IARJSET

ISSN (O) 2393-8021, ISSN (P) 2394-1588

International Advanced Research Journal in Science, Engineering and Technology

Vol. 8, Issue 10, October 2021

DOI: 10.17148/IARJSET.2021.81004

Role of metabolites of *Alternaria alternata* (Fr.) Keissl. on some growth parameters of *Triticum aestivum L.*

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Abstract: *Alternaria alternata (Fr.) Keissl.* is well known plant pathogen, food spoiler causing major damage to food commodities and also a common allergens in humans. It is reported as pathogens of wheat responsible for causing seed discoloration, seedling emergence, seed rots, seedling blight, rotting and a patent producer of variety of magnitude of metabolites of medical importance. In present study, attempts were made to grow the pathogen in artificial culture broth for period of 20 days for metabolite production. The culture filtrate thus obtained was graded in different concentrations and each was tested against wheat seeds and studied parameter such as seed germination rate seedling emergence and green biomass production. The rate of seed germination; the shoot length; biomass of shoot was declined against untreated control. Control seeds did not express many changes. The inhibitory effect was more pronounced for these parameters in response to increase in concentration of culture filtrate. Absolute culture filtrate had greater phytotoxic effect against control and the seed soaked in Czapek's broth nutrition medium enhanced seed germination, seedling emergence and green biomass production.

Keywords: metabolite, viability, seedling, biomass, metabolites, inhibition.

I. INTRODUCTION

Metabolites are the intermediate products of naturally occurring metabolic reactions within functional cells [1]. The primary metabolites of fungal origin are indispensable for primary growth, development and reproduction [2] while production and secretion of microbial secondary metabolites is thought to give the competitive advantage in native environments through suppressed growth of neighboring species, more efficient foreign or some other mechanism (3). Moreover, secondary metabolites disturb the normal cell division in plants causing chromosomal abnormalities leads to declining seed germination, seedling emergence and development of abnormal seedling at its higher concentration [4].

Wheat (*Triticum aestivum* L.) is the first strategic cereal crop consumed globally as most important staple food of about 36 per cent of the world population. Its grains are major storage of carbohydrates; moderate protein, negligible content of fat, and 20 per cent food calories. The wheat grains are rich source of vitamins B, multiple essential nutrients (dietary fibre, manganese, phosphorus and niacin); other dietary minerals and phytochemicals [5].

Wheat grains have great food value and its flour is used for making chapatti, some by-products and other worldwide dishes including burger, pizza, pasta and smoked mozzarella. The consumption of hybrid wheat grains enhance milk production, milk quality and maintains the level of energy requirement level of cow. The heat generated in response to digestion of infested straw by an herbivore can be useful in maintaining body temperature in cold climates. The straw is used as a substitute for the biofuel and biogas production and cultivation of Oyster mushroom without using nutrient supplement [6].

The world's total wheat production was estimated at 649,759 thousand tons in 2020. India ranks second largest producer on the globe, producing 106.21 million metric tons gains (International Grains Council, 2021). Punjab has emerged as the biggest wheat producer state followed by U.P., Haryana. M.P. and Rajasthan [7].

Wheat is an efficient source providing all required nutrients and medicinal benefits for healthy and rejuvenating body and help in maintaining level of haemoglobin [5]. The constituent of wheat grass possess antibacterial, antioxidant, anticancer, antiulcer property. It is recommended that the wheat grass exhibited anticancer activity on oral cancer [8]. Chemically, it neutralizes environmental pollutants, enhances immunity, restore energy and vitality [9].

Wheat (*Triticum aestivum* L.) is prone to attack by diverse group of fungal pathogens causing several diseases including stem rust, blotches and head blight scab cereal rusts. These diseases contribute to cause losses of grains to the extent of 10-20% [10].

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The response of fungal metabolites of different duration have been reported in plant system and confirmed by several researchers [11, 12, 13, 14, 15, 16]. Presently response of metabolites secreted in culture filtrate of *Aspergillus niger* against wheat plant has so far not been reported. It seemed to be worthwhile to study parameters in laboratory concerning to seed germination, length of shoot & root; biomass of fresh and dry shoot & root in using *Aspergillus niger* metabolites with *Triticum aestivum* L.

II. MATERIAL AND METHODS

A healthy wheat seeds have been collected from retailers. *Alternaria alternata (Fr.*) Keisel was obtained from laboratory of P.G. Department of Botany, RTM Nagpur University, Nagpur and it was propagated on agar jelly in aseptic condition [17]. An inoculum of isolate obtained from 8 days old culture was transferred aseptically under laminar flows into one liter Czapek'sbroth medium and allowed to incubate for a 20 days in B.O.D. at 25±1^{0}C temperature with continuous shaking. Separate sterilized broth and sterile distilled water were kept as control.

Healthy seeds sterilized with aqueous solution of 0.1% mercuric chloride were soaked for three hour in sterile distilled water to soften seed coat. The 20 days old culture filtrate containing metabolites of *A. alternata* (Fr.) Keissl, was graded to 20, 40, 60, 80 and 100 percent. For metabolite treatment, the water socked seeds were immersed in culture filtrate of different grades for 3 hours. The seeds soaked in sterile distilled water and Czapek's broth medium was served as control. After treatment for each metabolite, immediate seed washing was performed 5 times in a row. Treated and neglected moistened seeds were transferred to sterile blotter paper bars in germination areas and seedling growth studies. The spaces containing the seeds were covered with a glass cabinet to prevent seed contamination. The moisture of the blotter paper was retained by the addition of plain water when necessary. The harvest was taken on 8th days. Growing seeds are calculated at a percentage time while the length of the shoot and the root length are measured in centimeters for each metabolite treatment and untreated control. Seedlings raised in untreated control and treated seed metabolites were classified as normal and abnormal [5].

In order to record new raw biomass, seedlings raised from untreated control and metabolite-purified seeds were cut to separate the shoot and root system. Both shoots and roots were measured in an electronic balance and were recorded in terms of grams of each metabolite treatment and immersed immersion control.

III. RESULTS AND DISCUSSION

The metabolites collected in the *Alternaria alternata (Fr.) Keissl* culture filter for 20 days were estimated to reach different grades and each was tested for parameters such as seed germination, seedling emergence and green biomass *Triticum aestivum* L. On day 8, the number of germinated and ungerminated seeds was recorded as a percentage of each metabolite treatment and control (Table 1).

1) Seed germination

Untreated water soaked in seed results in 86.7 percent germination. Seed germination rate improved by 15.3 percent over control when the seeds were soaked into Czapek's broth while marked by a decrease of 4.0 and 7.7 percent by 20 and 40 percent metabolic treatment respectively. In addition, the metabolic treatment of the 60 and 80 percent culture filtrate was recorded to decrease by 12 and 18.5 percent. It is forbidden to reduce by metabolic treatment of high concentration which has resulted in a decrease in seed germination rate. The higher inhibition to extent of 34.6 percent was recorded with absolute culture filtrate treatment (Table 1). Low doses of culture filtrate have created a small phytotoxic effect while being seen gradually increasing with the concentration of the metabolite. Seeds with a hard seed coat failed to germinate due to the non-diffusion of water from the outside to the inside of the seed with protected seed coat (Table 1).

2) Effect on seedling emergence

The seedlings height from untreated and treated seeds was measured on eighth day for length of shoot and length of root (table 1).

(a)Shoot length

The seedlings raised from the control seed show a shoot length of 11.7 cm. It was increased by 1.7 percent when the seeds were soaked in the Czapek's broth. A 20% metabolic treatment for moderate phytotoxic effect reduced shoot length by 0.9 percent against untreated control. And 40% and 60 percent metabolic treatment filtrate culture shows an additional phytotoxic effect that reduces shoot length by 1.7 percent and 12.0 percent against untreated control. Absolute culture filtrate has a significant phytotoxic effect on the emergence of shoots. Decreased trend over control has been recorded by a plant receiving high-dose metabolic treatment showing 13.7 percent and 21.4 percent with 80 percent and absolute culture filtrate

(b) Root length

The seedling raised from untreated water soaked control exhibited 8.8 cm root length. It was enhanced to 3.4 percent with Czapek's broth treatment. The metabolic treatment 20 – 60 percent culture filtrate induced moderate

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phytotoxic effect on root length against the untreated control. Inhibitory effect was more pronounced with higher doses of culture filtrate. (Table 1).

(c) Emergence of normal and abnormal seedlings

The germinating seeds from untreated control and Czapek's broth transformed 83.4 and 100% standard seedlings with well-developed, root, shoot and no symptoms. It was reduced by 8.0 percent with low culture filtrate rates. Higher doses showed a greater phytotoxic effect that reduced the number of common seedlings and was up to 60 percent with complete cultural treatment. Significant reductions in this parameter have been reported in the conc. filtering culture. Seeds receiving treatment for the conversion of portable metabolites in abnormal seedling associated with disruption of a series of metabolites mutations in meristematic cells [5].

Seeds soaked in Czapek's broth did not show any change but untreated water soaked in germination seeds turned into abnormal seedling up to 3.3 percent. The number of abnormal seedling has increased with high doses of metabolite treatment. A significant proportion of rare seeds have been reported for treatment of cultural filtrate metabolite of 20 & 40 percent. The phytotoxic effect was pronounced with higher doses of culture filtrate increasing count of the abnormal seedling by 10 -23.4 percent against the untreated control (Table 1).

3) Effect of culture filtrate on biomass of seedlings:

The biomass of an untreated seedling of shoot weighs about 2.5gm. It was increased by 8.0 percent when the seeds were soaked in the Czapek broth medium. Treated seedlings had a negative or adverse impact on the biomass production of shoots and roots. The inhibitory effect on both parameters was most evident in the increase in the values of the custom filtrate (Table 1).

The phytotoxic effect on parameters such as seed germination, seedling germination and green biomass was important for seed treated with culture filtrate. This is in line with the findings of Bhajbhuje and Shastri [2] who reported potential of metabolites from culture filtrate of *Alternaria padwiki*(G) Ellis against *Oryza sativa* L. filter system. of sequential control, in which the seeds are soaked in a Czapek nutrient source. This is in line with the findings of Bharatwajand Ram Lal [18] who reported that improved seed germination and seed germination rates in Papaya. Other [15, 16] confirmed these parameters in *Triticum aestivum* L. and *Hordeum vulgare* L. that It appears to be the discovery of micronutrients in the Capek's Das broth which stimulates the immune and biological functions of cells.

Table 1: Effects of metabolites of *Alternaria alternate* on seed germination rate; seedling emergence and green biomass production of seedling of *Triticum aestivum* L.

Sr. No.	Conc of culture filtrate (%)	Percent seed germination	Nature of seedlings (%)		Seedling growth (length in cms)		Biomass of seedling (gms)	
			Normal	Abnormal	Shoot length	Root length	Shoot	Root
1	20	83.3 (-04.0)	76.7 (-08.0)	06.6	11.6 (-0.9)	8.3 (-05.7)	2.3 (-08.0)	1.7 (-05.6)
2	40	80.0 (-07.7)	73.3 (-12.1)	06.7	11.5 (-01.7)	7.6 (-13.6)	2.1 (-16.0)	1.2 (-33.3)
3	60	76.3 (-12.0)	66.3 (-20.5)	10.0	10.3 (-12.0)	7.3 (-17.0)	1.5 (-40.0)	0.9 (-50.0)
4	80	70.7 (-18.5)	55.3 (-33.7)	15.4	10.1 (-13.7)	7.1 (-19.3)	1.1 (-56.0)	0.8 (-55.6)
5	100	56.7 (-34.6)	33.3 (-60.1)	23.4	9.2 (-21.4)	6.9 (-21.6)	0.9 (-64.0)	0.6 (-66.7)
6	Czapek's Broth	100 (+15.3)	100 (+20.0)	-	11.9 (+01.7)	9.1 (+03.4)	2.7 (+08.0)	1.9 (+05.6)
7	Control(D.W.)	86.7	83.4	03.3	11.7	8.8	2.5	1.8

IV. CONCLUSION

The results of present investigation revealed that toxicity of fungal metabolites was intensified on high concentration of treatment attributed to release of secondary metabolites exhibit harmful effect. These metabolites may serves as growth inhibitors, exhibiting reduction in these parameters undertaken and enhanced abnormal seedlings transformation. It is concluded culture filtrate of *Alternaria alternate* contain hazardous chemical constituents hence it may be used for the preparation of eco-friendly pesticides for the management of population of weeds that unable to grow and develop crop plant to maturity.

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ACKNOWLEDGMENT

Author is thankful to Dr. N. M Dongarwar, Prof & Head, P.G.Department of botany, RTM Nagpur University, Nagpur and Principal, Jawaharlal Nehru Arts, Commerce & Science College, Wadi, Nagpur for providing the necessary facilities

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