RESEARCH ARTICLE

Response of metabolites of *Alternaria solani* on seed germination seedling vigour of *Solanum nigrum* L.

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Manuscript details:

Received: 24.10.2016
Accepted: 24.12.2016
Published : 06.01.2017

Editor: Dr. Arvind Chavhan

Cite this article as:
Bhajbhuje MN (2016) Response of metabolites of *Alternaria solani* on seed germination seedling vigour of *Solanum nigrum* L., *International J. of Life Sciences*, 4 (4): 615-618.

ABSTRACT

Fungal microbes secrete secondary metabolites in infested host tissues are known to create disturbances in the normal cell metabolism and cell division of somatic cells causing cytological abnormalities. The toxic metabolites obtained in nutrient medium at different growth intervals by *Alternaria solani* (Ellis & Martin) Jones & Grout were isolated from culture medium and tested for their cytological effects on somatic cells of the test material. An increase in per cent seed germination and seedling emergence without any abnormalities were recorded with seven days old metabolites treated seeds. The seed germination rate and seedling growth decreased while percent abnormalities increased with metabolites of longer duration. Many abnormalities such as anaphase bridge, laggards, diagonal metaphase and mis-orientation of mitotic spindles were observed in treated seeds.

Keywords: *Fungal metabolite, Solanum nigrum* L*, Alternaria solani*, *mutagenic.*

INTRODUCTION

The fungi of diverse group are known to secrete or excrete a variety of a multitude of low molecular weight bioactive organic compounds during their growth that are either non-toxic or toxic to host cells. The non-toxic metabolites induced stimulatory effect while toxic ones directly act on living host protoplasm, and damage the somatic cells to influence the course of disease development or symptom expression (Brakhate and Schroeckh, 2011).

Solanum nigram L. commonly known as nightshade, is a warm-season, non-tuberous medicinal plant, of *Solanaceae* family, native to Africa and widely grows as weed in moist habitat in various kinds of soils and cultivated tropical and subtropical agro climatic regions (Anonymous, 2011). It is used as medicinal plant to treat pneumonia, aching teeth, stomach ache, tonsillitis, wing worms, pain, inflammation and fever and also as hepaprotective, diuretic antipyretic (Wikipedia, 2016)

Solanum nigram L is affected with several seed borne diseases, among them *Alternaria* early blight is a serious, caused by *Alternaria solani* causing damping-off, collor rot, stem cancer, leaf blight and fruit rot

resulting in premature defoliation, reduction in size & quality of fruits, and reported to reduce the productivity to the extent of 20-30%. The pathogen also attack tomato, potato, pepper, horse, cabbage, cucumber, and zinnia (IHD. 2012). It was found to grow on stored seeds as internal seed borne and causes physiological damage to the seeds. The present paper reports response of metabolites of test pathogen on seed germinate rate and seedling vigour of *Solanum nigrum* L.

MATERIAL AND METHOD

The seeds *Solanum nigrum* L. were obtained from infested mature fruits. *Alternaria solani* was isolated from these seeds as internal seed borne pathogen employing the method suggested by ISTA (2016). Czapek's broth medium were used for toxin production. The toxin was isolated from culture filtrate in different duration (Lynch et al, 1991)

The same fungus was transferred aseptically into 35ml Czapek's broth medium in 150 ml conical flask and incubated for a period between 7 to 35 days at an interval of seven days at 28 ± 1^0C. Separate sterilized broth medium and sterile distilled water were kept as control. Seeds were soaked for 6 hours in sterile distilled water and in different duration of *Alternaria solani* metabolites in triplicate. Washing of the seeds was carried out immediately after the metabolic treatment. The parameters such as seed germination and seedling growth, cytological effects were undertaken for study.

RESULT AND DISCUSSION

A toxin is microbial metabolites released by pathogen which at very low conc. directly toxic to microbes but at higher dosage creates disturbances in normal cell division resulted to alteration or lethality in somatic cells of plants and animals (Holensein and Stoessi, 2008). The toxin isolated from the culture filtrate gave a yellow fluorescence under U.V. light either in the presence or absence of ammonia vapour. The colour of the spot on chromatogram was brick red with alkaline diazotized sulphuric acid, blue with Folin-Ciocaten reagent and yellow with alcoholic bromophenol blue. It gave no colour reaction with alcoholic ferric chloride, alkaline hydrogen phthalate and ninhydrin reagent. The reaction of the spot to various detection reagents and the U.V. absorption spectrum revealed that the toxin isolated might presumably be phenolic in nature. Similar type of toxin was isolated from *Alternaria brassicae* (Bhajbhuje *et al.*, 1992); *Alternaria solani* (Holensein and Stoessi, 2008; Carneiro *et al.*, 2010) and *Alternaria alternata* (Chung, 2012*).*

The data on percent seed percent seed germination, seedling height and per cent abnormalities were recorded for each treatment and control (Table 1). Per cent seed germination and seedling height was found to be enhance by 6.5% and 9.2% respectively over control when seeds treated with seven days old metabolite without cytological abnormalities (Table 1). An increase in seed germination and seedling growth rate were reported in *Brassica oleracia & B.*

Table 1: Effect of *Alternaria solani* metabolites on seed germination and seedling virour on three varieties of *Solanum nigrum*L.

Duration of treatment (Days)	Per cent germination	Seedling height (cm)	Per cent abnormalities
7	89.25 (+06.57)	5.11 (+09.18)	-
14	76.50 (-09.90)	4.12 (-11.97)	7.14 ± 0.02
21	62.25 (-21.33)	3.62 (-22.65)	9.21 ± 0.03
28	56.75 (-25.67)	3.22 (-31.19)	10.86 ± 0.04
35	47.50 (-43.28)	2.98 (-36.32)	16.42 ± 0.03
42	42.75 (-48.95)	2.42 (-48.39)	
Czepak's medium	84.50	4.72	-
Control (D.W.)	83.75	4.68	-
C.D. (0.05)	1.2	1.3	

1. Values in parenthesis indicate per cent reduction or increase in term of control.
2. ± indicates standard error

Campestris (Bhajbhuje *et al.*, 1992) with five days metabolite treatment. Holensein and Stoessi (2008) reported secretion of metabolites by *Alternaria solani* in very dilute conc. at early stages of growth that may act as growth promoter to host. In the present study, the increase in seed germination rate and seedling emergence may be attributed to secretion of non-toxic primary metabolites by the pathogen at early stages of its growth that may acts growth promoters.

The decrease in rate of seed germination and seedling height was recorded to the extent of 48% whereas the abnormalities were increased by 7.17 – 16.4% over the control when seeds treated with 14 to 42 days old metabolites (Table 1). Control seeds did not express any change. These results confirmed with the results obtained by Lynch et., al (1991) in *Solanum tuberosum* L. and Bhajbhuje *et al.*, (1992) in *Brassica. Oleracia* L. *& B. campestris* L. The phenomenon indicates that metabolites are both toxic and mutagenic as far as the present plant material is concerned.Many cytological abnormalities were observed in the treated seeds such as anaphase bridge, fragment, diagonal metaphase and mis-orientation of the mitotic spindle

Mutagenic effect of mycotoxin has been highlighted by Bhajbhuje and Thakre (1989), Carneiro *et al.* (2010); Brakhate and Schroeckh (2011); Chung (2012). Holensein and Stoessi (2008) has reported the secretion of Altersolarol-A and alternaric acid by *Alternaria solani* during its mycelial & reproductive growth in culture medium. The pathogen also found to secrete dibenzopyron, tetranic acid, altertoxin-I & II as major toxic mutagens and other 36 chemically known metabolites in culture medium (Bemnann, 1986). Carneiro *et al.*, (2010) reported the toxicity of metabolites produced by *Alternaria solani* on early blight of *Lycopersicon esculentum* Mill. In present study, seed germination rate and seedling growth were found to be decreased in treated seeds with 10 to 30 days metabolites. The toxicity of fungal metabolites was intensified on longer duration of the treatment may be attributed to the more accumulation of secreted secondary metabolites in culture medium for longer duration of fungal growth induced inhibition of seed germination and seedling emergence with chromosomal abnormalities (Bhajbhuje *et al.*, 1992; Holensein and Stoessi, 2008; Imazaki, *et al.*, 2010; Chung, 2012).

The chemicals are known to cause chromosomal breakage and create disturbances in the normal mitotic cell division that alter regular metabolism of somatic cell (Chung, 2012). From the present study it is revealed that the toxin produced is of phenolic in nature which disturbed the normal cell division in the active growing root tip. Lynch *et al.*, (1991); Bhajbhuje *et al.*, (1992); Carneiro *et al.*, (2010) and Chung (2012) have also reported close relationship between the duration of treatment and per cent chromosomal aberrations in *Triticum aestivum*, *Trigonella foenum-graceum, Solanum tuberosum, Brassica. oleracia & B. campestris, Lycopersicon esculentum* respectively.

Acknowledgements:
The author records his appreciation to the Head, P.G. Deptt. of Botany, RTM, Nagpur University in the matter of providing facilities and Dr .R.P. Thakre, Ex- Professor, for valuable guidance & encouragement during the tenure of which this work was done.

Conflicts of interest: The authors stated that no conflicts of interest.

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