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Potential of metabolites from culture filtrate of *Alternaria padwickii* (G) Ellis against *Oryza sativa L*

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ABSTRACT

A species of a mitosporic genus *Alternaria* is notoriously known plant pathogen as well as food spoiler, causing damage to food commodities, cooked food and grains in storage. Among these, *Alternaria padwickii* is reported as seed borne pathogen of rice responsible for causing seed discoloration, pre- and post-emergence, seed rot, seedling blight, sheath-rotting and a potent producer of variety magnitude of metabolites of medicinal importance. In present study, an attempts were made to isolate pathogen from infested stored rice seeds and allowed to grow for a period of 30 days in artificial Czapek's nutrient broth for metabolite production. The culture filtrate was graded in different concentrations and each was tested against rice seeds to study seed germination rate, seedling emergence and green biomass production. The rate seed germination was declined by 4.5-39.8 percent; the shoot length by 16.1-66.1 percent; root length by 15.5-44.0 percent; the biomass of fresh shoot reduced by 24.5-50.7 percent; biomass of fresh root declined to the extent of 8.7-49.1 percent; per cent normal seedling reduced by 5.8-55.8 percent over the control when seeds treated with 20 to 100 per cent culture filtrate containing metabolites. Control seeds did not express any change. The metabolites from 20 per cent culture filtrate induced marginal reduction in these parameters over the control. 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. The seed soaked in Czapek's broth enhanced seed germination, seedling emergence and green biomass production.

Keywords: culture filtrate, toxicity, phytotoxic, inhibition, biomass, metabolites, enhance

INTRODUCTION

Metabolites are confined products of enzyme – catalyzed reactions occurs within functional cells during growth and development. Moreover, pathogenic micro-fungi of diverse group are known to secrete or excrete a variety multitude of low molecular weight bioactive organic compounds during a period of growth in infested host tissues may be either non-toxic

or toxic to host cells (Holensein & 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 disturbance in normal cell metabolism to influence the courses of disease development or symptom expression (Bhajbhuje, 2016). Several species of *Alternaria* pro-duce more than 70 phytotoxins was reported to play a crucial role in determining host specificity and contri-buting to disease development (Holensein and Stoessi, 2008). Toxin of secondary metabolites caused damage rapidly dividing meristematic cells (Bhajbhuje, 2020).

Rice (*Oryza sativa* L.) is one of the world's main widely planted staple food crop and most widely consumed nutritious food for a major part of the world's human population including Asia. It is the most important grain with regard to human nutrition providing 21 per energy, 15 percent protein, minerals, vitamins and fibers. Rice flour and starch are used in batters and breading to increase crispiness. Rice starch is career of pharmaceutical, often used as a cosmetic dusting powder, a laundry stiffening agent in cold-starching of fabrics and a custard. Rice porridge is commonly consumed as a breakfast food, and a traditional food for the sick. It is easily digestible hence used in the preparation of delicious South Indian breakfast products such as *Idli, Dosa, Uttapam, Dhokla* etc (Wikipedia, 2021).

Rice world's production has risen steadily from about 200 million tons in 1960 to over 530 million tons with dedication of 162.1 million hectors land in 2020. Asia-Pacific Regions contributed over 90 percent of the world's rice production and consumed as nutritious food. India is second leading rice producer on the globe accounting nearly 158 metric tons in 2020 after China with 211 metric tons annual production. Moreover, India is the top exporting country with 13 million of tons, accounting for 30% of the global exports followed by Vietnam (15 percent) and Thailand (13 percent). India was expected to be the leading global producer and to harvest about 43.8 million hectares of rice in 2021(Wikipedia, 2021).

Majority species of the asexually producing genus *Alternaria* are ubiquitous pathogens, saprophytes, and entophytic. They can deteriorate quality and quantity of food commodities such as fruits, vegetables, cereals, oil plants etc. and decrease their nutritive profile by producing some potent toxic metabolites. They remains as an increasing threat to several crops around the globe causing several diseases including Alternaria leaf blight, damping off seedlings producing brown to black leaf spots lead to a reduction of leaf count and rate of photosynthesis. Several *Alternaria* species are known producers of various sorts of toxic secondary metabolites during their active growth and causes severe diseases in many parts by limiting their productivity to the extent of 20-30% (Serfbe *et al*., 2016; Meena *et al*., 2017).

Among the species, *Alternaria padwickii* is most serious, infecting rice seeds causing seed discoloration, pre- and post-emergence seed rot, seedling blight and has also been detected as a sheath-rotting pathogen and reported to be seed-borne; carried at high levels and likely to be introduced on imported seeds (Yusuf *et al*., 2019). It has an undermined ability to survive as sclerotia in plant debris and soil, as conidia on seed surface or as mycelium inside seed coat and produced both nontoxic as well as toxic metabolites in storage. Deteriorated grains become dark colored, chalky, brittle, shriveled, small black sclerotic appearance in center of lesions with reduced viability (Serfbe *et al*., 2016).

Literature survey reveals that fungal metabolites of primary nature enhanced seedlings growth (Bhajbhuje, 2020) while secondary metabolites becomes toxic to host cells, damage cell components of actively growing cells to influence the course of symptom expression in host plant (Ahmad, *et al*, 2020; Terna *et al*., 2020). Some phylloplane & endophytic fungi are involved in protecting plants from pathogens & produce antimicrobial compounds (Darota and Franscois, 2020). Several researchers have made investigation on role metabolites of *Alternaria* in plant system (Shazia *et al*., 2019; Ahmad *et al*., 2020; Bhajbhuje, 2020). Presently potential of culture filtrate of *Alternaria padwickii* against rice plant has so far not been reported. It seemed to be worthwhile to study parameters concerning to seed germination potential, length of shoot & root; green biomass production of seedlings in using *Alternaria padwickii* metabolites with *Oryza sativa* L.

MATERIALS AND METHODS

Rice (*Oryza sativa* L.), an important food grain with excellent source of nutrient of high caloric intake has been selected as experimental material. A composite

seed sample in storage of rice has been collected in cotton bags from different cultivators and screened for apparent deformities. *Alternaria padwickii*, an insistent of early leaf blight was isolated from infested rice seeds as an internal seed borne pathogen employing the technique of Gutierrez *et al*., (2010) and ISTA (2020). An inoculum of isolate obtained from 6 days old culture was transferred aseptically under laminar flow into one liter Czapek's broth medium and allowed to incubate for a period of 30 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.

Before metabolic treatment, healthy seeds of rice were soaked for 3 hours in water to make soften. The 30 day old culture filtrate containing metabolites of *Alternaria padwickii* was graded to 20, 40, 60 and 80 percent. For metabolite treatment, the water soaked seeds were immersed in culture filtrate of different grades for 5 hours. The seeds soaked in sterile distilled water and Czapek's broth was served as control. After metabolite treatment, the treated seeds were washed thoroughly for five consecutive times. The moistened treated and untreated control seed were transferred to sterile blotter paper folds in slots for germination and seedling growth studies. Harvest was taken on 8th day. The germinated seed were counted in term of percentage while length of shoot/ root of seedling was measured in term of centimeter for each metabolite treatment and untreated control. The seedlings raised from untreated control and metabolite treated germinating seeds were graded as normal and abnormal (Bhajbhuje, 2016).

To record the green fresh biomass, the seedlings raised from untreated control as well as metabolite treated seeds were cut to separate shoot system from the root. Both shoot and root was weighed on electronic balance and recorded in terms of grams for each metabolite treatment and untreated water soaked control.

RESULTS AND DISCUSSION

The metabolites accumulated in culture filtrate of *Alternaria padwickii* for 30 days was graded to 20, 40, 60, 80 percent and each was tested to study various parameters like seed germination, seedling emergence and green biomass production of rice. After 8th day a count of germinated as well as ungerminated seeds was recorded in terms of percentage for each metabolites treatment and untreated water control (Table 1).

1). Seed germination

The untreated control water soaked seeds induced 88 percent germination. The rate of seed germination was enhanced by 4.5 per cent over control when seeds soaked in Czapek's Dox broth while it was recorded to decline by 4.5 and 11.4 per cent with 20 and 40 per cent metabolite treatment respectively. It was confined to reduce by metabolic treatment of higher concentration resulted lowering of the seed germination rate. The higher inhibition to the extent of 39.8 percent was recorded with absolute culture filtrate treatment (Table 1). The metabolic treatment of 20 percent culture filtrate induced little phytotoxic effect while it was gradually significant with increase in metabolite concentration. The seeds having hard seed coat failed to germinate in response to non-diffusion of water from outer medium to inside seed through protective seed coat.

2) Effect on seedling emergence.

The seedlings of raised from untreated and treated rice seeds with culture filtrate of different concentration containing metabolites of *Alternaria padwickii* were measured on eighth day for length of shoot and root of seedling and recorded in table 1.

(*a) Shoot length*

The seedling raised from control seeds exhibited 6.6 cm shoot length. It was increased by 6.5 percent when seeds soaked in Czapek's broth medium. The metabolic treatment of 20 percent culture filtrate induced moderate phytotoxic effect reducing the shoot length by 16.1 percent against untreated control. Absolute culture filtrate had greater phytotoxic effect on shoot emergence. The declining trend over co ntrol was recorded for seedling receiving metabolic treatment of higher doses exhibiting inhibition of shoot length by 45.2 and 66.1 percent with 80 percent and absolute culture filtrate (Table 1).

(b) Root length:

The seedling raised from untreated water soaked control exhibited 6.1cm root length. It was enhanced to 5.2 percent with Czapek's broth treatment but reduced by 15.5 percent with seedling receiving treatment of 20 percent culture filtrate against untreated control.

Table 1: Effect of metabolites of *Alternaria padwickii* on seed germination rate; seedling emergence and green biomass production of seedling of *Oryza sativa* L.

Sr. No.	Conc. of culture filtrate	Per cent Seed germination	Nature of seedlings		Seedling growth (length in cms)		Biomass of seedling (gms)	
			Normal (%)	Abnormal (%)	Shoot length	Root length	Shoot biomass	Root biomass
1	20%	84.0[1] (-4.5)	81.0[2] (-5.8)[3]	3.0 (-96.5)	5.2 (-16.1)	4.9 (-15.5)	0.524 (-24.5)	0.147 (-8.7)
2	40%	78.0 (-11.4)	74.0 (-13.9)	4.0 (-95.3)	4.1 (-33.9).	4.4 (-24.1)	0.493 (-28.9)	0.126 (-21.7)
3	60%	69.0 (-21.6)	62.0 (-27.9)	7.0 (+91.9)	3.9 (-35.0)	4.1 (-29.3)	0.458 (-34.0)	0.107 (-33.5)
4	80%	64.0 (-27.3)	55.0 (-36.1)	9.0 (-89.5)	3.3 (-45.2)	3.8 (-34.5)	0.416 (-40.1)	0.094 (-41.6)
5	100%	53.0 (-39.8)	38.0 (-55.8)	15.0 (-82.6)	2.1 (-66.1)	3.2 (-44.0)	0.342 (-50.7)	0.082 (-49.1)
6	Czapek's Broth medium	92.0 (+4.5)	92.0 (+6.9)	0.0 (0.0)	6.6 (+6.5)	6.1 (+5.2)	0.741 (+6.8)	0.176 (+9.3)
7	Control (D.W)	88.0	86.0	2.0	6.2	5.8	0.694	0.161

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

The inhibitory effect was more pronounced with higher doses of culture filtrate, reducing root length by 34.5 and 44.0 percent. The metabolites of 80 percent and absolute culture filtrate had significant inhibitory effect on root length (Table 1).

(c) Emergence of normal and abnormal seedlings:

The germinating seeds from untreated control and Czapek's broth transformed 86 and 92 percent normal seedlings with well-developed root, shoot and without any symptoms. It was reduced by 5.8 percent, over with treatment of 20 percent culture filtrate. The significant reduction in this parameter was reported with increase in conc. of culture filtrate. The seeds receiving treatment of absolute culture filtrate lowered a count of normal seedling to the extent of 55.8 percent.

The transformation of metabolites treated germinating to abnormal seedling related to disturbances in series of metabolites changes in the meristematic cells. The soaked in Czapek's broth medium as well as untreated water soaked germinating seeds transformed into abnormal seedling to the extent of 2.0 percent having under developed roots, shoot with small lesions on the leaves respectively. A count of abnormal seedlings increased with higher dosages of metabolite treatment. Significant count of abnormal seeding was reported with metabolite treatment of 60 & 80 percent culture filtrate.

3) Effect of culture filtrate on biomass of seedlings:

a) Biomass of fresh shoot:

The biomass of shoot of seedling raised from untreated weighted about 0.694gm. It was increased by 6.8 percent when seeds soaked in Czapek's broth medium. The seeds soaked in 20 percent culture filtrate, reduced shoot biomass to extent of 24.5 percent and it was further declined by 28.9 percent with 40 percent culture filtrate treatment. The significant reduction was reported with higher dosages of metabolite treatment. Absolute culture filtrate had greater inhibitory effect lowering shoot biomass to the extent of 50.7 percent.

b) Biomass of fresh root:

The untreated control seedling exhibited 0.161gm root biomass. It was enhanced by 9.3 percent with Czapek's broth nutrients but reduced by 8.7 percent when seedling treated with 20 percent culture filtrate. The seedlings received treatment of 40 and 60 percent culture filtrate, reduced root biomass by 21.7 and 33.5 percent respectively. The culture filtrate of 80 and 100 percent had greater inhibitory effect, decreasing root biomass by 41.6 and 49.1 percent.

The rate of seed germination; 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 Bharatwaj (2014) who reported enhance seed germination rate and seedling vigour in Papaya. Bhajbhuje and Pathode (2014); Bhajbhuje (2020) confirmed these parameters in *Triticum aestivum* L, and *Hordeum vulgare* L. It may possibly seem to be availability of micronutrients in the Czapek's Dox broth which stimulate the metabolic, physiological and biochemical activities of the cells.

The results on seed germination declined by 4.5-39.8 percent; the shoot length by 16.1-66.1 percent; root length by 15.5-44.0 percent; the biomass of fresh shoot reduced by 24.5-50.7 percent; biomass of fresh root declined to the extent of 8.7-49.1; per cent normal seedling reduced by 5.8-55.8 percent over the control when seeds treated with 20 to 100 percent culture filtrate containing metabolites. Control seeds did not express any change.

These results were confirmed with earlier findings of Wagh *et al*., (2013) reported the typical symptoms of *Alternaria* leaf spot *in vitro* and *in vivo Alternaria alternata* inoculated plantlets and detached leaves of *Lepidium sativum*. Bhajbhuje (2015) 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*. Shazia *et al* (2019) reported complete inhibition in rate of seed germination of *Solanum lycopersicum* in response to the culture filtrate of *Alternaria alternata* and *Trichothecium roseum.* Ahmad *et al*., (2020) inhibitory effect of Alternaria metabolites on seed germination in rapeseed. Terna *et al*., (2020) reported responses of different tomato cultivars inoculated with culture filtrate of selected fungal pathogens including *Alternaria lycopersici*. This inhibitory effect of the culture filtrates can be attributed to the presence of certain toxic secondary metabolites that the test fungi have released in the nutrient medium.

Recent investigations have explored the availability of various mycotoxins from several microbes including fungi confined to different geographical regions of the world. Mycotoxin secretion by filamentous fungi has been reported in many crops including cereals, vegetables, oil-seed crops and pulses (Holensein and Stoessi, 2008; Wagh et a., 2013; Bhajbhuje, 2016; Meena *et al*., 2017; Shazia *et al*., 2019; Terna *et al*, 2020). The isolation of different mycotoxins provide information regarding the severity of the pathogen and its toxic effects caused by the cumulative action of all these toxins. The individual action of single mycotoxin for disease development is incurred by its efficiency and the degree of damages (Meena *et al*., 2017). 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). Several studies reported the occurrence of Alternaria species on cereals worldwide, and the capability of Alternaria strains to synthetize wide contents of mycotoxins in in vitro media Tsuge *et al*., 2013; Bhajbhuje, 2015). The most common Alternaria mycotoxins includes dibenzopyrone derivatives alternariol (AOH), alternariol mono-methyl ether (AME) and altenuene (ALT) from one side; the tetramic acid derivative Tenuazonic Acid (Masiello *et al.* 2020).

Phytotoxic and mutagenic and effect of mycotoxins has been highlighted by ESFA (2011)*; Meena et al (2017)* and Shazia *et al*.(2019); Dorota & Francois (2020). 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 meristemmatic cells of the plant system (Bhajbhuje, 2020). 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 (EFSA, 2011). Most *Alternaria* mycotoxins induced considerable cytotoxic effects; Altertoxin III is reported highly mutagenic while altertoxins I and II induced low mutagenicity. 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 (Brakhage and Schroeckh, 2011). TeA, iso-tenuazonic acid, and their salts exhibit herbicidal activity with broad spectrum properties,

quick killing, and high efficiency for plants. TeA was also found in lentils, and in recently found in beer and cereal foods (Meena *et al*., 2017). The addition of adjuvants improves the herbicidal activity of these compounds. Recently, many *in vitro* studies have reported that AOH causes DNA damage by inducing cell cycle arrest which leads to mutations in living beings. Furthermore, AOH also exhibits cytotoxic, foetotoxic, mutagenic and teratogenic effects that is responsible for the etiology of oesophageal cancer. It has been showed that both AME and AOH have potential carcinogenic, genotoxic and cytotoxic activity in both microbial and mammalian cell system (Masiello *et al*., 2020)

CONCLUSION

Alternaria padwickii produced several toxic metabolites in artificial medium during its static growth provided favorable environment. The toxicity of fungal metabolites was intensified on high concentration of treatment attributed to release of secondary metabolites, serves as growth inhibitors, exhibiting reduction in these parameters undertaken and enhanced abnormal seedlings transformation. It is concluded culture filtrate of *Alternaria padwickii* contain beneficial and hazardous chemical constituents. The isolation, purification and characterization of mycotoxins provides information regarding the severity of the pathogen and its toxic effects caused by the cumulative action of all these toxins. The individual action of single mycotoxins for disease development is incurred by its efficiency and the degree of damages. The elucidation of the functional pathway lies behind the biogenesis, action mechanism, signaling cascades involved and the relevant host mediated defense response in presence of these mycotoxins will assist pathogen controlling and disease development. Moreover, secondary metabolites may be used as structural lead for the preparation of ecofriendly pesticides for the management of population of 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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