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RESEARCH ARTICLE

Biology and Management of Aspergillus niger Van Tiegh. causing black mold rot of pear (Pyrus communis L.) in Kashmir Valley, India

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Abstract

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..... Pears are highly perishable fruits and are attacked by number of fungal pathogens causing various rot diseases. The threat of these diseases is influenced by the way these horticultural crops are handled and stored. The fungal rot diseases cause heavy losses to the fruits in storage, transit as well as in fields. Therefore, present study was carried out to study the incidence of fungal rot of pear. It was revealed from the study that pear fruits are attacked by Aspergillus niger Van Tiegh. causing black mold rot of pear. Study was also undertaken for the management of black mold rot of pear with some fungicides and plant extracts. It was revealed from the study that different concentration of fungicides brought about significant reduction in the mycelial growth and spore germination of Aspergillus niger under in vitro conditions. Amongst the tested fungicides, hexaconozole proved highly effective in inhibiting the mycelial growth and spore germination of Aspergillus niger followed by carbendazim, bitertanol and myclobutanil respectively. Higher concentration proved effective than lower concentrations. Amongst the plant extracts, Artemisia absinthium at highest concentration was found most effective against A. niger and cause highest inhibition in the mycelial growth and spore germination followed by R. obtusifolius, M. sylvestris, P. lanceolata and T. officinale at the same concentration. Other concentrations of plant extracts also bought about significant reduction in mycelial growth and spore germination of the test fungus but to a lesser extent.

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Introduction

Pear (*Pyrus communis* L.) belong to family Rosaceae, stands second in ranking after apple as the most important tree fruit grown allover the world. Pear is grown under temperate and subtropical conditions because of its wide climatic and soil adaptability. In India pear is primarily grown in Jammu and Kashmir, Himachal Pradesh, Uttaranchal, Punjab, Western Utter Pradesh and Arunachal Pradesh. In India total area under this crop is estimated to be only 38,600 hectares with a total production of 176,000 tonnes and a productivity of 4.6 t/ha (Ravindran et al., 2007). In Jammu and Kashmir, pear ranks second after apple with an annual production of 49.8 thousand metric tonnes during the year 2011-2012 (Rather et al., 2013). The rot fungi are known to cause huge losses to pear and other fruits during harvest and consumption in developed and developing countries (Parpia, 1976; Bashir et al., 2012).

Pear fruits are excellent source of carbohydrates, sugars, dietary fiber and a good source of vitamin C (Blattny, 2003). Many organic acids have been found in pear fruits that include malic acid, citric acid, quinic acid, α -

ketoglutaric acid, succinic acid, lactic acid, glycolic acid, shikimic acid, glyceric acid and mucic acid (Doyon et al., 1991; Hudina and Stampar, 2000; Colaric et al., 2005). Pear fruits are known to have pharmacological properties like anti-inflammatory, anti-tumour, antiallergic, etc. due to little amount of salicylates, benzoates present in the fruit (Macheix et al., 1990). Considering their medicinal importance, perishable nature and extent of losses caused by fungal rots to pears, an attempt was made to study the fungi causing decay of pear fruits in Kashmir Valley.

Materials and methods

To investigate the fungi which cause the rotting of pear fruits in Kashmir Valley, diseased pear fruits were collected in separate polythene bags from different fields, markets, godowns and storage houses of Kashmir valley. These samples were either used immediately or stored at 10° C in the laboratory for different pathological studies. Small portions of rotted tissues were isolated aseptically from the diseased pear fruits and transferred to Potato Dextrose Agar (PDA) medium. Pure colony cultures were obtained by sub-culturing the fungal growth in separate Petri plates containing the same medium. The pathogen was identified by their morphological, reproductive and cultural characteristics (Ellis, 1971; Barnett and Hunter, 1972; Watanabe, 2002; Gilman, 2008). For pathogenicity, pathogens were re-inoculated after isolation onto the healthy pear fruits (Tomkin and Trout, 1931). Then all the fruits were kept in clean polythene bags and incubated at $25\pm2^{\circ}$ C for ten days. These pathogenicity tests were used for the identification of plant pathogens and to confirm the detection of a particular disease. Identification of the disease and the pathogen was done following Koch's postulates. Different parameters such as symptoms caused by these fungi on the healthy pear fruits, cultural characteristics of the pathogens and microscopic studies of the pathogens were studied.

In the present study an attempt was made to study the effect of some selected fungicides and plant extracts under *in vitro* conditions for the control of black rot of pears caused by *Aspergillus niger*.

Preparation and evaluation of different concentrations of fungicides

Different concentrations such as 1000ppm, 800ppm, 600ppm, 400ppm and 200ppm of fungicides like carbendazim, hexaconozole, bitertanol and myclobutanil were prepared in sterilized distilled water. These different concentrations of fungicides were evaluated for their effect on mycelial growth of rot causing fungi, *Aspergillus niger* by food poisoning technique (Adams and Wong, 1991). Appropriate concentration (1ml) of fungicide solution was mixed with autoclaved and cooled PDA (9ml) just before pouring into Petri plates. The medium was then dispensed uniformly into 90 mm diameter Petri plates and inoculated with 5mm mycelial disc of the pathogen from 10 day old fungal culture. Three replicates were maintained for each concentration including control without any treatment. The Petri plates were incubated at $25\pm2^{\circ}$ C and observations of the mycelial growth of test fungus were recorded after seven days of incubation. The percent inhibition in mycelial growth due to various fungicidal treatments at different concentrations was computed as follows:

Mycelial growth inhibition (%) = $\frac{dc - dt}{dt} \times 100$

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

For studying the effect of fungicides on spore germination, spore suspension was prepared in sterilized distilled water. 0.5ml of spore suspension was mixed to 0.5ml of the fungicides of different concentrations in a test tube and then shaken. The mixture then contained the particular concentration of test fungicide. In case of control 0.5ml of spore suspension was mixed with equal volume of distilled water. A drop of the mixture (about 0.1ml) was then placed in the cavity slide and these were incubated for $25\pm2^{\circ}$ C in a moist chamber created in 100mm Petri plates by covering both sides of the Petri plate with moist filter paper to maintain enough humidity. Three replicates were maintained for each treatment including the control. The slides were examined after 24hrs by hand tally counts at different microscopic fields. Percent spore germination of each treatment was calculated by the formula given by Kiraly et al. (1974).

Percent spore germination =
$$\frac{\text{No.of spores germinated}}{\text{Total no.of spores examined}} \times 100$$

Preparation and evaluation of different concentrations of splant extracts

Different concentrations of aqueous extracts of leaves Artemisia absinthium L., Rumex obtusifolius L., Taraxacum officinale Weber ex Wiggers, Plantago lanceolata L. and Malva sylvestris L. were evaluated for their effect on the mycelial growth of A. niger isolated from decayed pear fruits. For the preparation of different concentrations of plant extracts, 200g leaves of all the plants were washed with sterilized distilled water, grinded in mortar and pestle using 200ml of sterilized distilled water (Bhat and Sivaprakasan, 1994). The material was homogenized for 5 minutes and filtered through double layered muslin cloth followed by Whattman's filter paper No. 1. The filtrate was then centrifuged at 5000 rpm for 10min and was considered as standard solutions (S). Then other concentrations such as S/2, S/10, and S/100 were obtained by adding appropriate amount of sterilized distilled water to the standard concentration. These concentrations were evaluated for their effect on the mycelial growth by food poisoning technique (Adams and Wong, 1991). 1ml from each concentration of the plant extract was mixed with 9ml of autoclaved and cooled PDA just before pouring into Petri plates. The medium was then dispensed uniformly into 90mm sterile Petri plates and then inoculated with 5mm mycelial disc of the pathogen from 10 day old fungal culture. Three replicates were maintained for each concentration including the control without any treatment. The Petri plates were incubated at $25\pm2^{\circ}$ C and observations of the mycelial growth of test fungus were recorded after seven days of incubation. The percent inhibition in growth due to various treatments at different concentrations was computed as follows:

Mycelial growth inhibition (%) = $\frac{dc - dt}{dt} \times 100$

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

These plant extracts were also evaluated for their effect on the spore germination of *A. niger*. Spore suspension was prepared from 10 days old fungal culture. A drop about 0.1ml of spore suspension was then placed in a cavity glass slide containing a drop (about 0.1 ml) of different concentration of plant extract and then incubated at $25\pm2^{\circ}$ C for 24 hours in a moist chamber created in 100mm Petri plates by covering both sides of the Petri plates with moist filter paper to maintain enough humidity. Three replicates were maintained for each treatment including the control. The slides were examined after 24hrs by hand tally methods at different microscopic fields. Percent spore germination for each was recorded using formula given by Kiraly et al. (1974).

Percent spore germination = $\frac{\text{No.of spores germinated}}{\text{Total no.of spore s examined}} \times 100$

Results and Observations

It was observed from the present study that pears in storage are infected by the fungus, *Aspergillus niger* Van Tiegh. resulting in black mold rot of pears. The casual pathogen was identified on the basis of symptoms caused by the fungus on pear fruits, cultural and microscopic characteristics. The infected fruits produce soft watery rot. The surface of the fruits becomes covered with black conidial heads of the pathogen. The growth of the fungus on the surface is very less. The epidermis showed cracks through which fungus comes out in small white tufts that later on due to formation of spores turns black (Fig. 1). The causal agent was isolated from the diseased fruits and cultured on Potato Dextrose Agar (PDA) medium. After 48 hours of inoculation at $24\pm2^{\circ}$ C, the fungus produced white colonies and then due to conidial production the colonies turn black in colour (Fig. 2). Microscopic studies of the fungus revealed that mycelium is septate and branched. Conidiophores arising from the mycelium are 400 μ m-1270 μ m x 13 μ m-17 μ m in diameter, swollen at the tips giving rise to vesicles which are 50 μ m-78 μ m in diameter. On vesicles sterile cells called metulae are formed, metulae support the conidiogenous cells called phialides that form conidia. Conidia are spherical, oval or globose and 3.0 μ m-4.5 μ m in diameter (Fig. 3).



Fig 1. Infected pear fruit, Fig 2. Culture of *Aspergillus niger* on PDA, Fig 3. *A. niger*: Conidiophore with conidia (100x).

Control of Aspergillus niger causing black mold rot of pear with fungicides and plant extracts

The present study was carried out to evaluate the effect of some fungicides and plant extracts on *Aspergillus niger* causing black mold rot of pear. Different concentrations of fungicides and plant extracts were evaluated for their effect on the mycelial growth and spore germination of the test fungi.

Effect of different concentrations of fungicides on the mycelial growth of Aspergillus niger

It was revealed from the results (Table 1, Fig. 4) that all the fungicides, viz. carbendazim, hexaconozole, bitertanol and myclobutanil at different concentrations (1000ppm, 800ppm, 600ppm, 400ppm and 200ppm) brought about significant inhibition in the mycelial growth of *Aspergillus niger* as compared to control. However, the most effective fungicide in inhibiting the mycelial growth of *Aspergillus niger* was hexaconozole. Hexaconozole and carbendazim at highest concentrations brought about maximum inhibition in mycelial growth (100%), followed by bitertanol (84.18%) and myclobutanil (78.11%) at the same concentration. Other concentrations also caused significant inhibition in mycelial growth but to a lesser extent. In different concentrations of hexaconozole the inhibition in mycelial growth varies from 100% - 65.50% and in carbendazim it varies from 100% - 49.79%. Likewise, the inhibition in mycelial growth varies from 84.18% - 29.20% in bitertanol and in myclobutanil the inhibition varies from 78.11% - 14.06% in different concentrations of the fungicides.

Concentration	Mycelial growth (mm)					
Treatment	200ppm	400ppm	600ppm	800ppm	1000ppm	Control
Carbendazim	32.27±0.64* (49.79)	16.03±0.45 (75.06)	7.80±0.53 (87.86)	1.33±1.15 (97.93)	0.00±0.00 (100)	64.27±1.10
Hexaconozole	22.17±0.76 (65.50)	10.93±0.90 (82.99)	5.03±1.05 (92.17)	0.00±0.00 (100)	0.00±0.00 (100)	64.27±1.10
Bitertanol	45.50±0.62 (29.20)	23.77±0.93 (63.01)	17.80±0.72 (72.30)	12.60±0.53 (80.39)	10.17±0.76 (84.18)	64.27±1.10
Myclobutanil	55.23±1.10 (14.06)	29.37±1.30 (54.30)	23.30±0.65 (63.75)	17.60±0.53 (72.61)	14.07±1.10 (78.11)	64.27±1.10

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*Each value is mean of 3 replicates \pm SD

Figures in parenthesis is the mycelial growth inhibition (%)



Fig 4: Effect of different concentrations of fungicides on the mycelial growth of Aspergillus niger

Effect of different concentrations of fungicides on the spore germination of Aspergillus niger

It was revealed from the results (Table 2, Fig. 5) that all the fungicides, viz. carbendazim, hexaconozole, bitertanol and myclobutanil at different concentrations, viz. 1000ppm, 800ppm, 600ppm, 400ppm and 200ppm caused significant reduction in the spore germination of *Aspergillus niger* as compared to control. However, the maximum inhibition in spore germination was brought about by hexaconozole at highest concentrations (1000ppm) followed by carbendazim, bitertanol and myclobutanil at the same concentration. The other concentrations also brought about significant reduction in spore germination but to a lesser extent. In hexaconozole the inhibition in spore germination varies from 48.43% - 11.27% in different concentrations. In carbendazim, the reduction in the spore germination varies from 50.81% - 15.14% and in bitertanol it varies from 57.80% - 19.66% respectively in different concentrations. Likewise, the reduction in the spore germination varies from 61.22% - 23.36% in different concentrations.

Concentration								
	Spore germination (%)							
Treatment	200ppm 400ppm 600ppm 800ppm 1000ppm Control							
Carbendazim	50.81±1.15*	32.82±1.00	27.00±1.15	24.19±1.00	15.14±0.58	86.15±0.58		
Hexaconozole	48.43±1.53	31.35±1.00	26.21±0.58	19.68±0.58	11.27±0.58	86.15±0.58		
Bitertanol	57.80±0.58	48.50±0.58	43.74±1.15	27.89±0.58	19.67±0.00	86.15±0.58		
Myclobutanil	61.22±1.53	55.22±0.58	44.11±1.00	33.82±1.15	23.36±0.58	86.15±0.58		

 Table 2: Effect of different concentrations of fungicides on the spore germination of Aspergillus niger

*Each value represents the mean spore germination % age of 3 replicates \pm SD



Fig 5: Effect of different concentrations of fungicides on the spore germination of Aspergillus niger

Effect of different concentrations of plant extracts on the mycelial growth of Aspergillus niger

It was revealed from the results (Table 3, Fig. 6) that different concentration of plant extracts, viz. Artemisia absinthium L., Rumex obtusifolius L., Taraxacum officinale Weber ex Wiggers, Plantago lanceolata L. and Malva sylvestris L. caused significant inhibition in the mycelial growth of Aspergillus niger as compared to control. However, the maximum inhibition in mycelial growth was found at highest concentration 'S' followed by lower concentrations S/2, S/10 and S/100 of the plant extracts. Among the plant extracts used A. absinthium at highest concentration 'S' was found most effective against A. niger and cause highest inhibition in the mycelial growth (61.83%) followed by R. obtusifolius (58.09%), M. sylvestris (55.73%), P. lanceolata (49.46%) and T. officinale (44.88%) at the same concentration. Other concentrations of plant extracts also bought about significant reduction in the mycelial growth ranges from 61.83% - 22.90% and in R. obtusifolius the inhibition ranges from 58.09% - 14.97% respectively. Likewise, the inhibition in mycelial growth in different concentrations of M. sylvestris ranges from 55.73% - 11.22% and in P. lanceolata the inhibition ranges from 49.46% - 8.47% respectively. Whereas, the inhibition in mycelial growth ranges from 44.88% - 3.07% in different concentrations of T. officinale.

Concentration	Mycelial growth (mm)					
Treatment	S	S/2	S/10	S/100	Control	
A. absinthium	16.67±0.42* (61.83)	22.43±0.55 (48.93)	28.00±0.20 (35.88)	33.67±0.61 (22.90)	43.67±1.60	
R. obtusifolius	18.30±1.04 (58.09)	25.03±0.86 (42.68)	30.23±0.58 (30.78)	37.13±1.10 (14.97)	43.67±1.60	
T. officinale	24.03±0.85 (44.88)	31.83±1.62 (27.11)	38.40±1.06 (12.07)	42.33±0.95 (3.07)	43.67±1.60	
P. lanceolata	22.07±0.66 (49.46)	28.37±0.66 (35.03)	36.60±0.56 (16.25)	39.97±0.95 (8.47)	43.67±1.60	
M. sylvestris	19.33±1.16 (55.73)	25.47±0.70 (41.68)	31.90±1.95 (25.42)	38.77±0.25 (11.22)	43.67±1.60	

Table 3: Effect of different concentrations of plant extracts on the mycelial growth of Aspergillus niger

*Each value is mean of 3 replicates \pm SD

Figures in parenthesis is the mycelial growth inhibition (%)



Fig 6: Effect of different concentrations of plant extracts on the mycelial growth of Aspergillus niger

Effect of different concentrations of plant extracts on the spore germination of Aspergillus niger

It was revealed from the results (Table 4, Fig. 7) that different concentrations (S, S/2, S/10 and S/100) of plant extracts, viz. Artemisia absinthium L., Rumex obtusifolius L., Taraxacum officinale Weber ex Wiggers, Plantago lanceolata L. and Malva sylvestris L. caused significant reduction in spore germination of A. niger compared to control. Among the plant extracts used, A. absinthium at highest concentration (S) was found most effective and caused highest reduction in spore germination followed by R. obtusifolius, M. sylvestris, P. lanceolata and T. officinale respectively at the same concentration. The other concentrations of plant extracts also bought about significant reduction in spore germination but to a lesser extent. In A. absinthium, the inhibition in spore germination varies from 70.66% - 30.00% in different concentrations. In different concentrations of R. obtusifolius the inhibition varies from 77.34% - 39.34% and in M. sylvestris the inhibition varies from 80.66% - 43.34% respectively. Likewise, the inhibition in the spore germination varies from 85.34% - 47.34% and from 88.66% - 50.66% in different concentrations of P. lanceolata and T. officinale respectively

Concentration	Spore germination (%)					
Treatment	S	S/2	S/10	S/100	Control	
A. absinthium	30.00±1.00*	43.34±1.53	55.34±1.53	70.66±1.53	90.00±3.00	
R. obtusifolius	39.34±1.53	47.34±1.53	63.34±1.53	77.34±1.53	90.00±2.00	
T. officinale	50.66±1.53	66.66±1.53	82.00±1.00	88.66±0.58	90.66±0.58	
P. lanceolata	47.34±2.08	54.66±0.58	75.34±1.53	85.34±0.58	96.66±1.53	
M. sylvestris	43.34±1.53	51.34±0.58	67.34±0.58	80.66±1.53	91.34±3.05	

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*Each value represents the mean spore germination % age of 3 replicates \pm SD





Discussion

It was clear from the results that pear fruits are attacked by Aspergillus niger Van Tiegh and is responsible for black mold rot of pear fruits. Such studies on fungal rot of pears have been carried out for the first time in Kashmir Valley. However, some study has been carried out on the fungal rot of pear in India and all over the world. The occurrence of fungal rot of pears and other fruits due to various fungi have been reported allover the world by several workers (Sugar and Spotts, 1992; Mari et al., 2000; Lennox et al., 2004; Xiao and Boal, 2004; Spotts and Castagnoli, 2010). Aspergillus niger have been reported to cause fruit rot of cherry (Thomidis and Exadaktylou, 2012), Aspergillus rot of amla (Fatima et al., 2012), black mold rot of onion (Wani and Taskeen-Un-Nisa, 2011). In the present study some fungicides and plant extracts were evaluated for their antifungal activity against the fungus, Aspergillus niger. From the results it is clear that all the tested fungicides proved highly effective in reducing the mycelial growth and spore germination of Aspergillus niger. In the previous work, similar findings were reported by Imtiaj et al. (2005); Mondall et al. (2009); Rathod et al. (2010); Taskeen-Un-Nisa et al. (2011) and Schmidt-Heydt et al. (2013). Amini and Sidovich (2010) used six different fungicides, viz. benomyl, carbendazim, prochloraz, fludioxonil, bromuconazole and azoxystrobin aganist phytopathogenic fungi Fusarium oxysporum f. sp. lycopersici and reported that all the fungicides were found effective in reducing the disease caused by Fusarium oxysporum. Parveen et al. (2013) tested various chemical fungicides, systemic and non-systemic, against fruit rot pathogens, viz. Alternaria alternata and Mucor piriformis for the evaluation of inhibition of mycelial growth and observed hexaconozole as effective fungicide in reducing the mycelial growth. It is also clear from the above study that the plant extracts of all the tested plants proved effective against black mold rot pathogen, Aspergillus niger. In the similar studies, several reports stated that the extracts of medicinal plants play an important role in controlling many phytopathogenic fungi (Okemo et al., 2003; Imtiaj et al., 2005; Khalil et al., 2005; Lee et al., 2007; Abd-El-Khair and Haggag, 2007; Baka, 2010; Znini et al. 2011; Ogbebor et al., 2007; Taskeen-Un-Nisa et al., 2010; 2011; Jeyaseelan et al., 2012 Senguttuvan et al., 2013; Znini et al. 2013; Parveen et al., 2013). Magro et al. (2006) reported the fungistatic activity of six aqueous plant extracts including Malva sylvestris L. against postharvest fungi, viz. Aspergillus candidus, Aspergillus niger, Penicillium sps. and Fusarium culmorum and reported that all the plant extracts brought about significant reduction in the mycelial growth with Malva sylvestris at higher concentrations being most effective. Oyelana et al. (2010) reported the antimicrobial activity of Ficus leaf extracts against some fungal and bacterial phytopathogens including A. niger. Raji and Raveendran (2013) reported the antifungal activity of selected plant extracts against phytopathogenic fungi, Aspergillus niger. Pawar (2013) reported the antifungal activity of nine plant extracts against five phytopathogenic fungi, viz. Alternaria alternata, Aspergillus niger, Curvularia lunata, Fusarium moniliforme and Trichoderma viride. He concluded that these plant extracts can possibly be exploited in the management of pathogenic fungi to prevent biodeterioration in an eco-friendly way.

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