International Journal of Multidisciplinary Research and Development
Online ISSN: 2349-4182, Print ISSN: 2349-5979
Impact Factor: RJIF 5.72
Received: 10-02-2018; Accepted: 11-03-2018
www.allsubjectjournal.com
Volume 5; Issue 3; March 2018; Page No. 53-56

Evaluation of selected botanicals essential oil against black mold disease of Pomegranate (*Punica granatum)* caused by *Aspergillus niger*

Sayed Reza Sadat, Sobita Simon, Abhilasha A Lal
Department of Plant Pathology, SHIATS, Allahabad, Uttar Pradesh, India

Abstract
Black mold is an important storage disease of pomegranate caused by the fungus *Aspergillus niger*. The fungus *A. niger* is common as a saprophyte in soil and on decaying plant material. Survey of fungi associated with postharvest deterioration of pomegranate was conducted in three major markets in Allahabad district during January – February (2016). Rotten pomegranate obtained from the three selected markets *viz.* Mundera Mandi. Barana Mandi and Phulpur Manndi. *Aspergillus niger* was isolated from the infected pomegranate. Five essential oils *viz. Syzgium aromaticum*(Clove), *Eucalyptus globus* (Eucalyptus), *Azadirachta indica (*Neem*),Citrus lemon*(lemon),and *Mentha piperita* (Mint)were tested on *A. niger in vitro* condition. All the essential oils significantly inhibited the radial mycelia growth of the test pathogen (*A. niger*).The highest percentage growth inhibition was achieved with eucalyptus oil(100%) and clove oil (100%)essential oils against *A. niger* followed by Neem oil (22.66%), *citrus*(21.48), mint(17.4%). The present investigation showed that essential oils of the mention plants have antifungal effect on Pomegranate postharvest rot fungi.

Keywords: pomegranate, Postharvest disease, *Aspergillus niger* and botanicals oil

Introduction
The Pomegranate (*Punica granatum)* belongs to the family (*Punicaceae*) is an important fruit of tropical, sub-tropical and arid regions. And is believed to originate from the Middle East (Iran and adjoining countries) and spread to most tropical and subtropical countries of the world. It is extensively cultivated in Iran, Egypt, Pakistan, Spain, Morocco, Afghanistan, and India and in some place of Myanmar, China, Japan, California, South Italy and Bulgaria
(Mitra *et al.*, 1999) [13]. Pomegranate fruits are the good sources of carbohydrates and mineral such as Ca, Fe and S and a moderate source of pectin (Waskar, 2006) [20].
Pomegranates an ancient, mystical, unique fruit borne on a small, long-living tree cultivated throughout the Mediterranean region, as far north as the Himalayas, California and Arizona in the United States. In addition to its ancient historical uses, pomegranate is used in several systems of medicine for a variety of ailments. The synergistic action of the pomegranate constituents appears to be superior to that of single constituent. In the past decade, numerous studies on the antioxidant, anti-carcinogenic and anti-inflammatory properties of pomegranate constituents have been published, focusing on treatment and prevention of cancer, cardiovascular disease, diabetes, dental conditions, bacterial infections and antibiotic resistance, and ultraviolet radiation-induced skin damage. Other potential applications include infant brain ischemia, male infertility, Alzheimer's disease, arthritis, and obesity (Jurenka, 2014) [9].
The pomegranate has great antiquity and is known to have been cultivated in the Middle East, since more than 5000 years ago. It is found in various regions from Caucasus to Afghanistan. This fruit crop has attained commercial scale in Maharashtra state where majority of the farmers take Ambia bahar crop. As per Maharashtra State Agricultural Marketing Board, Maharashtra leads in pomegranate production in the country with 85per cent of total production. The area under cultivation of pomegranate in Maharashtra was around40, 970 ha with a production of 4, 09, 700tones in the year 2002-2003 (Anonymous, 2003a) [3].
In the last two decades, *A. niger* has been developed as an important transformation host to over-express food enzymes. Being pre-dated by older names, the name *A. niger* has been conserved for economical and information retrieval reasons and there is a taxonomical consensus based on molecular data that the only other common species closely related to *A. niger* in the *Aspergillus* series is *A. tubingensis. A. niger*, like other filamentous fungi, should be treated carefully to avoid the formation of spore dust. However, compared with other filamentous fungi, it does not stand out as a particular problem concerning allergy or mycopathology. A few medical cases, e.g. lung infections, have been reported but always in severely immune compromised patients. In tropical areas, ear infections (otomycosis) do occur due to *A. niger* invasion of the outer ear canal but this may be caused by mechanical damage of the skin barrier. *A. niger* strains produce a series of secondary metabolites, but it is only ochratoxin a that can be regarded as amycotoxin in the strict sense of the word. Only 3–10%of the strains examined for ochratoxin a production have tested positive under favorable conditions. New and unknown isolates should be checked for ochratoxin a production before they are developed as production organisms. It is concluded, with these restrictions, that *A.*

niger is a safe production organism.

Materials and Methods

Survey was carried out in three selected vegetable markets of Allahabad district *viz.* Mundera Mandi. Barana Mandi and Phulpur Manndi. In (January-February) 2016.Pomegranate showing symptoms of rot and discolorations were randomly collected from these selected markets. The Pomegranate samples were collected from various traders in the three selected markets by sorting and selecting the Pomegranate with symptoms of rot and discoloration. The infected Pomegranates were separated from the healthy ones. Samples were kept in a polythene bag and labeled separately and were taken to the laboratory, Department of Plant Pathology, Sam Higginbottom Institute of Agriculture, Technology & Sciences, Allahabad for further study. The percentage disease incidence (PDI) for each was determined by using the formula below (Abdulsalam *et al.*, 2015) [1].

$$\text{PDI} \quad \frac{\text{Number of infected Pomegranates}}{\text{Total number of Pomegranate}} \times 100$$

For all laboratory experiments, Borosil and Corning glass wares were used. The glass wares (Petri dishes, pipettes, conical flasks, test-tube, etc.) which were used in the experiment were thoroughly washed with detergent powder and air dried, then wrapped in clean paper and sterilized in hot air oven at 180^0 C for 3 hrs. PDA (Potato dextrose agar) medium was used to culture rot fungi of Pomegranate.

A small portion of the infected Pomegranates were transferred to PDA plates for microscopically examination. The fungal isolates were purified and identified, based on their cultural and morphological characteristics including shapes of spores or conidia (Gilman, 1957) [6]. The purified cultures were maintained on PDA medium for further studies. The frequency of isolation of fungal species was recorded. The percentage occurrence was calculated using the following formula:

$$\% \text{ Occurrence} \quad \frac{\text{Number of times a fungus was encountered}}{\text{Total fungal isolations}} \times 100$$

Extraction of oils from botanical extracts: (Soxhlet extraction)

Fresh leaves of *Eucalyptus globules*, *Syzygium aromaticum, Citrus aurantium,*

Azadirachta indica and *Mentha peperata* were obtained from the gardens of campus and surrounding area of the Sam Higginbottom Institute of Agriculture, Technology and Sciences, Allahabad. In this method the sample is dried, ground in the small particles and placed in a porous cellulose thimble. The thimble is placed in an extraction chamber, which is suspended above a flask containing the solvent and below a condenser. The flask is heated and the solvent evaporates and moves up into the condenser where it is converted in to a liquid that trickles in to the extraction chamber containing the sample. The extraction chamber is designed so that when the solvent surrounding the sample exceeds a certain level it overflows and trickles back down into the boiling flask. At the end of the extraction process.

Experimental design and Data analysis

A completely Randomized Design was used as describe by Gomez and Gomez (1984) [7]. The experiment was replicated 3 times and data obtained was analysis of variance (ANOVA). Per cent inhibition of mycelia growth will be calculated by using the formula given by Vincent (1947) [18].

$$\text{Per cent inhibition of colony} \quad \frac{\text{C-T}}{\text{C}} \times 100$$

Where

C = Colony diameter in control

T = Colony diameter in treatment

Results and Discussion

The results of present study entitled "Evaluation of selected botanicals essential oil against black mold disease of Pomegranate caused by *Aspergillus niger* was conducted in department of Plant Pathology, Sam Higginbottom Institute of Agriculture, Technology and Sciences, during 2015-2016. The effect of essential oils was studied in vitro. The present finding of the following tables and figure under appropriate headings.

Table 1: The effect of essential oil of botanicals on the mycelia growth (mm) of *A.niger*

S/N.	Treatments	Radial growth (mm) of *Aspergillus niger*					
		48 hrs.	72 hrs.	96 hrs.	120 hrs.	144 hrs.	168 hrs.
T_1	Clove oil	0.00	0.00	0.00	0.00	0.00	0.00
T_2	Eucalyptus oil	0.00	0.00	0.00	0.00	0.00	0.00
3	Neem oil	19.17	24.00	32.33	36.00	61.17	69.67
T_4	Citrus oil	19.33	24.00	33.67	36.67	64.17	70.67
T_5	Mint oil	18.17	24.33	35.50	41.33	70.17	74.67
T_0	Control (control)	23.00	27.50	38.17	48.17	79.83	90.00
F-test		S	S	S	S	S	S
S.Em.		0.71	0.37	0.56	0.65	0.46	0.20
CD (5%)		2.20	1.16	1.74	2.02	1.42	0.62

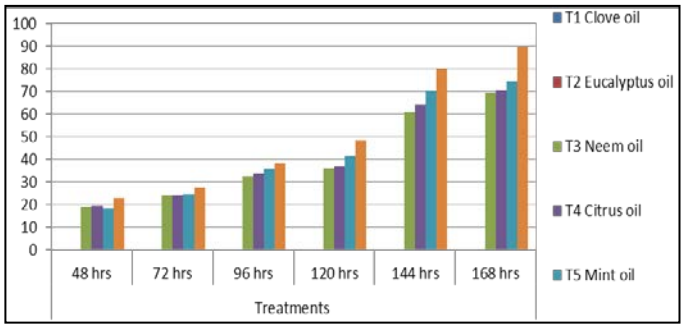

Fig 1: the effect of essential oil of botanicals on the mycelia growth of *A.niger*

Table 1: After 48 hours of incubation in B.O.D incubator at the temperature 28± 2^0c the radial mycelia growth of *A.niger* of pomegranate was found inhibited in T_1 clove oil (0.00 mm) and T_2 eucalyptus (0.00 mm) as compared to other treatments including control. The treatment T_5 mint oil (18.17mm), T_3 Neem oil (19.17 mm) and T_4 citrus oil(19.33 mm) were not

significantly differ the radial growth of *A.niger*, but they are significantly reduce the growth of *A.niger* as compared to control T_0 (23.00 mm).

Total inhibition of the radial mycelia growth of *A. niger* was observed after 72 hours in T_1 and T_2 (0.00 mm). In T_3 and T_4 (24.00mm) and T_4Mint oil (24.33 mm) was observed, however they are not significant among each other, but significantly reduce the growth of *A.niger* as compared to control T_0 (27.50 mm).

After 96, and 120 hours inhibition of rot pathogen of pomegranate was observed in T_1 clove oil (0.00 mm), T_2 eucalyptus oil (0.00) after incubation. At 96 hours after incubation the treatment T_3 (Neem oil (32.33 mm), T_4 citrus oil (33.67 mm) significantly reduced the radial growth of *A.niger* from T_5 mint oil (35.50 mm) and control T_0 (42.67 mm), whereas T_4 and T_3 are not significant among each other. The treatment mint oil significantly reduced the radial growth from control.

At 120 hours after incubation the treatment T_3 (Neem oil (36.00 mm) T_4 citrus oil (36.67mm) significantly reduced the radial growth of from T_5 mint oil (41.33mm) and control T_0 (48.17 mm), whereas T_4 and T_3 are not significant among each other. The treatment mint oil significantly reduced the radial growth of *A.niger* from control.

The radial mycelia growth of *A. niger* after 144 and 168 hours where total inhibition were observed and found in T_1 clove oil and T_2 eucalyptus oil (0.00 mm) as compared to T_3 Neem oil (69.67 mm, 69.67 mm), T_4 citrus oil (64.17 mm, 70.67 mm) and T_5 mint oil (70.17 mm, 64.70 mm) including control T_0 (69.83 mm, 90.00 mm).

Table 2: The effect of essential oil of botanicals on per cent inhibition of*A.niger* after 7 days of incubation (*in vitro)*

S/N.	Treatments	Concentration (%)	Mycelia inhibition (%)
T_1	Clove oil	3%	100.0
T_2	Eucalyptus oil	3 %	100.0
T_3	Neem oil	3 %	22.66
T_4	Citrus oil	3 %	21.48
T_5	Mint oil	3 %	17.04
T_0	Control (control)	--	00.00
F – test		--	S
S. Ed. (±)		--	0.20
C. D. (P = 0.05)		--	0.62

The effect of various plants essential oils using petroleum ether solvent on the radial mycelia growth of rot pathogen *A.niger* inhibition percentage is presented in (Table 2).The essential oils of clove and eucalyptus inhibited 100% percent the radial mycelia growth of the test pathogen at 168 hours after incubation in B.O.D The remaining treatments T_3, Neem oil (22.66 %), T4 citrus oil (21.48 %) and T_5, mint oil (17.04%) inhibited greater growth as compared to control (0%).

Table 3: Incidence of postharvest diseases % of pomegranate in three selected markets in Allahabad district during the dry season

Markets	Black mold
Mundera	20.0
Barna	15.0
Phulpur	10.0
Mean	15.0

In the survey during January-February (2016) of the markets to determine the incidence of postharvest disease of pomegranate, black mold disease was encountered. All the markets recorded very high incidence of the postharvest disease. The predominant disease during the survey was black mold. (Table 3).

Table 4: Percentage occurrence of isolated fungi associated with storage rots of pomegranate from the selected markets.

Fungus	Frequency of isolation from rotten pomegranate (%)
Aspergillus niger	60.0

A total 60% *Aspergillus niger*. Was isolated from the infected pomegranate collected from the selected markets (Table 4).

The results of *(*Hyldgaard *et al.*, 2012) [8] showed that the essential oils of clove, eucalyptus, Neem, citrus lemon oil Mint oil exhibited antifungal activity against *Aspergillus niger. Our findings are similar with their* results. They stated that the essential oils are aromatic and volatile liquids extracted from plants. The chemicals in essential oils are secondary metabolites, which play an important role in plant defiance as they often possess antimicrobial properties the exploitation of the essential oils as such would be more economical than a single component as fungi toxicant. The efficacy of the oils in controlling the rotting of the fruits during *in vivo* conditions clearly indicates their practical applicability as storage fungi toxicants of plant origin (botanical pesticides) for fruits and enhancing their market life for appreciable period.

Various scientist views reported the oil samples from spring planted crops had significantly higher menthol and lower terpinen-4-ol concentrations than those from autumn planted crops. The oil samples showed a different degree of inhibition against the twenty-five microorganisms tested. Peppermint oil exhibited a marked antifungal activity against *A.niger* (Marotti *et al.*, 1994) [11]. Essential oils of spices and herbs (thyme, origanum, mint, cinnamon, salvia and clove) were found to possess the strongest antimicrobial properties among many tested (Kalemba and Kunicka, 2003) [10].

(Moghtader, 2013) [14] find out that the antifungal effects of *Mentha piperita* essential oil under investigation compared to synthetic menthol on *A.niger* exhibited strong synthetic menthol. The high percentage of antifungal activities of Mentha oil is related to menthol, the main organic compound. This essential oil can be used for antifungal activity and as natural compound. The effectiveness of the oil concentration depends on the target pathogen and effects of natural compounds on fungus.

(Yousef, 2013) [2] showed that volatiles from Lemongrass oil was strongly active against spore germination; spore germination of *A.niger* and *A.fumigatus.* Volatile aromatic plant components have been widely shown to exhibit greater antimicrobial activity than do non aromatic volatile oils.

Velazquez-Nunez *et al*. (2013) [17] studied antifungal activity of citrus essential oils. They reported the minimum inhibitory concentration for the growth of *A. flavus* by direct addition 16.000 mg.L-1, while for the vapor contact 8000 mg of EO mg.L-1 in air. For the both studied methods, growth of *A. flavus* decreased with increasing EO concentration. Further,

studies have also documented that eucalyptus and lemon essential oils are effective even against fungal strains in vapor contact, e.g.: *Aspergillums niger, A. flatus, Penicillium chrysogenum and P.verrucosum* (Viuda-Martos *et al.,* 2008) [19], *A. clavatus, A.niger*, etc. (Su *et al.,* 2006) [15].

Our report are in agreement with the above scientists, reports of mycelia run of *A. niger* in their result Antimicrobial activity of four essential oils from lemon (*Citrus lemon*), mint (*Mentha piperita*), and was against *Aspergillus niger*,. The results of this study confirm that essential oils from aromatic plants such as lemon, mint, and antifungal activity. The most effective against all tested strains was the mint oil (Ferdes and Ungureanu, 2012) [5].

In our result mint oil was found maximum growth of A.niger comparison with other essential oils. In a report, made by (Aqil *et al*., 2000) [4], the essential oils from peppermint (*Mentha* sp.); clove (*Syzgium aromaticum*) and eucalyptus (*Eucalyptus globus*) were evaluated for their antifungal activity against *Aspergillus niger*, by agar well diffusion method. Maximum antifungal activity was detected in essential oil of clove oil followed by peppermint and eucalyptus. In their studies, the antifungal activity of essential oils *Syzygium aromaticum* (Linn.) Merrill & Perry, Eucalyptus globulus Label. *Citrus aurantium* Linn. *C. lemon* (Linn.) and *Mentha piperita* Linn. The highest and broadest activity was shown by the essential oils of *S. aromaticum, C.limon, C. aurantium* and M. piperita as compared to standard drug, Ketoconazole. The 5 ppm concentration of essential oils of *S. aromaticum, C. lemon* and *M. piperita* completely inhibited the mycelia growth of *A. niger*.

References

1. Abdulsalam AA, Zakari BG, Chimbekujwo IB, Channya FK, Bristone B. Isolation and control of fungi associated with neck rot disease of Pomegranates (*Allium cepa* L.) in Bama, Borno State, Nigeria. Global Journal of Biology, Agriculture & Health Sciences. 2015; 4(4):35-39.
2. Al Yousef SA. Antifungal activity of volatiles from lemongrass (cymbopogon citratus) and fungal species. International Journal of Clinical Biochemistry and Research. 2013; 2(2):73-76.
3. Anonymous. Pristine® Fungicide. BASF Corporation. Research Triangle Park, NC, USA, 2003.
4. Aqil F, Beng AZ, Ahmad I. *In vitro* toxicity of plant essential oils against soil fungi. J. Med. Arom. Pl. Sci. 2000; 23:177-181.
5. Ferdes M, Ungureanu C. Antimicrobial activity of essential oils against four food-borne fungal strains. *U.P.B. Sci. Bull. Series B.* 2012; 74(2):1454-2331.
6. Gilman JC. A manual of soil fungi. Oxford and IBH Publishing Company. New Delhi. 1957; pp. 229-230
7. Gomez KA, Gomez AA. Statistical procedures for agricultural research. 2nd Edition, John Wiley and Sons. 1984; pp. 680.
8. Hyldgaard M, Mygind T, Meyer RL. Essential oils in food preservation: Mode of action, synergies, and interactions with food matrix components. Frontiers in Microbiology. 2012; 3(.12):1-24.
9. Jurenka J. Therapeutic applications of pomegranate (*Punica granatum* l.): a review. Journal of clinical therapeutic. 2014; 13(2):128-144.
10. Kalemba D, Kunicka A. Antibacterial and antifungal properties of essential oils. *Curr Med Chem.* 2003; 10:813-829.
11. Marotti M, Piccaglia R, Giovanelli E, Deans SG, Eaglesham E. Effects of planting time and mineral fertilization on peppermint (*mentha x piperita* l.) essential oil composition and its biological activity. Flavour Fragr. J. 1994; 9(3):125-129.
12. Microbiology, 3(12):1-24.
13. Mitra SK, Farooqi AA, Sadhu MK. Tropical Horticulture. Naya Prakashan, (KOLKATA) India. 1999; 1:338-343.
14. Moghtader M. *In vitro* antifungal effects of the essential oil of *Mentha piperita* L. and its comparison with synthetic menthol on *Aspergillus niger*. 2013; 7(11):521-527
15. Su YC, Ho CL, Wang EI, Chang ST. Antifungal activities and chemical compositions of essential oils from leaves of four eucalyptus. Taiwan Journal For. Sciences. 2006; 21:49-61
16. Vapor contact. Food Control, 31(1):1-4.
17. Velazquez-Nunez MJ, Avila-Sosa R, Palou E, Lopez-Malo A. Antifungal activity of orange (*Citrus sinensis*var. Valencia) peel essential oil applied by direct addition, 2013.
18. Vincent JM. Distortion of fungal hypae in the presence of certain inhibitors. Nature. 1947; 159:239-241.
19. Viuda-Martos M, Ruiz-Navajas Y, Fernández-López J, Perez-Alvarez J. Antibacterial activity of lemon (*Citrus lemon* L.), mandarin (*Citrus reticulata* L.), grapefruit (*Citrus paradisi* L.) and orange (*Citrus sinensis* L.) essential oils. Journal of Food Safety. 2008; 28(4):567-576.
20. Waskar DP. Pomegranate (*Punica granatum*). In Advances in Arid Horticulture, International Book Distributing Company, Luck now (U.P.) India. 2006; 2:375-394.