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Infection and Phytopathological effects of Curvularia lunata in Sorghum.

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ABSTRACT

A study of 98 seed samples of sorghum procured from 14 locations revealed a high percent incidence of *Curvularia lunata*. The seeds were categorized into asymptomatic, moderately symptomatic and heavily symptomatic categories to examine the phytopathological effects of *C. lunata* using the Petri-plate method and test-tube seedling symptom test. The biochemical analysis was also assessed to identify the nutritional values in infected seeds with comparison to the healthy seeds. The percent of asymptomatic, moderately symptomatic and heavily symptomatic seeds (400 seeds/sample) were 36-120, 50-113 and 16-99%, respectively. 200 seeds per sample were sown on moist blotting papers (10 seeds/plate) and on 1% water agar medium in test tubes (1seed/test tube) and incubated at 25±2ºC for 12/12 h alternating cycles of light and darkness for up to 7 days and 14 days, respectively. On the 7th and 14th days, seed germination, ungerminated seeds with fungal growth, seedlings with symptoms and mortality were observed. The symptomatic seedling part kept on the fresh PDA plate to isolate *C. lunata*. The symptoms on seedlings were black spots on shoot and leaf, necrosis and rotting. The ungerminated seeds showed rotting with heavy growth of *C. lunata* on and around the seeds. The protein, carbohydrates, and lipid contents were decreased in infected seeds as compared to the healthy seeds, but in some infected seeds protein content was increased, may be due to activation of stress proteins. The phenol and tannin contents were comparatively increased to regulate the fungal stress condition. The finding suggests that *Curvularia lunata* is transferred to the next generations through infected seeds and decreased the nutritional values in sorghum seeds..

Keywords: *Sorghum, Seeds, Curvularia lunata, Transmission, Biochemical analysis*

INTRODUCTION

A diverse community of microorganisms is associated with the caryopses of all weed and crop plants in the Poaceae family (Pitty *et al.,* 1987; Ikeda *et al.,* 2006). Infection may happen by fungi, which can ultimately degrade the quality of the product during pre- and post-harvest as well during storage. fungal contamination not only spoil and ruin the quality of the produce but also can cause adverse effects on health due to their ability to produce a range of metabolites known as mycotoxins (Alkuwari *et al.,* 2022). Mycotoxins are estimated to be present in 25% of the world's harvested crops leading to five billion dollars in losses annually in the United States and Canada only (Eskola *et al.,* 2020; Bartholomew *et al.,* 2021). Overall, over 400 compounds are defined as mycotoxins, while 30 of them are given more importance since they are deleterious to human and animal health (Agriopoulou *et al.,* 2020).

Cereals are the principal nutritional source for a large part of the world's population (Vicente *et al.,* 2019). According to a recent FAO forecast, one of the main targets of global agricultural production is staple foods. FAO's forecast for world cereal production in 2023/24 has been raised marginally and now stands at 2.841 million tonnes, marking a 1.1 percent (32 million tonnes) rise from the previous year's level (https://www.fao.org/worldfoodsituation/csdb/en). But cereals are the target of many diseases, mostly caused by fungi, and therefore, a risk for both food security and safety. Plant pathogenic fungi are the main causes of serious diseases affecting plants (Vicente *et al.,* 2019), leading to significant reductions in yield quantity and quality, and consequently, economic losses worldwide. However, devastating plant epidemics in less developed countries frequently affect crops destined directly for human consumption and not for trade and have a social impact

definitely outstripping their economic impact (Vurro *et al.,* 2010). It is estimated that around 30% of emerging diseases are caused by fungi (Giraud *et al.,* 2010). Among all the causes that can be attributed to decreasing crop productivity, yield loss due to plant pathogens plays a crucial role since plant diseases are, directly or indirectly, responsible for losses of an estimated 40 million every year (Roberts *et al.,* 2016), corresponding to 20–40% of total losses in crop yield (Savary *et al.,* 2012). Deleterious fungal communities, in particular, differ depending upon grass host species, environment, geographical location, and level of resistance to grain mold, weathering, post-harvest seed deterioration and associated disease complexes (Little *et al.,* 2011).

Sorghum bicolor is the fifth most significant crop in throughout the world after maize, rice, wheat and barley (FAOSTAT, 2015). It is the main staple grain in Burkina Faso to human nutrition (Gilles *et al.,* 2021). Sorghum cultivation is helpful to suppress weeds growth, promote nutrient cycling and prevent soil erosion (Mohanty *et al.,* 2024). The nutritional value of sorghum is highest and as compared to other grains it is a cheapest crop. It has fiber, protein, sugar, starch and phenolic compounds in rich amount (Shen *et al.,* 2018). In addition, it is a good source of minerals (especially Zn, P, K and Mg) and vitamins (especially vitamin B, A, E, K and D) (Martino *et al.,* 2012; USDA, 2019). Sorghum has phenolic compounds that beneficial to prevent human diseases such as cellular damage due to activation of reactive oxygen species and cancer (Gonz´alez-Montilla *et al.,* 2012).

Diseases of sorghum have significantly reduced the number of functional leaves and have led to global yield reduction of about 50% (Ogolla *et al.,* 2019). Sorghum is hampered by a number of pathogens including fungi, bacteria, nematodes, and viruses (Dania *et al.,* 2020). Sorghum production is significantly affected by numerous fungal pathogens, which reduce its quality and yield (Khaskheli *et al.,* 2025). According to Funnel and Pedersen (2006) fungal diseases in sorghum are enhanced by favorable environmental conditions. However, it is not exempted from parasitic attacks, especially of fungi, which can lead to yield losses and also serve as a reservoir for fungal agents that could induce the secretion of mycotoxins harmful to human and animal health through their metabolism (Sanon *et al.,* 2023).

Curvularia is one of the known genera that is responsible for causing various diseases in sorghum and many other crops. Loss of seed viability is the major resultant effect of *Curvularia* infection. Different species of Curvularia cause more severe diseases (Brecht *et al.,* 2007). The uncommon leaf spot and leaf fleck caused by *C. eragrostidis, C. pallescens, C. lunata, C. penniseti, C. sorghina* and *C. verruculosa* were recorded on sorghum (Leslie, 2002). *Curvularia lunata* is main dominant fungal species that cause severe infection on sorghum (Yago *et al.,* 2011). It causes grain mold and leaf spot diseases in sorghum and affects productivity and quality of grain (Abbas and Agag, 2024). *C. lunata* was recognized in the aleurone layer, pericarp, embryo, seed coat, and endosperm by microscopic examination. Mycelium and mycelia of fungi are heavily colonized in scutellum, followed by in coleorhiza, coleoptile and embryonic axis (Deshmukh and Raut, 1993). These fungi naturally cause both intra- and extra- embryonal infections in sorghum seeds by deformation of seed parts and lysis of several tissues (Rastogi *et al.,* 1990). The major routes of grain infection by these fungi are likely to be via the floret and developing grain on the field (Navi *et al.,* 2005). It is also possible that grain can be infected systemically from plants grown from infected grain or infected through roots or crowns, as has been observed in other grains (Al-Sadi and Deadman, 2010; MurilloWilliams and Munkvold, 2008). To date no report has been analyzed on disease transmission and biochemical parameters of sorghum infected with *Curvularia lunata*, therefore in the present study phytopathological effects of *C. lunata* were studied to determine the symptoms of infection on seed and seedlings with percent incidence as well as biochemical parameters were also evaluated with comparison to the healthy seed.

2. MATERIALS AND METHODS

Collection of seed samples

A total of 98 sorghum seed samples were collected from 14 locations of farmer's fields for isolation of the pathogen *Curvularia lunata*. Seed samples were kept individually in brown paper envelops, followed by in polyethylene bags, labeled and given laboratory accession numbers.

Dry seed inspection and Percent incidence

Dry seed inspection per (400 seeds/sample) was done on the basis of seed discolourations. Percent incidence of the pathogen was calculated using standard blotter method (ISTA, 1985).

Isolation of the pathogen

Curvularia lunata was isolated by using agar plate method. Initially sorghum seed samples were thoroughly washed with running water for approximately 10 min., followed by surface sterilized with 2% sodium hypochlorite (NaOCl) for 3 min., and then washed with sterile distilled water for twice times. Sterilized seed samples (10 seeds per Petri-plate) were placed on fresh potato dextrose agar (PDA) plate having streptomycin (50µg/ml) as anti-bacterial drug (Otero *et al.,* 2002). PDA plates were incubated at 28ºC for 3 days. Pure culture of different out growing fungi was obtained by transferring fungal colonies to new PDA plates and then incubated for 5-7 days at 28°C. The experiment was performed in triplicate form.

Phytopathological effects

Naturally infected sorghum seed samples carrying asymptomatic, moderately symptomatic and heavily symptomatic seeds

infected with *C. lunata*, were selected for transmission and phytopathological effects. 200 seeds per sample were sown on moist blotters (10 seeds/plate) and 1% water agar medium in test tubes (1seed/test tube) and incubated at 25±2ºC for 12/12 h alternating cycles of light and darkness up to 7 days and 14 days respectively. Phytopathological effects were observed by examine seed germination, ungerminated seeds with fungal growth, seedlings with symptoms and mortality. Infected plant parts were used to isolate the pathogens.

Biochemical Analysis

The biochemical estimations including total soluble proteins, total lipids, total soluble sugars, starch, total phenol and tannin of healthy and infected seeds were carried out by using standard protocols.

Extraction

100 mg seed powder was macerated in 10 ml of ethanol and allowed to stand overnight at room temperature (RT) for proper extraction. The extracted solution was centrifuged at the speed of 1000 rpm tentatively for 30 min. The supernatant was collected to estimate total soluble sugars and total phenol. The pellet was separated to starch analysis. The methods to evaluate total soluble proteins, total lipids and tannin were described separately.

Total Soluble Protein

Concentration of total soluble protein was identified using Lowery *et al.* (1951) method with modifications. 100 mg of each seed powder was mixed with 10 ml of ethanol: ether (2:1) solution and kept overnight. The mixture was centrifuged at 2500 rpm for 5 min. and residue was collected and dried. Dried residue was dipped in 10 ml of 5% trichloro acetic acid (TCA) and homogenized properly using mortar-pestle, followed by again centrifuged it at 2500 rpm for 5min. Residue was separated and dried. Dried residue was mixed with 10 ml of 10% TCA and stirred. The solution was centrifuged at 2500 rpm for 5 min. The supernatant was discarded and pellet was boiled for 10 min. with 10 ml of NaOH (1N). The solution was cooled under water-bath and then filtered with the help of Whatman filter paper No. 42. A regression curve was prepared. A stock solution of BSA (Sigma Chem. Co., St. Louis, USA) was prepared in 1N NaOH (1mg/ml). Concentrations ranging from 100 to 1000 µg were separately measured in test tube and volume of each sample was made to1 ml by adding distilled water. 0.1 ml aliquot was used for protein estimation and mixed with 5 ml of alkaline solution (Prepared by mixing 50 ml of 2% Na_2CO_3 in 0.1 N NaOH and 1 ml of 0.5 % $CuSO_4$.$5H_2O$ in 1% Sodium potassium tartarate). After 10 min. of incubation, 0.5 ml of Folin-Ciocalteau reagent (1N) was added and shaken properly. The solution was incubated for 30 min. thereafter absorbance was read at 750 nm using spectrophotometer.

Total Lipid

1 gm of each powdered seed sample was crushed properly with 10 ml of distilled water using mortar-pestle (Jayaraman, 1981). 30 ml of chloroform: methanol (2:1) solution was added in homogenized sample and mixed thoroughly. The mixture was kept overnight at RT, followed by 20 ml of chloroform and distilled water was added. The solution was centrifuged at 10,000 rpm for 10 min. Three layers were visualized: upper coloured aqueous layer of methanol has all the water-soluble materials; a thick pasty interphase; and a clear lower layer of chloroform that contains all lipids was formed. Lower (organic) layer was collected in pre-weighed Petri-plate and kept it for evaporation. The methanol layer and interphase were discarded. After complete evaporation, the final weight was calculated. The total lipid content per gram of sample was measured by subtract the final weight of Petri-plate with pre-weighted Petri-plate.

Total Soluble Sugars

Total soluble sugar was estimated using phenol-sulphuric acid method (Dubois *et al.,* 1951). A standard regression curve of standard sugar (glucose) was prepared. A stock solution of glucose (1mg/ml) was prepared in distilled water. From this solution, 100-1000 µg was pipette out into separate test tubes and volume was made up to 1 ml with distilled water. 0.5 ml of supernatant was mixed completely with 1 ml of 5% phenol and then 5 ml of H_2SO_4 was added gradually into each test tube. During addition of H_2SO_4 each tube was gently agitated. All tubes were kept in water-bath for 20 min at 30ºC. The intensity of colour complex was measured at 490 nm.

Starch

Mc Cready *et al.* (1950) method was used to estimate starch content. Each pellet obtained after extraction of total soluble sugar was suspended in 5 ml of distilled water separately and subsequently 6.5 ml of 52 % perchloric acid was added and stirred. The solution was centrifuged at the speed of 1000 rpm for 30 min. The supernatant was collected. The whole procedure was repeated thrice and then supernatant was collected in each step. Thereafter total volume of supernatant was maintained up to 100 ml with distilled water. The solution was filtered through Whatman filter paper No. 42. 0.5 ml of filtrate was used to analysis the starch content following the same procedure as that of total soluble sugar.

Total Phenol

Total phenol was estimated using Bray and Thorpe (1954) method. A stock solution of tannin acid was prepared according 1mg/ml in 80% ethanol. Concentrations ranging from 100-1000µg were prepared in the test tube and volume was raised to 1ml by addition of 80% ethanol. 0.5 ml of supernatant was mixed with 1 ml of phenol reagent (20 ml of follin's reagent and

20 ml of distilled water), followed by 2 ml of 20% Na_2CO_3 was added. The mixture was allowed to stand for 1 min. in water-bath, cooled and then 21 ml of distilled water was added. The centrifugation was done for 10 min. at 1000 rpm. The absorbance was read at 650 nm against standard of tannin acid (1mg/ml).

Tannin

One gram of each sample was mixed with 100 ml DW and boiled on hot plate at 100 ºC for around 1 h. The mixture was cooled and then filtered with the help of Whatman 42 filter paper. 1mg/ml stock tannic acid in distilled water was used as standard. Varying concentrations (1-10µg) of standard was taken. Addition of 5 ml Folin-Denis reagent (LOB Chemie Pvt. Ltd.) and 10 ml of Na_2CO_3 solution into 50 ml of distilled water and 10 ml of diluent extract was taken out. All tubes were incubated at 25ºC temperature for 30 min. in a water-bath. Absorbance was read at 700 nm against blank (Amadi *et al.,* 2004; Ejikeme *et al.,* 2014).

Statistical Analysis

Each treatment was conducted in triplicate form and represented as mean values. Duncan's multiple range tests was used to calculate the all data. P values of <0.05 and Student's t-test p values of <0.001 were known to be statistically important (Montgomery, 1976).

RESULTS AND DISCUSSION

Dry seed inspection revealed seeds with black spots, red spots, grey spots, brown discolouration, black discolouration, red discolouration, damaged seeds, healthy seeds, and shriveling seeds (Figure 2 A, B, C). The incidence range of seed categories: asymptomatic, moderately symptomatic and heavily symptomatic (400 seeds/sample) was 36-120, 50-113 and 16-99%, respectively. Black spots and discoloration on seed surface gave pure colony of the *Curvularia lunata* on PDA media (Figure 1B). The occurrence of *C. lunata* was in 86 samples, out of 98 seed samples. Percent incidence range of *C. lunata* was varied from 3% to 100%. Highest incidence % was recorded with Laboratory accession no. of S-1 and S-2 that were used for further experiments.

3.1 Phytopathological effects

The maximum seed germination was 70% in asymptomatic and 25% in moderately and heavily symptomatic sorghum seed samples in Petri-plate method (Figure 2 D, E, F). The germinated asymptomatic category showed 15% *C. lunata* growth in black/greyish black colour on and around seeds, while 20% and 50% infection recognized in moderately and heavily symptomatic seeds, respectively. The ungerminated seed categories were also contaminated with *C. lunata* with different percentage as described in Table 1. The infected seedlings showed mortality 5%, 20% and 25% in the three categories, respectively.

On water agar test the seed germination was 65%, 25% and 15% in the three seed categories respectively on 14th day (Figure 2 G, H, I). Growth of *C. lunata* on and around the germinated and ungerminated seed samples and seedling symptoms in percentage were described in Table 2. Mortality of seedlings was highest 10% in moderately and heavily symptomatic samples as compared to the asymptomatic.

The seedling symptoms including black dots, necrosis and rotting of seedlings in Petri plate method and water agar test were observed in 9th day and 5th day, respectively. The fungus caused blackening on radical base of the shoot and leaves. Due to the *C. lunata* infection, the average whole length of seedling in Petri plate method and water agar test was highly reduced in symptomatic samples with respected to the asymptomatic category (Table 2).

The symptomatic plant parts were surface sterilized and plated on PDA agar, which later yielded colonies of *C. lunata* (Figure 2 N, O). The study proved that the infected seed with *C. lunata* transfer in next generation and cause reduction in the germination rate and whole length of seedling as well as increase mortality. Phytopathological effects of *C. lunata* on sorghum seeds have been firstly reported in the present study. Our results are similar to Singh and Singh (2022) study, which showed black discolored of pearl millet seed due to naturally infection of the *Curvularia lunata* that also caused pre- and post-emergence mortality. In their study asymptomatic seeds also carried infection as like in the present study. Infection of *Curvularia* spp. is common within the plant species in the grass family (i.e., Poaceae) such as maize, millet and sorghum, causing diseases such as seedling blight, root rot, etc. (Chahal *et al.,* 1994; Iftikhar *et al.,* 2003; Rana *et al.,* 2022). *Curvularia lunata* had been reported as seed-borne pathogen of sorghum (Girish *et al.,* 2011; Deanna *et al.,* 2013). Bhat and Shishupala, 2024 performed pathogenicity test and found significant reduction in vigor index and length of sorghum root by infection of *Curvularia* spp. Browning of the aerial parts of the cowpea plant was identified as *Nigrospora sphaerica* and *Culvularia penniseti* in the study of Obisesan and Ojo (2023). Buzdar *et al.* (2023) reported deleterious effects of *C. lunata* on seed health in soybean and suggests the use of diseased-free seeds for better germination and vigorous crop.

Table 1: Phytopathological effects

Parameters	Seedling symptom Test					
	Petri-plate method			Test tube method		
Seed categories →	Asympto-matic	Moderately symptomatic	Heavily sympto-matic	Asympto-matic	Moderately symptomatic	Heavily symptomatic
Seed germination	70%	25%	25%	65%	25%	15%
Growth of *C. lunata* on ungerminated seed	10%	25%	30%	10%	25%	55%
Growth of *C. lunata* on germinated seed	15%	20%	50%	10%	20%	20%
Seedling symptoms	5%	10%	25%	10%	20%	20%
Seed mortality	5%	20%	25%	5%	10%	10%
Average Whole Length (cm)	10.6	6.73	3.43	18.06	9.97	6.7

3.2 BIOCHEMICAL ANALYSIS

3.2.1 Total Protein

The impact of *Curvularia lunata* infection on protein content in sorghum seed samples revealed significantly reduction in S-2 with 105.243 µg/mg/ml over healthy seeds (136.433 µg/mg/ml in S-7 and 124.749 µg/mg/ml in S-8) as shown in Figure 3. Similar to our results, Ndife *et al.* (2013) reported prominently reduction in protein content of cocoa beans when infected with *Phytopthora capsici* and *phytopthora megakanya*. Kandhare (2014) revealed that all common and dominant seed-borne fungi caused reduction in protein content, proves their proteolysis efficacy. However, significantly augmentation in protein concentration in infected seed of S-1 was also observed comparatively that may be occurs due to the activation of stress proteins. According to the Gupta *et al.,* 2015 study, plant secret some essential proteins that are responsible to the plant defense system against pathogens. Jimoh and Abdullahi, 2017 reported that the crude protein content increases because of the degradative activities of the *Sorghum bicolor* seeds by the fungi. Additionally, Darwiche *et al.,* 2017 described that during the pathogenesis, to survive and cause the disease, the fungi produce specialized proteins to suppress the plant's immune response. Independent t-test analysis revealed that the total protein content of infected seeds (S-1 and S-2) did not differ significantly from that of healthy seeds (S-7 and S-8) (123.19 ± 19.87 vs 130.59 ± 6.04 µg/mg/ml; $t = -0.88$, $df \approx 6$, $p > 0.05$).

Table 2: Total protein content in *Curvularia lunata* infected and healthy sorghum seed samples

Seed Samples	Laboratory accession no.	Total Protein content (µg/mg/ml)
***Curvularia lunata* Infected Sorghum Seed Samples**	S-1	141.128±3.304
	S-2	105.243±0.311
Healthy Sorghum Seed Samples	S-7	136.433±0.251
	S-8	124.749±0.277

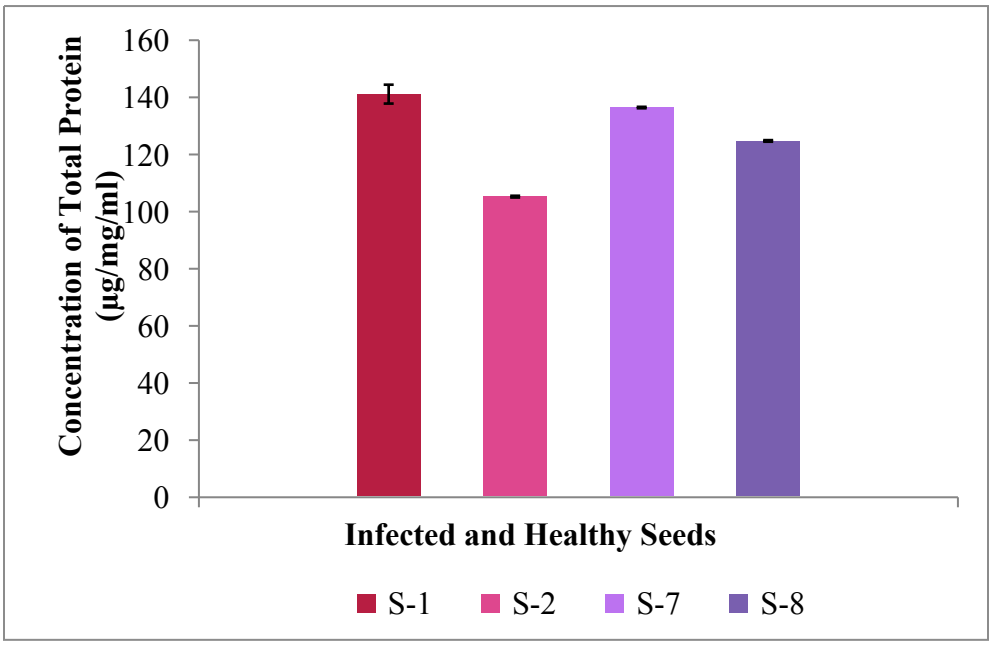

Figure 3: Total protein content in *Curvularia lunata* infected and healthy sorghum seed samples

3.2.2 Total Lipid

Curvularia lunata infection in sorghum seed samples: S-1 and S-2 cause drastic reduction 0.022 and 0.0273 g/g, respectively in the total lipid content with comparison to the healthy seeds that have 0.1087 g/g in S-7 and 0.0953 g/g in S-8) (Figure 4). Likewise, Bandara *et al.,* 2017 found reduction in macronutrients i.e. protein, fat, and starch by 15, 12 and 13%, respectively in sorghum genotypes after stalk inoculation with fungal pathogens (*Fusarium thapsinum, F. andiyazi, F. proliferatum,* and *Macrophomina phaseolina*) as compared to the controls. Fatty acid metabolic pathways contribute to plant defense against pathogens (Kachroo and Kachroo, 2009). However, fungal infection employs different strategies to subvert plant defense responses by altering plant physiology, which results in fungal colonization of seeds. They use these strategies to counteract plant defense responses to complete the life cycle and produce viable progeny. The main fungal strategies for inhibiting plant defense responses include subverting reactive oxygen species (ROS) damage, manipulating tissue pH, inhibiting host proteases, and subverting hormone signaling (Rodriguez-Moreno *et al.,* 2018). Independent t-test analysis showed that total lipid content was significantly lower in infected seeds (0.0247 ± 0.0063 g/g) compared with healthy seeds (0.1020 ± 0.0125 g/g) ($t = -13.57$, $df \approx 7$, $p < 0.0001$).

Table 3: Total lipid content in *Curvularia lunata* infected and healthy sorghum seed samples

Seed Samples	Lab accession no.	Total Lipid content (g/g)
***Curvularia lunata* Infected Sorghum Seed Samples**	S-1	0.022±0.0035
	S-2	0.0273±0.0081
Healthy Sorghum Seed Samples	S-7	0.1087±0.0094
	S-8	0.0953±0.0128

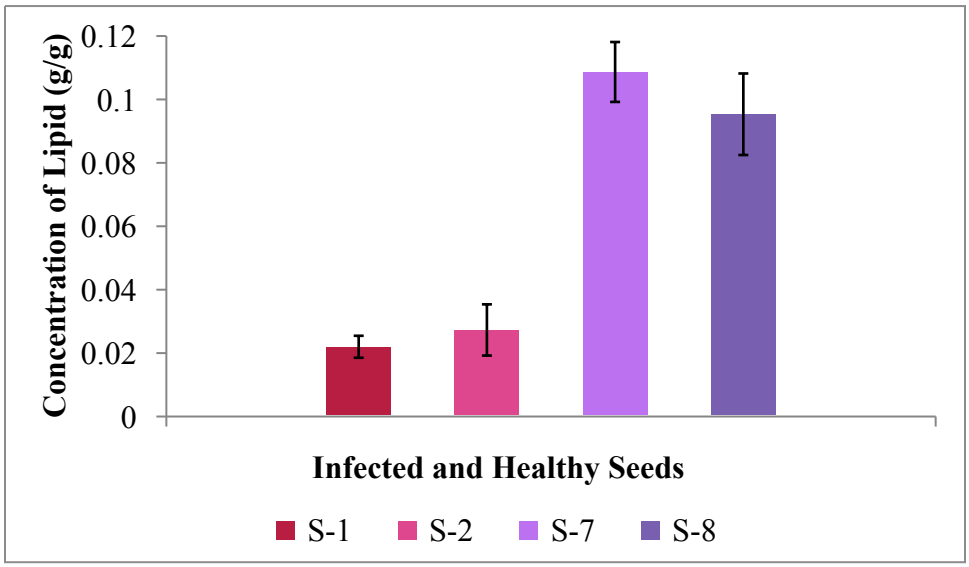

Figure 4: Total lipid content in *Curvularia lunata* infected and healthy sorghum seed samples

3.2.3 Carbohydrates

As shown in Figure 5 and 6, total soluble sugar (TSS) and starch contents decreased in seed samples infected with *C. lunata* as compared to the healthy seeds. With respect to the starch, drastically reduction in TSS content was observed. There was a significant ($P<0.05$) reduction 47.21%, 45.48%, 44%, 42.60% in carbohydrate content of *Irvingia gabonensis* seeds infected with *Rhizopus stolonifer, Aspergillus flavus*, *A. niger* and *Penicillium italicum*, respectively relative to the uninfected seeds (49.41%) in the study of Emiri and Enaregha (2021). Sugars play a key function in plant defense responses against fungal pathogens. Fungi reduce the level of sugars through their uptake and consumption for energy and structural purposes (Morkunas and Ratajczak, 2014). The degradation of storage reserves is vital for the germination of mature seeds, and the sugars obtained from the hydrolysis of starch are the main energy source for seedling emergence (Beck and Ziegler, 1989). It has also been shown that carbohydrates and proteins are required to provide energy and substrates for the seed germination. Therefore, the low germination in infected seeds may be explained by the decreased availability of sugars and proteins (Liu *et al.,* 2021). Sanyaolu *et al.* (2014) reported that depletion in carbohydrate content of fungal infected seeds could occur due to the relative deduction in protein content in the infected seeds may be caused by the degradation and dissolution of the seed tissue by the fungi. Independent t-test analysis indicated that total soluble sugar content was significantly lower in infected seeds (-1.28 ± 0.65 µg/mg/ml) compared with healthy seeds (2.01 ± 0.13 µg/mg/ml) ($t \approx -12.1$, $df \approx 6$, $p < 0.0001$). And starch content was also significantly lower in infected seeds (3.69 ± 0.59 µg/mg/ml) compared with healthy seeds (9.90 ± 0.92 µg/mg/ml) ($t \approx -13.9$, $df \approx 7$, $p < 0.0001$).

Table 4: Total soluble sugar content in *Curvularia lunata* infected and healthy sorghum seed samples

Seed Samples	Lab accession no.	Total Soluble Sugar content (µg/mg/ml)
***Curvularia lunata* Infected Sorghum Seed Samples**	S-1	-1.871±0.0015
	S-2	-0.692±0.0015
Healthy Sorghum Seed Samples	S-7	2.148±0.0061
	S-8	1.880±0.0181

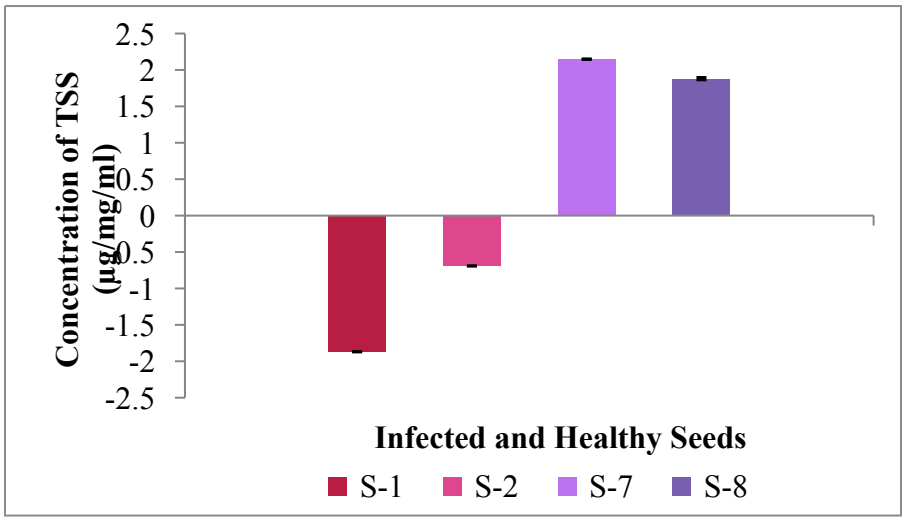

Figure 5: Total soluble sugar content in *Curvularia lunata* infected and healthy sorghum seed samples

Table 5: Starch content in *Curvularia lunata* infected and healthy sorghum seed samples

Seed Samples	Lab accession no.	Starch content (µg/mg/ml)
Curvularia lunata Infected Sorghum Seed Samples	S-1	4.229±0.0481
	S-2	3.156±0.0793
Healthy Sorghum Seed Samples	S-7	10.732±0.2373
	S-8	9.077±0.0735

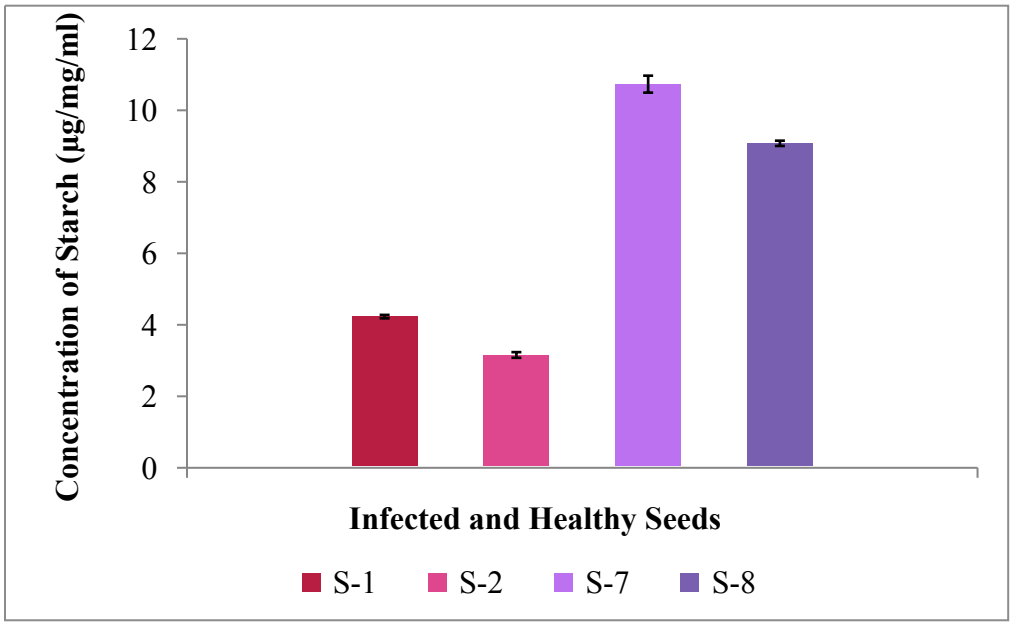

Figure 6: Starch content in *Curvularia lunata* infected and healthy sorghum seed samples

3.2.5 Total Phenol

Total phenol contents increased in *C. lunata* infected seed samples as compared to the healthy seed samples: S-7 and S-8 that have 18.98 and 18.84 µg/mg/ml, respectively (Figure 7). The phenol concentration in infected seed samples i.e., S-1 and S-2 was recorded 34.063 and 56.677 µg/mg/ml. Surovy *et al.* (2020) reported significantly increase (up to 491.70 µg gallic acid/g) total phenolic concentration in 1 to 59% infected wheat grains with *Magnaporthe oryzae Triticum* with comparison to the healthy wheat grains (356.93 µg gallic acid/g). Phenolic acids play a key role in seed development and germination and also contribute to the protection of seeds against pathogens (Granger *et al.,* 2011; Corso *et al.,* 2020). In plants, the defense function of phenolic compounds is partially attributed to their antioxidant properties (Lattanzio, 2013; Krõl *et al.,* 2014). Independent t-test analysis revealed that total phenol content was significantly higher in infected seeds (45.37 ± 12.38 µg/mg/ml) than in healthy seeds (18.91 ± 0.11 µg/mg/ml) ($t \approx 5.23$, $df \approx 5$, $p < 0.01$).

Table 6: Total phenol content in *Curvularia lunata* infected and healthy sorghum seed samples

Seed Samples	Lab accession no.	Total Phenol content (µg/mg/ml)
***Curvularia lunata* Infected Sorghum Seed Samples**	S-1	34.063±0.1242
	S-2	56.677±0.1365
Healthy Sorghum Seed Samples	S-7	18.98±0.1
	S-8	18.84±0.0529

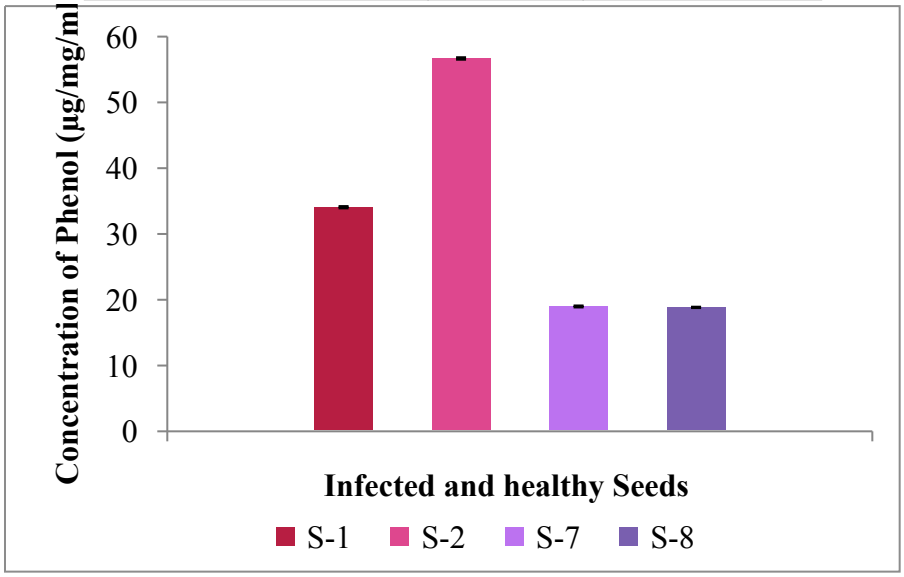

Figure 7: Total phenol content in *Curvularia lunata* infected and healthy sorghum seed samples

3.6.6 Tannin

Tannin contents in infected seed samples were found in highest amounts with the concentration of 36.33 and 40.57 µg/g/ml in S-1 and S-2 as compared to the healthy seeds (33.15 µg/g/ml in S-7 and 29.22 µg/g/ml in S-8) as described in Figure 8. Emiri and Enaregha (2021) revealed augmentation 0.71%, 0.60%, 0.82%, and 1% of tannin contents in the *Rhizopus stolonifer, Aspergillus flavus*, *A. niger* and *Penicillium italicum* fungi infected *Irvingia gabonensis* seeds relative to uninfected seeds (0.48%). Tannins have antimicrobial activity against multiple genera of microorganisms, including fungi, yeasts and bacteria (Zhu *et al.,* 2019). The mode of antimicrobial action of tannins is potentially due to inactivation of microbial adhesins and cell envelope transport proteins (Haslam, 1996; Ya *et al.,* 1998; Saura-Calixto and Perez-Jimenez, 2009). Tannins greatly reduce the fungal enzymatic activities of cellulases and pectinases which lead to the reduction of the intensity of fungal diseases. These results demonstrate that tannins have diverse mechanisms against microorganism, and the action mode of tannins probably depends on the individual species (Achmad *et al.,* 2015). Independent t-test analysis showed that tannin content was significantly higher in infected seeds (38.60 ± 2.09 µg/mg/ml) than in healthy seeds (31.22 ± 2.07 µg/mg/ml) ($t \approx 6.12$, $df \approx 10$, $p < 0.001$).

Table 7: Tannin content in *Curvularia lunata* infected and healthy sorghum seed samples

Seed Samples	Lab accession no.	Tannin content (µg/mg/ml)
Curvularia lunata Infected Sorghum Seed Samples	S-1	36.33±0.3614
	S-2	40.57±0.0818
Healthy Sorghum Seed Samples	S-7	33.15±0.1137
	S-8	29.22±0.0923

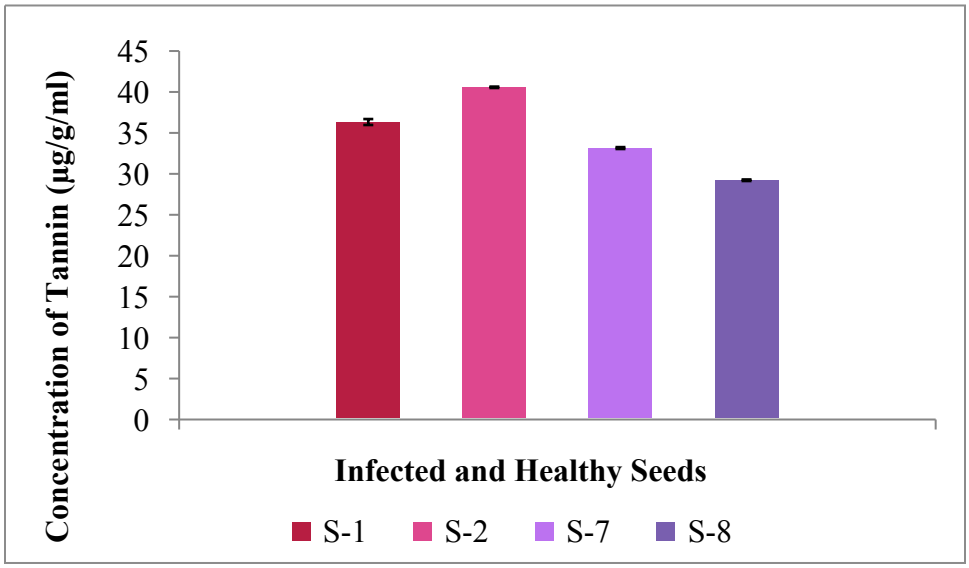

Figure 8: Tannin content in *Curvularia lunata* infected and healthy sorghum seed samples

Overall, the finding results suggest that *Curvularia lunata* present a threat to the cultivation of sorghum. Therefore, there is a need to find appropriate ecofriendly control, such as plant extracts on the basis of future prospects, have proven to be effective in combating fungal infection (Kareem *et al.,* 2018; Obisesan *et al.,* 2022).

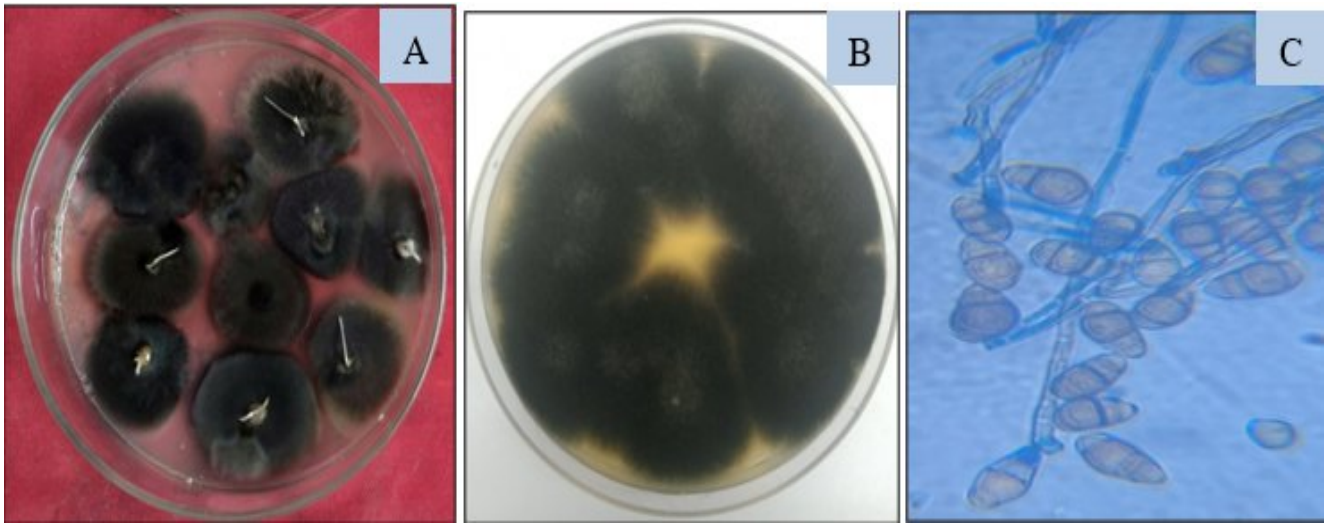

Figure 1: Isolation of *Curvularis lunata* A) Studied seeds on PDA plate B) Pure culture C) Identification under microscope

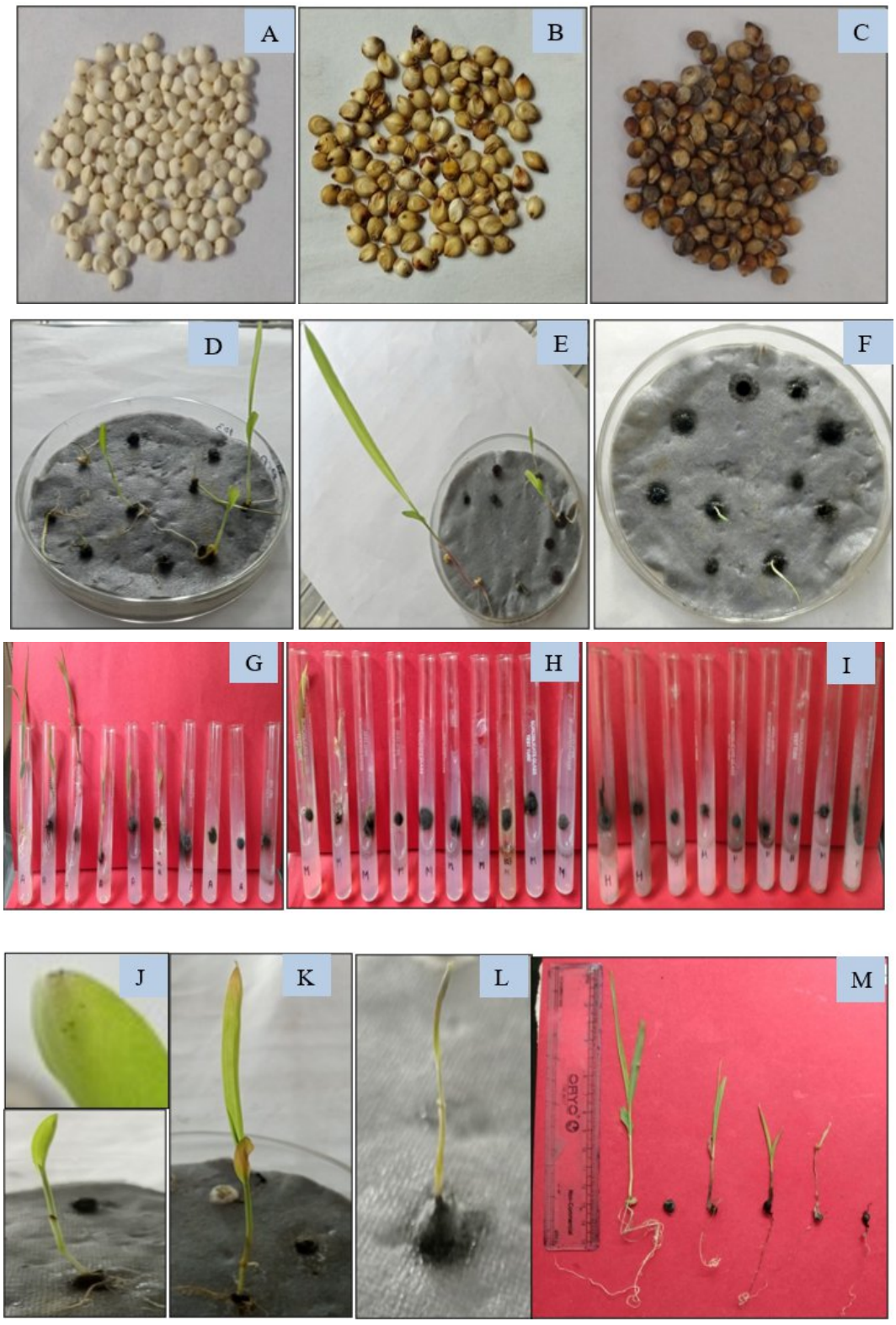

Figure 2: Dry seed inspection: A) asymptomatic seeds, B) moderately symptomatic seeds C) heavily symptomatic seeds; Phytopathological effects (Petri plate method: D) asymptomatic seeds, E) moderately symptomatic seeds F) heavily symptomatic seeds after 7 days, and Water agar test: G) asymptomatic seeds, H) moderately symptomatic seeds I) heavily symptomatic seeds after 14 days); Seedling symptoms: J) black dots on leaf and stem, K) necrosis, L) rotting of seedlings; M) Phytopathological parameters N) Infected seedling parts on PDA plate O) Identification of *Curvularis lunata* under microscope

4. CONCLUSION

The present study concluded that *Curvularia lunata* infection on sorghum seeds cause major loss in seed germination and nutritional values. The incidence of *Curvularia* in sorghum was recorded with maximum percent. Phytopathological results revealed that the infection of *C. lunata* can be observed by appearance of black dots on shoot, leaf necrosis, rotting of seeds/seedlings and increasing mortality. The biochemical parameters significantly reduced due to *C. lunata* infection. Overall, it was observed that seed borne fungal infection play a significant role in transmission and develop symptomatic disease from seed to the growing crop as well as reduce nutrients. Therefore, on the basis of future prospects, On the basis of future prospects, it is necessary to use ecofriendly, cost-effective antifungal drugs to control fungal infection in sorghum.

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