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### In-Vitro Evaluation of Plant Extracts against Sudden Death Disease in Mango Trees

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**Abstract:** Mango is a major fruit crop of Sindh. In recent past the outbreak of sudden death in mango has caused massive losses in its production. In order to find the preventive measures for this disease efficacy of plant extracts was evaluated in this experiment. Botanical extracts of five plants i.e. neem (*Azadirachta indica*), ak (*Calotropis procera*), dhatura (*Datura stramonium*), tobacco (*Nicotiana tabacum*) and onion (*Allium cepa*) were used. These extracts were tested in vitro in four concentrations 2ml, 5ml, 10ml, 20ml per liter of nutrient media in three replicates using completely randomized design. The results revealed that plant extracts considerably inhibited mycellial growth of test fungus as compared to control. Neem extract was the most effective and it produced 84.29% inhibition followed by tobacco with 74.84% reduction in fungal growth. Minimum reduction (53.428%) of *Ceratocystis fimbriata* was observed in dhatura extracts. The use of these extracts especially neem can be fruitful in eco-friendly management of sudden death in mango.

**Key words:** Sudden death, Mango, Plant extracts, Management

#### Introduction

Mango is an important fruit crop in Pakistan. It is grown successfully in the tropical conditions of Sindh. The area under mango cultivation in the province is 62732 hectares with an annual production of 399214 tones. (GoP, 2014-15). Due to its unique taste and aroma, Pakistani mangoes have a great demand worldwide. Therefore special care is taken ensure the quality production of this fruit. It makes mango an important source of foreign exchange for the country. However lack of awareness of latest technologies at farm level hinders the sustainable production. Apart from this, increasing production costs and attack of pests and disease affects mango crop both

quantitatively and qualitatively. Similarly less fruit setting, more fruit drop and presence of stress conditions in orchards is important hurdle in achieving its potential yield. In recent past the outbreak of sudden death in mango severally affected its production in the country. The sudden death basically constitutes a blend of slow and quick decline in mango, gummosis and bark splits. (Malik et al., 2005). In addition to this blackening of root system and oozing of blackish mass from infected portions are the characteristics symptoms. Due to the vascular nature of this menace blockage of xylem vessels results in wilting, leaf shedding and ultimately drying and death of

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mango plants. (Khuhro et al., 2005; Fateh et al., 2006). Number of a-biotic factors contributes in the dissemination of this disease along with bark beetle and improper irrigation system in the orchards. (Mailk et al., 2005; Al-Adawe et al., 2006). Due to its uncertain etiology mango production remained challenged for a considerable period of time. (Kazmi et al., 2005). The association of certain fungal flora, nematodes, bark beetles, phytoplasma and nutrient deficiencies has been reported in literature previously. In Pakistan Fateh et al., 2006 & Malik et al., 2005 reported the association of *Ceratocystis fimbriata* and *Ceratocystis manginecans* with sudden death in mango. The success in finding the exact cause of this disease proved very effective in devising its management program. Keeping in view the importance of mango in Sindh province present work was carried out to assess the performance of plant extracts against *Ceratocystis fimbriata*. Disease management in plants mostly depends on the use of fungicides. But the extensive and injudicious use of chemicals has led to environmental problems. Their residual deposition on fruits and vegetables is very harmful for human life. There is a need to use some alternates of these chemicals in plant disease management programs. Plant based bio pesticides provide a better choice in this regard as an effective and eco friendly control. Antifungal properties of a number of plants have been reported in literature. Therefore plant based products especially neem has gained importance against a number of pests and diseases. The presence of biologically active nimbin and azadirachtin in different parts of neem plant is responsible for its antimicrobial properties. (Pennigton et al., 1981). Therefore this study was planned to evaluate the response of *Ceratocystis fimbriata* against botanical extracts of five different plants.

## Materials and Methods

In present work in vitro the efficacy aqueous extracts of neem (*Azadirachta indica*), dhatura (*Datura stramonium*), ak (*Calotropis procera*), Tobacco (*Nicotiana tabacum*) and onion (*Allium cepa*) against *Ceratocystis fimbriata* was tested. Food poison method was used to evaluate these extracts. Dhingra & Sinclair, (1985). The extracts of were prepared following the methods of Shetty et al., (1989) and Mondali et al., (2009). Plant material i.e. leaves of neem, ak, dhatura, tobacco and onion bulbs were collected. These were washed thoroughly in tap water and air-dried. One hundred gram (100 g) of each plant material was crushed in a sterile mortar. The paste was mixed with 100 ml of sterile distilled water in 250 ml beaker and stirred vigorously. The mixture was kept overnight and then filtered. Four concentrations i.e. 2ml, 5ml, 10ml, 20ml of each filtrate were added to the melted potato dextrose agar media. The experiment was conducted under completely randomized design with three replications of each treatment. Five petri dishes per replication in each treatment were used. The petri plates without extracts served as control. After solidification of nutrient media, a piece of actively growing *Ceratocystis fimbriata* colony (5-mm disc) was placed aseptically on PDA. These petri dishes were incubated at  $27^{\circ}\text{C} \pm 2^{\circ}\text{C}$  and mycellial growth was recorded on daily basis till the control treatment was fully covered with fungus. The percent inhibition of fungal growth by fungicides and plant extracts was calculated according to following formulae. Shreesha and Venkateswarlu (2013).

$$\text{Inhibition Percentage (I \%)} = \frac{\text{Growth in control} - \text{Growth in treatment}}{\text{Growth in control}} \times 100$$

The data were analyzed statistically with Statistix 8.1

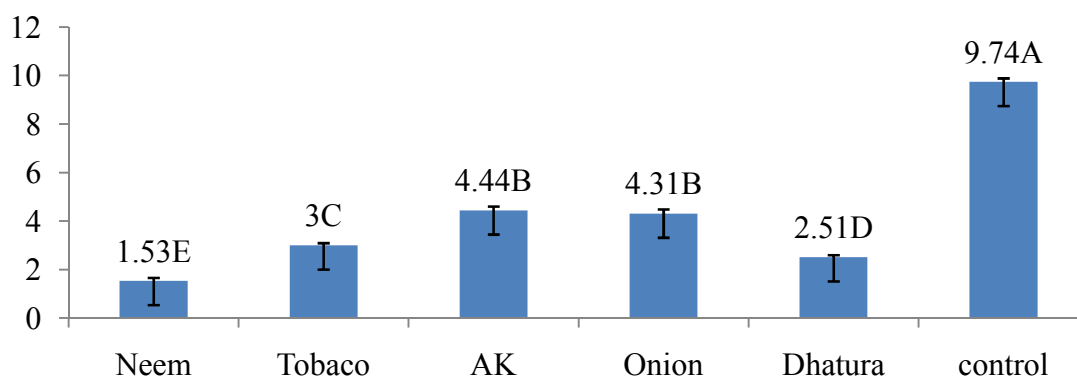
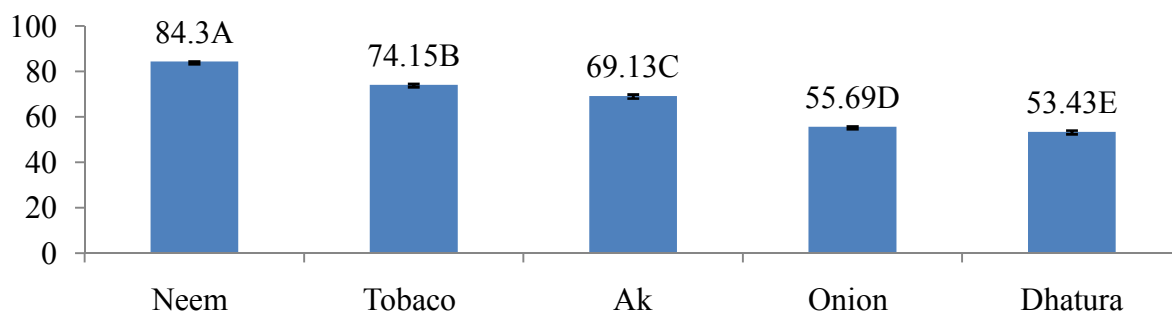
## Results and Discussion

Table 1 shows the performance of plant extracts against *Ceratocystis fimbriata* at different concentrations. Among the tested botanicals, aqueous extracts of neem leaves showed maximum inhibition (84.29%) of the pathogen. The fungal growth in petri dishes containing neem extracts was slow as compared to the vigorous mycellial mat in control. The antifungal potential of neem and its by products has been exploited greatly which proved very effective for disease management in plants. (Al-Abed et al., 1993). Tewari and Nayek, (1991) and Gurjar et al., (2012) found neem extracts very useful against a number of fungal pathogen in plants. The presence of a variety of biologically active compounds in neem plays a key role in reducing the fungal growth on nutrient media. (Amadioha 1998 & 2003; Pennigton et al., 1981). The tobacco extracts in present results produced 74.15% inhibition of *Ceratocystis fimbriata* growth over control. These results are in accordance with Suleiman (2011) who reported that higher concentrations of neem and tobacco extracts completely inhibited the development of *Aspergillus*, *Penicillium* and *Rhizopus* on nutrient media. The presence of osmotin in tobacco has a key role in its antifungal properties against different plant pathogenic fungi. (Abad et al., 1996). In current results ak extracts were found more effective than those of onion and dhatura. An inhibition 69.13% of target pathogen over control was seen in ak treatment which comparatively higher than 55.69 % and 53.43% reduction in *Ceratocystis fimbriata* growth in onion and dhatura extracts respectively. It was observed that mycellial growth of fungus in extracts was prominent in onion and dhatura extracts as compared to the fungal growth in ak treatment. Sharma et al., (2014) found dhatura extracts very useful against different

species of plant pathogenic *Rhizoctonia* and *Fusarium*. Similarly onion bulbs as a good source of flavonoids and polyphenols are effective in the suppression of both bacteria and fungi. (Jeffery and Jeffery, 2003; Hendrich, (2006); Etaweel (2013). The toxicity in plant extracts is related to the presence of active compound and their methods of extraction in particular. The type of plant material and its age also play a key role in the quantification of these compounds. (Nicolls, 1969 and Qasem et al., 1996; Mondali et al., 2009). However environmental conditions of an area influence the exact quantity of these compounds in particular plant species. Thus the availability of alkaloids, quinones, flavonoids etc in plants determine their antimicrobial activity. (Sales et al., 2016). It is evident from present results that neem and tobacco extracts suppressed the growth of *Ceratocystis fimbriata* than ak, onion and dhatura. The difference among these extracts is due to their constituent composition. Similarly their solubility, pH and volatility of these plant materials also affect their antimicrobial properties, apart from this response of nutrient media and the target pathogen is also very important. (Gillitzer et al., 2012; Talibi et al., 2012.). In some previous studies Vijaya et al., 2007; Tagoe et al., 2009; Gurjar et al., 2012; Shrivastava and Swarankar 2014 have suggested the use of neem and other plant extracts for disease management in different plants. Ahmad et al., 2005 found the application of plant extracts very effective against anthracnose in mango. As sudden death in mango has caused economic losses but the extensive use of chemicals against this menace and their residual effects in fruits has become a major concern. There is need to use plant based product with minimal side effects to ensure chemical free mango production. The application of botanical extract is gaining importance at farm level but the use of

**Table 1.** Effect of plant extracts on mycellial growth (mm) of *Ceratocystis fimbriata*

	2ml	5ml	10ml	20ml	Mean
Extracts					
Neem	2.44E ± 0.11 (74.76)	1.89E ± 0.11 (80.57)	1.33D ± 0.19 (86.33)	0.44D ± 0.11 (95.52)	1.53E ± 0.12 (84.3)
AK	4.44C ± 0.11 (63.80)	3.67C ± 0.00 (69.16)	2.55C ± 0.22 (77.18)	1.33C ± 0.19 (86.46)	3.00C ± 0.08 (74.15)
Dhatura	6.33B± 0.00 (54.03)	5.44B ± 0.11 (62.28)	3.89B ± 0.40 (73.75)	2.11B ± 0.11 (78.55)	4.44B ± 0.16 (69.13)
Onion	6.5B ± 0.09 (32.93)	5.41B ± 0.26 (44.39)	3.57B ± 0.48 (63.44)	1.77BC ± 0.29 (81.99)	4.31B ± 0.17 (55.69)
Tobacco	3.50 D ± 0.09 (31.18)	3.00D ± 0.19 (44.00)	2.22CD ± 0.11 (60.00)	1.33 C ± 0.00 (86.46)	2.51D ± 0.08 (53.43)
Control	9.67 A ± 0.00	9.73A ± 0.60	9.73A ± 0.6	9.84A ± 0.09	9.74A ± 0.15
LSD	0.26	0.45	0.89	0.49	
SE	0.12	0.21	0.41	0.23	

**Fig. 1.** Mean Mycellial growth (mm) of *Ceratocystis fimbriata* on PDA with different concentration of plant extracts.**Fig. 2.** Percent reduction in mycellial growth of *Ceratocystis fimbriata* by plant extracts.

chemicals still is the foremost choice of farmers. (Malik et al., 2011). But the over and extensive application of these chemicals has led to environmental degradation. There is also a need to create awareness about the use of these botanicals at farm level. The results of this study show that neem based products along with other botanical extracts like tobacco can be effective in the prevention of sudden death in mango. The preventive as well as curative control of fungal pathogens with these botanicals has been reported by Sales et al., (2016). As an integral part of integrated management of this disease in mango plant extracts along with the judicious use of fungicides can promote the environment friendly management for sudden death in mango. Mass production and commercialization of these botanicals with the help of latest technological advancements will help to increase their utilization in plant disease management.

### References

- Abad, L. R., M. P. D'Urzo, D. Liua, M. L. Narasimhan, M. Reuveni, J. K. Zhu, X. Niu, N. K. Singh, P. M. Hasegawa and R. A. Bressan. 1996. Antifungal activity of tobacco osmotin has specificity and involves plasma membrane permeabilization Plant Sci. 118 II-23.
- Ahmed I., S. A. Rahman, S. ul-Alam, R. Parvin, K. Mursheda, Farhana, S. B. Kim and T. S. Lee. 2005. Effect of fungicides and plant extracts on the conidial germination of *Colletotrichum gloeosporioides* causing mango anthracnose. Mycobiol. 33(4): 200-205.
- Al-Abed, A.S., J. R. Qasem and H.A. Abu-Blam. 1993. Antifungal effects of some common wild plants species on certain plant pathogenic fungi. Dirasat (Pure & Appl.Sci.) 20:149-158.
- Al-Adawi, A. O., M. L. Deadman., A. K. Al-Rawahi., Y. M. Al-Maqbali., A. A. Al-Jahwari., B. A. Al-Saadi., I. S. Al-Amri and M. J. Wingsfield. 2006. Etiology and causal agents of mango sudden decline disease in Sultanate of Oman. Eur. J. Plant Pathol. 116 (4): 247-254.
- Amadioha, A. C.1998.Fungitoxic activity of extracts of *Azadirachta itidica* and *Xylopiya aethiopia* on *C. lindemuthianum* in cowpea. J. Herbs, Spices and Medicinal Plant 6: 33-40.
- Amadioha A.C. 2003. Fungitoxic effects of extracts of *Azadirachta indica* against *C. miyabeanus* causing Brown Spot of Rice. Acta. Phytopath. Pflanz. 35: 37-42.
- Dhingra, O. D. and J. B. Sinclair. 1985. Basic plant pathology methods. Boca Raton, FL, USA, CRS Press.
- Eltaweel. M. Assessment of Antimicrobial Activity of Onion Extract (*Allium cepa*) on *Staphylococcus aureus*; *in vitro* study 2013 International Conference on Chemical, Agricultural and Medical Sciences (CAMS-2013) Dec. 29-30, Kuala Lumpur (Malaysia)
- Fateh, F. S. M. R. Kazmi, I. Ahmad, and M. Ashraf. 2006. *C. fimbriata* isolated from vascular bundles of declining mango trees in Sindh, Pakistan. Pak. J. Bot. 38(4): 1257-1259.
- Gillitzer P, A.C. Martin, M. Kantar, K.Kauppi, S. Dahlberg and D. Lis. 2012. Optimization of screening of native and naturalized plants from Minnesota for antimicrobial activity. J. Med. Plants Res. 6(6): 938-49.
- GoP. 2014-15. Fruit, vegetable and condiments statistics of Pakistan. Ministry of national food security &

- research. Economic wing, Islamabad. Pp10
- Gurjar, M. S., S. Ali, M. Akhtar, K. S. Singh. 2012. Efficacy of plant extracts in plant disease management. *Agri. Sci.* 3(3):425-433
- Hendrich, A. 2006. Flavonoid-membrane interactions: possible consequences for biological effects of some polyphenolic compounds. *Acta Pharmacologica Sinica* 27(1): 27–40.
- Jeffrey, B. and B. Herbert. 2003. Photochemical Dictionary. A hand book of bioactive compounds" 1st edition, Tavlror and Francis London Washington D.C. pp 234-245.
- Khuhro, R.D, S.M. Nizamani, Q.D. Abbasi, G.S. Solangi and M.M. Jiskani. 2005. Mango Tree Mortality due to Asian ambrosia beetle, *Xylosandrus crassiusculus* Mot. (Coleoptera: Scolytidae) Pak. J. Agri. Agril. Engg. Vet. Sc. 21(1): 39-42.
- Malik, M. T., C. A. Haq, A. G. Grewal, M. I. Khan and M. Nisar. 2001. An integrated approach to recover naturally infected mango plants with dieback (*Diplodia natalensis*). *J. Agric. Res.* 39: 51-58.
- Malik, M. T., S. M. Khan., A. A. Dasti and M. R. Kazmi. 2005. First record of *Ceratocystis manginecans* causal organism of mango sudden death in Pakistan. *Pak. J. Phytopathol.* 17 (2): 187-191.
- Malik, M.T., A. A. Dasti and S. M. Khan. 2005. Mango decline disorders prevailing in Pakistan. Proc. International conference on mango and date palm. culture and export. June 20-23, (Eds.): A.U. Malik, M.A. Pervez, K. Zia. University of Agriculture, Faisalabad, Pakistan.
- Malik, M.T., C.N. Akem, M. R. Kazmi and F.S. Fateh. 2011. Towards chemical management of mango sudden death disease in Pakistan. Int. conf. on "New Frontiers in Plant Pathology for Asia and Oceania, Darwin-Australia" 26-29 April [Abstract no 157 pp 1141].
- Mondali, N. K., A. Mojumdar, S. K. Chatterje, A. Banerjee, J. K. Datta, S. Gupta. 2009 Antifungal activities and chemical characterization of Neem leaf extracts on growth of some fungal species in vitro culture medium. *J. Appl. Sci. Envi. Manage.* 13(1): 49-53.
- Pennington, T. D. 1981. *Flora Neotropica*, New York Botanical Garden, NY, Monogr. No. 28.
- Sales, M. D. C., H.B. Costa, P. M. B. Fernandes, J. A. Ventura and D. D. Meira. 2016. Antifungal activity of plant extracts with potential to control plant pathogens in pineapple Asian Pac. *J. Trop. Biomed.* 6(1): 26–31
- Sharma, B., K. K. Srivastava, N. Verma, R. Niwas and M. Singh. 2014. Antifungal potential of leaf extract of *Datura stramonium* L., against some important plant pathogenic fungi. *Acta Biologica Indica* 3(2):659-662
- Shetty, S. A. H. S. Prakash and H. S. Shetty. 1989. Efficacy of certain plant extracts against seed-born infection of *Triconiella padwickii* in paddy (*Oryza sativa*). *Can. J. of Bot.* 67(7): 1956-1958.
- Shrivastava, D. K. and K. Swarnkar. 2014. Antifungal Activity of leaf extract of Neem (*Azadirachta Indica* Linn) Int. *J. Curr. Microbiol. App. Sci* (3(5): 305-308
- Sireesha, O. and N. Venkateswarlu. 2013. In-vitro evaluation of botanicals and panchagavya against leaf blast fungus *Pyricularia grisea*. *Asian J. Pharm. & Clin. Res.* 6(5):84-86.

- Suleiman, M. N. 2011. Antifungal properties of leaf extract of neem and tobacco on three fungal pathogens of tomato (*Lycopersicon Esculentum* Mill) Adv. Appl. Sci. Res.2 (4): 217-220
- Tagoe, D., S Baidoo, I Dadzie, V Kangah, H Nyarko. 2009. A Comparison Of The Antimicrobial (Antifungal) Properties Of Garlic, Ginger And Lime On *Aspergillus Flavus*, *Aspergillus Niger* And *Cladosporium Herbarum* Using Organic And Water Base Extraction Methods. The Internet Journal of Tropical Medicine. Volume 7 Number 1.
- Talibi, I., L. Askarne, H. Boubaker, E. H. Boudyach, F. Msanda and B. Saadi. 2012. Antifungal activity of some Moroccan plants against *Geotrichum candidum*, causal agent of postharvest citrus sour rot. Crop Prot. 35(1): 41-6.
- Tewari, S.N. and M. Nayek 1991. Activity of four plant leaf extracts against three fungal pathogens of rice. Trop. Agric. (Trinidad), 68: 373-375.
- Vijaya, H. K., S. Kulkarni, Y. R. Hegde. 2007. Evaluation of plant extracts against *Ceratocystis paradoxa* causing sett rot of sugarcane, Karn. J. Agric. Sci. 0(1): 168-169.
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