RESEARCH ARTICLE

Role of fungal metabolites on seed viability and seedling emergence of *Triticum aestivum* L.

Bhajbhuje MN[1]*and Pathode Punam R[2]

[1] *Dept. of Botany, Jawaharlal Nehru Mahavidyalaya, Wadi, Nagpur-23 (M.S.) India*

[2]*P. G. Department of Botany, RTM Nagpur University, Nagpur 440 033 (M.S.) India.*

**Corresponding Author Email: dr_mnbhajbhuje@rediffmail.com*

Manuscript details:

Date of publication 18.10.2014

Available online on http://www.ijlsci.in

ISSN: 2320-964X (Online)
ISSN: 2320-7817 (Print)

Editor: Dr. Arvind Chavhan

Cite this article as:
Bhajbhuje MN and Pathode Punam R (2014)Role of fungal metabolites on seed viability and seedling emergence of *Triticum aestivum* L., *Int. J. of Life Sciences,* Special issue A2: 6-10.

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

ABSTRACT

Fungal metabolites are well known low molecular weight, biconcave organic compounds created or secreted or excreted by diverse group of fungal organis- ms as results of diverse beneficial or detrimental activities or chemical reactions occurring in every functional cell during its growth and metabolism. The metabolites produced in growth nutrient medium by *Alternaria triticina*, a serious causal pathogen of leaf blight of *Triticum aestivum* L. were isolated from culture filtrate for a period between 5 to 25 days at an interval of five days in Czapek's broth medium and tested for their effects on seed viability and seed emergence of wheat. An increase in per cent seed germination and shoot length of seedling over control were recorded with five days old metabolites treated seeds. The rate of seed germination and seedling emergence declined while percent dead seeds and abnormal seedlings increased with metabolites of longer duration. The seed coat of treated hard seeds becomes soft, but seeds did not germinate. The metabolites from five day old culture filtrate served as growth promoter while metabolites of longer duration are toxic and acts as growth inhibitor.

Key words : *Alternaria triticina*, fungal metabolites, seed viability, seedling emergence, phytotoxic.

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). Majority of microbes release or excrete various active metabolites during their static growth and proliferation in favourable environment due to constantly occurring diverse metabolic reactions in every functional cell, which at low concentration enhance growth of plant seedlings and serve as growth promoter. Higher dosages of metabolites of fungal origin induce stunted growth, creating disturbances in normal karyokinesis of cell cycle, leads to chromosomal alteration and cause lethality (Bhajbhuje, 2013) and also may acts as

mutagens resulting to mutants that exhibit appearance of some phenotypic variations in resultant seedlings in subsequent generation (Venda *et al*., 2012).

Wheat (*Triticum aestivum* L.), one of the world's main widely planted staple nutritious food crop for more than one third of the world population is grown extensively in every continent around the globe except Antarctica for its amber-coloured non-dehiscent caryopsis, a single seeded fruit, as it is proved as an excellent health-building food and leading source of vegetable protein, minerals, Vit-B and dietary fibre in human diet, contributing 20% of all calories and proteins to the world diet than other major cereals (Wikipedia, 2014). Wheat seed is known for its potential longevity and has multiple applications as whole grain to improve nutrition, boost food security, foster rural development, support sustainable land care and for its value added products (Taylor and Koo, 2011). India is second leading producer of bread wheat on the globe, contributing 14.1% of the World's total annual output. Lion's share of India's production, accounting for over 32.77% of the nation's total output is contributed by Uttar Pradesh followed by Punjab. Whole grain provides nearly 55% of carbohydrate and 20% of the food calories and mostly used as animal feed as well as raw material for ethanol production, brewing of wheat beer, for cosmetics while white flour from seed endosperm is used for making of bread, preparing zero cholesterol confectionary products, biscuits, pasta, noodles, yeast breads; cakes, cookies, crackers and pastries Asides from being used as food, wheat has several medicinal virtues including anticancer property (Wikipedia, 2014).

Deuteromycetous ubiquitous notoriously destructive plant pathogen, *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 *Triticum aestivum* L.is serious incited by *Alternaria triticina* 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% (Mamgain *et al*., 2013). *Alternaria triticina* has been first recorded from India and initially isolated from wheat leaves as parasite and later on from stored wheat seeds as saprophyte and has shown to be seed transmitted in wheat. Moreover, 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. The infected seeds are often shriveled, reduced in size with a brown discolouration of the seed surface and loss the weight to the extent of 46-75% (Prabhu and Prasada, 1966). 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) have studied role metabolites by *Alternaria* species in plant system. Presently specific role of fungal metabolites on seed germination and seedling emergence has so far not been reported from wheat. It seemed to be worthwhile to study parameters concerning to seed germination and seedling vigour using *Alternaria triticina* metabolites with wheat.

MATERIALS AND METHODS

A composite seed sample in storage of bread wheat (*Triticum aestivum* L) collected in cotton bags from different cultivators has been screened for apparent deformities or discoloration. *Alternaria triticina*, an incitant of early *Alternaria* leaf blight of wheat was isolated from infested wheat seeds as an internal seed borne pathogen following the technique of ISTA (2014). An inoculum of *Alternaria triticina* isolate obtained from 6 days old culture was transferred aseptically into 35ml Czapek's broth medium and incubated for a period between 5 to 25 days at laboratory temperature and shaken every day. Separate sterilized broth and sterile distilled water were kept as control. After an interval of 5 days, the culture filtrate containing metabolites was tested for seed germination and seedling growth of wheat.

Healthy seeds sterilized with aqueous solution of 0.1% mercuric chloride were soaked for one hour in sterile distilled water to soften the seed coat. Hundred water soaked seeds were placed for 3 hours in 5 to 25 days old culture filtrate containing metabolites of *Alternaria triticina* in triplicate. Washing of the seeds was carried out immediately after the metabolic treatment. 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). Recently upgraded standard agar plating method of ISTA (2014) was used for detection of *Alternaria triticina* on stored wheat seeds. It is in agreement with the earlier findings of Mathur and Kongsdal (2003) who recommended agar plating method for detection of *Alternaria triticina* in wheat seeds. However, the agar plate method was proved superior in respect of isolation of deeply seated fungal pathogen and sporulation as this medium is a jelly and rich source of carbon as well as other essential micronutrient for fungal proliferation (Chung, 2012).

The results of present investigation reveals that seed germination rate and shoot length of seedlings was recorded to enhance by 11.8% and 13.1%; per cent normal seedling increased by 40.8% whereas a count of dead seeds was confined to reduce by 38.8% over control respectively in with five 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); and *Vigna mungo* (Bhajbhuje, 2014) with five to 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*. Moreover, a conidial suspension of $1.0x10^4$/ml induced the highest growth stimulating effects on the total plant length in cucumber. Metabolites of *Trichoderma harzianum* induced germination wheat seeds with hard seed coat (Mokhtar and Dehimat, 2013) while *Fusarium oxysporum f. sp. lycopersici* and *Alternaria solani* metabolites enhanced seed germination rate of tomato (Raithak and Gachande, 2013). Several researchers reported secretion primary metabolites and some growth regulating factors in filtrate by *Alternaria alternata* and *A. solani* at early stages of fungal growth that enhanced the seed germination rate, seedling emergence (Chung, 2012; Raithak and Gachande, 2013; 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).

Table 1: Record of per cent seed viability and shoot length of seedlings receiving metabolic treatment to wheat seeds (*Triticum aestivum* L.)

Duration of treatment (Days	Seed viability			Seedling emergence		
	Per cent seed germination[1]	Ungerminated seeds		Shoot length of seedlings (cms)	Nature of seedlings	
		Dead seeds (%)	Hard seeds (%)		Normal seedlings (%)	Abnormal seedlings (%)
5	85.3 (+11.8)	11.2 (-38.8)	3.5 (-32.7)	14.32 ± 0.02 (+13.06)	90.0 (+40.8)	10.0 (-72.2)
10	73.3 (-4.1)	20.0 (+11.1)	6.7 (+21.8)	10.3 ± 0.03 (-6.4)	84.8 (+32.7)	15.2 (-57.9)
15	72.0 (-5.8)	20.7 (+15.0)	7.3 (+32.7)	9.38 ± 0.03 (-14.6)	73.6 (+15.2)	26.4 (-26.8)
20	64.5 (-15.6)	29.0 (+61.1)	6.5 (+18.2)	8.87 ± 0.02 (-19.4)	66.2 (+3.6)	33.8 (-6.8)
25	58.0 (-24.2)	32.3 (+79.4)	9.7 (+76.4)	8.31 ±0.05 (-24.5)	58.5 (-8.4)	41.5 (+14.9)
Czepak's medium	83.0 (+8.5)	11.3 (-37.2)	5.7 (+3.6)	12.03 ± 0.02 (+4.6)	63.8	36.2
Control (D.W.)	76.5	18.0	5.5	11.01±0.02	63.9	36.1

1. Average of 300 germinated seeds; 2. Values in parenthesis indicate per cent reduction or increase over control 3. ± indicates standard error

Moreover, the low concentration of these metabolites did not express any phenotypic variation in seedling receiving treatment. 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. Siderophores produced by microbes improve nutrient acquisition, hormonal stimulation, disease suppression and the induction of resistance (Sung *et al*., 2011).

The results of table 1 revealed that per cent seed germination declined by 4.1% to 24.2%; the shoot length of seedling reduced to the extent of 6.4% to 24.5%; per cent normal seedling declined by 8.4% to 40.8% whereas a count of dead seeds was found to increase by11.1% to 79.4% over the control when seeds treated with 10 to 25days old metabolites. Control seeds did not express any change. These results were confirmed with earlier findings of Madhavi *et al*., (2012) in *Allium cepa* L.; Bhajbhuje (2013) in *Solanum melongena* Mill.; and Venda kumari *et al*., (2014) in *Brassica carinata* & *B. braun*. Anand *et al*., (2008) investigated that *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. Wagh *et al* (2013) reported t *Alternaria* leaf spot *in vitro* and *in vivo* in *Alternaria alternata* inoculated plantlets and detached leaves of *Lepidium sativum*. The phenomenon indicates that metabolites are both phytotoxic and mutagenic as far as the present plant material is concerned.

Various factors are considered responsible for non-germination of seeds, among them, the hard seed, become barrier for seed imbibition. In present study, the ungerminated seeds have been categorized into hard and dead seeds. Percent hard seeds were reported declined with increase of dead seeds 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 seeds of cucumber and tomato. Jyoti and Malik (2013) reported the secretion of cell wall, cellulose tannin and other chemicals degrading enzymes by the fungal microbes, which may be induce softening of seed testa. It may be attributed to the softening of seed coat by series of chemical reactions on seed testa followed by diffusion of aqueous solution of metabolites to embryonic cells leading to increase in percent dead seeds.

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 (Wikipedia, 2014). *Alternaria alternata* produced several toxic metabolites of major toxicological importance including, HST-toxin, AAL-toxins, tenuazonic acid, alternariol monomethyl ether, alternariol, altenuene, and altertoxin I 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). The mycotoxins are known to cause chromosomal breakage, create disturbances in normal karyokinesis in mitotic cell division, alter regular metabolism & cell membrane permeability and also induced physiological and biochemical changes in host cells leading to rapid increase of electrolyte loss and decline in the membrane potential of metabolically active meristematic cells of the plant system (Sung *et al*, 2011; Bhajbhuje, 2013). 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 10-25 days metabolites (Table 1). 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, 2013). 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 & 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.

CONCLUSION

Alternaria triticina a leaf spot insisting fungal pathogen of wheat 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 and seedling vigour. The toxicity of metabolites was intensified on longer duration of treatment attributed to release of secondary metabolites, serves as growth inhibitor, reduced seed germination and seedling vigour 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 crop plants.

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. *J. of Plant Protection Res.,* 48(4) : 437-451.

Bhajbhuje MN (2013) Karyotoxicity of fungal metabolites in *Trigonella foenum-graceum L. Int. Res. J. of Sci. & 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.

Brakhage AA, Schroeckh V (2011) Fungal secondary metabolites in strategies of activate silent gene clusters. *Fun.Gene.Biol.,*8(1):15-22

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

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

Holensein JE, 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 J. Phytopath.*, 24(1):26-31.

ISTA (2014) International Rules for Seed Testing: *International ISTA News Bulletin* 2014. Zurich, Switzerland.

Jyoti, Malik CP(2013) Seed deterioration: A review. *International Journal of Life Sci. Biotech & 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.

Mamgain A, Roychoudhary, Jagatpati T (2013) *Alternaria* pathogenicity & its strategic controls. *Res. Jour. of Biology*, 1 : 1-9

Mokhtar H, Aid Dehimat (2013) Study of *Trichoderma harzianum* filtrate on viability of some hard wheat seeds and on their interior associated fungi. *Agric., Biol. Jour. North America*, 4(1) : 48-53.

Prabhu AS, Prasada R (1966) Pathological and epidemiological studies on leaf blight of wheat caused by *Alternaria triticina*. Indian Phytopathol., 19 : 95-111.

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 rowth of Cucumber and Harvest of tomato. *Mycobiology*, 39(1): 7-11.

Taylor RD, Koo WW (2011) Outbreak of the U.S. and World wheat industries 2010-2020. Centre of Agricultural policy and Trade studies, North State University, Fargo, North Dakota58108-6050.

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 (2014) Fungal metabolites, Org. en.wikipedia,org/wiki. Inc . (Retrieved April. 7, 2014).