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Metabolite effect of *Alternaria alternata* (Fr.) Keisler on seedling emergence of *Hordeum vulgare* L.

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Abstract

Diverse group of micro-fungal organisms are proved to secrete or excrete both primary non- toxic as well as secondary toxic metabolites during growth and metabolism. Metabolites of primary origin induce growth stimulating response while secondary metabolites appear to create disturbances in normal cell metabolism, growth and reproduction. The metabolites produced in growth nutrient medium at different growth intervals by *Alternaria alternata* (Fr.) Keissler, a serious causal pathogen of leaf spot of *Hordeum vulgare* L, were isolated, confirm chemical nature and evaluated for their effect on seed germination, seedling emergence and fresh biomass production. An increase in per cent seed germination, seedling emergence and green biomass without any abnormalities over control were recorded with seven days old metabolites treated seeds while same parameters were declined with metabolites of longer duration.

Keywords: *Fungal metabolite, Hordeum vulgare* L, *Alternaria alternata, green biomass, vigour.*

1. Introduction

Fungal metabolites are low molecular weight organic compounds secreted during growth and development. The toxic metabolites penetrate host tissues, directly act on living host protoplasm, and damage the somatic cells to influence the course of disease development [1]. The primary metabolites elicited growth stimulating response and serve as functional units in life process of the host cell while secondary metabolites are toxic to host tissues. Several species of *Alternaria produce* more than 70 phytotoxins was reported to play a crucial role in

determining host specificity was contributing to disease development [1] Toxin from secondary metabolites damage rapidly dividing meristematic cells 2.

Barley (*Hordeum vulgare* L.), one of the first domesticated, ancient, staple cereal crop of Fertile Crescent of Middle East origin belongs to family Poaceae in recorded history is cultivated for single seeded, non-dehiscent caryopsis fruit that has potential applications as whole grain to improve nutrition, boost food security, foster rural development and support sustainable land care and for its value added products, bran and flour for multiple food applications It ranks fourth among cereal crops on the globe in terms of annual seed production; yielded over 154.18 million metric tons seeds in 2019-20.

Barley grain is store house of 78% carbohydrate, 10% protein and negligible quantity of fat (1%). It is rich source of dietary fiber, niacin, propionic acid, riboflavin, vit-B_6, minerals, manganese, magnesium, phosphorus, trace elements, potassium, calcium in addition to indigestible cellulose which are essential for good health. The barley embryo remains free from sodium and cholesterol. A diet high in fiber can be useful for patients at risk for colon disease, heart disease, and diabetes. According to Health Canada and the US FDA, consuming at least 3 grams per day of barley can lower levels of blood cholesterol and minimize a risk for cardiovascular diseases. Consumption of whole-grain barley, reduce blood glucose response to a meal, improves cholesterol levels and glucose regulation 3.

A great number of species were recorded for genus *Alternaria* infecting different crops causing world-wide economic loss. The susceptible cultivars of *Hordeum vulgare L.* are affected with several seed borne diseases and some of them are seed transmitted. Zare 4 has isolated *Alternaria alternata* as Kernel blight black point pathogen from endosperm and seed coat. The pathogen caused the discoloration in the palea and lemma which remains adhered to outer seed coat. Black point causes a slight reduction in rate of germination and also responsible for spoilage of healthy grains 4.

The response of fungal metabolites of different duration have been reported in plant system and confirmed by Sung et al 5; Bhajbhuje 6. Sung et al. 5; Bhajbhuje 2 and Tsuge et al. 7 have studied role metabolites by *Alternaria* species in plant system. Presently response of metabolites secreted in culture filtrate of *Alternaria alternata* against barley plant has so far not been reported. It seemed to be worthwhile to study parameters concerning to seed germination, seedling emergence, biomass of fresh short & root and karyokinesis in mitotic cell division using *Alternaria alternata* (Fr.) metabolites.

2. Materials and Method

The opportunistic plant pathogen, *Alternaria alternata* (Fr.) Keissler was isolated from stored seeds samples of *Hordeum vulgare* L as an internal seed borne pathogen following the technique of ISTA 8. The metabolites were isolated from culture filtrate in different duration following method described earlier 6.

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 28 days at an interval of seven days at 25 ± 1^0C. Separate sterilized broth and sterile distilled water were kept as control. Seeds were soaked for three hours in sterile distilled water and in different duration of *Alternaria alternata* (Fr.) Keissler metabolites in triplicate. Washing of the seeds was carried out immediately after the metabolic treatment. Hundred seeds were then placed in blotting paper folds for germination and other fifty seeds were placed on moist filter paper in sterilized petri-plates for cytological study. The petri plates having seeds were allowed to incubate at 25±1^0C in B.O.D. incubator aseptically. The seedling height was measured and per cent seed germination was recorded on eighth day. The seedlings raised from germinating seeds were classified into normal and abnormal seedlings whereas ungerminated seeds were categorized into hard and dead seeds. The green biomass was estimated by weighing the shoot and root separately for treated and

untreated seedlings. The cytological studies were performed following the procedure described earlier 6.

3. Results and Discussion

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 9. These microbes either release or excrete many chemically known metabolites during their growth in favourable environment due to constantly occurring diverse metabolic reactions in every functional cell, which at low conc. directly toxic to microbes, may enhance growth of seedlings as growth stimulator. Its high dosages may induce stunted growth creating disturbances in normal karyokinesis of cell cycle leads to chromosomal alteration or cause lethality in eukaryotic plant cells 2 and also may acts as mutagens results to mutants that exhibit appearance of some phenotypic variations in resultant seedlings of plants in subsequent generation 11.

The various phytochemical confirmative tests and the U.V. absorption spectrum revealed that the toxins isolated might be organic compounds of phenolic in nature. Similar type of toxin was isolated from *Alternaria alternata* 12 and *Alternaria solani* 2. The per cent seed germination, seedling emergence, fresh biomass of seedlings and cytological abnormalities were recorded for each treatment and control (Table 1).

1. Effect of metabolites on seed germination of *Hordeum vulgare*:

The metabolites in culture filtrate from *Alternaria alternata* for a period of 7 to 28 days was tested on the seeds of *Hordeum vulgare* at an interval of 7 days and evaluated their role on seed viability.

Table 1: Effect of metabolites of *Alternaria alternata* on length of shoot of seedling of *Hordeum vulgare* L.

Sr. No.	Duration of treatment (Days)	Per cent Seed germination	Nature of seedlings		Seedling growth (length in cms)		Biomass of seedling (gms)		Percent abnormalities
			Normal (%)	Abnormal (%)	Shoot length	Root length	Shoot biomass	Root biomass	
1	7	87.9 (+1.7)[2]	87.0 (+2.1)[2]	13.0 (-12.2)	11.3 (+6.6)	11.9 (+2.3)	4.6 (+2.2)[2]	2.2 (+4.8)[2]	6.76 ± 0.06
2	14	78.6 (-9.0)	78.0 (-8.5)	22.0 (+49.0)	8.0 (-24.5)	9.6 (-17.2)	3.8 (-15.6)	1.1 (-47.6)	7.45 ± 0.03
3	21	75.2 (-12.1)	71.0 (-16.7)	29.0 (+96.5)	6.8 (-35.8)	7.1 (-38.8)	0.8 (-82.2)	0.4 (-80.1)	10.11 ± 0.04
4	28	72.9 (-15.6)	65.0 (-33.7)	35.0 (+137.1)	5.1 (-51.9)	5.4 (-53.5)	0.6 (-86.7)	0.1 (-95.2)	12.68 ± 0.06
5	Czapek's Broth medium	86.6(+0.2)	85.2 (0.0)	14.8 (0.0)	10.6 (0.0)	11.6 (0.0)	4.5 (0.0)	2.1 (0.0)	17.11 ± 0.05
6	Control (D.W)	86.4	85.2	14.8	10.6	11.6	4.5	2.1	-

1. Average of 300 germinated seeds;
2. Average of 100 seedlings;
3. Values in parenthesis indicate per cent reduction or increase in term of control.

± indicates standard error

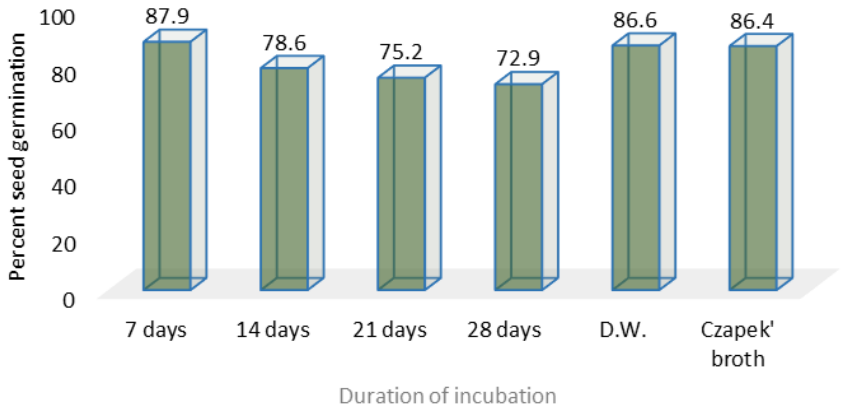

Fig. 1 Effect of *Alternaria alternata* metabolites on seed germination of *Hordeum vulgare* L

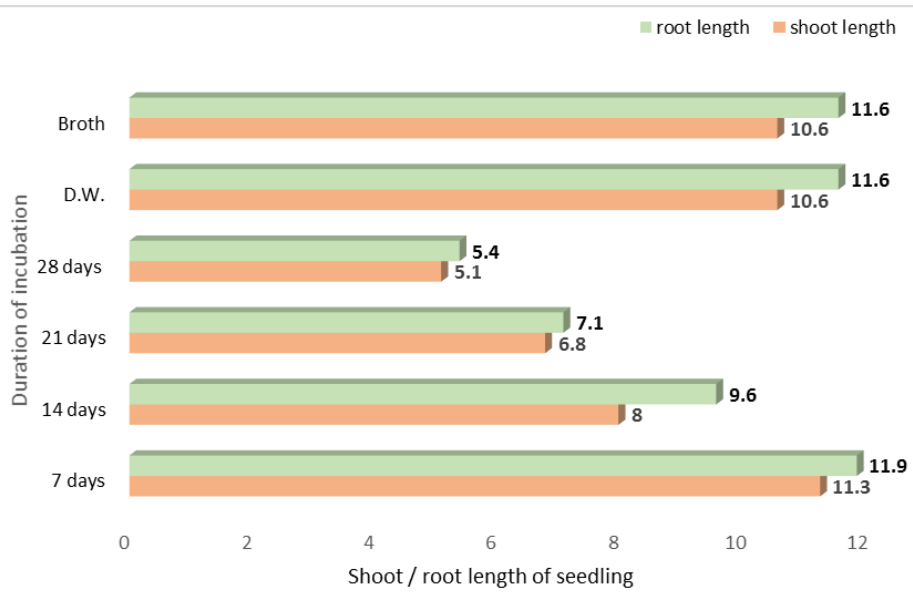

Fig. 2 :Effect of *Alternaria alternata* metabolites on seedling emergence of *Hordeum vulgare* L.

The control water-soaked seeds induced 86.4% germination. The seed germination rate was enhanced by 0.2% over control with seeds soaked in Czapek's Dox broth while it was recorded to increase by 1.7% with 7-days old metabolic treatment. The decrease in per cent seed germination was reported with metabolite treatment of longer duration. The rate of seed germination was confined to decline by 9.0% and 12.1%, when seeds treated with metabolites from 14-days and 21-days old culture filtrate respectively. The high inhibitory effect was reported in 28-days old culture which exhibited 15.6% germination. The untreated control seeds did not express any change (Table 1).

A count of hard seeds was reduced by 17.3% with 7-day old metabolic treatment. The rate of dead seeds over

control was enhanced with metabolite treatment of 14 to 28 days in duration. The metabolite treatment of 14-days old culture filtrate induced little while 28-days old culture filtrate had higher phytotoxic effect (Table 1).

2. Effect of metabolites on seedling emergence of *Hordeum vulgare* L.

The seedlings of *Hordeum vulgare* L. raised untreated and treated seeds with culture filtrate of different duration containing metabolites of *Alternaria alternata*, were measured on seventh day for length of shoot and root of seedling (Table. 1).

(a) Shoot length of seedlings:

The metabolites treatment of 7-days old culture filtrate induced significant effect increasing length of shoot by 6.6% against untreated control. The declining in length of shoot over control was recorded in seedlings receiving metabolic treatment from longer duration culture. Reduction in length of was reported when the seedlings raised from treatment of 14-days and 21-days old culture filtrate, inhibiting the shoot length by 24.5 % and 35.8 % respectively while treated seeds with 28 days old culture filtrate exhibited 51.9 % inhibition in shoot length over control (Table 1).

(b) Root length of seedlings:

The length of root was enhanced with treatment of 7-day old culture filtrate by 2.3% against untreated control. Inhibition in root length was recorded by 17.2% and 38.8% with 14 and 21-days old culture filtrate. The seeds treated with 28-days old culture filtrate had more than 50% inhibitory effect (Table 1).

(c) **Emergence of normal & abnormal seedlings:**

The water soaked control seeds was produced 85.2% normal seedling with well-developed root and shoot without any symptoms. The rate of transformation of germinating seeds to normal seedling was enhanced by 2.1 %, over untreated control, when seeds treated with metabolites from 7-days old culture filtrate. The metabolites accumulated for 14-28 days incubation are reported to be phytotoxic. Marginal reduction of normal seedlings was reported with 14 and 21-days old culture filtrate. The metabolites treatment of 28-days old culture filtrate reduced a count of normal seedlings to the extent of 33.7% (Table 1).

The seeds soaked in Czapek's broth medium as well as untreated water soaked germinating seeds transformed into abnormal seedlings to the extent of 14.8% having underdeveloped root, shoot with small lesions on the leaves respectively. A count of abnormal seedlings increased with longer duration of metabolite treatment. The transformation to abnormal seedlings was significant with metabolite treatment of 28-days old culture filtrate (Table 1).

3. **Biomass of seedlings:**

The seedlings raised from untreated control as well as metabolite treated seeds were cut to separate shoot system from the root. The green fresh biomass for both shoot and root was weighed on electronic balance (Table 1).

(a) Biomass of fresh shoot:

The biomass of shoot of seedling raised from untreated and Czapek's broth nutrients was same and weighted about 4.5gm. Inhibitory effect was pronounced, when seeds soaked in culture filtrate of different duration containing metabolites. The biomass of shoot was enhanced 7-days old culture filtrate by 2.2% and it was further declined by 15.6% of seedlings receiving treatment of 14-days old culture filtrate. Significant reduction by 82.2% and 86.7% was reported with 21 and 28-days old culture filtrate (Table 1).

(b) Biomass of fresh root:

The root biomass of untreated and treated seedlings was recorded in terms of grams. The untreated control and Czapek's broth nutrients seedling exhibited gm. root biomass and did not exhibit any change. The root biomass was enhanced marginally over control, when seedlings treated with 7-days old culture filtrate but it was declined with 14 to 28-days old culture filtrate (Table 1).

The results of present investigation revealed that seed germination rate; length of shoot/ root and fresh biomass of seedlings was recorded to enhance by 6.6%, 2.3%, 2.2% and 4.8% while count of normal seedling in

terms of percentage was increased by 2.1% whereas a count of dead seeds was recorded to decline by 17.3% over control respectively with 7-days old metabolites treatment (Fig. 1). These results are in conformity with the earlier findings for these parameters involving *Brassica oleracia & B. campestris 13; Triticum aestivum* L. 14; *Capsicum annum* L., 15 Chickpea 14 with five to seven days metabolite treatment. Rahman et al., 15 reported higher per cent seed germination and vigour index in chili receiving the metabolite treatment of filtrate of *Trichoderma harzianum* culture. Metabolites of *Fusarium oxysporum f. sp. lycopersici* and *Alternaria solani* enhanced seed germination rate of tomato 16.

The rate of seed germination rate; length of shoot/root and biomass of fresh shoot/roots were reported to enhance over control respectively, when seed soaked in Czapek's broth nutrient medium. This is in agreement with the findings of Bhajbhuje and Pathode 10 Bhajbhuje 13 who reported higher rate of seed germination of seedling vigour in *Triticum aestivum* L. and *Vigna mungo* (L.) Hepper. It may possibly seemed to be incorporation of sugar as carbon source and other micronutrient in the artificially prepared broth that satisfy the basis requirement for proliferation and sporulation of fungal organism.

The results on seed germination declined by 9.0-15.6%; the shoot length by 24.5-51.9%; root length by 17.2-53.5%; the biomass of fresh shoot reduced by 15.6-86.7%; biomass of fresh root declined to the extent of 47.6-95.24%; per cent normal seedling reduced by 8.5-33.7% whereas a count of dead seeds was found to increase by 39.6-96.2%over the control when seeds treated with 14 to 28-days old metabolites (Fig. 2).). Control seeds did not express any change. These results were confirmed with earlier findings of Bhajbhuje et al., 13 in *Brassica. oleracia & B. campestris;* Bhajbhuje & Pathode, 10 in *Triticum aestivum* L. . Wagh et al 17 reported the typical symptoms of *Alternaria* leaf spot *in vitro* and *in vivo Alternaria alternata* inoculated plantlets and detached leaves of *Lepidium sativum*. Bhajbhuje 14 reported inhibition in seed germination, seedling emergence and biomass production in *Triticum aestivum* L with longer duration metabolite treatment of *Alternaria solani* and *A. alternata*. 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., 5 who reported higher percent of ungerminated hard seeds of cucumber and tomato.

Mycotoxin secretion by several filamentous fungi has been reported in many crops including cereals, vegetables, oil-seed crops and pulses 1. 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 14. 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 18 in artificial nutrient medium during its growth period provided favourable climatic environment. The pathogen had seven pathogenic variants producing different host-specific toxins (HSTs) and cause diseases on different plants. The HSTs was reported release from germinating conidia of *Alternaria alternata* (Fr.) Keissler prior penetration of host cell 7.

Phytotoxic and mutagenic and effect of mycotoxins has been highlighted by Chung 12; Tharvanjit 19 and ESFA 20. 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 5; 14. 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 20. Most *Alternaria* mycotoxins induced considerable

cytotoxic effects; Altertoxin III is reported highly mutagenic while altertoxins I and II induced low mutagenicity 18. Tenuazonic acid had antitumor, antiviral and antibacterial activity and it initially inhibited the protein synthesis by suppression of the release of newly synthesized proteins from the ribosomes into a supernatant fluid. Alternariol and alternariol monomethyl ether had foetotoxic and teratogenic effects 9. 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 12. 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 5.

4. Conclusion

The present study reveals that the metabolites are organic compounds of phenolic nature produced by *Alternaria alternata* (Fr.) Keissler, a leaf spot insisting fungal pathogen of *Hordeum vulgare*. The primary fungal metabolites induced growth stimulating effect enhancing seed germination rate, seedling growth and green biomass production without chromosomal abnormalities. The toxicity of fungal metabolites was intensified on longer duration of treatment by releasing of toxic secondary metabolites that induced inhibitory effect on the parameter undertaken and also creates disturbances in normal karyokinesis in mitotic cell division. The primary metabolites secreted by pathogen induce enhancing growth parameters in present plant material and may prove beneficial in crop plant. The toxic metabolites may be used as mutagens in evolving high yielding mutant varieties of economically important crop plants.

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Conflict of interest

No conflict of interest influenced in this research.

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