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EXPLORATION OF ETHANOLIC EXTRACTS OF PARTHENIUM AND LEMON GRASS FOR MANAGEMENT OF CITRUS SCAB (*ELSINOE FAWCETTII*)

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A B S T R A C T

Citrus scab/blemishes caused by due to different fungal pathogens is very common in citrus orchards and affecting the Pakistani citrus export considerably. Use of plant extracts against fungal plant diseases is very common because they are eco-environment friendly and is a cheap source to manage diseases. The aim of this research was to check the antifungal activity of two important plant extracts against *Elsinoe fawcettii* the causal agent of citrus scab. Ethanolic extracts of lemongrass (*Cymbopogon citratus*) and parthenium *(Parthenium hysterophorus*) were evaluated in vitro. The best disease inhibition zone (3.2mm) was recorded when we use Lemon Grass at the concentration 50µg/ml followed by 2.83mm by parthenium plant extracts at the same concentration. Ethanolic extract of lemon grass and parthenium can be best option for disease management in field conditions.

Keywords: Citrus scab, ethanolic extracts, management, parthenium, lemon grass.

INTRODUCTION

The significant destroyers of fruits and vegetables are fungi which during storage make them unfit for human consumption by devaluing their nutritive value and mostly by producing harmful mycotoxins (Martin *et al.,* 1999). Citrus plants are vulnerable to a number of diseases one of them is scab of citrus and is a serious fungal disease caused by *Elsinoe fawcettii* infecting most of the commercial citrus cultivars worldwide (Cheema and Kapur, 1993).

Generally the disease causing fungi are normally controlled by different synthetic fungicides. But the extensive use of such synthetic chemicals is very harmful and is being restricted because of their harmful effects on the human health and its surroundings (Harris *et al*., 2001). Furthermore they are broad spectrum and high level use of such chemicals has toppled many problems such as soil, environmental, animal life and very important food contaminations (Stangarlin, 1999).

Plants extracts are considered as best substitute to synthetic chemicals because they are less hazardous to environment and consumer health. Keeping in view the importance of this work two important plants such as Parthenium and lemon grass were explore to know their antifungal properties against Citrus scab causing pathogens in vitro conditions which further will include the research program of plant disease diagnostic lab Department of Plant Pathology University of Agriculture Faisalabad for their biochemical analysis and field evaluations.

MATERIALS AND METHODS

Samples of citrus fruit affected with citrus scab symptoms were collected from thirty different orchards district Sargodha of Punjab province. The potato dextrose agar medium was prepared. The cut pieces (2-3 mm) of citrus fruit peel were surface sterilized with 70% ethyle alcohol and subsequently washing with sterilized water and then plating on the petri plates containing PDA medium for pathogen isolation. Agar slants/ test tubes were prepared with PDA (filled 1/3rd) and out growing colony from petri plates were purified under laminar flow chamber. The isolated fungus of *Elsinoe fawcettii* was later observed microscopically on the basis of morphology of the fungus (Barnett and Hunter, 1998).

Preparation of Plant Extracts: Fresh leaves samples of

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Parthenium hysterophorus and *Cymbopogon citrates* plants were collected from different localities in district Faisalabad and washed thoroughly under running tap water, dried under shade on blotting paper and then grind. After this twenty gram each sample was taken in a conical flask and extracted with solvent of pure ethanol (100 mL) for 6 hours at room temperature in an orbital shaker. The extracts were separated from the pellet by passing through filter paper. The residues were extracted twice and extracts were evaporated and freed of solvent at reduced pressure 45°C using rotary evaporator. The final extracts were stored in refrigerator at -4°C (Sultana *et al*., 2009). Different doses of both plant extracts were applied against isolated fungi by using poison food technique. Plant extracts were used at 5, 15, 25 and 50 µg/mL concentrations. PDA medium was prepared and different concentrations of plant extracts as mentioned above were made and added in PDA medium by using agar well method. Then culture of isolated fungi of about 5 mm discs was placed in the center of petri plates containing media and agar wells. Three replications of plant extract concentration were used and plates without plant extracts were served as control. The Petri plates were wrapped with parafilm tightly and incubated at 25± 2°C temperature and radial colony of tested fungus were recorded after 3, 5 and 7 days and growth were measured in mm. Standard errors of means of three replicates of each treatment were calculated using software STATISTIX 8.1. All the data were analyzed by analysis of variance (ANOVA) using similar software. Following the ANOVA, Tuckeys test was applied for further analysis.

RESULTS

The results of survey conducted in Sargodha and nearby localities revealed the presence of citrus scab disease with the incidence of up to 45%. The infection occurs when fruit size is small similar to the size of small size lemon. Samples of fruit, leave and twig were further processed in laboratory to isolate the associated pathogen. For further fungus growth and sporulation, the test tubes were incubated in an incubator at (28°C ± 2). The isolated fungi were identified as *Elsinoe fawcettii*, *Colletotrichum gloeosporioides*, *Alternaria alternata* and *Fusarium* spp. (Figure 1) were identified microscopically on the basis of morphological characters of the fungus.

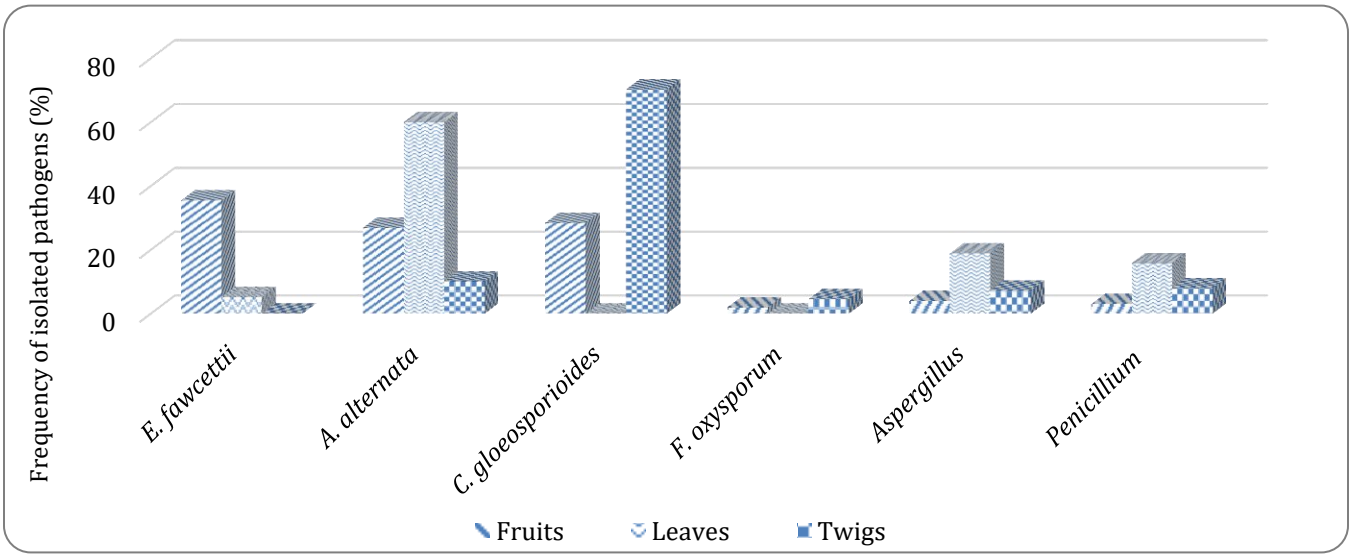

Figure 1.Frequency of isolated fungal pathogens from fruits twigs and leaves samples.

In the surveyed orchards the most prevalent pathogens was *E. fawcettii* (35.50%) on the fruit followed by *Colletotrichum gloeosporioides* (28.50%), *Alternaria alternata*(27.00%), *Aspegillus* spp. (4.00%), *Penicillium* spp (3.00%), *F. oxysporum* (2.00%). In case of isolations from leaves samples the most prevalent pathogen was *A. alternata* (60.00%) whereas *C. gloeosporioides* (70.32%) was the most prevalent pathogen isolated from twigs Figure 1.The data on the effect of ethanolic extracts is presented in figure 2. All the tested ethanolic plant extracts (Lemon Grass, Parthenium, Kasni and *Aloe vera*) inhibited the mycelia growth of *E. fawcettii* invariably and significantly. The best disease inhibition zone (3.2mm) was recorded when we use Lemon Grass at the concentration 50µg/ml followed by 2.83mm by Parthenium plant extracts at the concentration of 50µg/ml. Whereas Kasni and *Aloe vera* were found least effective against *E. fawcettii* (Figure 2).

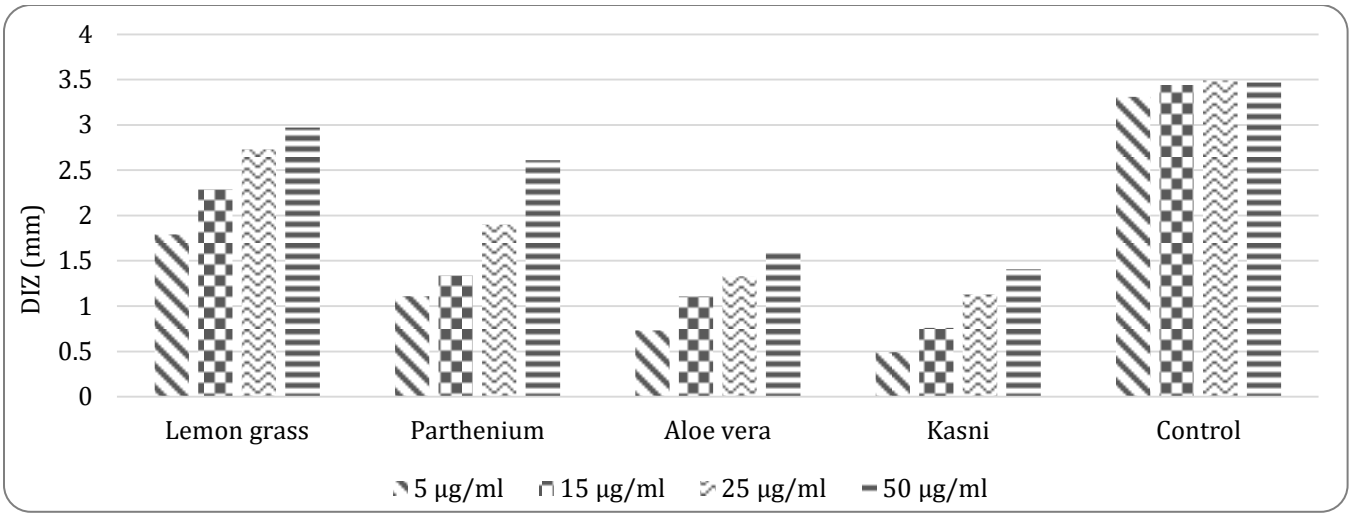

Figure 2. Effect of Aqueous extracts on mycelial growth of *E. fawcettii* over 7 days

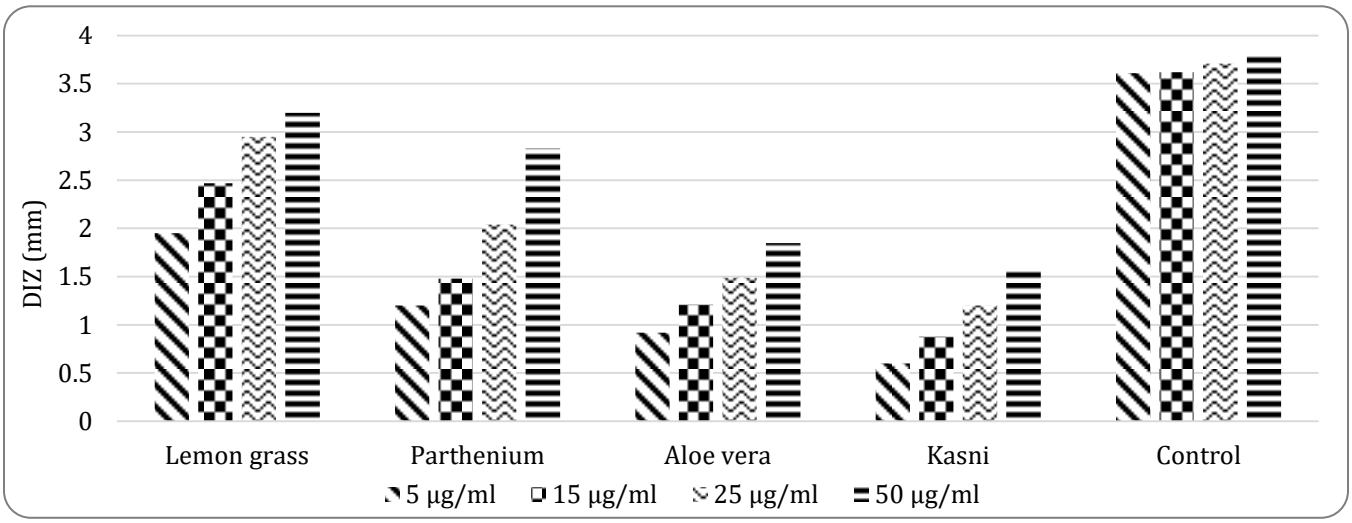

Figure 3. Effect of Ethanolic extracts on mycelial growth of *E. fawcettii* over 7 days.

In vitro evaluation of different ethanolic extracts of Lemon grass and Parthenium were done against *E. fawcettii* at different concentrations. Results showed that lemon grass was significantly effective against *E. fawcettii* followed by parthenium plant extracts at all concentrations. . There were 4 ethanolic plant extracts, Lemon Grass, Parthenium, Aloe vera and Kasni. There were 4 levels of concentrations viz, 5 µg/ml, 15 µg/ml, 25 µg/ml and 50 µg/ml. The petri plates without any extracts served as control. After 7 days the best disease inhibition zone seen was 3.2 by Lemon Grass @ 50 µg/ml followed by 2.83 by Parthenium, 1.85 by Aloe vera and 1.58 by Kasni plant at same concentration.(Figure 3).

DISCUSSION

The citrus fruit is vulnerable to different diseases by many pathogens (Bassanezi, *et al*., 2002). These diseases and disorders are more than hundred and are triggered by viral, most important fungal and some bacterial pathogens affecting the plants from seeding levels to mature fruit bearing stage and later on subsequently in significant losses (Gopal *et al*., 2014; Reddy, 1985). It is assumed that approximately 10% of fruits production is lost annually only due to the fungal pathogens. Citrus scab is caused by *E. fawcettii* and an important disease in all of the citrus growing countries particularly in New Zealand Argentina, Australia, U.S.A., India, and Pakistan (Rao, 1983), reported that citrus scab disease destroyed citrus nursery badly in India. The disease incidence in subcontinent and in India on citrus cultivars was also studied by Nirvan (Nirvan, 1961).

The external appearance and quality of citrus fruit which is produced for dietary purpose and for fresh market is reduced by citrus scab disease and caused 15 to 60 % losses in citrus fruit especially to mandarin in India

(Mukhopadhyay, 1985). Palanisivami *et al.,* (1993) reported that the scab disease was present in India long time ago but the pathotype responsible for cause is not clear. The prevalence of disease on all plant parts leads to premature leaf and fruit drop. In suitable environment 65 to 72 % losses in the form of fruit drop are reported causing heavy losses to growers (Huang and Huang, 1999). In highly susceptible cultivars pustules develop on young leaves results in distortion of twigs and emerging shoot apices and pustules are yellow in color which turn brown with time (Hyun *et al*., 2009). Emerging leaves and external surface of fruits are more susceptible to pustules and raised scabs. The external surface of the fruit is also badly affected due to the raised lesions and scabs (Nelson, 2008). On grapefruit the scab lesions are rough, immature and not smooth. Leaf distortion also takes place if scab infects leaf. Betancourt and Jenkins, (1937) reported that disease symptoms are prominent in five to seven days. Whiteside, (1981) reported that the ascospores of *Elsinoe fawcettii* are small as compared to those of *E. australis* pathotype. They mostly affect the premature fruits. The conidia serves as the inoculum source to infect new plants that s why to prevent new young fruits the old diseased parts should be removed as soon as possible. Hyun *et al.,* (2001) investigated and described a new type of citrus scab in South Korea. It affects citrus fruit but is different from *Elsinoea ustralis*.

Different plant extracts have been used till now to control the fungal diseases of the plants. Obagwa and Korsten, (2002) evaluated the ethanolic extracts of garlic and clove against citrus to control the fungal pathogens of *Penicillium* species. The results revealed that both plant extracts were effective to control mycelial growth of the fungus at 0.1 % v/v concentration. The ethanolic extracts of *Phlomis fruticose* against fungal diseases of citrus inhibited *Aspergillus spp., Penicillium spp., Fusarium spp.* and *Phomopsis spp*. Moreover these plants had very active fungicidal activity against *Colletotrichum spp* at a very low concentration (Prasad and Anamika, 2015). The results were highly significant. Yousef, (2013) reported the similar results. He revealed that the fungal inhibition was high by lemon grass at concentration of 15-20 µg/ml against many fungal pathogens including *E. fawcettii*. Tapwal *et al.,* (2011) also reported that diseases inhibition zone was high by application of lemon grass followed by parthenium plant extracts at 5, 10, 15 and 20% concentrations against *A. solani*, *A. zinnia* and *E. fawcettii.*

Falah *et al.,* (2008) and Singh *et al*., (2012) studied the thyme, lime, chilly, ginger and camphor plant extracts against *P. digitatum*and *Fusarium spp*. The results revealed that all plants showed best results at 10% concentration and chilly extracts inhibited mycelial growth 100 %. Singh *et al*., (2012) evaluated ethanolic extracts of ruthenium, veronica, eucalyptus, nerium, lantana and osmium against *Alternaria*disease of citrus. The results revealed that highest inhibition of disease was recorded by nerium followed by parthenium, ocimum, lantana, veronica and eucalyptus respectively. The aim of the following research was to evaluate the antifungal activity of the ethanolic extracts of lemon grass and parthenium plant extracts against *E. fawcettii* causing citrus scab.

In present study, a survey was conducted for collection of citrus fruits affected with citrus scab from thirty orchards in Sargodha District and nearby citrus growing localities. Sargodha is famous for its citrus production worldwide. All the collected samples showed the prevalence of citrus scab disease. During the months of March and April the disease severity was high because of rising temperature. The fruit size is like small lemon and disease progress more. The symptoms of citrus scab are complex with other fungal diseases. The disease is more severe when weather is relatively warm and humid during young fruit setting. In cold weather conditions the disease is not a big problem and fruit produced for fresh market is not affected badly (Whiteside, 1975).

Different fungal pathogens were isolated from the diseased samples. The results showed that the most prevalent fungal pathogen was *E. fawcettii* (35.50%) on the fruit surface followed by *C. gloesporioides* (28.50%), *A. alternata* (27.00%) *Aspergillus spp*. (4.00%), *Penicillium spp.* (3.00%) and *F. oxysporum* (2.00%) was least prevalent. When pathogens isolated from leaves the most prevalent pathogen was *A. alternata* (60.00%) followed by *Aspergillus spp.* (18.90%), *Penicillium spp.* (15.85%) and least was *E. fawcettii* (5.25%). From twigs the most prevalent pathogen isolated was *C. gloesporides* (70.32 %) followed by *A. alternata* (10.00 %), *Penicillium spp.* (8.00%), *Aspergillus spp.* (7.18 %) and *F. oxysporum* (4.50 %). Singh and Deverall, (1984) also reported the similar results. They concluded that *A. alternate, G. candidum, P. digitatum and E. fawcettii were isolated from citrus tree showing typical scab symptoms in preharvest stage.*

In present study the effect of antifungal activity of ethanolic plant extracts was observed on colony growth

of *E. fawcettii*. The results indicated that the disease inhibition zone was high by lemon grass ethanolic extract followed by parthenium. Yousef, (2013) reported the similar results. He revealed that the fungal inhibition was high by lemon grass at concentration of 15-20 µg/ml against many fungal pathogens including *E. fawcettii*. Tapwal *et al.,* (2011) also reported that diseases inhibition zone was high by application of lemon grass followed by *Cannabis sativa* and *Adiantum venustum* extracts at 5, 10, 15 and 20% concentrations against *A. solani*, *A. zinnia* and *E. fawcettii.*

In present study the effect of antifungal activity of aqueous plant extracts were observed on colony growth of *E. fawcettii*. The results indicated that the disease inhibition zone was high by application of lemon grass and was statistically significant. (Bokhari, 2009; Madan *et al.,* 2011; Singh *et al.,* 2014), also reported the similar results. They revealed that disease inhibition zone was high by lemon grass extracts followed by parthenium at higher concentrations against *Fusarium spp*., *E. fawcettii* and *Aspergillus spp*.

CONCLUSION

Citrus scab caused is a challenging threat for citrus industry of Pakistan. It was concluded that plant extracts used in present studies were found effective against citrus scab pathogen. By the addition of pathenium and lemon grass in management schedule of scab pathogen in field conditions we can reduce the losses considerably. Plant extracts are affordable and environment friendly method for citrus scab management as compare to others.

REFERENCES

Bassanezi, B. R., F. B. Armando., L. Amorim, N. G. Fernandes, R. T. Gottwald and M. B. Joseph. 2002. Spatial and Temporal Analyses of Citrus Sudden Death as a Tool to Generate Hypotheses Concerning Its Etiology. J. Epidemiology

Barnett H.L. and B.B. Hunter. 1998. Illustratted genera of imperfect fungi.(4thed) APS, Minnesota, USA. 218pp

Bitancourt, A. A. and A. E. Jenkins. 1937. Sweet orange fruit scab caused by *Elsinoeaustralis*. Journal of Agricultural Research Center, 54: 1-18

Bokhari, Fardous. M. 2009. Antifungal activity of some medicinal plants used in Jeddah, Saudia Arabia. Mycopath. 7(1): 51-57.

Cheema S. S., and S. P. Kapoor. 1993. Citrus diseases and their management progress of plant pathological research, Ind. Soc. Plant pathologist: 267-285.

Falah, S., T. Suzuki and T. Katayama. 2008. Chemical constituents from *Swietenia macrophylla* bark and their antioxidant activity. Pak. J. Biol. Sci. 11: 2007-2012.

Gopal, K.L., M. Lakshmi, G. Sarada and T. Nagalakshmi. 2014. Citrus melanose (*Diaporthe citri* Wolf): A review. Int. J. curr. Microbiol. appl. sci. 3: 113–24.

Harris, C.A., M.J. Renfrew, and M.W. Woodridge. 2001. Assessing the risk of pesticide residues to consumers: recent and future developments. Food Additives and Contamination 18:1124-1129.

Huang, H. and H. M. Huang. 1999. The occurrence of citrus scab and its control. South China Fruits, 28: 18.

Hyun, J. W., L. W. Timmer, S. C. Lee, S. H. Yun and K. S. Kim. 2009 Molecular analysis of *Elsinoe* isolates and *Elsinoeaustralis* causing scab diseases of citrus in Jeju island in Korea. Plant dis. 85: 1013-1017.

Hyun, J. W. Timmer, L. W., Lee, S. C., Yun, S. H. Ko, S. W. and K. S. Kim. 2001. Pathological Characterization and Molecular Analysis of *Elsinoe* isolates Causing Scab Diseases of Citrus in Jeju Island in Korea.

Madan.H., S. Gogia and S. Sharma. 2011. Antimicrobial and spermicidal activities of *Partheniumhysterophorus* Linn. and*Alstoniascholaris* Linn. Ind. J. Natural prod. Resour. 2(4): 458-463.

Martin, S., Homedes, V., Sanchis, V., Ramos, A.J. and N.Magan. 1999. Impact of *Fusarium moniliforme* and *F. Proliferatum* colonization of maize on calorific losses and fumonisinproction under different environmental conditions. Journal of Stored Product Research.35 :15-26.

Mukhopadhyay, S. 1985. The dieback of mandarin oranges in Darjeeling district, West Bengal, India. Bidhan Chandra Krishi Viswavidyalaya: 12

Nelson, S. 2008. Citrus scab. Plant Disease. 60: 1-6.

Nirvan, R.S. 1961. Annual report of the horticultural research, citrus scab and Its control. Saharanpur, India.

Obagwa, J and L. Korsten. 2002. Control of citrus green and blue molds with garlic and clove extracts. Plant Pathol. 109: 221–25.

Palanisivami, A., T. Naryanaswany and R. Jeyarajan. 1993. Sweet orange (*Citrus sinesis* L.) a new host for scab caused by *Sphacelomaaustralis*. Ind. J. Mycol. And Plant Pathol, 23: 217-218.

Prasad. R. H. and Anamika. 2015. Effects of Plant Leaf Extracts against *Colletotrichumgloeosporioides*causing citrus diseases. J. Agri. Sci. 7(5): 195-198.

Singh H, Al-samarai G, M. Syarhabil 2012. Exploitation of Natural Products as an Alternative Strategy to Control Postharvest Fungal Rotting of Citrus. International Journal of Scientific and Research Publications.2 (3).

Singh, V. and B. J. Deverall. 1984. *Bacillus subtilis* as a control agent against fungal pathogens of citrus fruit. 83(3): 487-490

Singh G., S. Gupta and N. Sharma. 2014. In vitro screening of selected plant extracts against *A. alternata.* Journal of Experimental Biol. and Agri. Sci. 2(3): 344-351.

Stangarlin J. R., Schwan-Estrada K. R. F, Cruz MES, M. H. Nozaki. 1999. Medicinal plants and alternative control of phytopathogens. BiotecnologiaCiência&Desenvolvimento. ; 11:16-21.

Rao, Raghavindra N. N. 1983. Efficacy of two copper based fungicides for the control of citrus scab pesticides 1: 31-33.

Reddy, G.S. 1985, Citrus diseases and their control. Indian council of agricultural research publication, New Delhi.79pp

Sultana, B., F. Anwar and M. Ashraf. 2009. Effect of extraction solvent/technique on the antioxidant activity of selected medicinal plant extracts. *Molecules*. 14: 2167-2180

Tapwal, A., Nisha, S. Garg, N. Gautam and R.Kumar. 2011. In Vitro Antifungal Potency of Plant Extracts Against Five Phytopathogens. Brazilian arch.Bio. Tech. 54(6): 1093-1098.

Whiteside,J.O. 1975. Biological characteristics of *E. fawcettii* pertaining to the epidemiology of sour orange scab. J. phytopathol. 65: 1170-1175.

Whiteside, J.O. 1981. Evolution of current methods for citrus scab control. Proc. Fla. State. Hort. Soc. 94: 5-8.

Yousef, S. A. A. 2013. Antifungal Activity of Volatiles from Lemongrass (*Cymbopogoncitratus*) and Peppermint (*Menthapiperita*) oils against Some Respiratory Pathogenic Species of Aspergillus. Int.J.Curr. Microbiol. App. Sci. 2(6): 261-272.