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Quantitative Changes in Primary Metabolites in Sorghum (*Sorghum bicolor)* Due to Charcol Rot Disease, Causal Agent *Macrophomina phaseolina*

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A B S T R A C T

Charcoal rot, a root and stem disease, is one of the most important disease caused by the fungus *Macrophomina phaseolina* in sorghum that limits their productivity worldwide. Fungal infection caused both quantitative and qualitative damage to the seeds. An attempt was made to study the quantitative changes in primary metabolites on the infected seeds and seedlings of sorghum at different stages after sowing. Observations revealed that content of total sugars and starch were highest in healthy (control) seeds and lowest in heavily infected seeds and were continuously increased in healthy (control), weakly and in moderately infected seedlings and decreased in heavily infected seedlings from 10 to 30 days after sowing. Phenols and proteins decreased as the severity of infection increased and were highest in weakly infected and lowest in heavily infected seeds among naturally infected seeds. Proteins in seedlings decreased as the infection increased and the total phenol contents were increased throughout from 10 to 30 days after germination.

***Key words*:** Quantitative changes, Primary metabolites, Sorghum, *Macrophomina phaseolina*

Sorghum bicolor (L.) Moench commonly known as "Jowar" is one of the most important millets of India belonging to the family "Poaceae" and is a worldwide widespread C4 crop. It is a subsistence crop and the main food for populations in arid or semiarid regions [1-2]. It is used for the production of gluten-free products, in alcoholic breverages, pet foods. The major sorghum cultivating states are Maharashtra, Karnataka, Rajasthan, Tamil Nadu and Andhra Pradesh. Charcoal rot disease in sorghum is a root and stem disease caused by the soil-borne fungus *Macrophomina phaseolina* (Tassi) Goid. *Macrophomina phaseolina* is a polyphagous, necrotrophic fungus that causes charcoal rot disease in more than 500 agriculturally important crops including common bean, soybean, chickpea, sunflower, maize, geranium, tomato and sorghum [3-4]. Charcoal rot is a disease with a high economic impact especially in sorghum, soyabean and maize [5]. In sorghum, the onset of charcoal rot usually occurs after flowering and at the grain filling stage, when the plants are at drought stress. High temperature and low soil moisture are the important factors predisposing sorghum plants to infection by *M. phaseolina*. Charcoal rot is a major disease, causing sorghum yield loss and reduced sorghum seed quality, altering the level and profile of seed composition nutrients [6]. During infection the host plant defend itself against potential pathogens by means of number of physical and chemical factors which may already present in the host or may be produced to the response of infection [7]. The current research focuses on characterizing the effect of one of the major diseases (charcoal rot) on seed and seedling sugars, protein and phenols.

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MATERIALS AND METHODS

Seeds and seedlings of healthy (control) and naturally infected (three categories weakly, moderately and heavily) with *Macrophomina phaseolina* after 10, 20 and 30 days of sowing were taken for conducting studies. The seeds were grown in Petri plates on blotter and earthen pots (height 30 cm, diameter 20 cm) filled with sterile coarse sand (pH 8.3). the emerging healthy and naturally infected seedlings were excised for the estimation of primary metabolites at 10, 20 and 30 days after sowing.

Estimation of primary metabolites

Total sugars and starch were estimated by the method of [8]. Total phenols were determined by [9] method and total proteins were measured according [10].

Statistical data analysis

All experiments were performed in 3 different sets with each set-in triplicate. The data are expressed as mean, ± SEM (standard error of the mean). Statistical analysis of data was done by using BioStat 2009 professional 5.8.4 software in a completely randomized design. All data obtained by subjected to one way analysis of variance (ANOVA). Values of p which were ≤ 0.05 were considered as significant. Graphs were drawn by using Microsoft Excel software.

RESULTS AND DISCUSSION

Total sugar contents were increased from healthy to weakly but after that it get decreased and lowest in heavily infected seeds among all three categories of naturally infected seeds. Sugars were continuously increased in healthy weakly and in moderately infected seedlings and decreased in heavily infected seedlings from 10 to 30 days after sowing. Total starch contents were also increased from healthy to weakly but after that it gets lowered down and lowest in heavily infected seeds among all three categories of naturally infected seeds. Starch contents were slightly increased in seedlings up to the 20 days and followed by decreased to the 30 days stage after sowing. Proteins were slightly increased from healthy to weakly and followed by continuously decrement and lowest in heavily infected seeds. In seedlings, protein contents were continuously decreased in healthy, weakly and heavily infected seedlings throughout from 10 to 30 days after sowing. Phenols were decreased as the severity of infection increased in seeds. In seedlings, phenol contents were continuously increased in healthy, weakly and heavily infected seedlings throughout from 10 to 30 days after sowing.

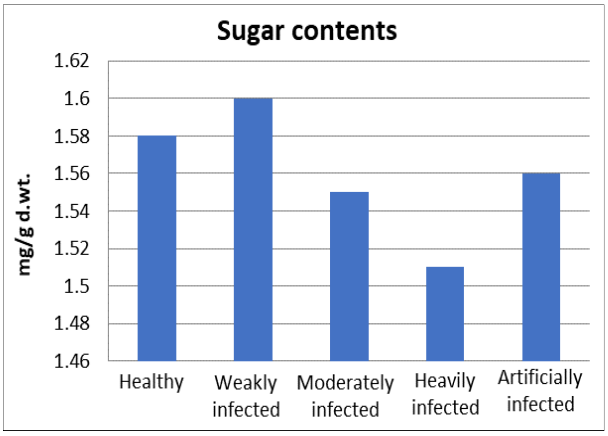

Fig 1 Amount of sugars in seeds of healthy(control) and naturally infected (weakly, moderately and heavily)

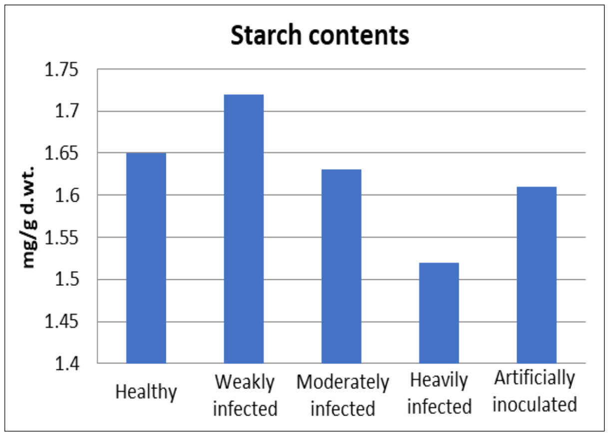

Fig 2 Amount of starch in seeds of healthy(control) and naturally infected (weakly, moderately and heavily)

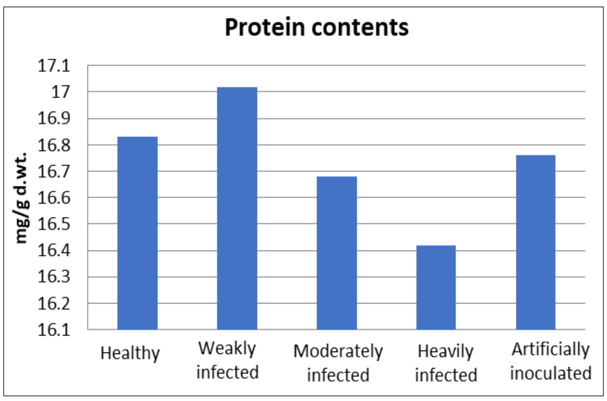

Fig 3 Amount of protein in seeds of healthy(control) and naturally infected (weakly, moderately and heavily)

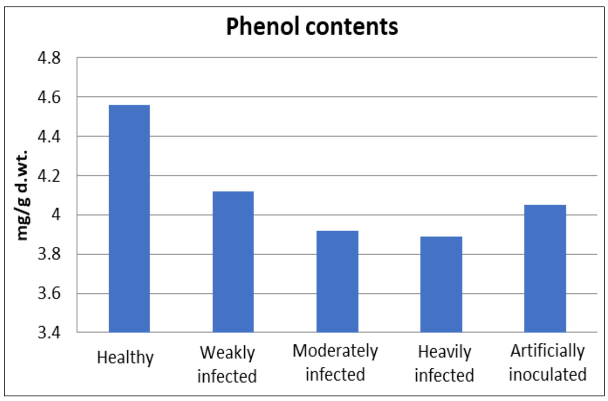

Fig 4 Amount of phenol in seeds of healthy(control) and naturally infected (weakly, moderately and heavily)

Sugar decreased after infection in seeds because it is a good source of food and carbon and is easily digested by the fungus. [11] reported the effect of seed borne *Fusarium oxysporum fungus* on cowpea. It concluded reduction in sugars. As we reported in seedlings the gradual increase in the level of sugars and starch (weakly and moderately infected seedlings) because high level of sugars in plant tissues enhances plant resistance and the increased sugar content is one of the adaptive strategies of plants for the maintenance of structure and function during stress. Sugars enhance oxidative burst at early stages of infection, increasing lignifications of cell walls, stimulate the synthesis of flavonoids. The primary metabolic process provides abundant cellular energy and substrates for plant defense responses in the context of plant-pathogen interactions, and some metabolites perform their function as molecules signalling to trigger defense response through signal transduction and pathogen recognition [12-13]. [14-15] also finds, in vitro cultured embryo axes of yellow lupine become less affected by the infection of hemibiotrophic fungus Fusarium oxysporum through external supply of sucrose due to enhanced generation of superoxide anions, which may be one of the causes for the greater resistance. [16] reported in *A. thaliana* tolerance to biotic stress is strongly enhanced by the accumulation of sucrose. A similar behaviour was observed by [17] in leaves of alfalfa subjected to drought. Soluble sugars, especially fructose and glucose in higher plants can accumulate in tissues as a consequence of different types of stress [18], and can contribute to regulate cellular turgidity [19].

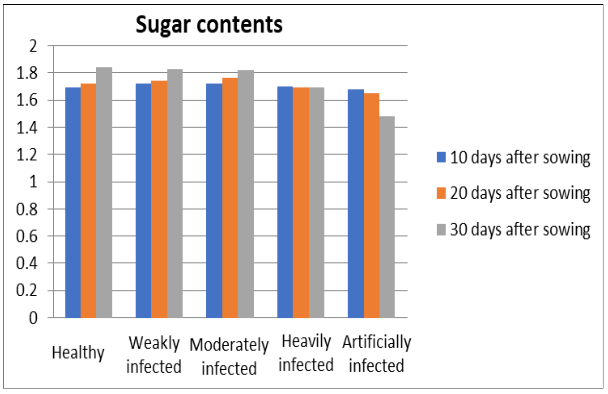

Fig 5 Amount of sugars in seedlings of healthy (control) and naturally infected (weakly, moderately and heavily) on 10th, 20th and 30th day of sowing

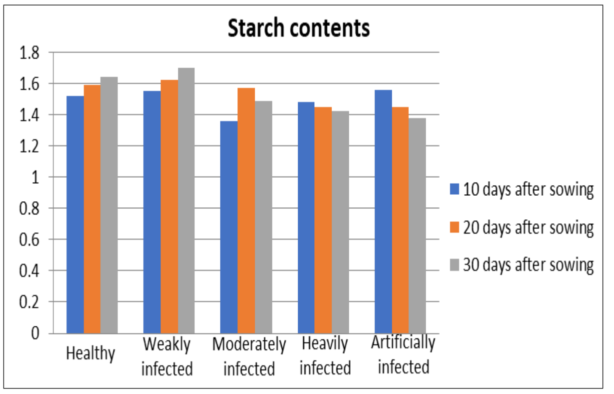

Fig 6 Amount of starch in seedlings of healthy (control) and naturally infected (weakly, moderately and heavily) on 10th, 20th and 30th day of sowing

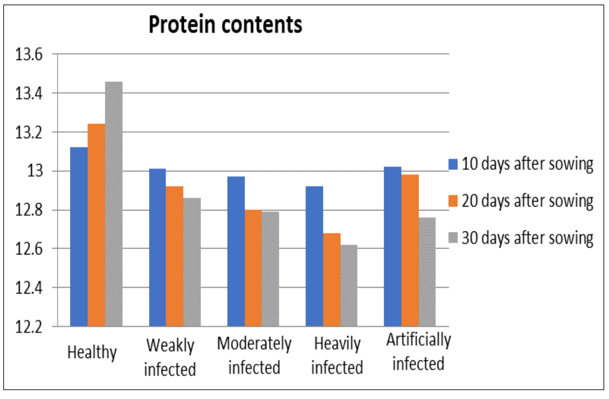

Fig 7 Amount of protein in seedlings of healthy (control) and naturally infected (weakly, moderately and heavily) on 10th, 20th and 30th day of sowing

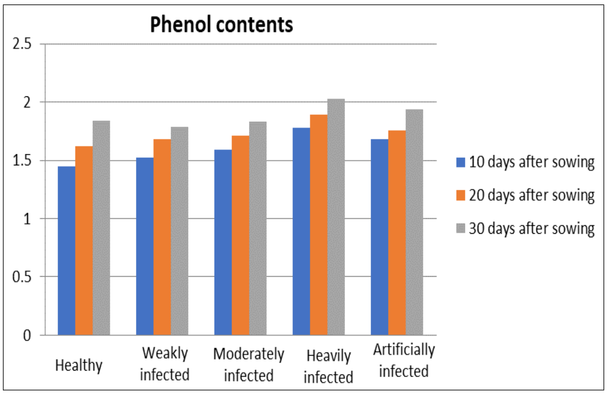

Fig 7 Amount of phenol in seedlings of healthy (control) and naturally infected (weakly, moderately and heavily) on 10th, 20th and 30th day of sowing

Protein content was drastically reduced in both seeds and seedlings as the days of storage increases. This agrees with the findings of [20-21]. The possible reason may be that the fungus hydrolyses the proteins present in seeds by proteolytic enzymes. This is supported by [22] who reported that the crude protein of melon seeds was reduced in fungal contaminated seeds when compared with healthy seeds. [23] observed decline in the protein content of sesame and sunflower seeds artificially inoculated with *Alternaria alternate*, *Aspergillus flavus*. The presence of higher levels of phenols in infected tissues may be implicated in resistance of the host to infection. Accumulation of phenolic compounds at the infection site has been correlated with the restriction of pathogen development. [24] observed induction of phenol content in sesame by the inoculation of charcoal rot fungus *M. Phaseolina*. [25] also reported total phenol content imparting resistance against soft rot pathogen in potato and found significantly higher total phenol content in resistant variety than susceptible.

CONCLUSION

Charcoal rot is a major disease of sorghum (*Sorghum bicolor*) caused by *Macrophomina phaseolina* and results in significant loss in yield and seed quality. The present study demonstrates that a combination of charcoal rot infestation in the soil, and drought results in the infection of sorghum plants, can alter seed protein, sugars, fat and phenols. It concluded that charcoal rot can alter seed composition, but its effect will depend on the type of seed composition nutrient. Since there was a lower protein content in seeds and seedlings but it shows different response in sugars, starch and phenols.

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