



Efficacy of some botanicals against the fungal rot of *Prunus amygdalinus* Batsch in Kashmir valley

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Abstract

The antimycotic efficacy of *Solanum nigrum* L., *Euphorbia helioscopia* L., *Fragaria ananassa* Duchesne ex Rozier, *Salix alba* L. and *Datura stromonium* L. were screened against the *Aspergillus niger* Van Tieghem, *Fusarium solani* (Mart) Sacc., *Alternaria alternata* (Fr.) Keissl. and *Trichothecium roseum* (Pers.) Link that cause rot nut diseases which degrades the quality and yield of nuts after harvest. It was observed from the results that higher concentrations of plant extracts caused significant inhibition in fungal pathogen mycelial growth. However, maximum inhibition was caused by the higher concentrations than lower concentrations. Plant extracts of *Euphorbia helioscopia* at higher concentrations caused more inhibition in the growth of nut rot pathogenic fungi followed by other plant extracts. The extracts from plants have been found to be antifungal in nature, thus gained popularity for developing eco-friendly integrated pest management.

Keywords: *Aspergillus Niger*, *Fusarium solani*, *Alternaria alternata*, *Trichothecium roseum*, antimycotic efficacy, plant extracts

Introduction

Prunus amygdalus Batsch is one of the most important nut crop cultivated in temperate regions of the world. In Kashmir, it mainly grows in Pulwama, Budgam, Shopian, Gandarbal districts and other hilly areas. The seeds are eaten fresh or as a dessert in confectionary. Almonds are most nutritious, cholesterol free food and its kernel contains fiber, vitamin E, fat, protein, potassium, magnesium, phosphorus, calcium and iron (Ahmad and Verma, 2009) [1]. The almond seeds also possessed medicinal properties, viz. anti-inflammation, immunity boosting, antihepatotoxicity, improved complexion, improved movement of food through colon and prevention of cancer (Davis and Iwahashi, 2001; Puri, 2003) [24, 15]. However, these almond nuts are susceptible to different rot diseases caused by fungi. These fungi are responsible for loss of fresh produce on a large scale (Mitcham and Mitchell, 2002) [7]. Rot disease not only degrades the fruit quality in the field and nuts after harvest but also reduces their yield. The pathogens which cause rot of almonds include *Aspergillus niger* Van Tieghem, *Fusarium solani* (Mart) Sacc., *Alternaria alternata* (Fr.) Keissl. and *Trichothecium roseum* (Pers.) Link which not only reduce the quality and market value of nuts but also consumption value of the affected nuts is reduced. The fungal rot of nuts has been controlled by spraying chemicals such as fungicides. Use of fungicides as control agents not only has harmful impact on environment but also gives rise to resistant strains of pathogens (Spotts and Cervantes 1986) [18]. The less harmful substances such as whole plant extracts are being used as alternative to fungicides (Jobling, 2000) [28]. The extracts from plants have been found to be antifungal in nature, thus gained popularity for developing eco-friendly integrated pest management. The different concentrations of five plant extracts like *Solanum nigrum* L., *Euphorbia helioscopia* L., *Fragaria ananassa* Duchesne ex Rozier, *Salix alba* L., and *Datura stromonium* L. were first time evaluated for their antifungal activities in the present study.

Materials and Methods

The diseased samples of *Prunus amygdalinus* Batsch were collected from different fields and markets in Kashmir valley to investigate the rot causing fungi. The samples were taken to the laboratory for pathological studies. A small portion of rotted tissue was taken aseptically from the fruit of *Prunus amygdalinus* and transferred to culture plates containing Potato Dextrose agar media to isolate the rot causing fungi. Pure culture of the isolated fungi was maintained by sub culturing the isolated pathogen in separate petri plates containing the same medium. The pathogens isolated were identified by cultural, morphological and reproductive characteristics following Koch's postulates (Gilman, 2008) [25]. Furthermore, during the present study some botanicals such as *Solanum nigrum* L., *Euphorbia helioscopia* L., *Fragaria ananassa* Duchesne ex Rozier, *Salix alba* L., and *Datura stromonium* L. were assessed for their antimycotic activity.

Preparation and evaluation of different plant extracts

Different concentrations of aqueous extracts of leaves of *Solanum nigrum* L., *Euphorbia helioscopia* L., *Fragaria ananassa* Duchesne ex Rozier., *Salix alba* L. and *Datura stromonium* L. were assessed against *Aspergillus niger* Van Tieghem, *Fusarium solani* (Mart) Sacc., *Alternaria alternata* (Fr.) Keissl. and *Trichothecium roseum* (Pers.) Link isolated from rotted *Prunus amygdalinus* fruit. Different concentrations of plant extracts were prepared by taking 200 g leaves of each plant. The fresh disease free leaves (about 200g) of plant species were thoroughly washed with double distilled H₂O and macerated with 200 ml sterile distilled water in a blender (Bhat and Sivaprakasan, 1994) [22]. The macerate was homogenized for 5 min filtered through double layered muslin cloth, then centrifuged at 4000 rpm at about 15 minutes, filtered through Whatmann No. 1 filter paper and was labeled and stored at 4°C in storage vials for further experimental use. The solution obtained was considered as standard and S,

S/2, S/5 and S/10 concentrations obtained were then assessed for their efficacy on rot fungi by food poisoning technique (Grover and Moore, 1962) [26]. Different concentrations of plant extracts prepared after adding adequate quantity of distilled water to standard concentration were mixed with potato dextrose agar medium and about 15 ml mixtures were poured into sterile Petri-plates. The Petri-plates were then allowed to solidify in laminar airflow chamber. After solidification, these Petri-plates were inoculated by placing 5mm mycelial discs of the individual fungus in the center of each plate. The discs were cut from the actively growing colonies. The 3 replicates maintained for each concentration were incubated at 25 ± 2 °C and observations were made after 7 days of incubation. PDA plates without aqueous extracts served as control. The percentage inhibition in fungal growth at different concentrations was calculated as follows:

$$\text{Percentage inhibition (\%)} = \frac{dc-dt}{dc} \times 100$$

Where dc=average diameter of fungal colony in control, and dt=average diameter of fungal colony.

Results

Mycoflora analysis revealed the presence of four fungal genera from the seeds of almond. The major fungal flora isolated from the almond seeds were *Aspergillus niger* Van Tieghem, *Fusarium solani* (Mart) Sacc., *Alternaria alternata* (Fr.) Keissl. and *Trichothecium roseum* (Pers.) Link. During the present study it was revealed that the antimycotic efficacy of some botanicals, viz. *Solanum nigrum* L., *Euphorbia helioscopia* L., *Fragaria ananassa* Duchesne ex Rozier., *Salix alba* L. and *Datura stromonium* L. showed significant variation in mycelial growth against almond rot fungi. However, plant extracts of *Euphorbia helioscopia* at higher concentrations caused more inhibition in the growth of nut rot pathogenic fungi followed by other plant extracts.

Effect of botanicals on the mycelial growth of *Aspergillus Niger* Van Tieghem.

Results revealed (Table1, Figure1) that plant extracts caused significant mycelial growth inhibition in *Aspergillus Niger* at different concentration. Plant extracts of *Euphorbia helioscopia* were found more effective at higher concentrations (S) with maximum mycelial growth inhibition (88.33%) followed by *Datura stromonium* (77.66%), *Solanum nigrum* (73.66%), *Fragaria ananassa* (72.66%), *Salix alba* (67.33%).

Table 1: Effect of different concentrations of botanicals on the mycelial growth of *Aspergillus Niger*

Plant extracts	Mycelial growth inhibition (%)			
	S	S/2	S/5	S/10
<i>Solanum nigrum</i>	73.66±1.52	54.66±2.51	49.66±1.52	45.33±0.57
<i>Euphorbia helioscopia</i>	88.33±2.08	62±2.00	51±1.00	43±1.00
<i>Fragaria ananassa</i>	72.66±1.52	59±1.00	57.33±2.08	54.33±1.52
<i>Salix alba</i>	67.33±2.08	65.33±0.57	55.33±2.08	45.33±1.52
<i>Datura stromonium</i>	77.66±2.51	69.66±2.08	62.33±1.52	52±2.00

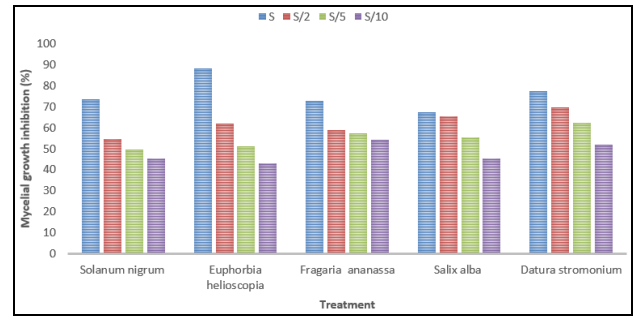


Fig 1: Effect of different concentrations of botanicals on mycelial growth of *Aspergillus niger*

Effect of botanicals on *Fusarium solani* (Mart) Sacc.

It was observed from the results (Table 2, Figure 2) that plant extracts at different concentration caused significant inhibition in the mycelial growth *Fusarium solani*. Plant extracts of *Euphorbia helioscopia* were found more effective at higher concentrations (S) with maximum mycelial growth inhibition (87%) followed by *Datura stromonium* (70.66%), *Salix alba* (60.66%), *Solanum nigrum* (53.66%), *Fragaria ananassa* (40.66%).

Table 2: Effect of different concentrations of botanicals on the mycelial growth of *Fusarium solani*

Plant extracts	Mycelial growth inhibition (%)			
	S	S/2	S/5	S/10
<i>Solanum nigrum</i>	53.66±1.52	50.00±2.00	41.66±1.52	37.33±2.08
<i>Euphorbia helioscopia</i>	87±1.00	76.66±1.52	71±1.00	63.66±1.52
<i>Fragaria ananassa</i>	40.66±0.57	37.66±1.15	25.00±2.00	20.33±0.57
<i>Salix alba</i>	60.66±1.15	48.00±1.00	44.66±1.15	41.66±1.52
<i>Datura stromonium</i>	70.66±1.15	67.00±2.00	61.33±1.15	54.66±1.52

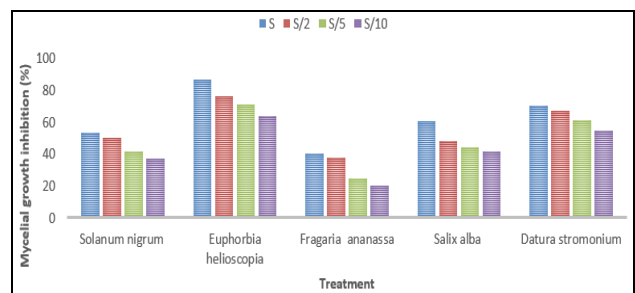


Fig 2: Effect of different concentrations of botanicals on mycelial growth of *Fusarium solani*

Effect of botanicals on the mycelial growth of *Alternaria alternata* (Fr.) Keissler

It was observed from the results (Table 3, Figure 3) that plant extracts at different concentration caused significant inhibition in the mycelial growth *Alternaria alternata*. Plant extracts of *Euphorbia helioscopia* were found more effective at higher concentrations (S) with maximum mycelial growth inhibition (77.33%) followed by *Fragaria ananassa* (76.66%), *Solanum nigrum* (63.33%), *Datura stromonium* (57%), *Salix alba* (45.66%).

Table 3: Effect of different concentrations of botanicals on the mycelial growth of *Alternaria alternata*

Plant extracts	Mycelial growth inhibition (%)			
	S	S/2	S/5	S/10
<i>Solanum nigrum</i>	63.33±1.52	61.66±0.57	59.00±1.00	40.00±1.00
<i>Euphorbia helioscopia</i>	77.33±2.08	70.00±1.00	65.33±1.52	44.66±0.57
<i>Fragaria ananassa</i>	76.66±1.52	72.66±0.57	64.33±0.57	49.00±2.00
<i>Salix alba</i>	45.66±0.57	43.66±0.57	34.66±1.52	22.66±1.15
<i>Datura stromonium</i>	57.00±2.00	55.33±0.57	43.66±0.57	34.00±2.00

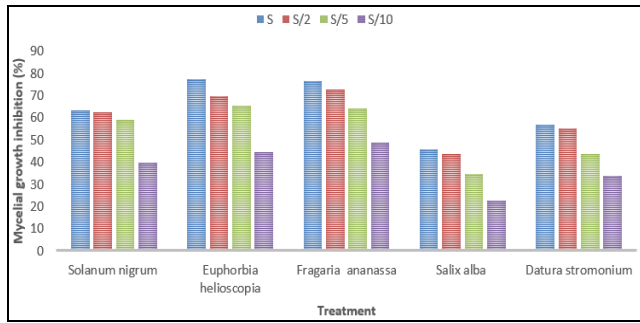


Fig 3: Effect of different concentrations of botanicals on mycelial growth of *Alternaria alternata*

Effect of botanicals on the mycelial growth of *Trichothecium roseum* (Pers.) Link

It was observed from the results (Table 4, Figure 4) that plant extracts at different concentration caused significant inhibition in the mycelial growth *Trichothecium roseum*. Plant extracts of *Fragaria ananassa* were found more effective at higher concentrations i.e. S with maximum mycelial growth inhibition (83%) followed by *Euphorbia helioscopia* (77%), *Solanum nigrum* (73.33%), *Datura stromonium* (60.66%), *Salix alba* (49.66%).

Table 4: Effect of different concentrations of botanicals on the mycelial growth of *Trichothecium roseum*

Plant extracts	Mycelial growth inhibition (%)			
	S	S/2	S/5	S/10
<i>Solanum nigrum</i>	73.33±1.52	68.33±0.57	62.66±0.57	51.00±1.00
<i>Euphorbia helioscopia</i>	77.00±1.00	72.66±1.15	70.00±0.57	53.33±1.52
<i>Fragaria ananassa</i>	83.00±1.73	76.00±1.00	68.33±0.57	57.33±0.57
<i>Salix alba</i>	49.66±1.54	47.00±2.00	39.33±0.57	28.33±1.15
<i>Datura stromonium</i>	60.66±1.15	59.00±1.00	47.00±2.00	43±1.73

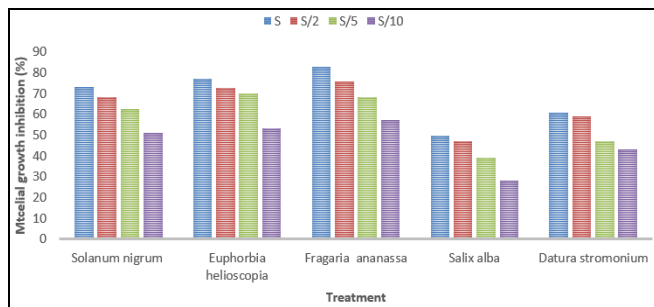


Fig 4: Effect of different concentrations of botanicals on mycelial growth of *Trichothecium roseum*

Discussion

The present study revealed the presence of four fungal genera from the seeds of almond. The major fungal flora isolated from the almond seeds were *Aspergillus niger*, *Fusarium solani*, *Alternaria alternata* and *Trichothecium roseum*. Presence of *Aspergillus niger*, *Aspergillus flavus*, *Fusarium nivale*, *Mucor mucedo* and *Penicillium expansum* was recorded by (Pandey and Sing 2014) [9]. Similar results were reported by (Karande et al. 2007; Patil et al. 2014) [2, 14]. It was also obvious from the results that all the tested plant extracts significantly inhibited fungal pathogen mycelial growth. Also, higher concentrations proved more effective than lower concentrations. Several similar studies reported that plant extract play a role for the control of phytopathogenic fungi (Mares et al. 2004; Khalil et al.

2005; Nisa et al. 2010, Znini et al. 2011; Raji and Raveendran, 2013; Maria et al. 2016; Parveen et al. 2016, 2017) [5, 3, 19, 21, 16, 6, 11, 16]. Maria et al. (2016) [6] reported that *M. balsamum*, *P. heptaphyllum*, *G. glabra*, *R. mangle*, *A. sativum*, and *A. vera* showed significant antimycotic activity against *C. paradoxa* and *F. guttiforme*. However, Satish et al. (2007) reported the antimycotic efficacy of aqueous extract of *Punica granatum*, *Lawsonia inermis*, *Emblica officinalis*, *Syigium cumini*, *Datura stromonium*, *Achras zapota*, *Mimusops elengi*, *Peltophorum pterocarpum*, *Polyalthia longifolia*, *Prosopis juliflora*, *Acacia nilotica* and *Eucalyptus globules* against tested *Aspergillus* species. Similarly, Tatiana et al. (2015) [20] reported the antimycotic activity of extracts of *Hyssopus officinalis*, *Satureja hortensis*, *Allium sativum*, *Tagetes patula* and *Mentha* against *Botrytis cinerea*. Paola et al. (2011) [10] reported that three medicinal plants, viz. *Salvia sclarea*, *S. officinalis* and *R. officinalis* showed the antimycotic activity against *Alternaria* spp. Kumar and Kumar (2015) reported the antifungal activity of *Solanum indicum*, *Azadirachta indica*, *Oxalis latifolia* against *Fusarium oxysporum*. Buch and Arya (2015) [23] reported the antifungal activity of plant extracts *Annona reticulate*, *Balanites roxburghii*, *Cochlospermum religiosa*, *Gliricidia sepium*, *Limonia acidissima*, *Sapindus emarginatus*, *Tephrosia jamnagarensis* against *Fusarium oxysporum*, *Alternaria alternata* and *Chaetomium globosum*. Gwa et al. 2018 [27] Studied the antifungal activity of *Zingiber officinale* Rosc., *Piper nigrum* Linn., *Azadirachta indica* A. Juss., *Nicotiana tabacum* Linn. and *Carica papaya* Lam. medicinal plants against *Penicillium expansum* causative agent of yam tuber rot. It was revealed from the results *P. nigrum* was the most effective in inhibiting the growth of pathogen followed by *Z. officinale*. However, *C. papaya* was least effective plant extract in inhibiting the growth of the pathogen. Furthermore, higher concentrations proved more effective than the lower concentrations. Olatinwo et al. (2019) [8] reported the antifungal potential of fungal endophyte *Acaromyces ingoldii* and evaluated its inhibitory properties against *Gloeophyllum trabeum* and *Trametes versicolor* mycelial growth. Their results showed significant inhibition on these rot causing fungi and also showed that increased concentration of *Acaromyces ingoldii* decreased the mycelial growth of both fungi. Koka et al. 2019 [4] studied the antifungal activity of aqueous extracts of *Ajuga bracteosa*, *Taraxacum officinale*, *Mentha arvensis* and *Iris kashmiriana* against *Trichothecium roseum* causing decaying of eggplant on inhibition of spore germination and mycelial growth.

Conclusion

It is concluded from the present investigation that all the plant extracts at higher concentrations caused significant inhibition of fungal pathogens causing rot nut diseases. However, *Euphorbia helioscopia* at higher concentrations caused potent antimycotic activity against nut rot pathogenic fungi followed by other plant extracts. Therefore, the extracts from plants have been found to be antifungal in nature, thus gained popularity for developing eco-friendly and effective therapeutic drug against fungal infection.

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Conflict of Interests

The authors have nothing to declare.

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