RESEARCH ARTICLE

Response of metabolites from culture filtrates of *Alternaria* species against *Triticum aestivum* L

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ABSTRACT

Metabolites are known products of enzyme-catalyzed reactions that occur naturally within cell. The potential of metabolites of two *Alternaria* species, *A. alternata,* and *A. solani* from culture filtrate in Czapek's nutrient broth, was investigated against *Triticum aestivum* L both in laboratory bioassays and in pots. In laboratory bioassays, the potential of culture filtrates of both *Alternaria* species was studied on seed germination and seedling emergence in blotter paper slots. The metabolites from 5-days culture filtrate of both *Alternaria* species enhanced seed germination rate by 9.6 - 10.2% while length of shoot, shoot fresh biomass, length of root and root fresh biomass of wheat seedlings was increased over control by 10-12%; 9-13%; 12-14% and 9-14% respectively. Rate of transformation of germinated seeds to normal seedlings was enhanced over control when treated with 5-days old culture filtrate. The toxicity of culture filtrate increases with longer duration of treatment. The toxicity appeared in 10-25 days old culture filtrates, significantly inhibited seed germination, shoot length, shoot fresh biomass, root length and root fresh biomass over control. In pot trials, foliar application of culture filtrates was made on 1-week and 2-week old wheat seedlings. The seedlings receiving 5-days old metabolites treatment of both *Alternaria* species markedly enhanced the shoot biomass while seedlings emerged from 10-25 days old metabolites treated seeds reduced the shoot biomass. The seedlings of 2-week age old were reported more susceptible to foliar spray than the 1-week old. The reduction in shoot biomass was more significant to 2-week old wheat seedlings when treated with 10-25 days old metabolites. It is revealed that metabolites from 5-days old culture filtrate of both *Alternaria* species resulted enhance effect, may be able to be used as alternative growth promoter while other treatments had inhibitory effects, and may be beneficial for destroying the weeds which reduce the productivity of economical important crops.

Key words: *Alternaria alternata, A. solani, metabolites, growth promoter, toxicity, Triticum aestivum* L.

INTRODUCTION

Pathogenic fungal microbes of diverse group are known to secrete or excrete a variety of a multitude of low molecular weight bioactive organic compounds during a period growth, in infested host tissues, may be either non-toxic or toxic to host cells (Holensein and Stoessi, 2008). The metabolites of non-toxic nature are reported to be beneficial to host but toxic ones directly act on living host protoplasm creating disturbances in normal cell metabolism to influence the course of disease development or symptom expression (Bhajbhuje, 2013; Bhajbhuje and Pathode, 2014; Kandhare, 2015).

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 all continents around the globe except Antarctica for its amber-coloured, non-dehiscent single seeded caryopsis, as it is proved to be an excellent health-building food and leading source of vegetable protein, minerals, Vit-B and dietary fibre, contributing 20% of all calories and proteins to the world human diet (Wikipedia, 2015). 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 32.8% of the nation's total output is contributed by Uttar Pradesh followed by Punjab. Whole grain provides 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, 2015).

Majority species of *Alternaria* remains as an increasing threat to several crops around the globe causing several diseases including Alternaria leaf blight, damping off of seedlings, producing brown to black leaf spots lead to a reduction of leaf count and rate of photosynthesis. The infected seeds are often shrivelled, reduced in size with a brown discolouration on seed surface and loss seed germination potential that adversely affect annual productivity of vegetables and other crop plants to the extent of 20-30% (Mamgain *et al*., 2013). The pathogen can survive as conidia on seed surface or as mycelium inside seed coat and produced both non-toxic as well as toxic metabolites in storage. Literature survey reveals that fungal metabolites of primary nature enhanced seedlings growth (Sung *et al*., 2011; Chung, 2012; Bhajbhuje, 2013; Bhajbhuje and Pathode, 2014) while secondary metabolites becomes toxic to host cells, damages cell components of actively growing cells to influence the course of symptom expression in host plant (Brakhage and Schroeckh, 2011; Madhavi *et al*., 2012; Bhajbhuje, 2013; Venda Kumari *et al*., 2014; Khandare, 2015). Several researchers have made investigation on role metabolites of *Alternaria* in plant system (Tsuge *et al*., 2013; Bhajbhuje and Pathode, 2014; Bhajbhuje, 2015). Presently response of metabolites secreted in culture filtrate of *A. alternata* and *A. solani* against wheat plant has so far not been reported. It seemed to be worthwhile to study parameters in laboratory and in pot trials concerning to seed germination, length of shoot and root; biomass of fresh shoot and root in using *Alternaria alternata* and *A. solani* metabolites with *Triticum aestivum* L.

MATERIALS AND METHODS

Preparation of cultural filtrates of test fungi

The isolation of leaf blight causing pathogens, *Alternaria alternata* and *A. solani was* made on Czapek's Dox agar nutrient medium from infested seeds of vegetables as an internal seed borne pathogens employing the technique of ISTA (2014). An inoculum (5 mm agar discs) of test isolates from 6 days old culture was transferred aseptically into sterile 35ml Czapek's broth and incubated for a period between 5 to 25 days under static conditions at 25±1^{0}C. Separate sterile broth and distilled water were kept as control. The metabolites were isolated from culture filtrate in different duration following method described earlier (Bhajbhuje, 2014). The cultures were filtered through sterilized muslin cloth followed by Whatman filter paper No.1. These filtrates containing metabolites were preserved at 4 °C in a refrigerator and used for treatment within a period of a week of filtration to avoid chances of any contamination or chemical alteration (Akbar and Javaid, 2010).

Laboratory bioassays

Healthy seeds sterilized with aqueous solution of 0.1% mercuric chloride were soaked for one hour in sterile distilled water to soften seed coat. Hundred water soaked seeds were placed for 3 hours in 5 to 25 days old culture filtrate containing metabolites of *Alternaria* *alternata* and *A. solani* in triplicate. After each metabolite treatment, immediately washing of seeds was carried out for 5 consecutive times. The moistened treated and untreated control seeds were transferred to sterile blotter paper folds in slots for germination and seedling growth studies. The slots containing seeds were covered with glass cabinet to avoid spoilage of seeds by any saprophyte contaminants. The moisture content of blotter paper was maintained by addition of sterile distilled water when required. Harvest was taken on 8th day. Data regarding seed germination, root/shoot growth in terms of length and fresh biomass was recorded (Bhajbhuje, 2015).

Pot trials

A pot experiment was conducted in a field using plastic pots of 8 cm diameter and 12 cm deep containing 350 g sandy loam soil supplemented with farm yard manure. Ten seeds of wheat were sown in each pot. After seed germination, pots were divided into two sets to perform the foliar spray on 1-week & 2-weeks old seedlings. Pots containing seeds were watered, when required and kept these pots under natural environmental condition where sufficient light is made available. The culture filtrates of both *Alternaria alternata* and *A. solani* were sprayed 3 times with interval of 5 days on 1-week and 2-weeks old wheat seedlings in triplicates. Plants of the control treatment were sprayed with sterile distilled water. After 30 days growth, plants were carefully uprooted and washed under tap water. Roots were separated from shoots. Result on length as well as dry biomass of shoot and root was recorded (Bhajbhuje, 2015).

RESULTS AND DISCUSSION

Metabolites are intermediate products of metabolism having multifold functions, including fuel, signaling, stimulatory and inhibitory effects on enzymes, catalytic activity, defense, and interactions with other organisms. Metabolites of primary nature are directly involved in normal growth, development, and reproduction while a secondary metabolite usually has several important ecological functions (Wikipedia, 2015). Many new general techniques for both bio-control and for causing enhancement of plant growth have recently been developed. *Trichoderma* spp. possesses innate resistance to most agricultural chemicals, including fungicides, although individual strains differ in their resistance. Majority species of *Alternaria* including *A. alternata* and *A. solani* are known to cause an early blight disease in vegetables producing small, darkened lesions on plant parts that spread into growing black spots of dead tissue, often killing most of the plant in the long run. Seeds infected with the disease may even damp off during germination (Mamgain *et al*., 2013).

Laboratory bioassays

Leaf blight causing pathogens, *Alternaria alternata* and *A. solani* isolated on infested seeds of vegetables were allowed to grow in Czapek's broth nutrient medium for a period between 5 to 25 days in static climate. The metabolites of different duration from culture filtrates of these isolates were tested against *Triticum aestivum*. Results concerning to seed germination, seedlings growth; count of normal seedlings and fresh biomass of treated and untreated control plants is tabulated in Table 1. The metabolites from 5-days old culture filtrates of both the isolates exhibited significant enhancing effect on seed germination, length of shoot and root as well as biomass of fresh shoot and root while 10-25 days old culture filtrate had insignificant effect on these parameters undertaken. The rate of seed germination was confined to enhance by 9.6 – 10.2%; length of shoot and root shoot of seedling by 9.6 – 12.3% and 12.3 – 13.6% while biomass of fresh shoot and root was reported to increase by 8.7 – 13.0% and 9.2 – 14.3% over control for *A. solani* and *A. alternata* respectively with five days old metabolites treatment (Table 1).

The response of metabolites from 10 to 25 days old culture filtrates of test fungal isolates against the parameters understudy was significant. The seed germination rate was declined by 5.7% to 25.5% and 4.5% to 27.4% over control when seeds treated with 10 to 25days old metabolites of *A. alternata* and *A. solani* respectively. Control seeds did not express any change. It was noticed that the seedlings growth was suppressed when seeds treated with metabolites of longer duration. Moreover, majority of treated germinating seeds were transformed into abnormal seedlings. The count of normal seedlings declined to the extent of 2.8 – 38.4% and 4.8 – 40.6% while count of abnormal seedlings rose from treated seeds was significantly enhanced (Table 1).

Table 1: Record of per seed viability, length of shoot & roots of metabolite treated and untreated seed of *Triticum aestivum* L in laboratory bioassay.

Duration of treatment (Days)	Seed viability		Seedling height				Biomass of Fresh seedling				Nature of Seedlings			
	Per cent Seed germination		Shoot length (cm)		Root length (cm)		Shoot fresh weight (mg)		Root fresh weight (mg)		Normal seedlings (%)		Abnormal seedlings (%)	
	AA[1]	AS[2]	AA	AS	AA	AS	AA	AS	AA	AS	AA	AS	AA	AS
5	86.5 (+10.2)[3]	86.0 (+9.6)	8.2 (+12.3)	8.0 (+9.6)	9.2 (+13.6)	9.1 (+12.3)	5.2 (+13.0)	5.0 (+8.7)	2.4 (+14.3)	2.3 (+9.2)	89.0 (+13.2)	87.5 (+11.3)	11.0 (-48.6)	12.5 (-41.6)
10	74.0 (-5.7)	75.0 (-4.5)	6.9 (-5.5)	6.7 (-8.2)	7.6 (-6.2)	7.4 (-8.6)	4.2 (-8.7)	4.1 (-10.9)	1.9 (-9.5)	1.8 (-14.3)	84.8 (+7.9)	82.6 (+5.1)	15.2 (-28.9)	17.4 (-18.7)
15	71.5 (-8.9)	71.0 (-9.6)	6.3 (-13.7)	6.1 (-16.4)	7.1 (-12.3)	6.9 (-14.8)	3.8 (-17.4)	3.6 (-21.7)	1.7 (-19.0)	1.6 (-23.8)	76.4 (-2.8)	74.8 (+4.8)	23.6 (+10.8)	25.2 (+17.8)
20	66.0 (-15.9)	65.5 (-16.6)	5.8 (-20.5)	5.7 (-21.9)	6.5 (-19.8)	6.3 (-22.2)	3.5 (-23.9)	3.4 (-26.1)	1.6 (-23.8)	1.6 (-23.8)	62.7 (-20.2)	59.2 (-24.7)	37.3 (+74.3)	40.8 (+90.7)
25	58.5 (-25.5)	57.0 (-27.4)	5.2 (-28.8)	5.0 (-31.5)	5.8 (-28.4)	5.5 (-32.1)	3.1 (-32.6)	2.8 (-39.1)	1.4 (-33.3)	1.3 (-38.1)	48.4 (-38.4)	46.7 (-40.6)	51.6 (+141.1)	53.3 (+149.1)
Czapek's broth	84.0 (+7.0)	84.0 (+7.0)	7.6 (+4.1)	7.6 (+4.1)	8.5 (+4.9)	8.5 (+4.9)	4.8 (+4.3)	4.8 (+4.3)	2.2 (+4.8)	2.2 (+4.8)	80.8 (+2.8)	80.8 (+2.8)	19.2 (-10.3)	19.2 (-10.3)
Control (D.W.)	78.5	78.5	7.3	7.3	8.1	8.1	4.6	4.6	2.1	2.1	78.6	78.6	21.4	21.4

1. AA - *Alternaria alternata*; 2. AS – *Alternaria solani;* 3. Values in parenthesis indicate per cent increase or decrease over control

Length of shoot had significant response to metabolites from 10-25 days old culture filtrate, inhibited shoot length by 5.5 to 28.8% and 8.2 to 31.5% over control for *Alternaria alternata* and *A. solani* respectively as compared to control. The effect of these metabolites was significant on shoot biomass. Metabolites of this duration of these two *Alternaria* species declined biomass of fresh shoot over control by 8.7 - 32.6% and 10.9 – 39.1% respectively (Table 1). Length of root exhibited an significant response to these metabolite treatments, significantly inhibited root length to the extent of 6.2 to 28.4% and 8.6 to 32.1% compared to control. The adverse effect of these metabolite treatments on root biomass was significant. Root biomass was reduced to the extent of 9.5 – 33% and 14 – 38% over control (Table 1).

Pot trials

A pot experiment was conducted in a field using plastic pots. Ten seeds of wheat were sown in each pot containing sandy loam soil supplemented with farm yard manure and were allowed to germinate the seeds under natural environmental condition. The culture filtrate of 5-25 days duration of *A. alternata* and *A. solani* was sprayed 3 times on 1-week and 2-weeks old wheat seedlings in triplicates. Plants of the control treatment were sprayed with sterile distilled water. After 30 days growth, result on length as well as dry biomass of shoot and root was recorded (Bhajbhuje, 2015).The effect of metabolites from culture filtrates of shorter duration was reported insignificant and it was significant with metabolites of longer duration. The treatment with metabolites from 5-days old culture filtrates of test fungal isolates enhanced length of shoot of 1-week and 2-week old seedlings by 11.9% and 9.6% respectively (Fig 1). Similar stimulatory effect was recorded for length of root with same metabolite treatment (Fig.2). The root length was enhanced over control by 13.6% and 12.3% for both test fungal isolates respectively (Table 2).

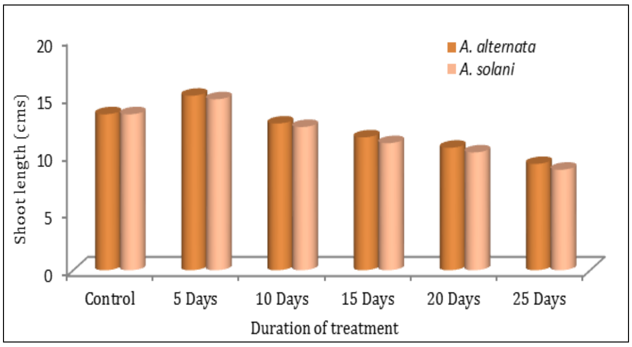

Fig. 1(a) Effect of foliar spray of metabolites on shoot length of 1-week old seedling of wheat

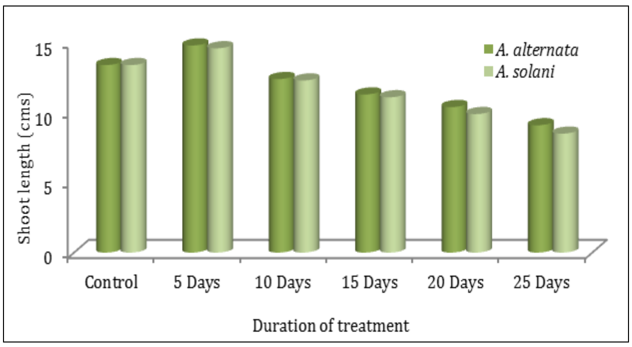

Fig. 1(b) Effect of foliar spray of metabolites on shoot length of 2-week old seedling of wheat

Table 2: Effect of foliar spray of metabolites from culture filtrate of *Alternaria alternata* and *A. solani* on growth of 1- & 2-week old wheat seedlings in pot trials after 30 days growth.

Duration of treatment (Days)	1-week old seedlings								2-week old seedlings							
	Seedling height				Dry Biomass of Seedling				Seedling height				Dry Biomass of Seedling			
	Shoot length (cm)		Root length (cm)		Shoot dry weight (mg)		Root dry weight (mg)		Shoot length (cm)		Root length (cm)		Shoot dry weight (mg)		Root dry weight (mg)	
	AA	AS	AA	AS	AA	AS	AA	AS	AA	AS	AA	AS	AA	AS	AA	AS
5	15.1 (+11.9)	14.8 (+9.6)	9.2 (+13.6)	9.1 (+12.3)	5.2 (+13.0)	5.0 (+8.7)	2.4 (+14.3)	2.3 (+9.2)	14.8 (+9.6)	14.6 (+8.1)	8.9 (+9.9)	8.7 (+7.4)	4.9 (+6.5)	4.8 (+4.3)	2.3 (+9.5)	2.2 (+4.8)
10	12.7 (-5.9)	12.4 (-8.2)	7.6 (-6.2)	7.4 (-8.6)	4.2 (-8.7)	4.1 (-10.9)	1.9 (-9.5)	1.8 (-14.3)	12.4 (-8.1)	12.3 (-8.9)	7.5 (-7.4)	7.3 (-9.9)	4.1 (-10.9)	3.9 (-15.2)	1.7 (-19.0)	1.6 (-23.8)
15	11.5 (-14.8)	11.0 (-18.5)	7.1 (-12.3)	6.9 (-14.8)	3.8 (-17.4)	3.6 (-21.7)	1.7 (-19.0)	1.6 (-23.8)	11.3 (-16.3)	11.1 (-17.8)	6.8 (-16.0)	6.5 (-19.8)	3.5 (-23.9)	3.3 (-28.3)	1.6 (-23.8)	1.4 (-33.3)
20	10.6 (-21.5)	10.2 (-24.4)	6.5 (-19.8)	6.3 (-22.2)	3.5 (-23.9)	3.4 (-26.1)	1.6 (-23.8)	1.6 (-23.8)	10.4 (-22.9)	9.9 (-26.7)	6.2 (-23.5)	5.9 (-27.2)	3.2 (-30.4)	3.0 (-34.8)	1.4 (-33.3)	1.3 (-38.1)
25	9.2 (-31.9)	8.7 (-35.6)	5.8 (-28.4)	5.5 (-32.1)	3.1 (-32.6)	2.8 (-39.1)	1.4 (-33.3)	1.3 (-38.1)	9.1 (-32.6)	8.5 (-37.8)	5.3 (-34.6)	5.1 (-37.0)	2.9 (-36.9)	2.6 (-43.5)	1.2 (-56.1)	1.1 (-47.6)
Czapek's broth	14.2 (+5.2)	14.2 (+5.2)	8.5 (+4.9)	8.5 (+4.9)	4.8 (+4.3)	4.8 (+4.3)	2.2 (+4.8)	2.2 (+4.8)	14.2 (+5.2)	14.2 (+5.2)	8.5 (+4.9)	8.5 (+4.9)	4.8 (+4.3)	4.8 (+4.3)	2.2 (+4.8)	2.2 (+4.8)
Control (D.W.)	13.5	13.5	8.1	8.1	4.6	4.6	2.1	2.1	13.5	13.5	8.1	8.1	4.6	4.6	2.1	2.1

1. AA - *Alternaria alternata*; 2. AS – *Alternaria solani;* 3. Values in parenthesis indicate per cent increase or decrease over control

In pot trials, the inhibitory effect of 10 to 25-days old culture filtrates containing metabolites of both *A. alternata* and *A. solani* was confined significant on both 1-week and 2-week old wheat seedlings. Moreover the inhibitory effect of *Alternaria solani* was pronounced against *A. solani* (Table 2). The foliar spray applications of these metabolite treatment reduced length of shoot of 1-week old seedlings over control by 5.9 – 31.9% and 8.2 – 35.6% respectively. The root length of seedlings was declined by 6.2-28.4% and 8.6 – 32.8% with same metabolite treatment (table 2). The shoot biomass was significantly declined by 8.7 – 32.6% and 10.9 – 39.1% in 1-week old seedlings receiving foliar spray of 10-25 days old culture filtrates of *A. alternata* and *A. solani* respectively (fig. 3). The inhibitory effect of culture filtrates of these fungal isolates was insignificant on 2-week old plants. The age of plant was considered important parameter for inducing resistance. The 1-week old seedlings were confined more resistance to foliar spray application where different fungal culture filtrate treatments reduced the root biomass by 32–39% against 47 – 56% declining in 2-week old seedlings (Fig.4).

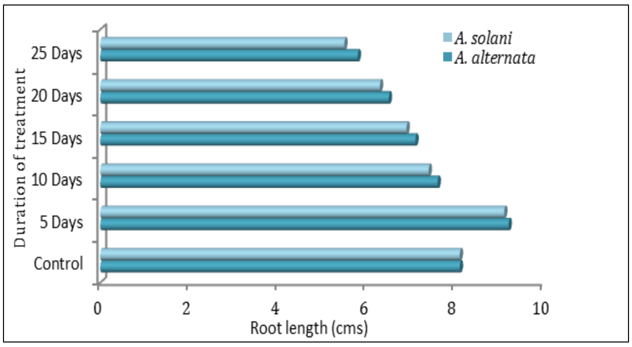

Fig. 2(a) Effect of foliar spray of metabolites on root length of 1-week old wheat seedling

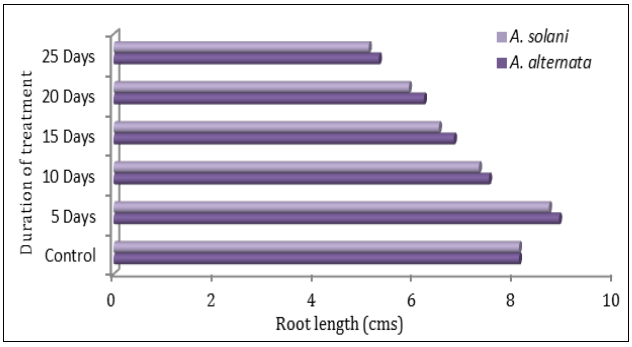

Fig. 2(b) Effect of foliar spray of metabolites on root length of 2-week old wheat seedling

In general the inhibitory effect of foliar spray on root length was much pronounced. Root dry biomass had more pronounced response to foliar spray application over root length. Metabolites from 10-25 days old culture filtrate of test fungal isolates significantly reduced the dry root biomass by 9.5 – 33.3% and 14.3 – 38.1% in 1-week old plants. The effect of these treatments on 2-week old seedlings was much significant. There were 19–56% and 24–48% reduction 2-week old plants, respectively (Fig.4).

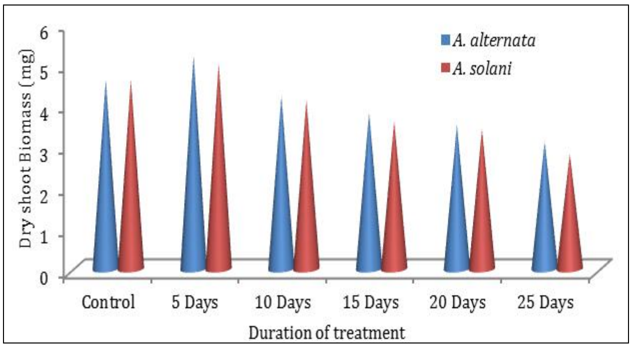

Fig. 3 (a) Effect of foliar spray of dry shoot biomass of 1-week old wheat seedling.

In general the inhibitory effect of foliar spray on root length was much pronounced. Root dry biomass had more pronounced response to foliar spray application over root length. Metabolites from 10-25 days old culture filtrate of test fungal isolates significantly reduced the dry root biomass by 9.5 – 33.3% and 14.3 – 38.1% in 1-week old plants. The effect of these treatments on 2-week old seedlings was much significant. There were 19–56% and 24–48% reduction 2-week old plants, respectively (Fig.4).

In laboratory bioassay, the metabolites from 5-days old culture filtrates of both the isolates exhibited significant effect on rate of seed germination, length of shoot and root as well as biomass of fresh shoot and root while 10-25 days old culture filtrate had insignificant effect on these parameters. The rate of seed germination was confined to enhance over control respectively with five days old metabolites treatment. In pot trials also, the effect of metabolites from culture filtrates of shorter duration was reported insignificant and it was significant with metabolites of longer duration. The treatment with metabolites from 5-days old culture filtrates of *Alternaria alternata* and *A. solani* enhanced length of shoot of 1-week and 2-week old seedlings. Similar stimulatory effect was recorded for other parameters undertaken. It is in agreement 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 enhancement in growth of seedling and higher rate of seed germination over control in Canola, cucumber and tomato plants receiving metabolic treatment of culture filtrate of *Shimizuomyces paradoxus*. Bhajbhuje and Pathode (2014) reported enhancement in these parameters over control in wheat seedlings receiving metabolite treatment of *Alternaria triticina*. Moreover, metabolites of *Trichoderma harzianum* induced germination wheat seeds with hard seed coat (Mokhtar and Dehimat, 2013); *Fusarium oxysporum f. sp. lycopersici* and *Alternaria solani* metabolites enhanced seed germination rate of tomato (Bhajbhuje, 2013). Literature survey revealed secretion of metabolites of primary nature and some growth stimulating factors by *A. alternata* and *A. solani* at early growth stages that enhanced the seed germination rate, seedling emergence (Chung, 2012; Bhajbhuje, 2014).

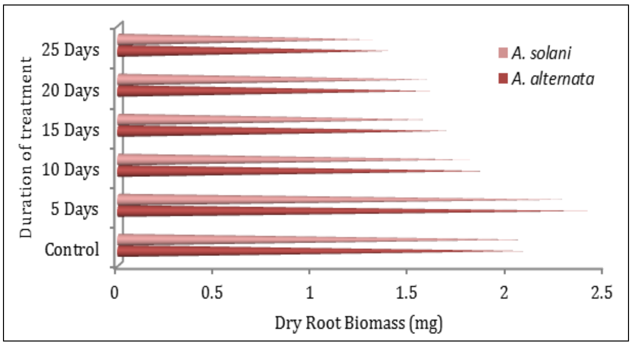

Fig. 4 (a) Effect of foliar spray of metabolites on dry root biomass of 1-week old wheat seedling.

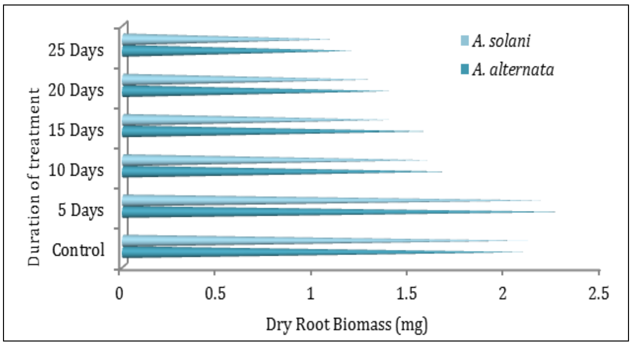

Fig. 4 (b) Effect of foliar spray of metabolites on dry root biomass of 2-week old wheat seedling.

These metabolites of primary nature may serve as growth promoter at low concentration and induced vigorous proliferation by stimulating phosphorylation in the host tissues in association of Ca^{2+} and Mg^{2+} (EFSA, 2011). It is noted that low concentration of these metabolites did not express any phenotypic variation in seedling receiving treatment (Bhajbhuje and Pathode, 2014). Moreover, the biomass of fresh shoot and roots as well as count of normal seedlings were significantly enhanced in seedlings receiving metabolite treatment from 5-days old culture filtrate of both test fungal isolates. A growth stimulating effect in response to seed germination rate and seedling emergence over control in present investigation may be attributed to secretion metabolites of primary nature by test fungal organisms at early stages of their growth that may serve as growth promoters.

In *laboratory bioassay*, the response of metabolites from 10 to 25 days old culture filtrates of test fungal isolates against the parameters understudy was insignificant. The per cent seed germination declined over the control when seeds treated with 10 to 25days old metabolites of *Alternaria alternata* and *A. solani* respectively. The seedlings emergence was suppressed when treated with culture filtrate of longer duration. The metabolite treated germinating seeds did not transform into normal seedlings. The count of normal seedlings was declined while count of abnormal seedling rose from treated seeds was significantly enhanced (Table 1).

Length of shoot had significant response to metabolites from 10-25 days old culture filtrate, declined this growth by 33% and reduced biomass of fresh shoot by 32 -39% over control respectively (Table 1). The root length and biomass exhibited an insignificant response to these metabolite treatments, significantly reduced root length by 28 to 32%; and root biomass to the extent of 33-38 % for *Alternaria alternata* and *A. solani* respectively as compared to control (Table 1).

In pot trials, the inhibitory effect of the metabolites from 10-25 days old culture filtrates of both *Alternaria alternata* and *A. solani* was confined significant in both 1-week and 2-week old plants treatment. Moreover the inhibitory effect of *Alternaria solani* was pronounced against *A. solani.* The foliar spray applications of these metabolite treatment reduced length of shoot, root and dry biomass of seedlings (Fig.1-4). The inhibitory effect of culture filtrates of these fungal isolates was insignificant on 2-week old plants. The 2-week old seedlings were more susceptible to foliar spray for these parameters undertaken.

The results of the present study were confirmed with earlier findings of Madhavi *et al*., (2012) in *Allium cepa* L.; Raithak and Gachande (2013) in *Lycopersicon esculentum* L and Venda Kumari *et al*., (2014) in *Brassica carinata* & *B. braun*; Bhajbhuje (2015) in *Vigna mungo.* Anand *et al*., (2008) confirmed production of nonspecific toxic metabolites in culture filtrate by *Alternaria alternata* and *Colletotrichum capsici* that induced inhibition of seed germination, length of shoot/root and vigour index of the seedlings of chilli, rice, mungbean, maize, cotton, groundnut, okra, eggplant, cucumber and tomato. Savitha *et al*., (2012) isolated toxin of *Alternaria semami* and same was tested on sesamum and tomato and reported greater inhibition of seed germination and length of shoot/ root at 2000 ppm conc. while 50 ppm conc. had least inhibition on these parameters. Wagh *et al* (2013) reported *Alternaria* leaf spot *in vitro* and *in vivo* in plantlets inoculated with *Alternaria alternata* 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.

Mycotoxin secretion by several filamentous fungi has been reported in many crops including cereals, vegetables, oil-seed crops and pulses (Holensein and Stoessi, 2008). Host-selective toxins (HSTs) produced by fungal plant pathogens are low-molecular-weight secondary metabolites with a diverse range of structures that function as effectors controlling pathogenicity or virulence in certain plant-pathogen interactions (Tsuge, *et al* 2013). *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 cereals, mandarins, peppers, apples, sunflower seeds, oilseeds rape, olives, various fruits and vegetable seeds (Wikipedia, 2015). Amongst other species, *Alternaria alternata* (Fr.) Keissler produced several toxic metabolites of major toxicological importance including, HST-toxin, AAL-toxins, tenuazonic acid, alternariol monomethyl ether, alternariol, altenuene, and altertoxin I (Helambe and Dande, 2012) in artificial nutrient medium during its growth period provided favourable climatic environment. Alternariol and alternariol monomethyl ether also have been produced by pathogen in artificially mould-infested building materials (Chung, 2012). The pathogen had seven pathogenic variants producing different host-specific toxins (HSTs) and cause diseases on different plants (Helambe and Dande, 2012). HSTs was reported release from germinating conidia of *Alternaria alternata* prior penetration of host cell (Tsuge *et al*., 2013).

CONCLUSION

The present study concludes that culture filtrates of the two tested *Alternaria* species contain beneficial and hazardous chemical constituents. Further studies are required to isolate and identify the potential of these constituents. Once identified, these natural compounds may be used as structural lead for the preparation of ecofriendly pesticides for the management of population weeds that unable to grow and develop crop plant to maturity and ultimately adversely helps to reduce the crop productivity to a greater extent.

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