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In vitro screening of *Aspergillus niger* van Teigh against *Fusarium oxysporum* f. sp. *melonis*, muskmelon wilt pathogen

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ABSTRACT: **Seven isolates of the antagonist *Aspergillus niger* van Teigh and three isolates of the muskmelon wilt pathogen *Fusarium oxysporum* f. sp. *melonis* were assayed for their *in vitro* interactions. The most virulent isolate of the pathogen was highly sensitive to antagonism compared to less virulent isolate. Variation existed among different *A. niger* isolates in their antagonistic potential. Observations on overgrowing ability of the antagonist, *A. niger* were more useful in screening isolates compared to other parameters such as inhibition zone, radial growth of the test pathogen and / or of the test antagonist in *A. niger* Vs *F. oxysporum* f. sp. *melonis* system. Isolate AN 27 was found promising based on its biocontrol capabilities.**

KEY WORDS: Antagonism, *Aspergillus niger*, *Fusarium oxysporum* f. sp. *melonis*, muskmelon, wilt

INTRODUCTION

Fusarium oxysporum Schlecht f. sp. *melonis* Sny. & Hans. is one of the three formae speciales of *F. oxysporum* causing vascular wilts in cucurbits. Earlier work indicated success in managing Fusarial wilts employing *Aspergillus niger* van Teigh. as an antagonist (Sen *et al.*, 1992). A successful biocontrol agent should be effective against several isolates of the pathogen and not be isolate specific. Further, while selecting the antagonist isolate, care needs to be taken to see that the antagonist or its metabolites do not have any deleterious/ phytotoxic effect on the plant. The present study was undertaken to select an isolate of the antagonist *A. niger* with wider biocontrol capabilities.

MATERIALS AND METHODS

Seven isolates of *A. niger viz.*, AN 2, AN 3, AN 4, AN 5, AN 27 and AN 1624, and three isolates of the muskmelon wilt pathogen, *F. oxysporum* f. sp. *melonis viz.*, MM 5 (less virulent), MM 40 (medium virulent) and MM37 (highly virulent) were obtained from the Division of Plant Pathology, IARI, New Delhi and studied for their *in vitro* interactions using dual culture method (Morton and Stroube, 1955). One 5mm agar disc of *A. niger* was inoculated

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at the center of Potato Dextrose Agar plate (90 mm) followed by four discs of *F. oxysporum* f. sp. *melonis* inoculated at the periphery. Inoculated plates were incubated at 28±1°C for 25 days. Three replications were maintained for each treatment. Appropriate monoculture plates were maintained as control.

To find the antagonistic efficiency of *A. niger*, 'rapid pathogenicity test' was conducted (Wensley and Mc Keen,1962). Muskmelon seeds cv. Pusa Madhuras were coated with *A. niger* spore suspension (10^7 spores / ml) and sown in fine sand in perforated aluminium trays at 28±2°C and water was added to maintain optimum moisture. Six-day-old seedlings were then placed in 30 ml vials (one seedling each) containing only test pathogen conidial suspension (10^7conidia/ ml) and observed for 10 days at 28±2°C. Disease reaction was scored on a 0-5 scale after Radhakrishnan and Sen (1982) as follows: 0=Cotyledons turgid; 1=Cotyledons flaccid; 2=1+Yellowing of the cotyledons; 3 =2+marginal necrosis; 4 =3+advanced marginal necrosis and 5 =Total wilt.

To examine the phytotoxic effects, if any, of the test antagonist on muskmelon plants, rapid pathogenicity test was conducted by placing six-day-old seedlings with fully opened cotyledons in 30 ml vials (one seedling each) containing spore suspension mixture of test antagonist and test pathogen (10^7 spores/ ml each). Observations were recorded till 10th day and the plants were graded for disease reaction as '+' for signs of phytotoxicity such as yellowing, necrosis and drying of the seedling, and '-' for no signs of phytotoxicity.

RESULTS AND DISCUSSION

Initial interactions between *A. niger* (henceforth referred as AN) and *Fusarium oxysporum* f. sp. *melonis* (henceforth referred as FOM) in dual culture-plates revealed formation of inhibition zone between the two test fungi. Campbell (1989) opined that such an inhibition zone could be taken as a clue for the production of antibiotics and thereby for screening and selecting effective antagonists or their isolates. Similar interactions involving *A. niger* and *Fusarium* spp. were reported by Sharma and Sen (1991). In all the above cases, the inhibition zone was interpreted as a cause of *A. niger* antibiosis rather than an interaction effect. The interactions of seven isolates of AN and three isolates of FOM revealed insignificant differences when the zone of inhibition was measured (Table 1). Length of inhibition zones was on a lower side and varied from 0.1 to 0.25cm in different interactions.

Table 1. *In vitro* interactions between *A. niger* and *F. o.* f. sp. *melonis*

A. niger (AN) isolate	Zone of inhibition (cm) 25 days after inoculation		
	FOM 5	FOM 37	FOM 40
AN 2	0.10	0.20	0.10
AN 3	0.20	0.20	0.15
AN 4	0.10	0.10	0.10
AN 5	0.10	0.20	015
AN 6	0.10	0.20	0.10
AN 27	0.25	0.25	0.20
AN 1624	0.10	0.10	0.00

Figures did not differ significantly at P=0.01

Close observations on pattern of the advancing line of growth of interacting fungi revealed a straight line in both the test fungi towards interaction zone instead of a usual concave peripheral line in monoculture. This indicated that the test pathogen too was exerting pressure on the antagonist.

When the cultures were allowed to interact for 27 days after inoculation, three phases in the growth of AN were observed. In check plates, where AN was inoculated alone, growth was found to be continuous with sporulation leading to black colony. However, in the interaction plates, AN growth showed initial active sporulation (phase I) followed by growth with suppressed sporulation (phase II)

at the point of interaction and then growth with revived sporulation (phase III) once the antagonist started overgrowing the test pathogen's colony.

Notable differences were observed among the seven isolates of AN in their overgrowing ability on FOM isolates (Table 2). Isolates AN 3, AN 5 and AN 27 could overgrow all the three isolates of FOM and were termed as 'group I' isolates. Isolates AN 6, AN 1624 and AN 2 could overgrow only one or two isolates of FOM and were termed as 'group II' isolates. Isolate AN 4 could not overgrow on any of the FOM isolates and hence categorized as 'group III' isolate.

Further, it was interesting to note that all the isolates except AN 4 (group III) could overgrow on the highly virulent isolate FOM 37, while only three AN isolates, *viz.*, AN 3, AN 5 and AN 27 could overgrow on the less virulent FOM 5. The colony of the medium virulent isolate FOM 40 was occupied by five AN isolates. This indicated that highly pathogenic isolates were more sensitive towards antagonism compared to less pathogenic isolates. This could be because virulence makes the pathogenic isolate highly specialized and thereby more sensitive towards antagonism.

To have a better understanding about *in vitro* antagonism, observations were recorded on (a) radial growth of FOM, (b) phase I growth of AN, (c) phase II growth of AN and (d) total AN growth.

All the three FOM isolates grew similar in check plates with a radius of 3.5 cm in 5 days of incubation (Table 3). Though significant reduction in test pathogens' growth was observed in dual culture plates compared to check plates, insignificant differences were obtained among FOM isolates over all AN isolates. This indicated that initial difference in growth and / or growth inhibition did not exist among FOM isolates up to the point of interaction.

Phase I growth of AN isolates indicated initial growth of AN from the point of inoculation to the interaction zone. Similar to FOM isolates, insignificant differences were observed among AN isolates over all FOM isolates regarding their initial growth before interaction (Table 4). The present investigation thus revealed that radial growth of the pathogen and/ or of the antagonist alone cannot be taken into consideration for evaluating different antagonist isolates for their efficacy or different pathogen isolates for their sensitivity.

Table 2. Isolate variation in *A. niger* for overgrowing *F. o.* f. sp. *melonis* isolates *in vitro*

A. niger (AN)isolate	Overgrowth of AN on FOM after 25 days of inoculation			
	FOM5	FOM 37	FOM 40	Group
AN 2	-	+	-	II
AN 3	+	+	+	I
AN 4	-	-	-	III
AN 5	+	+	+	I
AN 6	-	+	+	II
AN 27	+	+	+	I
AN 1624	-	+	+	II

Table 3. Interactions between *A. niger* and *F. o.* f. sp. *melonis* isolates

A.niger isolate	Radial growth of *F. o.* f. sp. *melonis* (in cm)			
	FOM 5	FOM 37	FOM 40	Mean
AN 3	1.55	1.60	1.50	1.55
AN 5	1.50	1.45	1.60	1.51
AN 27	1.50	1.60	1.40	1.50
AN 1624	1.80	1.75	1.50	1.68
AN 2	1.35	1.40	1.40	1.38
AN 6	1.35	1.60	1.60	1.58
AN 4	1.45	1.50	1.60	1.51
Check	3.50	3.50	3.50	3.50
Mean	1.78	1.80	1.76	

CD (P=0.01) = Between AN isolates 0.10, Between FOM isolates NS, Interaction effect 0.26

Table 4. Interactions between *A. niger* (AN) and *F. oxysporum* f. sp. *melonis* (FOM) isolates

A .niger Isolate	Radial growth of *A. niger* (cm)								
	A. niger X FOM 5			*A. niger* X FOM 37			*A. niger* X FOM 40		
	Phase I	Phase II	Total	Phase I	Phase II	Total	Phase I	Phase II	Total
AN 3	1.35	0.56 (1.03)	2.35	1.40	0.60 (1.05)	3.3	1.30	0.60 (1.05)	2.65
AN 5	1.35	0.67 (1.08)	2.55	1.20	0.94 (1.20)	3.1	1.25	0.56 (1.03)	1.95
AN 27	1.30	0.40 (0.95)	2.00	1.50	0.56 (1.03)	2.7	1.60	0.56 (1.03)	2.70
AN 1624	1.30	0.00 (0.70)	1.30	1.30	0.46 (0.98)	2.5	1.30	0.46 (0.98)	2.50
AN 2	1.35	0.00 (0.70)	1.35	1.25	0.71 (1.10)	1.3	1.30	0.00 (0.70)	1.30
AN 6	1.35	0.00 (0.70)	1.35	1.25	0.46 (0.98)	1.70	1.60	0.00 (0.70)	1.70
AN 4	1.45	0.00 (0.70)	1.45	1.35	0.00 (0.70)	1.20	1.20	0.00 (0.70)	1.20
Check	–	—	4.50	–	–	4.50	–	–	4.50
Mean	1.35	0.21 (0.84)	2.10	1.32	0.52 (1.01)	2.50	1.36	0.27 (0.88)	2.30

CD (P=0.01)	Between An Isolates	Between FOM isolates	Interaction effect
Phase I	NS	NS	NS
Phase II	0.07	0.04	0.125
Total AN growth	0.22	0.16	0.44

Phase II growth of AN indicated the interaction effect on the growth of AN by FOM. With isolate FOM 37, the highly virulent isolate, AN isolates had maximum mean phase II growth (0.52 cm) compared to FOM 40 (0.27 cm) and FOM 5 (0.21cm) - the least virulent isolate (Table 4). Individual comparisons revealed that group I AN isolates had maximum phase II growth compared to group II isolates. This is because all the isolates of group I could overgrow all three isolates of FOM. Similar results were obtained in phase III growth (total AN growth) which indicated overgrowth of test antagonist isolates (Table 4). Group I isolates had maximum mean total growth which did not differ significantly among themselves.

Thus the present investigation revealed that observations on inhibition zone, radial growth of the pathogen and/ or of the antagonist do not indicate about the efficacy of the antagonist. However, observations on overgrowth and total growth of AN are dependable parameters to select effective AN isolates *in vitro* against FOM

Of the three group I isolates, AN 3 was found to have phytotoxic effect on muskmelon seedlings by causing root rot when muskmelon cv Pusa Madhuras seedlings were dipped in AN spore suspension *in vitro*. Hence isolate AN 3 was deleted for further testing.

When the biocontrol capabilities of AN 5 and AN 27 were assayed by rapid pathogenicity test using seed coat method, it was found that AN 27 was more effective compared to AN 5 in decreasing wilt caused by the most virulent isolate FOM 37. Hence isolate AN 27 was selected as a potential isolate of *A. niger* (Table 5).

Thus the present investigation revealed that overgrowth of the antagonist AN on the colony of the test pathogen FOM is useful and dependable parameter. Further, most virulent isolate of the test pathogen was found more sensitive compared to less virulent isolate as all the AN isolates could overgrow. *A. niger* AN 27 was found potential with wider biocontrol capabilities.

Table 5. Disease reaction by *F. o.* f. sp. *melonis* isolate on muskmelon seedlings raised from *A. niger* spore coated seeds in rapid pathogenicity test

Isolate	Seeds coated with	Disease reaction on 0-5 scale			
		7 days	8 days	9 days	10 days
FOM 37	Check	1.5	2.6	3.2	5.0
	AN 5	0.3	0.8	1.0	2.0
	AN 27	0.0	0.3	0.4	0.5
	CD (P=0.05)	0.02	0.03	0.03	0.09

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