International Journal of Researches In Biosciences, Agriculture & Technology | Mar. 2015 Special Issue- I | ISSN No. (Online) 2347-517X

Potential of Metabolites of *Alternaria solani* Against *Sorghum vulgare* L. in Laboratory Bioassay

R. Fule[1] and M. N. Bhajbhuje[2]
[1]P.G. Department of Botany, RTM Nagpur University Campus, Nagpur
[2]Jawaharlal Nehru Mahavidyalaya, Wadi, Nagpur
dr_mnbhajbhuje@rediffmail.com

Abstract

Fungal metabolites are known organic compounds produced by diverse group of fungal organisms in infested host tissues as well as in nutrient growth medium as results of diverse metabolic activities or chemical reactions occurring in every functional cell during its growth. A leaf blight fungal pathogen, *Alternaria solani* produced metabolites in culture filtrate were isolated for a period between 7 to 28 days at an interval of seven days in Czapek's broth medium and studied for their potential on seed germination, seedling vigour and fresh biomass of seedlings of *Sorghum vulgare* L. An enhancement in seed germination; length of shoot/root and fresh biomass of seedlings over control was recorded with seven days old metabolites treated seeds. The toxicity of the culture filtrate increased with longer duration of treatment and exhibited inhibitory effect. The rate of seed germination, seedling vigour and fresh biomass of seedlings reduced while percent dead seeds and transformation of germinated seeds to abnormal seedlings increased with 14-28 days old culture filtrate containing metabolites treatment. The seed coat of treated hard seeds becomes soft, but seeds did not germinate. The metabolites from seven day old culture filtrate served as growth promoter while metabolites of longer duration are toxic and acts as growth inhibitor.

Keywords: *Alternaria solani*, metabolites, inhibition, enhancement, seed viability, seedling emergence,

Introduction

Microbes are ubiquitous and constitute largest group of living creatures with varying potentials in biochemical, physiological and nutritional mode and play a key role in numerous fields including agriculture, biotechnology and biological engineering (Brakhage and Schroeckh, 2011). During static growth and proliferation in favourable environment, several microbes release or excrete various active metabolites in response to regularly occurring diverse metabolic reactions in every functional cell. These metabolites at low concentration enhance growth of seedlings and serve as growth promoter but its higher dosage induce stunted growth, creating disturbances in normal metabolism and cell cycle (Bhajbhuje and Pathode, 2014) and may acts as mutagens resulting to mutants that exhibit appearance of some new phenotypic traits in subsequent generation (Venda kumari et al., 2012).

Sorghum vulgare is a principle food crop belongs to family Poaceae that can thrive in warm climate worldwide with little rainfall providing nutrients for millions of people. It is native to Australia, with some extending to Africa, Asia, Mesoamerica and certain islands in the Indian and Pacific Oceans. The small, round caryopsis seed is store house of carbohydrate, protein, minerals such as calcium, selenium, manganese, iron and B-complex vitamins hence used for bread making, food for cattle, horses and poultry birds and also in brewing industries. Apart from its use as food, it contains some chemical compounds that are protective against cancer, heart disease, heavy menstrual flow, and tumor growth. It contains no gluten

which makes it an excellent nutrient source for individuals with celiac disease. *Sorghum* ranks fifth most important cereal crop grown on the globe. U.S. ranks first in *Sorghum* production in the World with 9.8 million tons output followed by Mexico with 7.3 million tons. India realized 5.5 million tons in 2013/14 season (Wikipedia, 2015).

Alternaria is remained associated with a wide variety of substrates including seeds and its several species remains as an increasing threat to majority crops around the globe causing several diseases in plants (Wagh et al., 2012). Among these, *Alternaria* leaf blight of vegetables is serious incited by *Alternaria solani* causing damping off of seedlings, producing brown to black leaf spots lead to a reduction of leaf count, adversely affect annual productivity to the extent of 20-30% (Bhajbhuje, 2013). The pathogen can survive as conidia on the seed surface or as mycelium inside the seed coat and produced toxic metabolites during their growth in storage (Holensein and Stoessi, 2008). The toxin from secondary metabolites rapidly penetrates into the host as well as non-host plant tissues, directly acts on living host protoplasm and damages cell components of actively growing cells to influence the course of symptom expression in host plant (Brakhage and Schroeckh, 2011). Tsuge et al (2013); Bhajbhuje and Pathode (2014) have studied role metabolites by *Alternaria* species in plant system. Presently potential of metabolites on seed germination; seedling growth and biomass of shoot/root has so far not been reported from *Sorghum*. It seemed to be worthwhile to study these parameters using *Alternaria solani* metabolites with *Sorghum vulgare*.

Material and Methods:

The stored seed samples of *Sorghum vulgare* L has been collected in cotton bags from different stockiest and retailors and screened for apparent deformities or discoloration. An inoculum of *Alternaria solani* isolate obtained from 6 days old culture was transferred aseptically into 35ml Czapek's broth medium and incubated for a period between 7 to 28 days at laboratory condition. Separate sterilized broth and sterile distilled water were kept as control. At an interval of 7 days, the culture filtrate containing metabolites was tested for seed germination; seedling growth and fresh biomass of seedlings of *Sorghum vulgare*.

Healthy water soaked sterilized with aqueous solution of 0.1% mercuric chloride seeds were placed for 3 hours in 7 to 28 days old culture filtrate containing metabolites of *Alternaria solani* in triplicate. Control seeds were soaked in sterile distilled water. After washing for five consecutive times, the moistened treated and untreated control seeds were transferred to sterile blotting paper folds in slots for germination and seedling growth studies. The slots were covered with glass cabinet to avoid spoilage of seeds by any saprophyte contaminants. The moisture content of blotter paper containing seeds has been maintained by addition of sterile distilled water when required. The seedling height was measured and per cent seed germination was recorded on eighth day. The seedlings raised from germinating seeds were graded as normal and abnormal seedlings defined by Ismail et al., (2012).

Results and Discussion:

Seed is critical input for substantial agriculture as it is a container of embryos of a new generation and vehicle for the spread of new life (Saskatchewan, 2013). Leaf blight pathogen of vegetables, *Alternaria solani* was grown in artificial Czapek's broth nutrient medium for a period between 7 to 28 days at laboratory condition. The metabolites obtained from culture filtrate of pathogen at an interval of 7 days were tested for seed germination; seedling growth and fresh biomass of seedlings of *Sorghum vulgare*.

The results of present investigation reveals that seed germination rate; length of shoot/ root and fresh biomass of seedlings was recorded to enhance by 5.3%, 8.9%, 6.3% and 0.9%. while count of normal seedling in terms of percentage was increased by 3.5% whereas a count of dead seeds was recorded to decline by 21.5% over control respectively with seven days old metabolites treatment (Table 1). These results are in conformity with the earlier finding to these parameters involving *Aijung rice* (Islam and Borthakur, 2012) with seven days metabolite treatment. Sung et al., (2011) reported higher seed germination and seedling growth rates in Canola over control in cucumber and tomato plants receiving metabolic treatment of culture filtrate of *Shimizuomyces paradoxus*. Metabolites of Fusarium *oxysporum f. sp. lycopersici* and *Alternaria solani* enhanced seed germination rate of tomato (Bhajbhuje, 2013). Literature survey revealed that the secretion primary metabolites and some growth regulating factors in filtrate by *Alternaria solani* at early stages of fungal growth that enhanced the seed germination rate, seedling emergence (Chung, 2012; Bhajbhuje, 2014). These primary metabolites at low concentration served as growth promoter and induced vigorous growth by stimulating phosphorylation in the host tissues in association of Ca^{2+} and Mg^{2+}(EFSA, 2011). A growth stimulating effect in response to seed germination rate and seedling emergence over control in present study may be attributed to secretion of primary metabolites by pathogen at early stages of its growth that may serve as growth promoters (Sung et al., 2011).

The results of table 1 revealed that per cent seed germination declined by 2.1- 13.7%; the shoot length of seedling reduced to the extent of 8.9-51.8%; root length declined by 2.5-50.6%; the biomass of fresh shoot reduced by 2.6 – 37.1%; biomass of fresh root declined to the extent of 2.1-30.0%; per cent normal seedling reduced by 2.7-30.2% whereas a count of dead seeds was found to increase by 18 – 46%.1% over the control when seeds treated with 14 to 28-days old metabolites (Fig. 1-4). Control seeds did not express any change. These results were confirmed with earlier findings of Bhajbhuje (2013) in *Solanum melongena* Mill; and Venda kumari et al., (2014) in *Brassica carinata* and *B. braun*. Fungal isolates, *Alternaria alternata* and *Colletotrichum capsici* produced nonspecific toxic metabolites in culture filtrate which reduced seed germination, root length, shoot length and vigour index of the seedlings of chilli, rice, mungbean, maize, cotton, groundnut, okra, eggplant, cucumber and tomato (Anand et al., 2008). The phenomenon indicates that metabolites are both phytotoxic and mutagenic as far as the present plant material is concerned.

Percent hard seeds were reported to increase when treated with metabolites of longer duration (Table 1). The results are in agreement with earlier findings of Sung et al., (2011) who reported higher percent of ungerminated hard seeds of cucumber and tomato. Mycotoxin secretion by several filamentous fungi has been reported in many crops (Vedna kumari, et al., 2014). *Alternaria* species can invade crops at the pre- and post-harvest stage and cause considerable losses due to leaf spot, early blight, rotting of fruits and seeds, may results to secretion of a range of mycotoxins as well as other non-toxic metabolites under favourable environment in plants. Several toxic metabolites of major toxicological importance including, HST-toxin, AAL-toxins, tenuazonic acid, alternariol monomethyl ether, alternariol, altenuene, and altertoxin I produced by *Alternaria* in artificial medium during its growth period (Holensein and Stoessi, 2008).

Phytotoxic and mutagenic and effect of mycotoxins has been highlighted by Chung (2012) and Venda Kumari et al., (2014). Mycotoxin responds to inducing micro-mutation, cause carcinogenic disorders in experimental animals and also pose variety of health hazards in domestic animals and human beings (ESFA, 2011) . Alternariol-induced cytotoxicity is mediated by activation of the mitochondrial path-way of apoptosis. Higher dosages of tenuazonic acid had inhibitory effect on protein synthesis that lost seed viability (Chung, 2012). The low concentration of Altertoxin III, caused negligible damage at early stages, its higher concentration in the nutrient medium, reported causing more damage to the leaf surface at a later stage (Sung et al., 2011). Per cent seed germination and seedling height were found to be decline in treated seeds with 14-28 days metabolites (Fig. 1-3). The toxicity of fungal metabolites was intensified on longer duration of the treatment may be attributed to the more accumulation of secreted metabolites on longer duration, may induced inhibition in seed germination and seedling emergence (Sung et al., 2011; Bhajbhuje, 2014). The growth of the isolated pathogen results in changes associated with various cellular, metabolic and chemical alterations, including damage to the DNA, RNA and protein synthesis, enzyme degradation and inactivation, loss of membrane integrity, lowering of ATP, decline in sugar and protein content, inability of ribosomes to dissociate, starvation of meristematic cells, increase in seed leaches and fatty acid content, reduced respiration and accumulation of toxic substances which leads to spoilage of seeds(Jyoti and Malik, 2013). On the other hand, the prevalence of active fungal spores in seeds suggests an imminent public health danger since their mycotoxins produced in seeds may lead serious and devastating clinical conditions in the consumers (ESFA, 2011); Chung (2012); Tsuge et al., (2013). Sung et al., (2011) and Bhajbhuje (2014) have also reported close relationship between the duration of treatment and process of inhibition of seed germination and seedling emergence in crop plants.

Table. 1- Record of per seed viability, length of shoot and roots of metabolite treated and untreated seed of *Sorghum vulgare* L in laboratory bioassay.

S. No.	Duration of treatment ((Days)	Seed viability			Seedling height		Biomass of Fresh seedling		Nature of Seedlings	
		Per cent Seed germination	Non-viable seeds		Shoot length (cm)	Root length (cm)	Shoot fresh weight (g)	Root fresh weight (g)	Normal seedlings (%)	Abnormal seedlings (%)
			Dead seeds (%)	Hard seeds (%)						
1.	7	85.4 (+5.3)	-	14.6 (-21.5)	6.1 (+8.9)	8.4 (+6.3)	2.000 (+1.94)	0.904 (+0.89)	83.0 (+3.5)	17.0 (-14.1)
2.	14	79.4 (-2.1)	2.0	18.6 (0.0)	5.1 (-8.9)	7.7 (-2.5)	1.910 (-2.65)	0.877 (-2.12)	78.0 (-2.7)	22.0 (+11.1)
3.	21	72.1 (-11.1)	0.6	27.3 (+46.8)	4.4 (-21.4)	7.1 (-10.1)	1.762 (-10.19)	0.839 (-6.36)	67.0 (-16.5)	33.0 (-66.7)
4.	28	70.0 (-13.7)	8.0	22.0 (+18.3)	2.7 (-51.8)	3.9 (-50.6)	1.234 (-37.11)	0.627 (-30.02)	56.0 (-30.2)	44.0 (-122.2)
5.	Czapek's broth	81.4 (+0.4)	0.6	18.0 (-3.2)	5.7 (+1.8)	7.9 (0.0)	1.974 (+0.61)	0.896 (0.00)	80.2 (0.0)	19.8 (0.0)
6.	Control (D.W.)	81.1	0.3	18.6	5.6	7.9	1.962	0.896	80.2	19.8
Values in parenthesis indicate per cent increase or decrease over control										

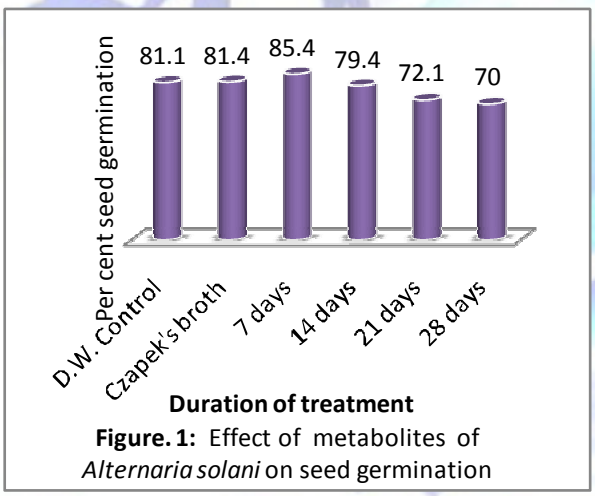

Figure. 1: Effect of metabolites of *Alternaria solani* on seed germination

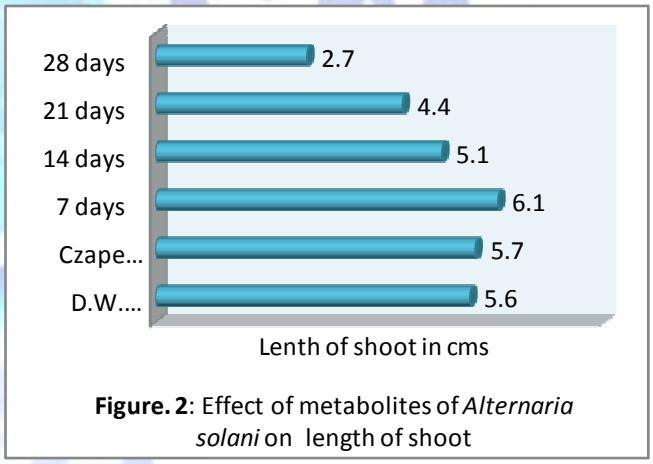

Figure. 2: Effect of metabolites of *Alternaria solani* on length of shoot

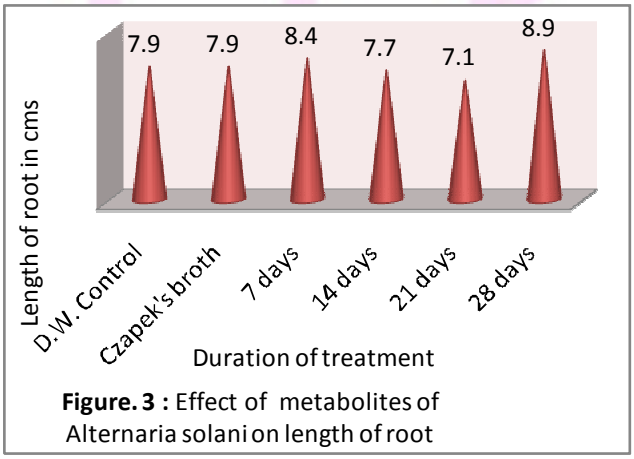

Figure. 3 : Effect of metabolites of Alternaria solani on length of root

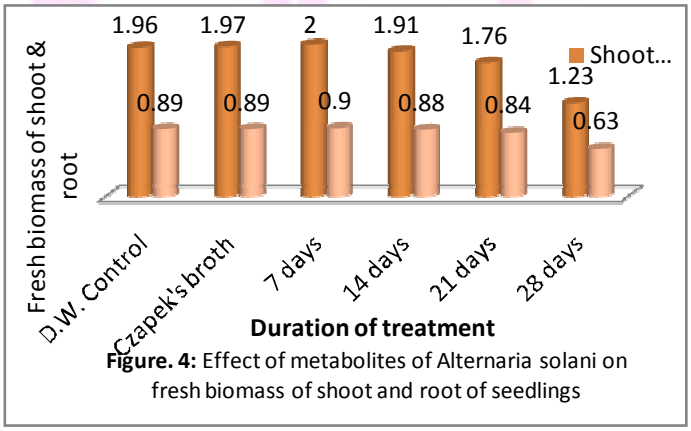

Figure. 4: Effect of metabolites of Alternaria solani on fresh biomass of shoot and root of seedlings

Conclusion:

Alternaria solani, a leaf spot insisting fungal pathogen of several vegetables produced metabolites in nutrient medium during its growth. Primary metabolites are secreted at early stages of growth may serve as growth promoter, and exhibited growth stimulating effect by enhancing seed germination rate; seedling vigour and also increased the biomass of fresh shoot and roots. The toxicity of metabolites was intensified on longer duration of treatment attributed to release of secondary metabolites, serves as growth inhibitor, inhibited seed germination; length of shoot/root and fresh biomass of seedlings with greater count of abnormal seedlings. Primary metabolites may be beneficial to crop plants as they enhance seedling growth in plants. The toxic secondary metabolites may be used as mutagens in evolving high yielding mutant varieties of economically important crops.

Acknowledgement:

The authors gratefully acknowledges the facilitation of this work by Prof. and Head, Dr Mrs. Alka Chaturvedi and Dr .R.P. Thakre, Ex- Professor, P.G. Department of Botany, RTM, Nagpur University, Nagpur.

References:

Anand T, Bhaskaran R, Raghuchander T (2008) Production of cell wall degrading enzymes and tons by *Colletotrichum capsici* and *Alternaria alternata* causing fruit rot of chilies. *Jour. of Plant Protection Res.*, 48(4) : 437-451.

Bhajbhuje MN (2013) Karyotoxicity of fungal metabolites in *Trigonella foenum-graceum L. Int. Res. Jour of Sci., and Engg.*, 1(2) : 47-54.

Bhajbhuje MN (2014) Response of fungal metabolites on meristematic cells from roots of *Vigna mungo* (L.) Hepper. *Asiatic Jour. Biotech Resources. Special issue*, 4(3) : 41-47.

Bhajbhuje, M. N. and Punam Pathode. 2014. Role of metabolites on viability and seedling emergence of *Triticum aestivum* L. *Int. Jour. of Life Sci.*, Special Issue A2 Oct. 2014 : 6-10)

Brakhage AA, Schroeckh V (2011) Fungal secondary metabolites in strategies of activate silent gene clusters. *Fungal Gene. Biol.*, 48(1): 15-22.

Chung Kung-Ren (2012) Stress response and pathogenicity of Necrotrophic pathogen *Alternaria alternata. Scientific,* 20(12): 635-641.

EFSA (2011). Scientific Opinion on the risks for animal and public health related to presence of *Alternaria*
toxins in feed and food. *EFSA Journal*, 9(10): 2407.

Holensein, J.E., and Stoessi, A. (2008). Metabolites of *Alternaria solani* Part IX: Phytotoxocity of Altersolarol- A. *Envi. Health Penlt.,*108(2) : 143-147.

Ismail M, Anwar SA, Ul-Haque MI, Iqbal A, Ahmad N, Arain MA (2012). Seed borne fungi associated with cauliflower seeds and their role in seed germination. *Pakistan Jour. Phytopathology*, 24(1) : 26-31.

Jyoti, Malik CP(2013) Seed deterioration: A review. *International Journal of Life Sci. Biotech and Pharma Res.*, 2(3): 373-386.

Madhavi M, Kavita A, Vijayalaxmi M (2012) Studies of *Alternaria porri* (Ellis) Ciferri pathogenic to Onion (*Allium cepa* L.). *Archives of Applied Sci. Res*., 4(1) : 1-9.

Saskatchewan (2013) Guideline for seed borne diseases of pulse crops. Agricultural Knowledge Centre at 1- 866-457-2377 www.agriculture.gov.sk.ca/seed-testing labs.(Retrieved April 10, 2014).

Sung GH, Bhushan S, Park KB, Park SK, Han JM. (2011). Enhancing effect of *Shimizuomyces paradoxus* on seed germination and seedling growth of Canola, Plant growth of Cucumber and Harvest of tomato. *Mycobiology*, 39(1): 7-11.

Tsuge T, Harimoto Y, Akimitsu K, Ohtani K, Kodama M, Akaqi Y, Equsa M, Yamamoto M, Otani H (2013). Host-selective toxins produced by plant pathogenic fungus *Alternaria alternata*, *Microbiol. Rev.*, 37(1): 44-66.

Vedna kumari, Kumar A, Choudhary HK, Prasad R, Jambhulkar, Sharma S. (2014). *In vitro* screening method: An efficient tool for screening Alternaria blight resistance/tolerance during early generations in Ethiopian mustards (*Brassica carinata , B. braun). African Jour. Agric. Res*., 9(1) : 137-143.

Wagh P, Sinha S, Singh HK, Khare UK (2013) Pathogenic behavior of *Alternaria alternata* and phytotoxicity of its culture filtrates on *Lepidium sativum*: A medicinal herb of immense pharmacological potential. *The Bioscan*, 8(2) : 643-647.

Wikipedia (2015) *Sorghum vulgare*, Org. en.wikipedia,org/wiki. Inc . (Retrieved March. 5, 2015).