ab	5.57 ± 0.06A
Fo x E	1.60 ± 0.06s-w	2.30 ± 0.06q-u	1.83 ± 0.03r-v	3.97 ± 0.12g-k	2.35 ± 0.29F
Fo x M	4.20 ± 0.10f-i	4.97 ± 0.17b-e	5.13 ± 0.20abc	4.33 ± 0.12fgh	4.66 ± 0.14BC
Fo x A	3.03 ± 0.09mno	0.80 ± 0.06yz	0.37 ± 0.09z	0.87 ± 0.03xyz	1.27 ± 0.31G
Fo x Chl	1.07 ± 0.07wxy	1.30 ± 0.06v-y	0.87 ± 0.03xyz	1.40 ± 0.06u-y	1.16 ± 0.07G

Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05). Small letters represent comparison among interaction means and capital letters are used for overall mean. Mp:Macrophomina phaseolina, ,Sr:Sclerotium rolfsii, Fo:Fusarium oxysporum, W:water, E:Ethanol, M:Methanol, A:Acetone, Chl: Chloroform

DISCUSSION

The present study revealed that all parts, leaves, shoot and root of *N. oleander* possessed antifungal activity. Ullah *et al*. (2014) reported that different parts of Ballotanigra possess photochemical to inhibit the growth of microbes. Similarly Yadav *et al*. (2013) reported alkaloids, flavanoids, carbohydrates, glycosides and tannins in different parts *N. oleander*. Rajendran (2011) in a study reported the presence of antifungal agents myricetin and rutin in the dried leaves of *N. oleander* and *Tecoma stans*. Elsadig *et al*. (2007) found isoflavonoid, Pentamethoxyflavone, terpenoids Amyrin and Ursolic acid or Oleanolic acid, constitutive antifungal compounds in leaves.

In another study *Rizwana et al*. (2012) reported that acetone, methanol and ethanol extract of different parts of *Withania somnifera* were effective in controlling several infectious diseases caused by *Klebsiella pneumonia* and methicillin resistant *Staphylococcus aureus* (MRSA). Similarly the antibacterial ability of different parts (fruits, stems plus leaves and roots) of Turkish and Iranian *Tribulus terrestris* was proved to be distinctive against *Enterococcus faecalis*, *S. aureus*, *E. coli* and *P. aeruginosa* (Kianbakht and Jahaniani, 2003; Al-Bayati and Al-Mola,2008). Antimicrobial and antifungal ability of *N.oleander* was also studied by Hadizadeh *et al.* (2009). During the study Hadizadeh *et al.* (2009) found that *N.oleander* possesses the inhibition on *F. oxysporum* and *F. solani*. In another study Hussain and Gorsi (2004) reported the antimicrobial activity of leaves and roots of *N.oleander* against *Bacillus pumilus*, *B.subtilis*, *Staphylocoocus aureus*, *Escherichia coli* and *Aspergillus niger*. Phani Deepthi

All the extracts whether the aqueous or organic solvents proved to be effective in extracting the antifungal compounds from different parts of the plant, but aqueous extract was the least effective one. In another study, Bokhari *et al*.(2013) displayed similar results with *Citrullus colocynthis* which was exploited for antifungal ability with different extracts **(**water, acetone, ethanol, methanol and chloroform) against *F. oxysporum*, *Alternaria alternata*, *M. phaseolina* and *Colletotrichum musae*. The most effective solvent in controlling the fungal growth was found to be ethanol extract and the least effective was aqueous extract.

However chloroform extract followed by acetone came out to be the promising ones among other extracts. In one of the study chloroform leaf extract of *Cissusm ultistriata* showed greater zone of inhibition for *Escerichia coli* as compared to methanol leaf extract (Adegoke *et al*., 2010). Similarly Ashraf *et al.* (2011) revealed chloroform extract of *Origanum vulgare*as most efficacious unlike methanol and aqueous extracts against *Aspergillus flavus*, *Aspergillus nigar and Aspergillus pterus*. Bhalodia *et al*. (2012) also reported the same results indicating that chloroform extracts of *Cassia fistula* fruit were strongly active against fungal strains of *Aspergillus niger*, *Aspergillus clavatus*, and *Candida albicans* than hydro alcohol. Bokhari *et al*. (2014) also clearly indicated that fruit and stem-bark chloroform extract of *Azadirachta indica*L successfully controlled soil borne pathogen *Rhizoctonia solani*. In another study Hussain and Gorsi (2004) reported that chloroform extracts of *Nerium oleander* showed high activity against *Bacillus subtilis***,** *Staphylococcus aureus***,** *Escherichia coli* and *Aspergillus niger***.**

Acetone extract of *Withania somnifera* were the most effective in inhibiting the growth of Bacillus subtilis ATCC 6633, Methicillin resistant *Staphylococcus aureus*(MRSA)ATCC 12498, *Streptococcus* pyogenes ATCC 19615, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25966,and hospital isolates of *Pseudomonas aeruginosa* and pneumonia (Rizwana *et al*, 2012). Similar investigations have been reported where acetone extracts showed pronounced inhibitory effect on the growth of pathogenic bacteria (Alagesaboopathi, 2011; Abdullahi *et al*., 2010).

All the three test fungi, *F. oxysporum*, *M. phaseolina and S. rolfsii* behaved separately towards the different organic plant extracts. Similar results were observed in a study where methanolic plant extracts from *Lantana camara*, *Salvadorapersica*, *Thymus vulgaris*, *Zingiber officinale* and *Ziziphusspinachristi* were evaluated for their antifungal efficiency on fungi, *Fusarium oxysporum*, *Pythium aphanidermatum* and *Rhizoctonia solani*.*S. persica* extract was found to be effective against *P. aphanidrematum* and *R. solani* and slightly inhibited mycelia growth of *F. oxysporum* respectively. Although, *L. camara* was found to be effective in controlling *P. aphanidermatumat* it was ineffective in controlling the other tested fungal species (Al-Rahmah *et al*., 2013)

A variation on fungitoxicity of the concerned plant extracts against phytopathogenic fungi may be due to considerable variations in their constituents and variation in fungal species itself (Manoranjitham *et al*., 2001; Narayana Bhat and Shukla, 2001).

Conclusion: The study has demonstrated that all parts of *N. oleander*, leaves, stem and roots have reduced the growth of the fungal colony. Individual part of the plant has responded differently to *M. phaseolina*, *S. rolfsii* and *F.oxysporum* respectively. Overall bioactive compounds from different parts of plant were more actively expressed in chloroform extract. Therefore it can be said that on commercial basis *N.oleander* is efficient in producing antifungal compounds which can be exploited against many economically important pathogenic fungi.

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REFERENCES

Al-Rahmah, A.N., A.A. Mostafa, A. A.-Mageed, S.M. Yakout, and S.A. Hussein (2013). Fungalcidal activities of certain methanolic plant extracts against tomato phytopathogenic fungi. Afr. J. Microbiol. Res.7 (6): 517-524.

Abdullahi M.I., I. Iliya, A. K. Haruna, M.I. Sule, A.M. Musa, M. S. Abdullahi (2010) .Preliminary phytochemical and antimicrobial investigations of leaf extracts of *Ochnaschweinfurthiana* (Ochnaceae). Afr.J. Pharm. Pharmacol.,4:083-086.

Abdel-Monaim, M.F (2010). Induced systemic resistance in tomato plants against Fusarium wilt disease. Pages 253-263. In Proc. 2nd Minia Conf. Agri. and Environ. Sci.., 22-25 March, 2010, Minia, Egypt.

Aboud, A. S. (2015). Antimicrobial activities of aqueous and ethanolic extracts from Nerium oleander used in the treatment of burns infections isolates. J. of Pharm., Chemical and Biol. Sci., 2(4):248-258.

Adegoke, S.A., O.M. Opata, J. E. Olajide (2010). Antimicrobial activity of the aqueous, methanol and chloroform leaf extracts of *Cissusmultistriata*. Afr. J. Biotechnol., 9(8): 1168-1172.

Alagesaboopathi, C. (2011). Antimicrobial potential of root, stem and leaf extract of Aristolochiabracteolata LAM. Int. J. Curr. Res., 3:019-021.

Al-Bayati, F.A., H.F. Al-Mola (2008). Antibacterial and antifungal activities of different parts of *Tribulusterrestris* L. growing in Iraq. J Zhejiang Univ Sci B., 9(2): 154–159.

Ali, S.S., S. Ali, S. Munir, T. Riaz (2008). Insecticidal and bactericidal effects of ethanolic leaf extract of common oleander, *Nerium oleander*. Punjab Univ. J. Zool., 23(1-2): 081-090

Alam, S. S, K. Sakamoto and K. Inubushi (2011). Biocontrol efficiency of Fusarium wilt diseases by a root-colonizing fungus *Penicillium sp*. Soil Sci Plant Nutr. 57: 204-212.

Ashraf, Z., A. Muhammad, M. Imran, A. H. Tareq (2011). *In vitro* antibacterial and antifungal activity of methanol, chloroform and aqueous extracts of *origanumvulgare* and their comparative analysis. Int. J. Org. Chem., 1: 257-261

Bhalodia, N.R., P. B. Nariya, R. N. Acharya, and V. J. Shukla (2012). In vitro antibacterial and antifungal activities of *Cassia fistula* Linn. fruit pulp extracts. Ayu., 33(1): 123–129.

Bhandari, G. (2014). An overview of agrochemicals and their effects on environment in Nepal. Appl. Eco. Environ. Sci. ,2(2): 66-73

Bokhari, N.A., K. Perveen, I. Siddiqui, I. Siddique, M.S. Alwahibi, and D.W.A. Soliman (2013). Antifungal Activity of *Citrullus colocynthis* against *Fusarium oxysporum*, *Alternaria alternata*, *Macrophomina phaseolina* and *Colletotrichum musae*. J. Pure Appl. Microbiol. Sci. 7(4):2981-2986.

Bokhari, N.A., I. Siddiqui, K. Perveen, I. Siddique, M.S. Alwahibi, D. W. A. Soliman, and M. Al-Subeie, (2014). Potential of different parts of Neem (Azadirachta indica) extracts in controlling Rhizoctoniasolani infestation. Int. J. Agri. & Biol., 16(3):639-643.

Boulenouar, N., A. Marouf and A. Cheriti (2009). Effect of Some Poisonous Plants Extracts on *Fusarium oxysporum* f. sp. *albedinis*. J. Biol. Sci., 9: 594-600.

Dissanayake, MLMC and JAN Jayasinghe (2013). Antifungal activity of selected medicinal plant extracts against plant pathogenic fungi; *Rhizoctoniasolani*, *Colletotrichummusea* and *Fusarium oxysporum.* Int. J. Sci. Inven. Today, 2(5):421-431.

Elsadig, E., Al B. Fatma, Al S. Iman and K. Tabisam (2007). Analysis of Phytoalexins by High Performance Liquid Chromatography-Mass Spectrometry (HPLC-MS) following Induction in *Nerium oleander*. Bot. Pl. Biol. Joint Congress:1019

Hadizadeh, I., B. Peivastegan, M. Kolahi (2009). Antifungal activity of nettle (*Urticadiocia* L.), colocynth (*Citrulluscolocynthis* L. Schrad), oleander (*Neriumoleande* L.) and konar (*Ziziphusspina-christi* L.) extracts on plantspathogenic fungi. Pakistan J. Biol. Sci.,12: 58-63.

Hahn, M. (2014).The rising threat of fungicide resistance in plant pathogenic fungi: Botrytis as a case study.J. Chem. Biol 7(4):133-41

Holder I.A, S.T. Boyce (1994). Agar well diffusion assay testing of bacterial susceptibility to various antimicrobials in concentrations non-toxic for human cells in culture. *Burns*.,**20:** 426-9.

Hussain, M.A., M.S. Gorsi (2004). Antimicrobial Activity of *Nerium oleander* Linn.. Asian J. Pl. Sci., 3(2): 177-180.

Ibrahim, F.A. A., N. Al-Ebady (2014). Evaluation of antifungal activity of some plant extracts and their applicability in extending the shelf life of stored tomato fruits. J. Food Process Technol., 5(6):34

Javaid A**,** D**.** Iqbal (2014). Management of collar rot of bell pepper (*Capsicum annuum*L.) by extracts and dry biomass of *Coronopusdidymus* shoot. Biol. Agri. Horti. 30(3): 164-172.

Javaid A**, S.** Rauf (2015). Management of basal rot disease of onion with dry leaf biomass of *Chenopodium album* as soil amendment.Int. J.Agri & Biol.17(1): 142-148

Jones J.B, J.P. Jones, R.E. Stall, T.A. Zitter (1991). Compendium of Tomato Diseases, American Phytopathological Society. St. Paul. MN.

Khokhar. K.M., K.S. Hooda, S.S. Sharma andV. Singh (2014). Post Flowering Stalk Rot Complex of Maize - Present Status and Future Prospects. Maydica Electronic Publication 59:226-242

Kianbakht, S., F. Jahaniani (2003). Evaluation of antibacterial activity of *Tribulusterrestris* L. growing in Iran. Iranian J. Pharmacol Ther.2:22–24.

Komarek, M., E. Cadkova, V. Chrastny, F. Bordas, J.C Bollinger (2010). Contamination of vineyard soils with fungicides: A review of environmental and toxicological aspects. Environ. Int.36; 138 – 151.

Manoranjitham S.K, V. Prakasam, K. Rajappan (2001). Biocontrol of damping-off of tomato caused by *Pythiumaphanidermatum*. Ind. Phytopathol. 54:59–61.

Mullen, J. (2001). Southern blight, Southern stem blight, White mold. The Plant Health Instructor.DOI:10.1094/PHI-I-2001-0104-01

Narayana Bhat M, B.K Shukla (2001). Evaluation of some leaf extracts against *Pythiumaphanidermatumin* vitro and pot culture. Ind. Phytopathol. 54:395–397

Ojha S, M. Chakraborty, N.C. Chatterjee (2012). Influence of salicylic acid and *Glomusfasciculatum* on fusarial wilt of tomato and brinjal. Arch Phytopathol Plant Prot. 45(13): 1599-1609.

Rajendran, A. (2011). Isolation, Characterization, Pharmacological and Corrosion inhibition Studies of Flavonoids obtained from Nerium oleander and Tecoma stans. Int. J. Pharm Tech Res. 3(2):1005-1013.

Rizwana, H., A.A.Al Hazani, A.I. Shehata and N.M.S. Moubayed (2012). Antibacterial potential of Withanias omnifera L. against human pathogenic bacteria. Afr. J. Microbiol. Res. 6(22): 4810-4815.

Stranger, R.N and P. R. Scott (2005). Plant Diseases: a threat to global food security; Annual Review of Phytopathol., 43:83-116

Smith, G.S.,T.D. Wyllie (1999). Charcoal rot. G.L. Hartman, J.B. Sinclair, J.C. Rupe (eds). Compendium of Soybean Disease (fourth ed.). American Phytopathological Society, St Paul,MN : 29-31

Tukey, J.W. (1977). Exploratory data analysis. Addison-Wesley, Reading

Talibi, I., L. Askarne, H. Boubaker, E.H. Boudyach, F. Msanda, B. Saadi, and A. Ait Ben Aoumar (2012).Antifungal activity of Moroccan medicinal plants against citrus sour rot agent Geotrichumcandidum. Lett. Appl. Microbiol., 55:155-161

Ullah, N., I. Ahmed, S. Ayaz (2014). In Vitro Antimicrobial and Antiprotozoal Activities, Phytochemical Screening and Heavy Metals Toxicity of Different Parts of *Ballotanigra*. Bio Med Res.Int :1-9.

Vinale, F., G. Manganiello, M. Nigro, P. Mazzei, A. Piccolo, A. Pascale, M. Ruocco, R. Marra N. Lombardi, S. Lanzuise, R. Varlese, P. Cavallo, M. Lorito, L.W. Sheridan (2014). A Novel Fungal Metabolite with Beneficial Properties for Agricultural Applications. Molecules 19:9760-9772

Yadav, CH. S. D. P. D., N. S. P. Bharadwaj, M. Yedukondalu, CH. Methushala, A.R. Kumar (2013). Phytochemical evaluation of *Nyctanthesarbortristis*, *Nerium oleander* and *Catharathnusroseus*. Ind. J. Res. Pharm. Biotechnol. 1(3): 333-338.

Zibbu.G., A.Batra (2010). A Review on Chemistry and Pharmacological activity of *Nerium oleander* L. *J. Chem. Pharm. Res.*, 2(6):351-358.

Zibbu, G., A. Batra (2012). In vitro and in vivo determination of phenolic contents and antioxidant activity of desert plants of Apocynaceae family. Asian J. Pharmaceut. Clinical Res. 5 (suppl 1):76-83.