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Interactions of *Aspergillus niger* isolate AN 27, a potential biocontrol agent, against soil borne plant pathogens

A.K. PATIBANDA[1] and B. SEN[2]
Division of Plant Pathology, Indian Agricultural Research Institute, New Delhi 110 012
[1]Agricultural Research Station, Tandur, R. R. Dt. 501 141

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Management of soil borne plant pathogenic fungi using antagonists is now an established approach in combating plant diseases. For successful utilization of the antagonist, it is essential to know the spectrum of antagonistic activity against varied soil borne plant pathogenic fungi. Success in the control of soil borne plant pathogenic fungi using *Aspergillus niger* van Teigh. is earlier demonstrated (4). The present investigation is aimed at knowing the spectrum and mechanism of *A. niger* isolate AN 27 antibiosis against six soil borne plant pathogenic fungi *in vitro*.

One isolate each of *Fusarium oxysporum* f. sp. *melonis* Sny. & Hans., *Fusarium solani* (Mart.) Sacc., *Rhizoctonia solani* (obtained from the Vegetable Pathology Laboratory, Division of Plant Pathology, IARI, New Delhi), *Pythium aphanidermatum* (Eds.) Fitz. (ITCC 123), *Sclerotinia sclerotiorum* (Lib.) de Bary (ITCC 3292) and *Sclerotium rolfsii* Sacc. (ITCC 3073) (obtained from Indian Type Culture Collection, IARI, New Delhi) were tested *in vitro* for their sensitivity towards the antagonist, *Aspergillus niger* AN 27 using dual culture method. One 5 mm disc of *A. niger* AN27 and four discs of the test pathogen were inoculated at the center and periphery respectively on 90 mm sterile PDA plates under aseptic conditions. Test antagonist or test pathogen alone inoculated plates served as checks.

Macroscopic and microscopic observations were recorded up to 25 days after inoculation. For specific interactions between *A. niger* and *F. oxysporum* f. sp. *melonis*, dual culture was done by inoculating single 5 mm disc each of *A. niger* and *F. o.* f. sp. *melonis* at a distance of 70 mm in a 90 mm PDA plate. pH of the solid medium was assayed using pH paper strips.

Antagonist *A. niger* AN 27 (hence forth referred as 'AN 27'), when inoculated at the center, attained a radial growth of 4.5 cm within 7 days in control plates (Table 1). Radial growth of *S. rolfsii* (3 days) and, R. *solani* and *S. sclerotiorum* (4 days) was faster compared to that of *A. niger* An 27. *P.aphanidermatum*, *F. solani* and *F. o.* f. sp. *melonis* were on par with that of *A. niger* AN 27 and took 7 days to completely occupy the plate.

Interactions of AN 27 and *F. o.* f. sp. *melonis* (hence forth referred as 'Fom') revealed normal growth of both the fungi without any inhibition for four days followed by static growth up to 12th day and then AN 27 started overgrowing Fom colony. An opaque zone (Zo) was formed around AN 27 colony, which was delimited by an inhibition zone (Zi) around Fom colony. Unlike in check plates, where AN 27 had continuous growth with sporulation leading to black colour colony, in dual culture plates – AN 27 attained three growth phases (Fig 1). Initial 'phase I' growth with normal hyphae and active sporulation as in check, followed by 'phase II' growth after 5 days with thinner hyphae and suppressed sporulation at the point of interaction and a final 'phase III' revived growth of normal hyphae and sporulation after 12 days. Phase III growth was initiated when the test antagonist AN 27 started overgrowing Fom colony. Medium pigmentation of Fom changed from violet to pink in interaction plates (Fig 2A). Microscopic examination revealed aggregation of substances exuded by AN

Present address: [2]B-21, Inderpuri, New Delhi 110 012

Table 1. Antagonistic action of *Aspergillus niger* AN 27 *in vitro.*

S. No.	Test Fungus	Radial growth in cm. 2 d	3 d	4 d	5 d	7 d	12 d	25 d	Lysis	Opaque Zone	Inhibition Zone	Pigmentation	Overgrowth
1.	AN 27 Ck	0.9	1.7	2.0	2.3	4.5	4.5	4.5	-	-	-	-	-
2.	Fom Ck	1.0	2.0	2.5	3.0	4.0	4.0	4.0	-	-	-	Violet	-
3.	Fs Ck	1.0	1.6	2.3	2.8	4.0	4.0	4.0	-	-	-	-	-
4.	Pa Ck	1.0	1.6	2.3	2.7	4.0	4.0	4.0	-	-	-	-	-
5.	Ss Ck	1.0	2.5	4.0	4.0	4.0	4.0	4.0	-	-	-	-	-
6.	Rs Ck	1.8	2.8	4.0	4.0	4.0	4.0	4.0	-	-	-	-	-
7.	Sr Ck	2.0	4.0	4.0	4.0	4.0	4.0	4.0	-	-	-	-	-
8.	AN 27 vs	0.9	1.3	1.6	1.7	1.7	1.7	4.5					+
	Fom	1.0	1.5	1.5	1.5	1.5	1.5	1.5	-	+	+	Pink	
9.	AN 27 vs	0.9	1.3	1.6	1.9	2.5	4.5	4.5					+
	Fs	0.9	1.3	1.3	1.4	1.4	1.4	1.4	-	+	-	Orange	
10.	AN 27 vs	1.0	1.7	1.8	2.0	3.0	4.5	4.5					+
	Pa	1.0	1.3	1.3	1.3	1.3	1.3	1.3	+	+	+	-	
11.	AN 27 vs	1.0	1.7	1.7	1.7	1.7	4.5	4.5					+
	Ss	1.0	2.0	2.3	2.3	2.3	2.3	2.3	+	-	-	-	
12.	AN 27 vs	1.0	1.3	1.3	1.3	2.4	4.5	4.5					+
	Rs	1.7	2.7	2.7	2.7	2.7	2.7	2.7	-	-	-	-	
13.	AN 27 vs	0.9	1.0	1.0	1.0	1.0	1.0	1.0					
	Sr	2.0	4.0	4.0	4.0	4.0	4.0	4.0	-	-	-	-	+

Ck – control plates; d – days after inoculation; AN 27 – *Aspergillus niger* AN 27; Fs – *Fusarium solani*
Pa – *Pythium aphanidermatum*; Ss – *Sclerotinia sclerotiorum*; Rs – *Rhizoctonia solani*; Sr – *Sclerotium rolfsii*
'-' absence of the character; '+' presence of the character; Fom – *F. oxysporum* f. sp. *melonis*
------ dotted lines indicate interaction effects
Values or characters on lower side of the dotted line represents pathogen isolate and on upper side of the dotted line represents AN 27.

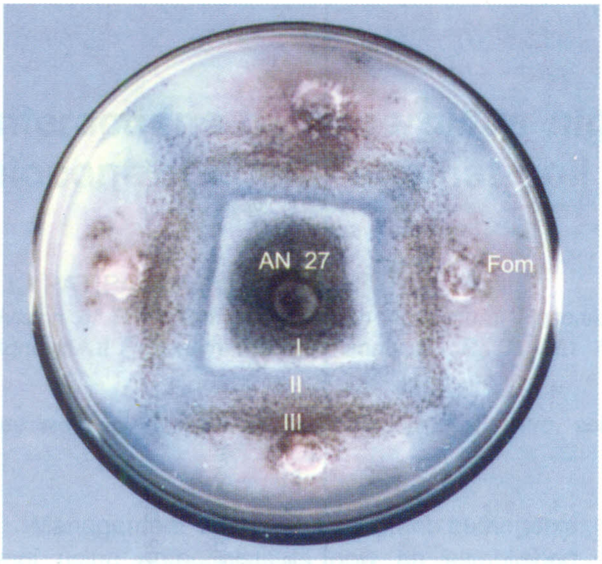

Fig. 1. Interactions of *Aspergillus niger* AN 27 (AN 27) and *F. oxysporum* f. sp. *melonis* (Fom) *in vitro*. Note three growth phases (I, II and III) of AN 27 in dual culture

27 in the opaque zone (Fig 2C) surrounded by a clear inhibition zone, thinner hyphae of AN 27 without sporulation in the interaction zone and formation of thick mycelial strands and chains of chlamydospores in the Fom colony (Fig 2 B, D). In AN 27 check plates, the medium pH has changed from an initial 6.8 to 2.0 within 7 days.

It is known that medium pigmentation of *Fusarium* is affected by pH (1) and some of the pigments produced by *Fusarium* were found to be toxic to other microbes (5). In the initial stages of interaction, Fom recognized change in medium pH due to AN 27 as evidenced from the change in colony and medium pigmentation. However, the toxic secretions of Fom delimited the movement of AN 27 'influence' such as organic acids and particulate matter (?) leading to formation of clear and transparent inhibition zone around Fom colony, opaque zone around AN 27 colony. Thus formation of inhibition zone and opaque zone are the manifestations of the pathogen reaction towards invading antagonist AN 27.

Dennis and Webster (2) opined that parasitic hyphae must be thinner than that of the host hyphae for easy penetration. These AN 27 parasitic hyphae were found to be 60% less in diameter compared to normal AN 27 hyphae (3). Thus in phase II growth, AN 27 overcame the challenge posed by Fom by formation of thinner hyphae. As the chlamydospores are produced under unfavorable conditions, the formation of thick mycelial strands

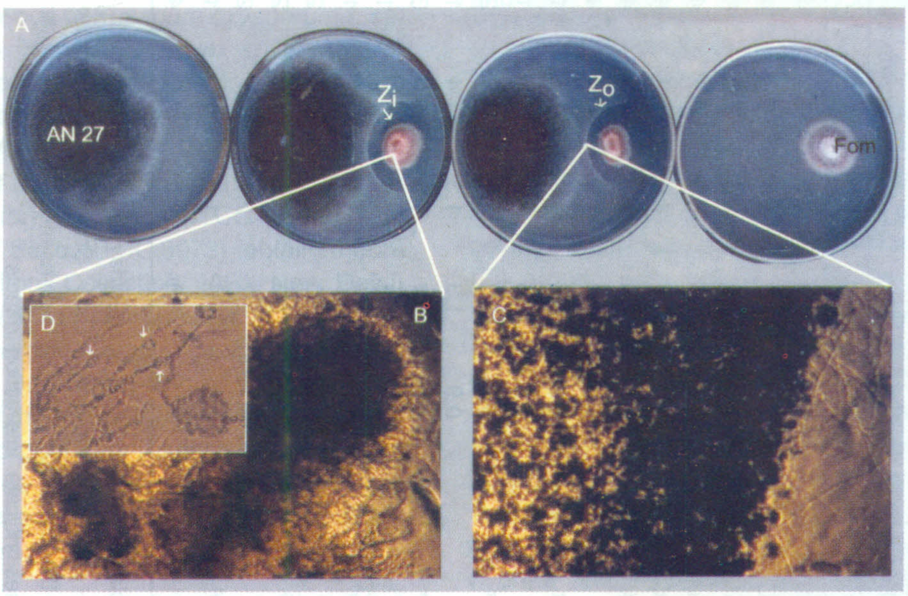

Fig. 2. A: Interactions of *A. niger* AN 27 (AN 27) and *F. oxysporum* f. sp. *melonis* (Fom) *in vitro*. Note Opaque Zone (Zo), Inhibition Zone (Zi) and change in colony colour of Fom from violet (in check plate) to pink (in dual culture plate). **B:** Mycelial strand in Fom colony during interactions under microscope. **C:** Microscopic observation of opaque zone where particulate matter from AN 27 aggregated around inhibition zone. **D:** Chains of chlamydospores in Fom colony.

and chlamydospores symbolized cessation of Fom metabolic activity and in turn its toxic metabolites. Such cessation of activity paved way to the revival of AN 27 growth in phase III.

In check plates, *F. solani* (henceforth referred to as 'Fs') had white cottony mycelium without any pigmentation. Colonies of Fs and AN 27 came close to each other by 3rd day after inoculation in dual culture plates (Table 1). After 5 days of interaction, Fs produced orange pigmentation signaling recognition system. However, absence of opaque zone indicated that AN 27 metabolites could freely diffuse into the medium. It was interesting to note lysis of Fs mycelium in advance of AN 27 penetration indicating that Fs couldn't offer enough resistance to the advancing AN 27 and succumbed to its metabolites.

Colonies of AN 27 and *P. aphanidermatum* (hence forth referred as 'Pa') came close to each other 3 days after inoculation (Table 1). A thin inhibition zone separated both the test fungi. An opaque zone was formed after 5th day and 12 days of incubation resulted in disappearance of opaque zone and lysis of Pa mycelium. AN 27 could then start overgrowing Pa colony. Thus similar to AN 27 – Fs interactions, Pa exerted initial resistance but succumbed later to AN 27 invasion.

In AN 27 – *S. sclerotiorum* (hence forth referred as 'Ss') dual cultured plates, both the colonies united by 4th day of inoculation (Table 1). Then up to 7th day both the colonies remained static. By 12th day, lysis was observed in Ss colony in advance of AN 27 growth. Inhibition zone and opaque zone were not observed indicating lack of resistance from Ss.

In interaction plates, AN 27 and *R. solani* (hence forth referred as 'Rs') came together by 3rd of inoculation (Table 1). Up to 5th day, both the colonies remained static in growth. Then An 27 started overgrowing Rs colony. Opaque zone, inhibition zone and or lysis of Rs mycelia in advance of AN 27 overgrowth were not observed. Microscopic examination of mycelial interactions revealed mycoparasitism signs such as appressoria formation and hyphal coiling by AN 27 around Rs hyphae. Similar signs of mycoparasitism were not observed in other interactions. This indicated that, AN 27 –Rs interactions involved mycoparasitism by AN 27 where as in other interactions it was antibiosis in nature.

AN 27 – *S. rolfsii* interactions revealed neutral association in which none of the test fungi were affected by the other. Reisolation of AN 27 and *S. rolfsii* from the dual culture plates gave colonies of both. In other interactions, reisolation after AN 27 overgrowth gave only AN 27 growth indicating that the test pathogenic fungi were lysed and killed.

The present study conducted *in vitro* indicated that *A. niger* AN 27 is a potential antagonist against all the test soil borne plant pathogenic fungi except *S. rolfsii*. The mechanism of antagonism varied from pathogen to pathogen. The efficacy of antagonism depended mainly on the defense arsenal exhibited by the test pathogen isolates.

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