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Aspergillus niger as Tomato Fruit (*Lycopersicum esculentum* Mill.) Quality Enhancer and Plant Health Promoter

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Abstract

In order to study the effects of *Aspergillus niger*, an experiment was carried out with application of nursery root-dip treatment with *A. niger* isolate (10 g/100 seedlings for 10 minutes), found significantly ($P \leq 0.001$) increased the yield and dry matter content of tomato plants. Application of all sixteen isolates of *A. niger* significantly increased the accumulation of salicylic acid, total phenolic and chlorophyll contents of plant, and lycopene, ascorbic acid (Vitamin C), Brix index, diameter of fruit skin, rate of pressure tolerance of tomato fruit compared to untreated control. Among the all isolates, *A. niger* SkNAn5 found to be the most efficient with increasing 54% yield and 59.8% dry matter of tomato plants. *A. niger* SkNAn5 also maximum significantly increased the salicylic acid of root, shoot with improved fruit quality of tomato having increased amount of vitamin C (35.59 g/100 g against control 23.9 g/100 g), lycopene (9.8 mg/100 g against control 8.3 mg/100 g), and also had increased rate of pressure tolerance of fruits (2.84 kg/cm against control 1.35 kg/cm). Increase in salicylic acid concentration may also increased the diameter of fruit skin with most efficient isolate SkNAn5 (0.49 mm) almost two fold compared to control (0.26 mm). Fruit Brix index of tomato plants treated with SkNAn5 significantly increased (8.75) compared to non-treated plants (5.81). These results suggest that nursery application of *A. niger* SkNAn5 may improve quantity and quality of tomato fruits.

INTRODUCTION

In recent years, more interest has been developed in using plant growth promoting microorganisms. Of these, phosphate-solubilizing microorganisms (PSM) such as *Pseudomonas fluorescens, P. stutzeri, P. striata, Bacillus subtilis, B. polymyxa,* etc. are efficient solubilizers among bacteria and *Aspergillus niger* and *Penicillium* spp., may proved to be efficient plant health promoters (Gaur, 1990, Rao, 1990; Khan et al., 2009). The PSMs provide essential nutrients to plants in addition to their antagonistic ability. Among various efficient phosphate solubilizers, *Aspergillus niger* was selected for the present study because of some most important attributes the fungus possesses. The fungus is not included in the toxigenic species of *Aspergillus* responsible for mycotoxins (ochratoxin A) (Khan and Anwer, 2007). Production of ribotoxin by *A. niger* was negative (Campbell, 1994) and the fungus is reported to decrease aflatoxin contamination (Boller and Schroeder, 1974; Wicklow et al., 1980; Horn and Wicklow, 1983). Two growth promoting compounds, 2-carboxymethyl 3-n-hexyl maleic acid (compound 1) and 2-methylene-3-hexylbutanedioic acid (compound 2) isolated from *A. niger* are responsible for increasing root and shoot length and biomass of crop plants (Mondal et al., 2000).

Several strains of bacteria like *Bacillus subtilis* and *Pseudomonas fluorescens* are well known to synthesize phytohormones such as indole acetic acid, gibberellins, cytokinins and zeatin which promote plant growth at various stages (Gracia de Salamone et al., 2001), but a very few fungi can promote growth. Evidences exists which indicate that some *A. niger* isolate also produce IAA and other phytohormones (Mostafa and Youssef, 1962) which

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significantly increased the growth and yield (Khan and Anwer, 2008).

The production of organic acid in the microenvironment around the root or in the culture media is considered the most important parameter to measure phosphate solubilization by microorganisms (Sperber, 1958; Hayman, 1975; Gaur, 1985 a, b). *A. niger* have been found to synthesize citric, gluconic, glycolic, oxalic and succinic acids (Sperber, 1958; Blumenthal, 2004; Ramachandran et al., 2008). The role of organic acids in solubilizing mineral phosphates and phosphorilated minerals is attributed to the lowering pH which helps in the formation of stable complex and later forms are more soluble and available form for plants (Jennings, 1989; Li et. al., 1991). It has been reported that *A. niger* reduce medium pH to 4.0, sufficiently low enough to solubilize phosphorus (Domich, et. al., 1980; Medina et al., 2007).

The metabolic changes that occur in plants leads to accumulation of phenolic compounds (Farkas and Kiraly, 1962; Bashan et al., 1985; Raju et al., 2008). Activation of systemic acquired resistance (SAR) in plants triggered by salicylic acid (SA) contributes to restrict invasion and infection of the pathogen (O'Connell and Panstruga, 2006; Firdous et al., 2007; Doehlemann et. al., 2008; Esmailzadeh et al., 2008). In chickpea and tomato, activation of SAR being a healthy plant results in a significant reduction of disease symptoms caused by *Fusarium oxysporum* (Houssien et al., 2010) and *Meloidogyne incognita* (Jayakumar et al., 2006). However, SA contents usually get increased in response of inoculation with antagonists (Singh et al., 2003; Saikia et al., 2006; Raju et al., 2008; Garcia-Limones et al., 2008; Gupta et al., 2010). It has been reported that systemic fungicides may first activate some defensive responses in the host (Guest, 1984; Jones et al., 1991; Molina et al., 1998). Garcia et al. (2001) determined that carbendazim (2.6 mM) increased salicylic acid (SA) as well as the accumulation of phenolics.

Salicylic acid (SA) or ortho-hydroxy benzoic acid and other salicylates are known to affect various physiological and biochemical activities of plants and may play a key role in regulating their growth and productivity (Hayat et al., 2010). Salicylic acid is considered to be an endogenous growth regulator of phenolic nature that enhanced the leaf area and dry mass production in corn and soybean (Khan et al., 2003). Dry matter accumulation was significantly increased in *Brassica juncea* when lower concentrations of salicylic acid were sprayed (Fariduddin et al., 2003). Khodary (2004) observed a significant increase in growth characteristic, pigment contents and photosynthetic rate in maize, sprayed with salicylic acid. Flowering is another important parameter that is directly related to yield and productivity of plants. Salicylic acid has been reported to induce flowering in a number of plants such as *Sinningia speciosa* (ornamental plant) flowered much earlier as compared to the untreated control (Martin-Max et al., 2005).

MATERIALS AND METHODS

Isolation, identification and pure culture of *Aspergillus niger* isolates

Extensive surveys were conducted in crop fields of different districts of Uttar Pradesh, India in a way that the entire state was covered. Soil was collected in sterilized polythene bags from different crop fields in an area. The samples were brought to the laboratory and stored inside the room away from the direct sunlight. The soil samples were processed within a couple of days using a standard serial dilution technique (Wakman, 1927) to isolate *Aspergillus niger* isolates. Ten gram soil was taken in 100 ml distilled water and stirred for 15 min. The suspension was left for 15 min and 1 ml was transferred to a test tube containing 9 ml distilled water. The

procedure was repeated three times to obtain a dilution of 1: 10^4, which was pipetted over PDA in a Petri plate (0.3 ml / plate) under a laminar flow. Three plates were maintained for each treatment. Inoculated plates were incubated at 25±2°C for 5 days in a BOD incubator. After incubation, the plates were examined and isolates belonging to *A. niger* aggregate were identified on the basis of cultural and morphological characters as described by Raper and Fennel (1965) and Gilman (2001). The pure culture of the isolates was prepared by inoculating culture tubes containing PDA slants with a spore from an identified colony of *A. niger*. The tubes were incubated at 25±2°C in a BOD incubator for 5 days. The isolates were also got identified from the National Bureau of Agriculturally Important Microorganisms (NBAIM), Mau, India, where they have been deposited.

Mass culture and dose of *Aspergillus niger* isolates

The fungus was mass cultured on sorghum seeds. The seeds were soaked overnight in a 5% sucrose and chloramphenicol (30 mg L^{-1}) solution. The seeds were then transferred to conical flasks of 500 mL capacity and autoclaved at 15 kg cm^{-2} pressure at 120ºC for 15–20 minutes two times with a gap of 12 hours to ensure sterilization. Thereafter, they were inoculated with the pure culture of *A. niger* under aseptic conditions and incubated for 8–10 days on a rotatory shaker (120 rpm) inside a BOD incubator at 25±2°C to provide optimum conditions for growth. To achieve uniform colonization and sporulation of the fungus, the flasks were shaken for 30 minutes daily during the incubation period. In pot study, tomato nursery root-dip treatment with *A. niger* isolates (10 g/100 seedlings) was done. Spore suspension of *A. niger* was made by grinding 10 g of colonized seeds in 100 ml distilled water. The roots of tomato seedlings were dipped in the suspension for 10 minutes. The colony forming unit load of *A. niger* per nursery was estimated using the dilution plate method (Table 1).

Crop culture

Certified seeds of tomato, *Lycopersicon esculentum* Mill. cv. Pusa Ruby were procured from an authorized dealer. The mycoflora examination of seeds (external and internal) through blotter paper test (Tempe, 1970) revealed absence of any seed borne pathogen (fungi/bacteria). The seeds were sown in raised bed and watered daily or after one day as per requirement. Five leaves stage seedlings of tomato (two weeks old) were transplanted at the center of the pots. Seedlings were irrigated with tap water immediately after transplanting and it continued as per requirement till harvest.

Treatment and plant culture

Clay pots (15 cm diameter and height) were filled with steam-sterilized soil amended with compost (3:1), and following 17 treatments were incorporated. Three replicates were maintained for all treatments. Additional 3 pots per treatment were taken to estimate salicylic acid (SA) of shoots and roots. The pots were arranged in a completely randomized design in an open space receiving uniform sunlight and irrigation.

01. Plant (Control)
02. Plant + AnC2
03. Plant + AnR3
04. Plant + AAn1
05. Plant + BAn4
06. Plant + BasAn5
07. Plant + BudAn3
08. Plant + GaAn1
09. Plant + JaAn2
10. Plant + LAn3
11. Plant + MeAn4
12. Plant + SkNAn3
13. Plant + SkNAn5
14. Plant + VAn4
15. Plant + BuAn3
16. Plant + ANAn1
17. Plant + ANAn4

Observations recorded

During the course of growth or at harvest following observations were recorded.

i. Estimation of leaf pigments:

Chlorophyll a, chlorophyll b and total chlorophyll were estimated by grinding 1 g of fresh leaves from interveinal areas of one month old tomato plants in 40 ml 80% acetone with the help of mortar and pestle. The suspension was decanted in a Buchner funnel having two Whatman filter paper No.1. The filtration was done with the help of suction pump. The residue was ground thrice by adding acetone. The suspension was decanted in Buchner funnel and filtered in vacuum. At last, mortar and pestle were rinsed with acetone and solution was transferred in Buchner funnel and filtered. The filtrate was transferred to 100 ml volumetric flask and the volume was made upto the capacity by adding acetone. Using spectrophotometer (Spectronic 20, USA), the optical density (OD) of the filtrate was read at 645 and 663 nm for chlorophyll. The chlorophyll contents were calculated by using the Arnon (1949) formulae.

Chlorophyll a (µg/ml) = 12.7 (A663) - 2.69 (A645)

Chlorophyll b (µg/ml) = 22.9 (A645) - 4.68 (A663)

Total chl (µg/ml) = 17.76 (A645) + 7.34 (A663)

Where, A663 is the OD at 663 ηm; A645 is the OD at 645 ηm.

ii. Estimation of total phenol:

Leaf samples (1g) from tomato (2 weeks after transplanting) were homogenized in 10 ml 80% methanol and agitated for 15 minutes at 70°C (Zieslin and Ben Zaken, 1993). The leaf samples from the three plants replicates of a treatment were processed separately. One milliliter of the methanol extract was added to 5 ml of distilled water and 250 µl of Folin-Ciocalteau reagent (1N) and the solution was kept at 25°C. The absorbance of the developed blue color was measured using a Spectrophotometer (Spectronic 20, USA) at 725ηm. Catechol was used as the standard. The amounts of phenolics were expressed as µg catechol /gm fresh weight of leaf sample (Sharma et al., 2005).

iii. Estimation of salicylic acid:

The salicylic acid (SA) of the leaves and roots of the tomato plant was estimated separately from 2 weeks old plant. The leaves and roots (5 g) were cut into small pieces of size 0.5-1.0 cm, soaked in water for overnight, than filtered through the Whatman filter paper no.1 and extracted in ethyl acetate. The ethyl acetate fraction was taken and sodium sulphate added to remove the moisture and filtrate was evaporated to dryness in water bath. The stock solution was prepared by the addition of 10 ml methanol. The stock solution was used for recording the absorbance in a spectrophotometer (SHIMADZU, 2450 PC, Japan) at 306 ηm. The absorbance was fixed at 306 ηm and the readings were recorded at different ppm of SA and standard curve was prepared for the estimation of SA concentration in the leaf sample (Pankaj et. al., 2005). From the standard curve the concentration of SA in the sample was calculated according to the formula $y = mx \pm c$ (Lowery et al., 1951).

iv. Estimation of lycopene in tomato fruit:

Extraction of lycopene was performed according to Fish et al. (2002). Fully ripe tomato fruits were first chopped and homogenized in a laboratory homogenizer. 0.5 g samples were weighed and 5 ml of 0.05% (w/v) butylated hydroxytoluene (BHT) in acetone, 5 ml ethanol and 10 ml hexane were added. The recipient was introduced in ice and stirred on a magnetic stirring plate for 15 min. After shaking, 3 ml of deionized water were added to each

vial and the samples were shaken for 5 min on ice. Samples were then left at room temperature for 5 min to allow the separation of both phases. The absorbance of the hexane layer (upper layer) was measured in a spectrophotometer (SHIMADZU, 2450 PC, Japan) at 503 ηm blanked with hexane.

A standard solution of lycopene in hexane (0.04 mg/ml) was also prepared, wrapped in foil, and stored in the dark at 4ºC. Working mixtures of relevant concentrations were made by appropriate combination and dilution with hexane. The absorbance was fixed at 503 ηm and the readings were recorded at different ppm of standard solution of lycopene. The standard curve was plotted and concentration of lycopene in the sample was calculated according to the formula y = mx ± c (Lowery et al., 1951) or the lycopene concentration in the sample was determined using the following formula (Vosburgh and Cooper, 1941)

Lycopene (mg/100g sample) =

$$\frac{3.1206 \times A\ 503 \times \text{Volume made up} \times \text{Dilution} \times 100}{1 \times \text{Wt of sample} \times 1000}$$

$$\text{mg of lycopene per 100g sample} = \frac{3.1206 \times A\ 503 \times \text{Volume made up} \times \text{Dilution} \times 100}{1 \times \text{Wt of sample} \times 1000}$$

Where, A 503 is the OD at 503 ηm.

v. Vitamin C (Ascorbic acid): Ascorbic acid was determined by titration using 2,6-dichloroindophenol (AOAC. 1990).

vi. Fruit soluble solids (Brix index): Brix index was determined by refractometer method using a digital refractometer (model 060279, Belgium) (AOAC, 1990).

vii. Fruit firmness: Firmness of fruit was measured by penetrometer (0) and diameter of fruit skin was measured by a digital calipers.

viii. Dry weight of plants: The tomato plants after collecting fruits were wrapped with paper and kept in an oven at 60°C for 2 days to determine dry weight of root and shoot.

Statistical analysis

Three replicates were maintained for each treatment with additional 3 pots/treatment for estimation of salicylic acid and leaf pigments from plants. The data on plant growth, yield etc. were subjected to single factor analysis of variance (ANOVA). Least significance difference (LSD) was calculated at $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$ for all variables to compare individual treatments. Duncan's multiple range test was applied to identify efficient isolates of *A. niger*. The data has been presented in tabulated form. Regression analysis among plant growth parameters, yield, chlorophyll content, phenolic content, salicylic acid content and lycopene content was performed and coefficient of regression was calculated and presented in graphical forms. All the experiments were conducted for consecutive two years and pooled data were subjected to analysis. Entire statistical analysis was done using SPSS-10 for Windows or Minitab-15.

RESULTS

Dry matter production and yield

All tomato plants applied with *A. niger* isolates as nursery treatment produced significantly ($P \leq 0.001$) more dry matter (17-60%) and yield (21-54%) in comparison to control (Table 2). This growth promoting effect was found greatest with *A. niger* SkNAn5 isolate with 60% increased dry shoot weight (58 g/shoot) and 54% more yield (554 g/ plant) in comparison to uninoculated control of 36.3 g/shoot and 360 g/plant, respectively (Table 2).

Leaf chlorophyll

Nursery treatments with *A. niger* isolates improved ($P \leq 0.05$) the leaf chlorophyll a

(from 0.83 to 0.917-0.954 mg/g fresh weight of leaf tissue), chlorophyll b (from 0.79 to 0.93-0.973 mg/g fresh weight of leaf tissue) and total chlorophyll (from 1.62 to 1.846-1.926 mg/g fresh weight of leaf tissue) of tomato plants (Table 3). Relative performance of *A. niger* isolates in improving leaf pigments varied significantly ($P \leq 0.05$) and the order of efficiency was SkNAn5 > VAn4 > AnC2 > AnR3 > ANAn4 > BuAn3 > rest of the *A. niger* isolates.

Leaf phenol

Total phenol content of tomato leaves of plants applied with *A. niger* isolates increased ($P \leq 0.05$) over control (from 90.3 to 113.2-122.9 mg/g catechol fresh weight of leaf tissue, Table 4). Significant variation in the performance of *A. niger* isolates was seen on tomato plant. Greatest increase in the phenol content was recorded with SkNAn5 (122.9 mg/g catechol fresh weight of leaf tissue) followed by AnC2, AnR3, ANAn4 and BuAn3.

Salicylic acid

Salicylic acid (SA) concentration in tomato root and shoot increased significantly ($P \leq 0.01$) due to nursery application of *A. niger* isolates in comparison with control (Table 4). Maximum increase in SA content was recorded with SkNAn5 (in leaf from19.3 to 27.5 and in root from 26.3 to 39.0 ppm/g fresh weight of tissue) followed by AnC2, AnR3, ANAn4 and BuAn3. Salicylic acid accumulation with the application of different isolates of *A. niger* in shoot was 29.5-42% more where as in root 33.8-48.3% greater than the control.

Lycopene content of tomato fruit

Lycopene content of ripen fruits of tomato (fully red) from the plants treated with the *A. niger* isolates increased ($P \leq 0.001$) over control, however, effect of the isolates varied to a great extent ($P \leq 0.001$, Table 4). Over all order of *A. niger* isolates increasing lycopene contents was SkNAn5 (increased from 8.3 to 9.8 mg/100 g fresh weight of fruit) > VAn4 > AnC2 > AnR3 > ANAn4 > BuAn3 > rest of the *A. niger* isolates.

Ascorbic acid

Ascorbic acid (vitamin C) content of ripen fruits of tomato from the plants treated with the *A. niger* isolates increased ($P \leq 0.001$) over control (Table 4). Over all order of *A. niger* isolates increasing ascorbic acid was SkNAn5 (35.59 g/100 g fresh weight of fruit) > VAn4 > AnC2 > AnR3 > ANAn4 > BuAn3 > rest of the *A. niger* isolates in comparison to control (23.9 g/100 g fresh weight of fruit).

Brix index (BI)

Plants treated with *A. niger* isolates had significantly higher ($P \leq 0.001$) Brix index (fruit soluble solids materials) compared to non-treated control plants (Table 4). Over all order of *A. niger* isolates increasing Brix index was SkNAn5 (with 8.75 BI) > VAn4 (with 8.65 BI) > AnC2 (with 8.61 BI) > AnR3 > ANAn4 > BuAn3 > rest of the *A. niger* isolates in comparison to control (with 5.81 BI).

Diameter of fruit skin

Plants treated with *A. niger* isolates had significantly higher ($P \leq 0.001$) diameter of fruit skin (0.45-0.51 mm) compared to non-treated control plants (0.26 mm, Table 4). Over all order of *A. niger* isolates increasing thickness of fruit skin was VAn4, SkNAn3, BuAn3, AnR3 (with 0.51 mm) > ANAn4, Ban4, BudAn3 (with 0.50 mm) > SkNAn5 (with 0.49 mm) >ANAn1, ANAn4, AnC2, LAn3 (with 0.48 mm) > rest of the *A. niger* isolates in comparison to control (with 0.26 mm).

Rate of Pressure tolerance

Plants treated with *A. niger* isolates had significantly higher ($P \leq 0.001$) rate of pressure tolerance (2.63-2.84 kg/cm) compared to non-treated control plants (1.35 kg/cm, Table 4). Over all order of *A. niger* isolates increasing rate of pressure tolerance was SkNAn5, AnR3 (with 2.84 kg/cm) > VAn4, Ban4 (with 2.82 kg/cm) > SkNAn3 (with 2.81 kg/cm) > AnC2 (with 2.79 kg/cm > rest of the *A. niger* isolates in comparison to control (with 1.35 kg/cm).

Table 1. Colony forming unit load of *Aspergillus niger* isolates at the time of nursery application.

***A. niger* isolates**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
	AAn1	**ANAn1**	**ANAn4**	**AnC2**	**AnR3**	**BAn4**	**BasAn5**	**BuAn3**
CFU count / nursery	1×10^9	5×10^8	5×10^9	6×10^9	6×10^9	2×10^9	3×10^9	5×10^9
A. niger isolates	9	10	11	12	13	14	15	16
	BudAn3	GaAn1	JaAn2	LAn3	MeAn4	SkNAn3	SkNAn5	VAn4
CFU count / nursery	3×10^9	5×10^9	4×10^9	1×10^9	2×10^9	5×10^9	10×10^9	8×10^9

Mean fallowed by similar letter(s) in each column, are not significantly different at 0.1% level of probability. *Significantly different from the control at $P \leq 0.001$.

Table 2. Effect of nursery treatment of efficient *Aspergillus niger* isolates on the dry matter production and yield of tomato in pots.

Treatment (*A. niger* isolate)	**Dry shoot weight (g)**		**Yield(g) /plant**	
Control	36.3f		360j	
AAn1	50.6b	(39.3)	468g	(30.0)
ANAn1	49.5bc	(36.4)	462g	(28.4)
ANAn4	54.7ab	(50.6)	493f	(36.9)
AnC2	55.7a	(53.4)	527c	(46.3)
AnR3	56.3a	(55.2)	513d	(42.4)
BAn4	51.3b	(41.4)	464g	(28.9)
BasAn5	45.9d	(26.5)	434i	(20.5)
BuAn3	53.9ab	(48.6)	503e	(39.6)
BudAn3	49.7bc	(37.0)	451h	(25.4)
GaAn1	48.8bc	(34.5)	446h	(24.0)
JaAn2	47.4c	(30.6)	438i	(21.6)
LAn3	49.7bc	(36.9)	452h	(25.5)
MeAn4	42.6e	(17.4)	440hi	(22.3)
SkNAn3	49.6bc	(36.5)	453h	(25.9)
SkNAn5	58.0a	(59.8)	554a	(54.0)
VAn4	56.0a	(54.3)	544b	(51.1)
LSD (P≤0.001)	2.4		7.3	
F-value (P≤0.001)				
Treatments (df=16)	184*		201*	

Each value is pooled data of two years with three replications per treatment per year.

Values in parenthesis are percent increase (+ve) over control.

Table 3. Effects of nursery treatment with *Aspergillus niger* isolates on chlorophyll a, b and total chlorophyll of tomato.

Treatment (*A. niger* isolate)	**Chlorophyll (mg/g fresh weight of leaf tissue)**		
	Chl. a	**Chl. B**	**Total Chl.**
Control	0.83g	0.790f	1.620i
AAn1	0.932e	0.947c	1.878e
ANAn1	0.929de	0.943c	1.872f
ANAn4	0.942c	0.959b	1.900c
AnC2	0.947b	0.964ab	1.911b
AnR3	0.943c	0.960b	1.902c
BAn4	0.935d	0.950bc	1.885d
BasAn5	0.937cd	0.953bc	1.889d
BuAn3	0.940c	0.956b	1.896cd
BudAn3	0.927e	0.941cd	1.867f
GaAn1	0.924e	0.939d	1.863fg
JaAn2	0.922ed	0.936d	1.859g
LAn3	0.923e	0.937d	1.861g
MeAn4	0.917f	0.930e	1.846h
SkNAn3	0.925e	0.940cd	1.865fg
SkNAn5	0.954a	0.973a	1.926a
VAn4	0.949b	0.967a	1.916b
LSD			

(P≤0.05)	0.004	0.006	0.005

Each value is pooled data of two years with three replications per treatment per year.

Mean fallowed by similar letter(s) in each c 043 are not significantly different at 0.5% level of probability; Chl – chlorophyll

Table 4. Effects of nursery treatment with *Aspergillus niger* isolates on total phenolic contents, salicylic acid, lycopene content, ascorbic acid (Vitamin C), Brix index, diameter of fruit skin and rate of pressure tolerance of tomato.

Treatment (*A. niger* isolates)	[x]Total Phenol content (mg/g catechol fresh weight of leaf tissue	[y]Salicylic acid (ppm/g fresh weight of tissue)		[z]Lycopene (mg/100 g fresh weight of fruit)	[z]Ascorbic acid (mg/100 g)	[z]Brix index	[z]Diameter of fruit skin (mm)	[z]Rate of pressure tolerance (kg/cm)
		Shoot	Root					
Control	90.4o	19.3h	26.3g	8.30c	23.90i	5.81j	0.26c	1.35c
AAn1	117.1g	26.0de	36.7de	9.50ab	33.45e	8.21f	0.50a	2.73ab
ANAn1	116.3h	25.84ec	36.4e	9.50ab	33.19ef	8.18f	0.48a	2.76ab
ANAn4	119.7d	26.6c	37.8c	9.60a	34.48c	8.46c	0.48a	2.78a
AnC2	121.1c	27.0b	38.3b	9.70a	34.91b	8.61b	0.48a	2.79a
AnR3	120.0c	26.7c	37.9bc	9.70a	34.56c	8.48c	0.51a	2.84a
BAn4	117.9f	26.2d	37.0d	9.60a	33.74e	8.31e	0.50a	2.82a
BasAn5	118.4e	26.3cd	37.3cd	9.60a	34.00d	8.38d	0.47b	2.66b
BuAn3	119.2e	26.5c	37.6c	9.60a	34.29cd	8.44c	0.51a	2.78a
BudAn3	115.7i	25.6e	36.2e	9.50ab	33.03f	8.12g	0.50a	2.75ab
GaAn1	115.2j	25.5ef	36.0ef	9.40b	32.83g	8.08g	0.46b	2.69b
JaAn2	114.7k	25.4ef	35.8ef	9.40b	32.63g	8.03h	0.47b	2.65b
LAn3	114.9k	25.4ef	35.9ef	9.40b	32.77g	8.06gh	0.48a	2.75ab
MeAn4	113.2l	25.0g	35.2f	9.30b	32.14h	7.88i	0.45b	2.63b
SkNAn3	115.5i	25.6e	36.1e	9.40b	32.92f	8.08g	0.51a	2.81a
SkNAn5	122.9a	27.4a	39.0a	9.80a	35.59a	8.75a	0.49a	2.84a
VAn4	121.6b	27.1b	38.5b	9.70a	35.09b	8.65b	0.51a	2.82a
LSD								
(*P≤0.05*)	2.62							
(*P≤0.01*)		0.25	0.32					
(*P≤0.001*)				0.21	0.28	0.04	0.03	0.06

Each value is pooled data of two years with three replications per treatment per year.
Mean fallowed by similar letter(s) in each column, are not significantly different at [x]5%, [y]1% and [z]0.1% level of probability.

DISCUSSION

Analysis of variance showed that nursery application of *Aspergillus niger* isolates significantly ($P \leq 0.001$) affected tomato yield with maximum increased with isolate SkNAn5 (54%) and dry matter of tomato plants highest with isolated SkNAn5 (59.8%). Earlier research showed, *A. niger* frequently enhances root growth and development, crop productivity and uptake and use of nutrients (Sen, 2000). Plant growth production of tomato can increase upto 467% after the addition of *A. niger* (Vassilev et al., 1996, 2006) greater than increased by application of *Trichoderma hamatum* or *T. koningii* (300%) of field crops (Chet et al., 1997). In an *in vitro* tests, *A. niger* PSBG-12 strain produced plant growth promoting substances such as indole acetic acid (IAA), gibberelic acid (GA) and under pot culture conditions showed better effect on plant growth parameters and nutrient uptake (Vaddar and Patil, 2007). In a field where muskmelon and watermelon crops were suffering from *Fusarium* wilt (sometimes *R. solani* and *Pythium* spp. were associated with the disease), treatment of seeds with *A. niger*

(Kalisena SD) @ 8 g per kg and soil with *A. niger* (Kalisena SL) @ 30 g per pit resulted in 81% control of the disease. The vines were more vigorous, and even with 15% incidence of disease, yield was approximately 5% greater as compared to that in disease-free areas (Chattopadhyay and Sen, 1996).

Among all isolates of *A. niger*, isolate SkNAn5 was the most efficient followed by VAn4, AnC2. Application of *A. niger* isolates resulted in significant increase of total phenolic content, salicylic acid of root and shoot, chlorophyll contents, Brix index of tomato, rate of pressure tolerance and diameter of tomato fruit skin. It has been reported that *A. niger* significantly checked the loss of chlorophyll content of leaves, lycopene content of tomato fruit and further increased the phenol content and salicylic acid of leaves and roots of chickpea and tomato caused by *F. oxysporum* f. sp. *ciceri* or *F. oxysporum* f. sp. *lycopersici* and *M. incognita* singly or concomitantly Sen (2000). These results are also supported by those of Kumar et al. (1999) who found that even foliar application of salicylic acid to soybean enhanced the flowering, pod formation and consequently yield of soybean. It was reported that salicylic acid application promotes cell division and cell enlargement (Hayat et al., 2005). Foliar application of salicylic acid increased the leaf area of sugarcane (Zhou et al., 1999). According to Shakirova et al. (2007) the positive effect of salicylic acid on growth and yield can be due to its influence on other plant hormones. Salicylic acid altered the auxin (IAA), cytokinin and ABA balances in wheat and increased the growth and yield under both normal and saline conditions. Increasing of yield under foliar application of salicylic acid could be ascribed to the well-known roles of salicylic acid on photosynthetic parameters and plant water relations. Fariduddin et al. (2003) reported that exogenous application of salicylic acid enhanced the net photosynthetic rate, internal CO_2 concentration and water use efficiency in *Brassica juncea*. In this study correlation analysis also revealed positive linear relationship between chlorophyll A and yield, dry shoot weight and yield, salicylic acid of root, leaf and lycopene content, lycopene content and total phenolic content, total phenol and salicylic acid of root and leaf, salicylic acid and rate of pressure tolerance, rate of pressure tolerance and diameter of fruit skin, diameter of fruit skin and salicylic acid indicating that an increase in salicylic acid, chlorophyll, phenolic content directly influenced the yield and quality of tomato fruit (Fig. 1). Khan et al. (2006) also reported that foliar application of GA significantly increased lycopene content of tomato fruits. In another study the highest fruit vitamin C was obtained in tomato plants treated with salicylic acid 10-2 M (32.5 mg per 100 g fruit fresh weight) compared to control plants (Javaheri et al., 2012). Exogenous application of salicylic acid increased the amount of fruit soluble solids materials (Brix index). Application of *A. niger* with increased salicylic acid would also contribute towards enhancing the capacity of the treated plants for biomass production as is reflected in the observed increase in fresh and dry weight of plants (Sen, 2000). Chandra et al. (2007) reported that application of salicylic acid increased total soluble sugar and soluble protein of cowpea plants. In cucumber and tomato, the fruit yield enhanced significantly when the plants were sprayed with lower concentrations of salicylic acid (Larque-Saavedra and Martin-Mex 2007). It was reported that the foliar application of salicylic acid to soybean also enhanced the flowering and pod formation (Kumar et al., 1999).

CONCLUSION

Application of *Aspergillus niger*, an efficient phosphate solubilizer, plant growth promoter can increase the accumulation of salicylic acid, total phenolic content,

chlorophyll content, lycopene content of fruit, ascorbic acid (Vitamin C) of fruit, fruit soluble solids materials (Brix index), diameter of fruit skin, rate of pressure tolerance of tomato fruit resulting increased yield with high quality of fruit. Phenolic contents considered to be a potent plant hormone because of its diverse regulatory roles in plant metabolism. It is well-established fact that salicylic acid potentially generates a wide array of metabolic responses in plants and also affects the photosynthetic parameters which enhance plant growth and yield. It may, therefore be concluded that the sustained increase in the observed parameters expectedly culminated in maximization of the process of biomass accumulation leading to higher productivity, lycopene, vitamin C content of tomato fruit and as well as fruit Brix index.

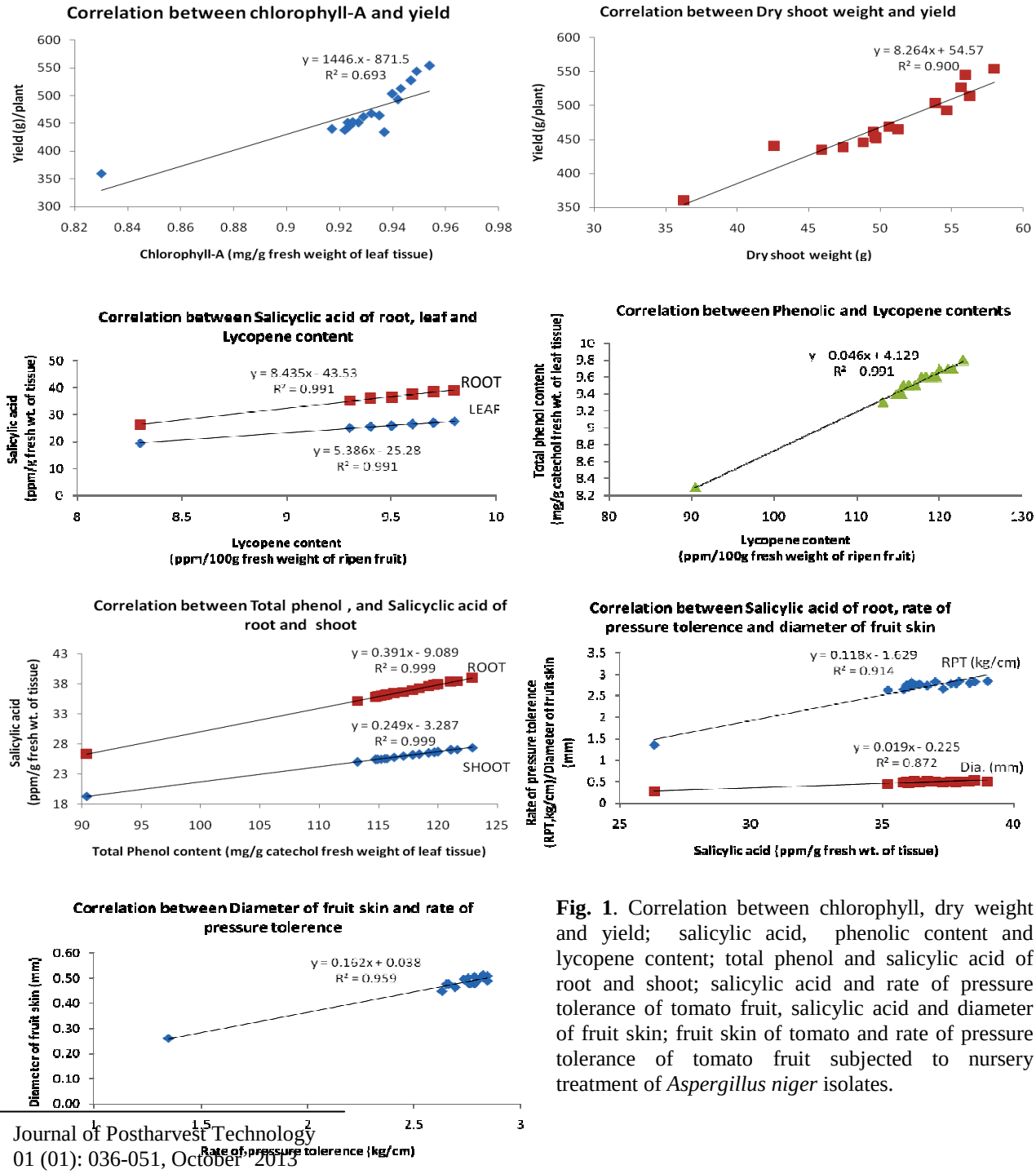

Fig. 1. Correlation between chlorophyll, dry weight and yield; salicylic acid, phenolic content and lycopene content; total phenol and salicylic acid of root and shoot; salicylic acid and rate of pressure tolerance of tomato fruit, salicylic acid and diameter of fruit skin; fruit skin of tomato and rate of pressure tolerance of tomato fruit subjected to nursery treatment of *Aspergillus niger* isolates.

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REFERENCES

AOAC. 1990. Official Methods of Analysis. 15th ed. Association of Official Analytical Chemists, Arlington, VA

Arnon, D. 1949. Polyphenoloxidase in Beta vulgaris. Plant Physiology, 24: 1-15.

Bashan, Y., Okon, Y., Henis, Y. 1985. Peroxidase, polyphenol oxidase and phenols in relation to resistance against Pseudomonas syringae pv. tomato in Tomato. Canadian Journal of Botany, 65: 366-372.

Blumenthal, C. Z. 2004. Production of toxic metabolites in Aspergillus niger, Aspergillus oryzae, and Trichoderma reesei: justification of mycotoxin testing in food grade enzyme preparations derived from the three fungi. Regulatory Toxicology and Pharmacology, 39: 214–228.

Boller, R. A. and Schroeder, H. W. 1974. Influence of Aspergillus candidus on production of aflatoxin in rice by Aspergillus parasiticus. Phytopathology, 64: 121-123.

Campbell, C. K. 1994. Forms of Aspergillosis. In: The Genus, Aspergillus (Eds. K. A. Powell, A. Renwick and J. F. Peberdy). Plenum Press, New York, pp. 313-319.

Chandra, A., Anand, A. and Dubey, A. 2007. Effect of salicylic acid on morphological and biochemical attributes in cowpea. Journal of Environmental Biology, 28: 193-196.

Chattopadhyay, C. and Sen, B. 1996. Integrated management of Fusarium wilt of muskmelon caused by Fusarium oxysporum. Indian Journal of Mycology and Plant Pathology, 26: 162-170.

Chet, I., Inbar, J. and Hadar, I. 1997. Fungal antagonists and mycoparasites. In: The Mycota IV: Environmental and microbial relationships (Eds. D. T. Wicklow and B. Soderstrom). Springervorla, Berlin, pp. 165-184.

Doehlemann, G., Ramon, W., Horst, R. J., Voll, L. M., Usadel, B. et al. 2008. Reprogramming a maize plant: transcriptional and metabolic changes induced by the fungal biotroph Ustilago maydis. Plant Journal, 56: 181–195.

Domich, K. H., Gams, W. and Andeson, T. 1980. Compeniun of soil fungi (vol. 1 and 2). Acad.Press. London. pp. 405-859,

Esmailzadeh, A., Soleimani, Z. M. J. and Rouhani, H. 2008. Exogenous applications of salicylic acid for inducing systemic acquired

resistance against tomato stem canker disease. Journal of Biological Sciences, 8(6): 1039-1044.

Fariduddin, Q., Hayat, S. and Ahmad, A. 2003. Salicylic acid influences net photosynthetic rate, carboxylation efficiency, nitrate reductase activity and seed yield in Brassica juncea. Photosynthetica, 41: 281–284.

Farkas, G. L. and Kiraaly, Z. 1962. Role of phenolic compounds in the physiology of plant diseases and disease resistance. Journal of Phytopathology, 44(2): 105–150.

Firdous, S. S., Ashgar, R., Haque, M. I. and Afzal, S. N. 2007. Development of hypersensitive response by Xanthomonas campestris pv. sesami on Lycopersicon esculentum L., and Solanum Tuberosum L., leaves . Pakistan Journal of Botany, 39(6): 2135-2139.

Fish, W., Pearkins-Veazie, P. and Collins, J. 2002. A quantitative assay for lycopene that utilizes reduced volumes of organic solvents. Journal of Food Composition and Analysis, 15: 309-317

Garcia, P.C., Rivero, R. M., Lopez-Lefebre, L. R., Sdnchez, E., Ruiz, J. M. and Romero, L. 2001. Direct action of the biocide carbendazim on phenolic metabolism in tobacco plants. Journal of Agricultural Food Chemistry, 49: 131-137.

Garcia-Limones, C., Dorado, G. , Navas-Cortes, J. A., Jimenez-Diaz, R. M. and M-Tena, M. 2008. Changes in the redox status of chickpea roots in response to infection by Fusarium oxysporum f. sp. ciceris: apoplastic antioxidant enzyme activities and expression of oxidative stress-related genes. Plant Biology, 11(2): 194 -203.

Gaur, A. C. 1985a. Phosphate solubilizing microorganisms and their role in plant growth and crop yields. In: Proceeding, National Sympsoi Soil Biology, Hissar. pp. 125-1[illegible]047

Gaur, A. C. 1985b. Phosphate solubilizing bacteria as bio-fertilizer. In: Proceeding, National Seminar on Development and Use of Biofertilizers, Ministry of Agriculture, New Delhi.

Gaur, A. C. 1990. Phosphate solubilising microorganisms as biofertilizers. Omega Scientific Publishes, New Delhi. pp. 176.

Gilman, J. C. 2001. A manual of soil fungi Biotech Books, Delhi. pp. 195-196.

Gracia de Salamone, I. E., Hynes, R. K. I. and Nelson, L. M. 2001. Cytokinin production by plant growth promoting rhizobacteria and selected mutants. Canadian Journal of Microbiology, 47: 404-411.

Guest, D. I. 1984. Modification of defence responses in tobacco and capsicum following treatments with fosetyl-Al [aluminium tris (o-ethyl phosphonate)]. Physiology and Plant Pathology, 25: 125-134.

Gupta, S., Chakraborti, D., Sengupta, A., Basu, D. and Das, S. 2010. Primary metabolism of chickpea is the initial target of wound inducing early sensed Fusarium oxysporum f. sp. ciceri race I. PLoS ONE 5(2): e9030. doi:10.1371/journal.pone.0009030.

Hayat, Q., Hayat, S., Irfan, M. and Ahmad, A. 2010. Effect of exogenous salicylic acid under changing environment: A review. Environmental and Experimental Botany, 68: 14-25.

Hayat, S., Fariduddin, Q., Ali, B. and Ahmad, A. 2005. Effect of salicylic acid on growth and enzyme activities of wheat seedlings. Acta Agron. Hung. 53: 433–437.

Hayman, D. S. 1975. Phosphorus cycling by soil microorganisms and roots. In: Soil Microbiology. A critical review (Ed. N Walker). Butterworths, London and Boston. pp. 67-92.

Horn, B. W. and Wicklow, D. T. 1983. Factors influencing the inhibition of aflatoxin production in corn by Aspergillus niger. Canadian Journal of Microbiology, 29: 1087-1091.

Houssien, A. A., Ahmed, S. M. and Ismail, A. A. 2010. Activation of tomato plant defence response against Fusarium wilt disease using Trichoderma harzianum and salicylic acid under greenhouse conditions. Research Journal of Agriculture and Biological Sciences, 6(3): 328-338.

Javaheri, M., Mashayekhi, K., Dadkhah, A. and Tavallaee, F. Z. 2012. Effects of salicylic acid on yield and quality characters of tomato fruit (Lycopersicum esculentum Mill.). International Journal of Agriculture Crop Sciences, 4(16): 1184-1187.

Jayakumar, J., Rajendran, G. and Ramakrishnan, S. 2006. Evaluation of salicylic acid as a systemic resistance inducer against Meloidogyne incognita on tomato cv. Co3. Indian Journal of Nematology, 26(1): 77-80.

Jennings, D. H. 1989. Some perspectives on nitrogen and phosphorus metabolism in fungi. In: Nitrogen, Phosphorus and Sulphur Utilization by Fungi (Eds. L. Boddy, R. Marchant and D. J. Read). Cambridge University Press, Cambridge.

Jones, J. B., Woltz, S. S., Jones, J. P. and Portier, K. L. 1991. Population dynamics of Xanthomonas compestris pv. vesicatoria on tomato leaflets treated with copper bactericides. Phytopathology, 81:714-719.

Khan, B., Khan, A. A. and Khan, M. R. 2003. Pathogenic variability among isolates of Meloidogyne javanica on Capsicum annum. Journal of Nematology, 35(4): 430-432.

Khan, M. R. and Anwer, M. A. 2007. Molecular and biochemical characterization of soil isolates of Aspergillus niger and assessment of antagonism against Rhizoctonia solani. Phytopathologia Mediterranea, 46: 304-315.

Khan, M. R. and Anwer, M. A. 2008. DNA and some laboratory tests of nematode suppressing efficient soil isolates of Aspergillus niger. Indian Phytopathology, 61(2): 212-225.

Khan, M. R., Altaf, S., Mohidin, F. A., Khan, U. and Anwer, A. 2009. Biological control of plant nematodes with phosphate solubilizing microorganisms. In: Phosphate solubilizing microbes for crop improvement (Eds. M. S. Khan and A. Zaidi). Nova Science

Publisher Inc., New York, USA. pp. 395-426.

Khan, M. M. A., Gautam, A. K. C., Mohammad, F., Siddiqui, M. H., Naeem, M. and Khan, M. N. 2006. Effect of Gibberellic Acid Spray on Performance of Tomato. Turky Journal of Biology, 30: 11-16.

Khodary, S. F. A. 2004. Effect of salicylic acid on the growth, photosynthesis and carbohydrate metabolism in the salt stressed maize plants. International Journal of Agriculture Biology, 6: 5-8.

Kumar, P., Dube, S. D. and Chauhan, V. S. 1999. Effect of salicylic acid on growth, development and some biochemical aspects of soybean (Glycine max L. Merrill). International Journal of Plant Physiology, 4: 327-330.

Larque-Saavedra, A. and Martin-Mex, R. 2007. Effect of salicylic acid on the bio-productivity of plants. In: Acid. A Plant Hormone (Eds. Hayat, S. and Ahmad, A). Salicylic Springer Publishers. Dordrecht. The Netherlands.

Li, S. L., George, E. and Marschner, H. 1991. Phosphorus depletion and pH decrease at the root-soil and hyphae-soil interfaces of VA mycorrhizal white clover fertilized with ammonium. New Phytologist, 119: 397-404.

Lowery, O. H., Roseenbrough, N. J., Farr, A. L. and Randall, R. H. 1951. Protein measurement with the Folin-phenol reagent. Journal of Biological Chemistry, 193: 265-275.

Martin-Mex, R., Villanueva-Couob, E., Herrera-Campos, T. and Larque-Saavedra A. 2005. Positive effect of salicylates on the flowering of African violet. Science of Horticulture, 103: 499-502.

Medina, A., Jakobsen, I., Vasiilev, N., Azcon, K. and Larsen, J. 2007. Fermentation of sugar beet waste by Aspergillus niger facilitate growth and P uptake of external mycelium of mixed populations of arbuscular mycorrhizal fungi. Soil Biology and Biochemistry, 39: 485-492.

Molina, A., Hunt, M. D. and Ryals, J. A. 1998. Impaired fungicide activity in plants blocked in disease resistance signal transduction. Plant Cell, 10: 1903-1914.

Mondal, G., Dureja, P. and Sen, B. 2000. Fungal metabolites from Aspergillus niger AN27 related to plant growth promotion. Indian Journal of Experimental Biology, 38(1): 84-7.

Mostafa, M. A. and Yovssef, Y. A. 1962. Studies on peritrophie mycorrhiza between Tropaeolum majus L. and two associated rhizospherie fungi. III. Effect of fungal metabolites on relative growth vigour of Tropaeoliem mains L. Egypt Journal of Botany, 111: 137-151.

O'Connell, R. J., Panstruga, R .2006. Tete and tete inside a plant cell: establishing compatibility between plants and biotrophic fungi and oomycetes. New Phytologist, 171: 699-718.

Pankaj, C. G., Shakil, N. A., Kishor, V. and Rohatgi, D. 2005. Estimation of salicylic acid and its role resistance mechanism in chickpea against Meloidogyne incognita. Indian Journal of Nematology, 35(2): 160-162.

Raju, S., Jayalakshmi, S. K., Sreeramulu, K. 2008. Comparative study on the induction of defence related enzymes in two different cultivars of chickpea (Cicer arietinum L.) genotypes by salicylic acid, spermine and Fusarium oxysporum. Australian Journal of Crop Science, 2(3): 121-140.

Ramachandran, S., Fontanille, P., Pandey, A. and Larroche, C. 2008. Stability of glucose oxidase activity of Aspergillus niger spores produced by solid-state fermentation and their role as biocatalysts in bioconversion reaction. Food Technology and Biotechnology, 46(2): 190-194.

Rao, A. B. 1990. Role of microorganisms in plant nutrition under acid conditions. In: Biofertilizers (Ed. LL Vyas). Scientific Publication, Jaudpur, Rajasthan, India. pp. 67-84.

Raper, K. and Fennell, D. 1965. The genus Aspergillus. Baltimore, MD: Williams and Wilkins Company.

Saikia, R., Yadav, M., Singh, B. P., Gogoi, D. K., Singh, T. and Arora, D. K. 2006. Induction of resistance in chickpea by cell wall protein of Fusarium oxysporum f. sp. ciceri and Macrophomina phaseolina. Current Science, 91(11): 1543-1546.

Sen, B. 2000. Biocontrol: A success story. Indian Phytopathology, 53(3): 243-249.

Shakirova, F. M. 2007. Role of hormonal system in the manisfestation of growth promoting and anti-stress action of salicylic acid. In: Salicylic Acid. A Plant Hormone (Eds. Hayat, S. and Ahmad, A.). Springer. Dordrecht. Netherlands.

Sharma, H. K., Prasad, D. and Sharma, P. 2005. Compatibility of Fungal bioagents as seed dressers with carbofuran in okra against Meloidogyne incognita. In: National Symposium on Recent Advances and Research Priorities in Indian Nematology, Dec. 9-11, 2005, IARI New Delhi. pp. 72.

Singh, R., Sindhu, A., Singal, H. R. and Singh, R. 2003. Biochemical basis of resistance in chickpea (Cicer arietinum L.) against Fusarium wilt. Acta Phytopathologica et Entomologica Hungarica, 38(1-2): 13-19.

Sperber, J. I. 1958. Solution of mineral phosphates by soil bacteria. Nature, 180: 994-995.

Tempe, J. de. 1970. Routine methods for determining the health condition of seeds in seed testing station. In: Proceedings of International Seed Testing Association, 35: 257.

Vaddar, U. B. and Patil, A. B. 2007. Studies on Grape Rhizosphere Microorganisms. Karnataka Journal of Agricultural Sciences, 20(4): 932.

Vassilev, N., Franco, I., Vessileva, M. and Azcon, R. 1996. Improved plant growth with rock phosphate solubilized by Aspergillus niger grown on sugarbeet waste. Bioresource Technology, 55: 237-241.

Vassilev, N., Vassileva, M. and Nikolaeva, I. 2006. Simultaneous P solubilizing and bio-control activity of microorganisms: potentials and future trends. Applied Microbiology and Biotechnology, 71: 137-144.

Vosburgh, W. C. and Cooper, G. R. 1941. Complex ions. 1. The identification of complex ions in solution by spectrophotometric measurements. Journal of American Chemical Society, 63(2): 437-442.

Wakman, S. A. 1927. Principles of soil Microbiology. Baltimore; Williams and Wilkins Co. pp. 600.

Wicklow, D. T., Hesseltine, C. W., Shotwell, O. L. and Adams, G. L. 1980. Interference, competition and aflatoxin levels in corn. Phytopathology, 70: 761-764.

Zhou, X. M., Mackeuzie, A. F., Madramootoo, C. A., Smith, D. L. J. 1999. Effects of some injected plant growth regulators with or without sucrose on grain production biomass and photosynthetic activity of field-grown corn plants. Agronomy and Crop Science, 183: 103-110.