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Antagonistic effects of *Aspergillus niger* against Plant pathogenic fungi isolated from *Solanum tuberosum.*

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ABSTRACT

The present study was aimed to find out the antagonistic effects of *Aspergillus niger*, isolated from compost against *Alternaria alternata, Alternaria sp., Geotrichum sp.,* and *Gliocladium sp.,* isolated from rotten potato (*Solanum tuberosum*). The antagonistic effects of *Aspergillus niger* were evaluated against pathogenic fungal isolates by dual culture experiments. The results demonstrated that *Aspergillus niger* showed 90.8%, 84.6%, 83.0% and 4.10% growth inhibition against *Alternaria alternata, Alternaria sp., Geotrichum sp., and Gliocladium sp*. respectively. Hence, *Aspergillus niger* could be used as biocontrol agent to control these pathogenic fungal species. However, it should be investigated extensively for food safety before commercialization.

Keywords: *Aspergillus niger*, Biocontrol.

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INTRODUCTION

Potato is a tuberous, starchy crop from the perennial *Solanum tuberosum* of the Solanaceae family. It is the fourth most important food crop in India after rice, wheat and maize. Several fungal plant pathogens cause serious damage at any stage of potatoes. *Alternaria alternata* and *Alternaria solani* are the causal agents of early blight, and then are important foliar pathogens of potato [1,2]. *Alternaria* are worldwide in their occurrence and cause diseases on host plants including apples, broccoli, cauliflower, carrots, potatoes, Chinese cabbage, tomatoes, citrus and a number of weeds [3]. *Geotrichum sp* were reported to cause rubbery rot on potatoes [4]. *Geotrichum* has been reported to cause sour rot of lemon (*Citrus limon* Burm, f.), mandarin (*C. reticulata* Blanco), and orange (*C. sinensis* (L.) Osbeck) worldwide [5]. Biological control is a good alternative method as compared to chemical control which refers to the purposeful utilization of introduced or resident living organisms, other than disease resistant host plants, to suppress the activities and populations of one or more plant pathogens[14,16] . It has limited impact on the environment and has more specific effect on the pathogen [7]. *Aspergillus niger* is a fungus and one of the most common species of the genus *Aspergillus*. Adebola and Amadi(2010) reported the antagonistic effect of *Aspergillus niger* against *Phytophthora palmivora,* causing cocoa black pod disease [8].

The objective of this research was to evaluate the antagonistic effects of *Aspergillus niger*, isolated from compost against *Alternaria alternata, Alternaria sp., Geotrichum sp.,* and *Gliocladium sp.,* isolated from diseased portion of rotten potato (*Solanum tuberosum*).

MATERIAL AND METHODS

Collection of Sample

Rotten potato samples were collected from different Cold storage houses around Jalandhar and compost sample was collected from Villages near LPU, Jalandhar.

Isolation and Identification of Aspergillus niger from Compost Sample

Fungal culture were isolated from compost by following serial dilution plate method using PDA medium supplemented with streptomycin sulphate and purified by point inoculation method [9]. The inoculated petri plates are incubated at 27° ± 1°C for 3-4 days. Pure cultures of fungi were raised from mixed culture by transferring small portion of fungal culture with the help of inoculating needle and inoculated in fresh PDA medium. The fungal isolates were maintained on PDA slants at 4^0 C until used for the study.

Identification of Fungal Forms

Pure cultures were identified by studying their macroscopic appearance, pigmentation and growth rate. Visual examination was done to study the important characters such as colour, macroscopic structures, growth zones. The fungal isolates subjected to certain morphological studies viz., conidiophores type and formation, spore morphology etc. The microscopic examination was made by observing the slide after staining with Lactophenol Cotton Blue. Based on these features identification was made following A Manual of Soil Fungi by Gilman[11] and A Manual of the *Aspergilli* [10].

Isolation and Identification of Plant Pathogenic Fungi from Rotten Potato

The diseased portion of rotten potato was surface sterilized with 1% sodium hypochlorite solution for 1 minute and rinsed in five successive changes of sterile distilled water and then blotted dry. The tubers were cut aseptically from healthy region to necrotic region. The discs of about 4mm diameter were made from the advancing edge of the rot as well as from middle of lesions and plated on media PDA into which 0.1% Streptomycin was added to suppress bacterial growth. The inoculated petri plates were incubated at 27° ± 1°C for 3-4 days.

Antagonistic Assay (Dual Culture Experiment)

The isolate of *Aspergillus niger* was evaluated against pathogenic fungal isolates from rotten potato tubers by dual culture technique[12]. Sterilized PDA media was poured into petriplates and allowed to solidify. The agar blocks were cut from actively growing margin of the individual species of *Aspergillus niger* and pathogenic fungal isolates and inoculated just opposite to each other approximately 3 cm apart on PDA in petriplates. Three replicates for each set were maintained. In control plates (without *Aspergillus niger)* a sterile agar disc was placed opposite side of the pathogenic isolates. The inoculated plates were incubated at 27° ± 1°C. The position of colony margin was recorded daily on the back of the disc. Assessments were made when the fungi has achieved an equilibrium after which there was no further alteration in the growth. Since both of the organisms in each Petri dish were mutually inhibited, the assessment was made for both organisms.

The percentage inhibition of growth was calculated as follow.

Percentage inhibition of growth = R-R1 x 100 / R

R = growth of the fungus from the centre of the colony towards the centre of the plate in the absence of antagonistic fungus.
R1 = growth of the fungus from the centre of the colony towards the antagonistic fungus.

RESULTS AND DISCUSSION

Fungal culture was isolated and morphological characteristics were evaluated after 90 hours incubation. Result has been depicted in Table 1 which clearly evidences that the cultures are *Alternaria alternata, Alternaria* sp*., Gliocladium* sp*., Geotrichum* sp*., Aspergillus terreus*. Pathogenic cultures were identified on the basis of published literature [1-6]. The pure cultures of fungi and microscopic structures of conidia are shown in Figs. 1, 2, 3, 4 and 6.

The results of dual cultures of *Aspergillus niger* and its interaction with pathogenic fungal cultures demonstrated that *Aspergillus niger* showed remarkable antagonistic effect on *Alternaria alternata, Alternaria* sp*.,* and *Geotrichum* sp*.* In control plates, the fungus grew rapidly and covered the entire agar surface of plates after 5 days of incubation. In co-inoculated cultures of *Aspergillus niger* and pathogenic fungal cultures, the colony margin of pathogenic fungal cultures zones opposite the colonies of *Aspergillus niger* gradually became flattened and bent, with clear inhibition after incubation for 5 days. Thus isolated culture of *Aspergillus niger* has antagonistic effects on pathogenic fungal cultures. The type of interactions between *Aspergillus niger* and each of the test fungus was recorded based on Wheeler and Hocking (1993), adapted from Magan and Lacey (1984)[13]. Fig. 7 shows the interaction between *Aspergillus niger* and *Alternaria alternata* in dual culture experiment. *Aspergillus niger* continued to grow through the surface of *Alternaria alternata* colonies and covered the whole surface after 5 days of incubation. Fig. 8 shows the interaction between *Aspergillus niger* and *Alternaria* sp*.* in dual culture experiment. *Aspergillus niger* inhibiting the growth of *Alternaria* sp. at the surface of contact and then continued to grow at a reduced rate through the surface of *Alternaria sp.* colonies. Fig. 9 depicts the interaction between *Aspergillus niger* and *Geotrichum sp.* in dual culture experiment. *Aspergillus niger* inhibiting the growth of *Geotrichum* sp*.* at the surface of contact and then continuing to grow at a reduced rate through the surface of *Geotrichum* sp*.* colonies. Fig. 10 depicts the interaction between *Aspergillus niger* and *Gliocladium* sp*.* in dual culture experiment. *Gliocladium* sp. inhibiting the growth of *Aspergillus niger* on contact and then continuing to grow at a reduced rate through the surface of *Aspergillus niger* colonies.

The results are depicted in Table 2 and percentage inhibition is shown in Fig. 5. The data shows that *Aspergillus niger* has 90.8%, 84.6%, 83.0% and 4.10% growth inhibition against *Alternaria alternata, Alternaria* sp*., Geotrichum* sp*.,* and *Gliocladium* sp. respectively.

According to results obtained from this *in vitro* antagonistic study, *Aspergillus niger* has showed strong potential to reduce the mycelial growth of *Alternaria alternata* followed by *Alternaria sp.,* and *Geotrichum sp*. and very less potential to reduce the mycelial growth of *Gliocladium sp.* Similarly, Tiwari 2011[15, 19, and 20] has also reported potential of *Aspergillus niger* and *Trichoderma viride* as biocontrol agents of wood decay fungi.

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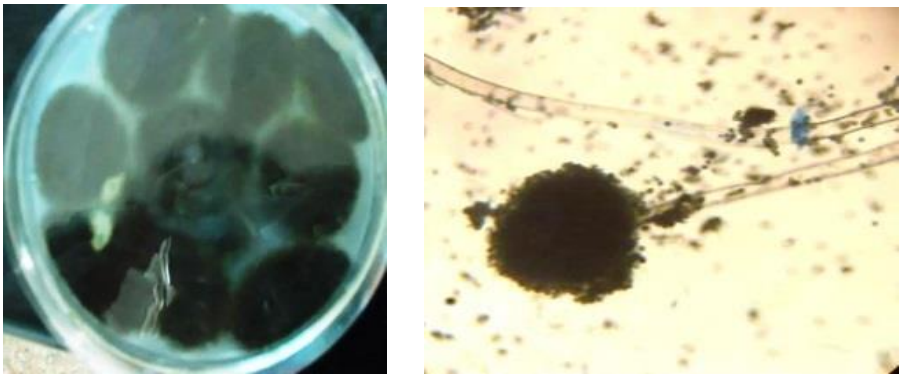

Figure 1: Pure culture of *Aspergillus niger* and microscopic structure of conidiophore and conidia at 40X magnification

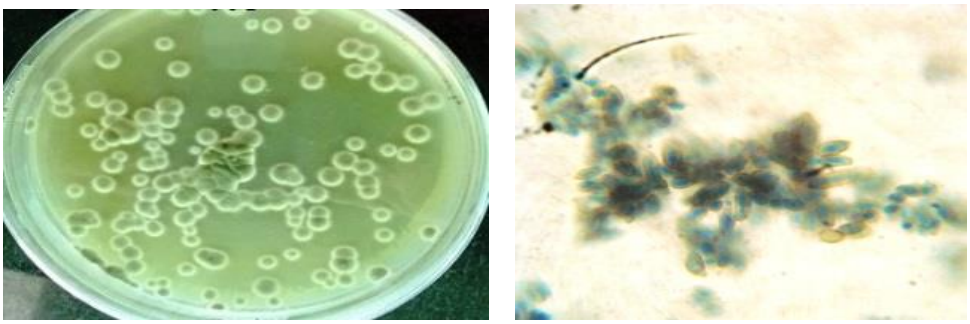

Figure 2: Pure culture of *Alternaria alternata* and microscopic structure of conidia at 40X magnification

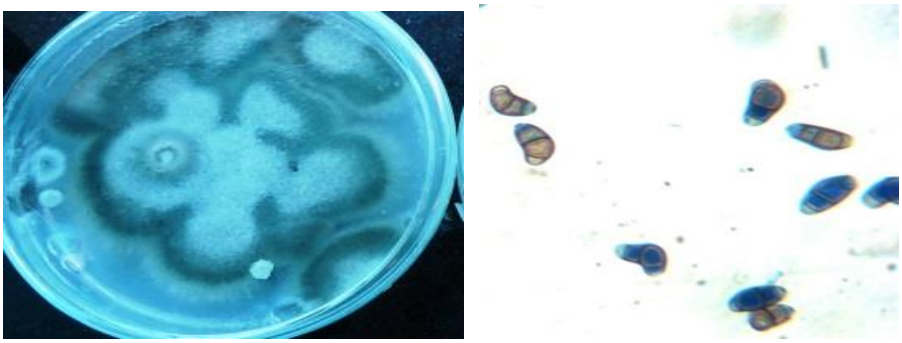

Figure 3: Pure culture of *Alternaria* sp. and microscopic structure of conidia at 40X magnification

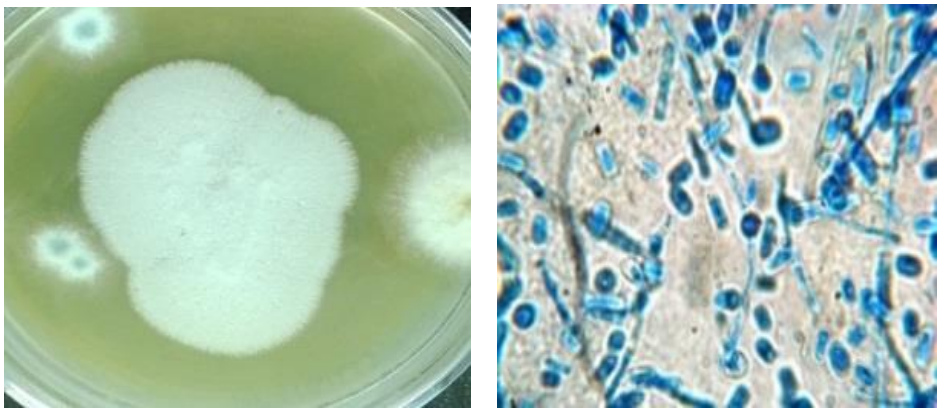

Figure 4: Pure culture of *Geotrichum* sp. and microscopic structure of Arthospores at 40X magnification

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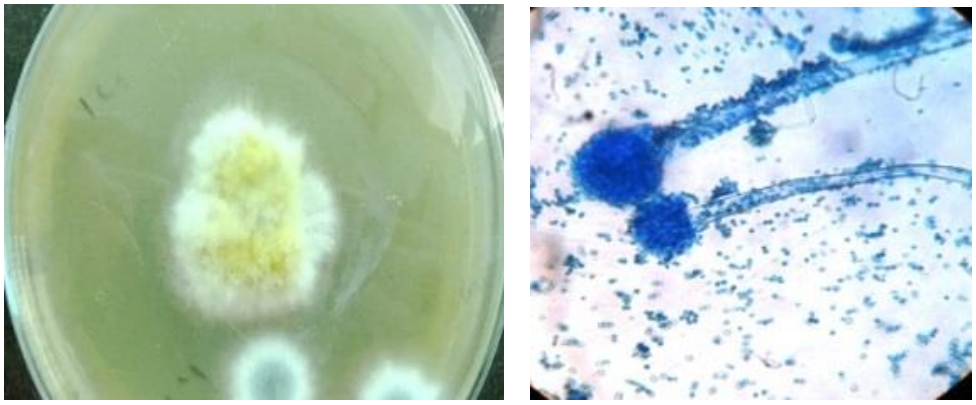

Figure 6: Pure culture of *Gliocladium* sp. and microscopic structure of conidiophores and conidia at 40X magnification

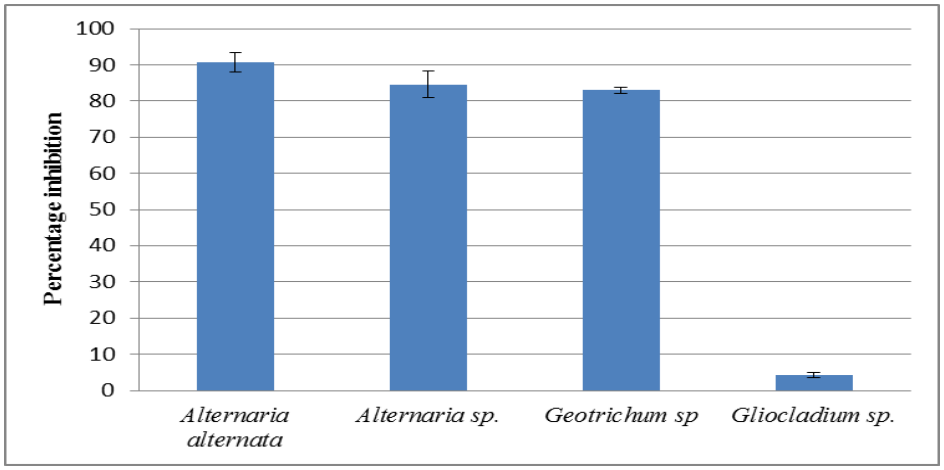

Figure 5: Antagonistic Effect of *Aspergillus niger* (Percentage Inhibition) on the Radial Growth of Pathogenic Fungal Cultures

Figure 7: Dual culture of *Aspergillus niger* with *Alternaria alternata,* on PDA observed after 5 days of incubation at 28°C.

Figure 8: Dual culture of *Aspergillus niger* with *Alternaria* sp. on PDA observed after 5 days of incubation at 28°C.

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Figure 9: Dual culture of *Aspergillus niger* with *Geotrichum* sp. on PDA observed after 5 days of incubation at 28°C.

Figure 10: Dual culture of *Aspergillus niger* with *Gliocladium* sp., on PDA observed after 5 days of incubation at 28°C.

Table 1: Morphological and Microscopic Characteristics of Fungal Isolates

	Fungal Cultures				
	Pathogenic fungi				Non Pathogenic fungi
Features	*lternaria alternata*	*Alternaria sp.*	*Gliocladium sp.*	*Geotrichum sp.*	*Aspergillus terreus*
lour of the colony	Bronze olive	Grey with white mycelium	White at first, becoming pale to dark green with sporulation.	hite, subsurface colonies, resembling yeast	nsist of a compact yellow basal felt covered by a dense layer of yellow conidial heads
Reverse	rk black with normal mycelial growth	Dark black with normal mycelial growth	rescent yellow with normal mycelial growth	ale yellow with normal mycelial growth	Light yellow to dark yellow
nidiophore	Simple and short	Branched and elongated	sely penicillate with phialides which bear conidia in heads or columns.	Arthroconidia	nidial heads were large, globose, and dark brown. Conidiophores were smooth-walled.
Conidia	Short conical or cylindrical beak, pale brown, smooth-walled	psoidal, often with a short, cylindrical beak, pale brown, smooth walled	Slimy, one-celled hyaline to green, smooth-walled conidia.	phae showed on slide culture. Septate and hyaline	onidia were globose and rough-walled.

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Table 2: Antagonistic Effect of *Aspergillus niger* on the Radial Growth of Pathogenic Fungal Cultures

No	Test isolates	Growth of Fungal Cultures (mm)			rcentage Inhibition of Growth (%)
		R(Control)	R1		
				Mean	
1	*Alternaria alternata*	21	5.5	5.5	90.8±2.70
			3.9		
			7.1		
2	*Alternaria sp.*	52	8.0	8	84.6±3.75
			6.1		
			9.9		
3	*Geotrichum sp.*	56	9.0	9.5	83.0±0.90
			9.5		
			10		
4	*Gliocladium sp.*	24	23	23	4.1±0.69
			22		
			24		

Potato is a tuberous, starchy crop and fourth most important food crop in India after rice, wheat and maize. Several fungal plant pathogens cause serious damage at any stage of potatoes as well as other host plants including apples, broccoli, cauliflower, carrots, Chinese cabbage, tomatoes, citrus and a number of weeds. Biological control is an effective method as compared to chemical control which destroys a range of macro and microorganisms. It has limited impact on the environment and has more specific effect on the pathogen. The antagonism between different microbial strains can be expressed by competition, direct parasitism, and production of metabolites. The objective of this work was to obtain and test biocontrol agents for controlling plant pathogenic fungi. *Aspergillus niger,* isolated from compost was tested as biocontrol agent against *Alternaria alternata, Alternaria sp., Gliocladium sp.* and *Geotrichum sp.* According to the present study, *Aspergillus niger* has shown strong potential to reduce the radial growth of *Alternaria alternata* followed by *Alternaria sp.,* and *Geotrichum sp*. and very less potential to reduce the radial growth of *Gliocladium sp.*

CONCLUSION

Biological control is an effective method as compared to chemical control which destroys a range of macro and micro-organisms. It has limited impact on the environment and has more specific effect on the pathogen. According to present research work, *Aspergillus niger* has showed strong potential to reduce the radial growth of *Alternaria alternata* followed by *Alternaria sp.,* and *Geotrichum sp*. and very less potential to reduce the radial growth of *Gliocladium sp.* Therefore, *Aspergillus niger* could be used as biocontrol agents against all tested pathogenic fungal cultures except *Gliocladium sp*. The presented data exhibit the antagonistic activity of *Aspergillus niger* and indicate the possibility of using this fungus as biocontrol agent to control tested pathogenic fungal cultures. Therefore, biocontrol agent tested in our study can be used for assessment of field biocontrol against tested pathogenic fungi and before commercialization it should be investigated extensively for food safety.

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REFERENCES

[1] Droby S, Prusky D, Dinoor A, Barkai-Golan R. Plant Dis 1984;68: 160-161
[2] El-Mongy NS. J Plant Protec Res 2009;49(1): 57-62
[3] http://anrcatalog.ucdavis.edu/Details.aspx?itemNo=8040
[4] http://www.teagasc.ie/research/reports/crops/4356/eopr-4356.pdf
[5] Hernández-Montiel LG, Holguín-Peña RJ, Latisnere-Barragan H. Plant Dis 2010;9(4): 488
[6] Rich AE. 1983. Potato Diseases, Academic Press Inc, New York.
[7] Sigee DC. 1993. Bactenol Plant Pathology: Cell and Molecular Aspects Cambridge: Cambridge University Press
[8] Adebola MO, Amadi JE. Agric Biol J N Amer 2010;1(3): 362-365
[9] Johnson LF, Curl AE. 1972. Method for the research on ecology of soil borne plant pathogens, Burgess Publishing Company, Minneapolis
[10] Thom C, Raper KB. 1951. A Manual of the Aspergilli, *Waverly Press Inc, The William and Willkins Company*
[11] Gilman JC. 1994. A manual of Soil Fungi, 2nd edition, Oxford and IBH publishing Co
[12] Skidmore AM, Dickinson CM. 1976. Colony interactions and hyphal interferences between Septoria nodorum and phylloplane Fungi. Trans Brit Mycol Soc 66: 57-64.
[13] Wheeler KA, Hocking AD. J Appl Bacteriol 1993;74: 164 – 169
[14] Vyas A, Bhardwaj P, Kumar M, Pachouri UC, Garg S, Singh J. Nat Acad Science Lett 2014;37(5):435–439
[15] Tiwari CK, Parihar J, Verma RK. J Indian Acad Wood Sci 2011;8(2):169–172
[16] Brimner TA, Boland GJ. Agric Ecosyst Environ 2003;100(1): 3–16
[17] Mondal G, Dureja P Sen B. Ind J Exp Biol 2000;38:84-87
[18] Mondal G. 1998. In vitro evaluation of Aspergillus niger AN27 against soil borne fungal pathogens and field testing against Macrophomina phaseolina on Potato Ph D Thesis IARI, New Delhi, 1998
[19] Grayston SJ, Vaugham D, Jones D. Appl Soil Ecol 1996;5:29–56.
[20] Schirmbock M, Lorito M, Wang YL, Hayes CK, Arisan-Atac F, Scala F, Harman GE Kubicek CP. Appl Environ Microbiol 1994;60:4364–4370.