INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 14, No. 1, p. 231-237, 2019

RESEARCH PAPER OPEN ACCESS

Antibacterial potential of ethanolic and methanolic extracts of herbal plants against *Xanthomonas campestris* pv. *mangiferaeindicae* the cause of black spot disease in mango

Hasnain Sajjad[1], Abdul Rehman[1], Khizar Razzaq[1*], M. Waqar Alam[2], Saira Mehboob[3], Nasir Ahmed Rajput[1], Nasir Ahmad Khan[1], Samia Anam[1]

[1]*Department of Plant Pathology, University of Agriculture Faisalabad, Pakistan*

[2]*Institute of Agricultural Sciences, University of Punjab Lahore, Pakistan*

[3]*Institute of Plant Pathology, Ayub Agriculture Research Institute, Pakistan*

Key words: Bacterial black spot disease, *X. campestris* pv. *mangiferaeindicae*, Mango, herbal/medicinal plants, ethanolic and methanolic extracts.

http://dx.doi.org/10.12692/ijb/14.1.231-237 Article published on January 26, 20199

Abstract

Mango (*Mangifera indica*) is an ever-green tree and native to south Asia. Every year different types of pre and post-harvest diseases cause huge losses to mango crop. Susceptible germplasm and favorable environmental conditions contribute toward wide epidemic of diseases. Bacterial black spot disease of mango is caused by*X. campestris* pv. *mangiferae indicae*is a big hazard to mango crop when it occurs in severe condition. In the present investigation different types of ethanolic and methanolic plant extracts clove (*Syzigum aromaticum*), hermal (*Peganum hermala*), dhaneya (*Coriandrum sativum*), kasni (*Cichorium intybus*), swanjna (*Moringa oleifera*) and cinnamon (*cinnamomum zylanicum*) were evaluated under laboratory conditions. Results of the experiment showed that Ethanolic and methanolic extracts of Hermal found to be most effective with a mean value of (2.10, 2.30) against *Xanthomonas campestris* pv. *Mangiferae indicae* followed by coriander and clove extracts at 50µg/mlconcentration with a mean value of (1.23, 2.26) (1.76, 2.16) respectively on nutrient agar medium.

* **Corresponding Author:** Khizar Razzaq ✉ mkhizar012@gmail.com

Introduction

Black spot of mango is endemic in the world especially through the major mango producing areas (western Oceania, Asia, southern and eastern Africa and Indian Ocean). Disease affects the quality and quantity of mango fruit that results into huge losses (Pruvost *et al.,* 1995). In 1915 disease was first described in South Africa (Doidge, 1915). Pathogen attacks on several marketable varieties and cause huge losses because of early fruit fall (Gagnevin and Pruvost, 2001). Typical symptoms of the disease can be observed on leaves and fruits, later on canker of twigs and branches were observed due to serious contamination of pathogen (Gagnevin and Pruvost, 2001). Black color Raised spots with Angular shaped lesions were observed on the leaves while erumpent and star shaped lesions were found on the fruit. Twig canker develops occasionally (Pruvost and Manicom, 1993). In 1940s pathogen was first recognized and later on classified in 1980s as *Xanthomonas campestris pv. Mangiferae indicae* (Patel*et al.,* 1948; Dye *et al.,* 1980; Ridgway, 1989; Johnson *et al.,* 1989).Today, many types of fungicides and other chemicals are used to control the diseases. Knowing the lethalness of these chemicals farmers community continuing the use of these type of chemicals, because there is no ecofriendly product is available to control the diseases(Ambridge and Haines 1987; Anon, 1998).

Due to the severe toxicity, ability to gathering in the foodstuff chain and their capability to destroy both harmful and beneficial pests many pesticides have been forbidden (Barnard *et al.,* 1997). With the passage of time several management strategies have been adopted by different scientist and all have significant effects in controlling diseases with the time span. Now a day trend was shifted toward the friendly environment products that have no effect on the human and animal health. Several plant extracts with known properties was used against several pathogens and satisfactory results was obtained. To handle the microbes that attack on plants this is an urgent requirement for substitute remedy (Mahajan and Das 2003). Some essential oils are also effective against many pathogens and considered environment friendly, essential oils distinguish the odors related with each plant (Pengelly, 1996). According to Kalemba & Kunicka 2003, several plant extracts and their chemical compound found to be effective against many pathogens like bacteria and fungi etc. Therefore, present experiment was planned to study the effect of different herbal plant extracts against bacterial black spot disease in mango.

Material and methods

Collection of diseased samples

Different areas of Punjab province such as Rahim Yar Khan, Shujabad, Multan and Muzaffargarah was selected and survey was conducted to collect the diseased samples of different plant parts showing typical symptoms of disease.

Isolation and purification of Pathogen associated with bacterial black spot disease of mango

Different plant parts like leaves, twigs and fruits showing typical symptoms of disease were collected from different mango orchards during survey. For further investigation samples were brought to the laboratory. Infected fruits were selected and cut into tiny pieces of 4-5mm and sterilized by using 1% sodium hypo chloride solution for one minute. Before placing them on filter paper they are washed twice with distilled water. After drying small pieces of samples were placed on petri plates using sterilized forceps. Petri plates were incubated at ±28°C with alternating light and dark period for one week and plates were observed on daily basis. After 96 hours of incubation yellow and round colonies were appeared. Pathogen was identified visually on the basis of morphological characters. Stock culture of bacteria was preserved at 4°C in refrigerator for further use. By using nutrient agar media culture was purified. For further growth plates were incubated at ±28°C. Whole experiment was done by using laminar flow hood chamber to avoid contamination.

Plant material

Different types of medicinal plant were selected such as kasni (*Cichorium intybus*), sohanjna (*Moringa*

oleifera), cinnamon (*cinnamomum zylanicum*),clove (*Syzigum aromaticum*), hermal (*Peganum hermala*) and dhaneya (*Coriandrum sativum*). Seeds were purchased from local market. Plant parts like stem and roots were collected from botanical garden, University of Agriculture, Faisalabad.

Preparation of plant extracts

For preparation of plant extracts, plant parts leaves were washed to remove the dust particles. After drying, plant parts were grounded into fine crushed form. Crushed material (20 gm) of each plant extract was weighed and added in conical flask separately, which have absolute ethanol and methanol. For shaking of flasks orbital shaker was used for 12 hours at room temperature. Filter paper was used to purify the contents of both ethanolic and methanolic extracts to remove the impurities and then extracts was mixed. Plant extracts were stored at 4°C for future use and to contamination (Sultana *et al.,* 2009). Different concentrations (5, 15, 25 and 50µg/ml) of ethanolic & methanolic plant extracts were prepared for further experimental use.

Test of antimicrobial activity

By using agar well diffusion method activity of different plant extracts was assessed against isolated pathogen *X. campestris* pv. *mangiferae indicae* (Chung *et al.,* 1990). Media was prepared and with concentration of 20ml was poured into 9cm petri plates. By mixing bacterium colony in sterilize water aqueous suspension was prepared. 100µl of suspension was poured in each petri plate. After solidification, a sterilize cork borer was to make four wells of 6mm diameter in each petri plate. Prepared concentrations of ethanolic and methanolic plant extracts were applied in each well by using micro-pipette. Wells without any plant extract were served as control. For whole experiment seven treatments and three replications was used. Plates were incubated at 37°C for 5 days. Antibacterial activity of plant extracts was measured by determining the inhibition zones of pathogen growth surrounding the plant extracts. Data was recorded after 3, 5 and 7 days of incubation, inhibition zones were measured in mm.

Statistical analysis

To analyze the data statistically MSTAT-C computer software was used (Russel and Eisen smith, 1983).analysis of variance technique was employed to test the overall significance while, (LSD) Least Significance Difference test (P≤ 0.l.) was used to compare the differences among treatment mean (Steel *et al.,* 1997).

Results

Different orchards of Punjab province viz. Shujabad, Muzaffargarh, Multan and Rahim Yar Khan were selected to measure the disease incidence and to collect the diseased specimens of leaves, fruit and twigs. During survey maximum disease incidence was observed on fruit as compared to the leaves and twigs. Disease incidence on fruit, leaves and twigs was measured from Shujabad (A), Muzaffargarh (B), Multan (C) and Rahim Yar Khan (D) and presented in figure 1.

Table 1. Effect of different concentrations of Ethanolic extracts of herbal plants against *X. campestris* pv. *mangiferae indicae*

Treatments	5 µg/ml (Mean)	15µg/ml (Mean)	25µg/ml (Mean)	50µg/ml (Mean)
Kasni	1.00	1.1	1.03	1.46
Hermal	2.06	1.76	1.86	2.10
Clove	1.06	1.2	1.23	1.76
Moringa	1.43	1.00	1.33	1.46
Coriander	1.26	1.2	1.2	1.23
Cinnamon	1.2	1.23	1.33	1.2
Control	0	0	0	0
Mean	1.14	1.07	1.14	1.31

Effect of different concentrations of Ethanolic and Methanolic extracts of Hermal plants against X. campestris.pv mangiferae indicae

Various types of medicinal plants ethanolic and methanolic viz. kasni, moringa,Hermal, clove, coriander and cinnamon extracts was tested against pathogen (*Xanthomonas campestris* PV. *mangiferae indicae*). Our results indicated that all extracts inhibit the pathogen at their maximum level but hermal was found to be most efficient against pathogen at various concentrations. As hermal showed highly significant results with 2.1mm average zone of inhibition at 50µg/ml concentration followed by other plant extracts kasni and moringa with average zone of inhibition (1.0mm and 1.0mm) at different concentrations respectively as presented graphically in figure 2 and table 1.At 50µg/ml Hermal (*Piganum hermala*) found to be most effective against pathogen with 2.30mm average zone of inhibition followed by coriander (*Coriandrumsativum*) and moringa (*Moringa oleifera)* with 0.77mm zone of inhibition at 15µg/ml respectively. In case of methanolic extract hermal found to be most effective against isolated pathogen with mean inhibited zone (2.30mm) followed by coriander (*Coriandrumsativum*) and moringa (*Moringa oleifera)* with (1.2mm, 1.00) at 15µg/ml respectively as presented in figure 3 and table 2.

Table 2. Effect of different concentrations of Methanolic extracts of herbal plants against *X. campestris* pv. *mangiferae indicae*.

Treatments	5 µg/ml (Mean)	15µg/ml (Mean)	25µg/ml (Mean)	50µg/ml (Mean)
Kasni	0.96	0.93	0.86	0.76
Hermal	1.60	2.40	2.03	2.30
Clove	1.90	2.16	2.16	2.16
Moringa	1.30	0.7	1.00	1.26
Coriander	2.30	2.20	2.23	2.26
Cinnamon	2.13	2.06	2.10	2.20
Control	0	0	0	0
Mean	1.46	1.49	1.48	1.56

Discussion

Many extract of plants such as clove, viscose, eucalyptus and tea tree have been used as topical antiseptics and antimicrobial due to the presence of their bioactive compounds. So present investigation was done to evaluate the significance of different plant extracts against *Xanthomonas campestris* PV. *mangiferae indicae* andresults of our experiment indicated that Hermal was found to be most effective against pathogen with 2.1mm average zone of inhibition at 50µg/ml concentration followed by other plant extracts kasni (1.0mm) and moringa with average zone of inhibition 1.0mm at different concentrations respectively.

In another experimentthe antimicrobial activity of different extracts *Feniculum vulgare,Pegnanum hermala,Ocimum basilicum, Ricinus communis, Azadirachata indica,* and *Cichorium intybus* was determined by disc diffusion method and results demonstrated that *Piganum hermala* extract was found to be most effective with 40±1.5mm zone of inhibition (Salahudin *et al.,* 2011).

These findings were also close agreement with other studies. In another experiment (Keskin & Toroglu, 2011) evaluated different plant extracts,*Capsicum annuum, Zingiber, Syzgium aromaticum, officinale, Alpinia ficinarum, Cuminum cyminum, Cinnamomun zeylanicum, Coriandrum sativum, Eugenia caryophyllata, Folium sennae, Flos tiliae, Origanum onites L, Folium menthae crispae* and *Piper nigrum* by using disc diffusion method and results indicated that *Syzgium aromaticum* was most

effective against Staphylococcus aureus and Escherichia coli with inhibition zone of (7-24mm 30µl^{-1}). Nandagopal and Kumari, (2007) evaluated *Chichorium intybus* against phytopathogenic bacteria and found that hexane and acetate root extracts are more effective than water extracts, petroleum ether and chloroform. Similar results were obtained by (Prabuseenivasan *et al.*,2006).

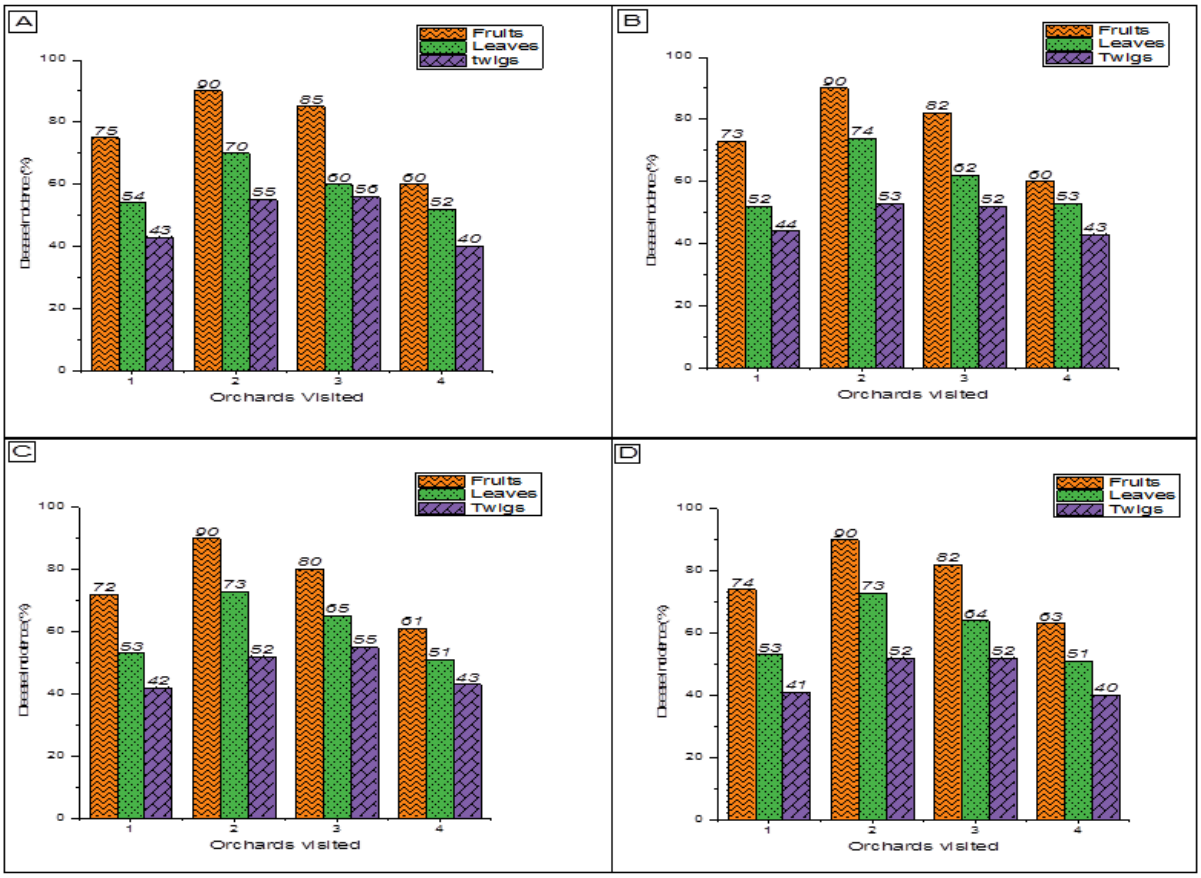

Fig. 1. Incidence of black spot disease of mango in different districts Shujabad, Muzaffargarh, Multan and Rahim Yar Khan (A, B, C, D) respectively.

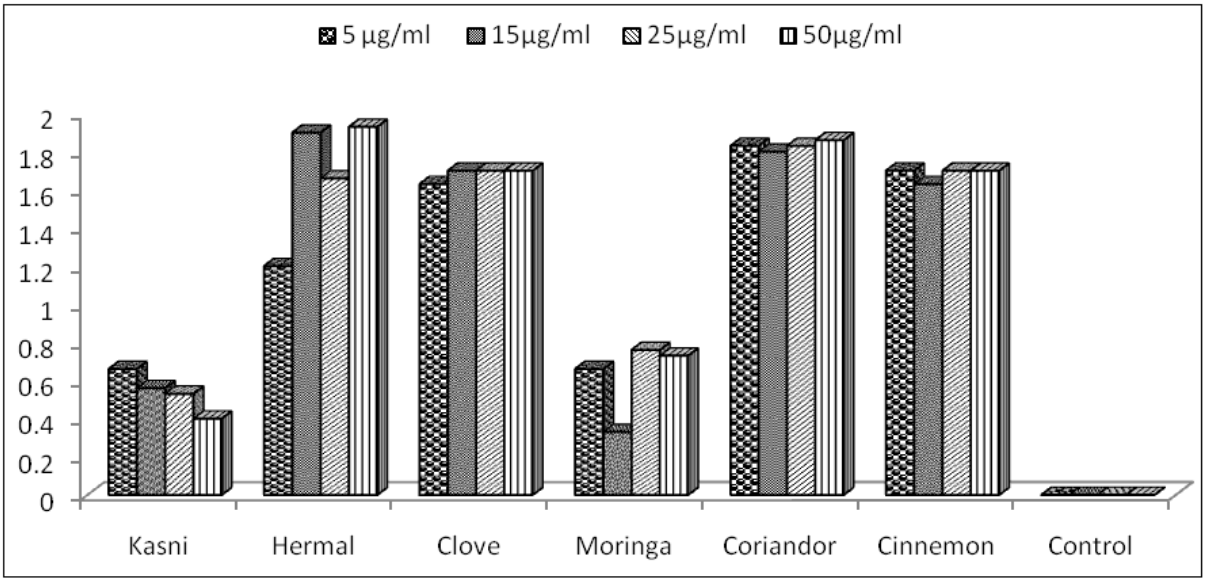

Fig. 2. Effect of different concentrations of Ethanolic extracts of herbal plants against *X. campestris* pv. *mangiferae indicae*.

Different plant extracts were evaluated against 2 gram positive and 2 gram negative strains of bacteria and they found that Cinnamon gave best results in inhibition of bacterial growth. Result of the present experiment closely related to the previous work. So, findings of the present experiment were helpful for managing destructive disease and are ecofriendly.

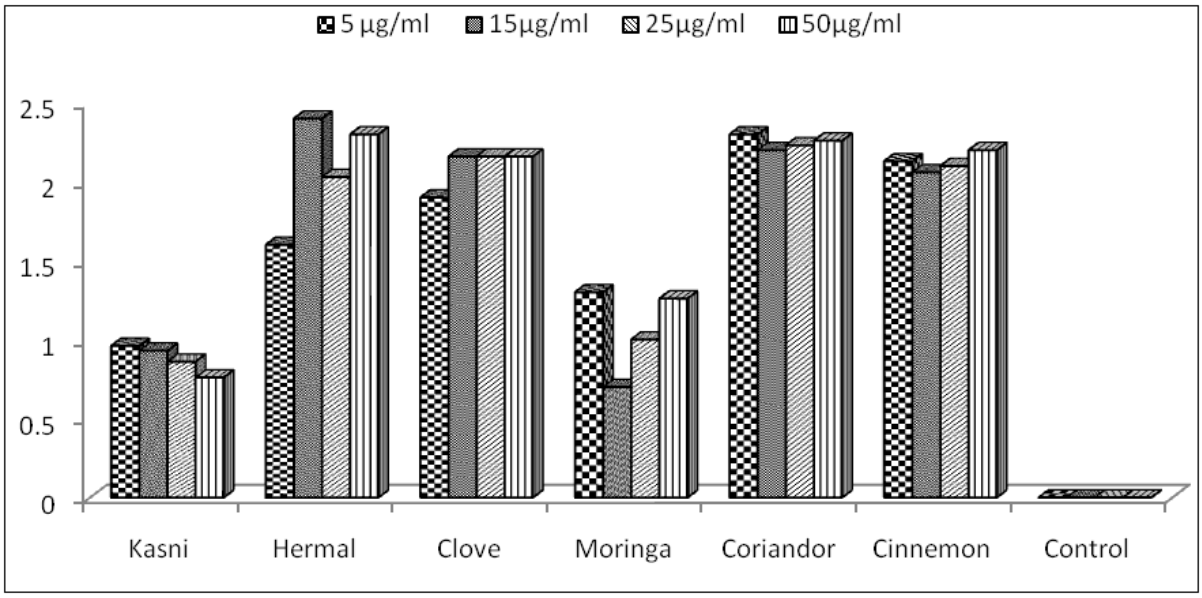

Fig. 3. Effect of different concentrations of methanolic extracts of herbal plants against *X. campestris* pv. *mangiferae indicae*.

Conclusions

Ethanolic and methanolic extracts of Hermal showed maximum inhibitory effects against the pathogens of black spot disease of mango. Further studies are needed to check the efficacy of different types of ethanolic and methanolic extract.

References

Ambridge EM, Haines IH. 1987. Some aspects of pesticide use and human safety in Southeast Asia. In 11. International Congress of Plant Protection, Manila (Philippines), 5-9 Oct 1987.

Anonymous. 1998. Pesticides incidents up for 1996/97 compared with previous year. International Pest Control **40,** 1-8.

Barnard C, Padgitt M, Uri ND. 1997. Pesticide use and its measurement. International Pest Control (United Kingdom).

Collins R, Dunne T, Campbell J, Johnson P, Malik AU. 2006. Constraints Analysis of Pakistan's Mango Supply Chains. Australian Centre for International Agricultural Research (ACIAR), Australia.

Dye DW, Bradbury J, Goto M, Hayward AC, Lelliott RA, Schroth MN. 1980. International standards for naming pathovars of phytopathogenic bacteria and a list of pathovar names and pathotype strains. Review of Plant Pathology **59(4),** 153-168.

Evans EA. 2008. World trends in world and US mango production, trade and consumption. Assessed 02/02/09.
http://edis.ifas.ufl.edu.document_fe718.

FAO. 2007. Food Agriculture Organization. [Online]. Available at: (accessed on 20th February, 2012).
http://www.fao.org

FAO. 2011 Food Agriculture Organization. [Online]. Available at: (accessed on 22th February, 2012).
http://www.fao.org

Fernald ML. 1950. Gray manual of Botany. 8th ed. American Book Co, New York.

Gagnevin L, Pruvos O. 2001. Epidemiology and control of mango bacterial black spot. Plant Disease, **85(9),** 928-935.

Johnson GI, Cooke AW, Mead AJ, Wells IA. 1989. September). Stem end rot of mango in Australia: causes and control. In III International Mango Symposium **291,** 288-295.

Kalemba DAAK, Kunicka A. 2003. Antibacterial and antifungal properties of essential oils. Current medicinal chemistry **10(10),** 813-829.

Keskin D, Toroglu S. 2011. Studies on antimicrobial activities of solvent extracts of different spices. Journal of Environmental Biology **32(2),** 251-256.

Doidge M, Ethel. 1915. A bacterial disease of the mango. Bacillus mangiferae n. sp. Annals of Applied Biology **2(1),** 1-45.

Mahajan A, Das S. 2003. Plants and microbes-Potential source of pesticide for future use. Pesticides information **28(4),** 33-38.

Nandagopal S, Kumari BR. 2007. Phytochemical and antibacterial studies of Chicory (Cichorium intybus L.)-A multipurpose medicinal plant. Advances in Biological Research **1(1-2),** 17-21.

Patel MK, Moniz L, Kulkarni YS. 1948. A new bacterial disease of Mangifera indica L. Current Science **17(6),** 189.

Pengelly A. 1996. The constituents of medicinal plants. Sunflower Herbals **2,** 109-112.

PPEA. 2003. Projet de Promotion des exportations agricoles s/c ministère de l'agriculture et de l'elevage du sénégal. Guide export – Mangue du Sénégal. Editions Techniques IFLEX 55.

Pruvost O. 1993. Xanthomonas campestris pv. mangiferaeindicae: cause of bacterial black spot of mangoes.

Pruvost O, Couteau A, Luisetti J, Vernière C. 1995. Biologie et épidémiologie de l'agent de la maladie des taches noires de la mangue. Fruits, **50(3),** 183-189.

Ridgway R. 1989. Mango Pests and Disorders. Department of Primary Industries, Queensland Government.

Russel DF, Eisensmith SP. 1983. MSTAT-C. Crop and Soil Science Department, Michigan State University, USA.

Silimela M. 2003. Evaluation of biocontrol and sunprotectors to control mango fruit diseases and disorders (Doctoral dissertation, University of Pretoria).

Steel RD, JH Torrie, Dicky DA. 1997. Principles and procedures of statistics. A biometrical Approach.

Sultana B, Anwar F, Ashraf M. 2009. Effect of extraction solvent/technique on the antioxidant activity of selected medicinal plant extracts. Molecules **14(6),** 2167-2180.

Sunita B, Mahendra R. 2008. Antifungal Activity of Essential Oils from Indian Medicinal Plants against Human Pathogenic Aspergillus fumigatus and A. niger. World Jour Med Sciences **3(2),** 81-88, 2008 ISSN 1817-3055.