

Antagonistic Efficiency of *Aspergillus giganteus* as a Biocontrol Agent against Aflatoxigenic *Aspergillus flavus* Infecting Maize

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Abstract

Aspergillus flavus is a fungal pathogen which infects maize crops and produces aflatoxin thus bringing about huge losses in crop production. Developing biocontrol agents against *Aspergillus flavus* has been the best strategy for the control of contamination in the fields. The aim of this study was to evaluate the biocontrol potential of *Aspergillus giganteus* against *A. flavus* by *in vitro* coculture studies. The effect of antagonism was studied by varying the carbon and nitrogen sources and under different interacting conditions of pH, temperature and water activities. The conidia production by *A. flavus* during coculture conditions favourable for antagonism was also assessed. A significantly notable growth inhibition of about 86.1% was brought about by *A. giganteus* in the coculture, which surrounded the mycelia of pathogenic *A. flavus*, arresting its growth. A maximum inhibition of 86.1% was observed when sucrose was used as the carbon source and a significantly higher inhibition of 90.93% was seen when beef extract was used as the nitrogen source. Among the different temperatures tested, the highest inhibition was observed at 30°C which was 87.43%. An increasing trend in the inhibitions were seen with decrease in pH and water activity (a_w), where, the highest inhibition was 89.75% for pH 6.0 and 94.03% for a_w 0.846. Drastic reductions in conidial number and halting of sclerotia production was observed in coculture clearly suggesting that *A. giganteus* will serve to be a potent and promising biocontrol strain under different environmental conditions.

Keywords: *Aspergillus flavus*, aflatoxin, pathogenic fungi, biocontrol, *Aspergillus giganteus*, antagonism

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INTRODUCTION

Plant diseases are caused by numerous organisms such as fungi, oomycetes, bacteria, viruses, protozoa, nematodes and so on. Among these, the most destructive and deleterious plant diseases are caused by fungal pathogens producing toxins which cause heavy losses in the crop production levels¹. The most dreaded challenge is to control the mycotoxigenic fungi to tackle issues of food safety as over 25% of the global food and feed crops are affected by accumulation of mycotoxins²⁻⁴. Corn is a major staple crop which is more often affected by *Aspergillus* species, predominantly by *A. flavus* and *A. parasiticus* which contaminate the kernels by producing aflatoxins which are highly carcinogenic⁵. The crops are prone to contamination during the pre-harvest stages and storage conditions where the environmental factors play a major role⁶. Hence, sustainable management of infectious *Aspergilli* has ever since been a necessity to control aflatoxin related health issues.

To keep these diseases at bay, many fast acting chemical based fungicides are being extensively used. These strategies have not been giving favourable results in controlling soil borne pathogens due to microbial degradation of fungicides or adsorption by soil colloids⁷. Moreover, the use of chemical pesticides has led to various environmental problems such as ground water contamination, disturbances in natural microflora and has caused negative impact on the terrestrial and aquatic ecosystems⁸.

Biological control has been an effective strategy for reduction of *Aspergilli* contamination in maize plants⁹. Competition is one of the successful mechanism of action for several biocontrol agents and a better understanding of the antagonist- pathogen interaction is needed in this case¹⁰. Competition for space, infection site and nutrients between the beneficial fungi and plant pathogenic fungi occur due to colonization of the shared habitat or substrate in the environment. This character of beneficial fungi is based on its inherent ability to adapt to the plant or the environment and maintain a high population density compared to the pathogen in order to outcompete them, forming the major bioactive principle¹¹.

Several bacterial species such as *Bacillus subtilis*, *Lactobacillus spp*, *Pseudomonas spp*, *Ralstonia spp* and *Burkholderia spp* have been found to inhibit the growth of *Aspergillus* in laboratory experiments⁴⁶. Also, fungi like *Aspergillus giganteus* and some marine fungi possess antifungal proteins which are capable of inhibiting the growth of several pathogenic filamentous fungi^{47,48}.

The survival and activity of such biocontrol agents mainly depend on key factors such as, temperature, water availability, pH and the presence of heavy metal ions¹². Most fungi have reduced biocontrol activity at low temperatures and hence choosing antagonists which will be effective at field temperatures is an important criteria for developing an efficient biocontrol agent¹³. Further, acidic pH conditions favour the activity of several antagonist fungi like *Trichoderma* and help in colonization¹⁴. Water availability is also a crucial factor in biocontrol as fungi have a high surface to volume ratio which makes them susceptible to dehydration. This in turn affects spore germination, hyphal growth and metabolite production^{15,16}. All these abiotic factors in turn trigger complex signaling pathways and metabolisms which act synergistically.

Hence, the present study was carried out to evaluate the inhibitory capacity of the antagonistic *Aspergillus giganteus* against aflatoxigenic *Aspergillus flavus* which infects the maize plants and to test the biocontrol efficacy in various environmental conditions by *in vitro* assays.

MATERIALS AND METHODS

Collection and maintenance of strains

The fungal strains chosen for the study were *Aspergillus flavus* (MTCC 2798) and *Aspergillus giganteus* (MTCC 8408). Lyophilized cultures of these fungi were procured from the Microbial Type Culture Collection, Institute of Microbial Technology (IMTECH), Chandigarh. *Aspergillus flavus* (MTCC 2798) is an aerobic fungi, with the special feature of production of aflatoxin. The strains were maintained in Czapek Yeast Extract Agar (CYA) at 28°C with regular subcultures done for every 30 days.

Co-culture in basal media to evaluate antagonism of *A. giganteus* against *A. flavus*

Competition between *A. giganteus* and *A. flavus* was assessed in Czapek Yeast Extract Agar (basal media) by co culture method as described by Weller *et al.*¹⁷. A mycelial agar plug of 5mm diameter was obtained from the peripheral region of a 5- 7 day old culture of pathogenic *A. flavus* and placed 3 cm from the center in a fresh CYA plate. Likewise, a 5mm mycelial plug was obtained from the peripheral region of a 5-7 day old culture of *A. giganteus* and was placed 3cm away from the inoculum of the pathogen. The control plates were inoculated with a 5mm mycelial plug from the culture of *A. flavus*. The plates were incubated at 28°C for 4-5 days for the mycelia to grow after which the interactions were scored for degree of antagonism based on Bell's Ranking scale of classes: 1- 5 (Bell *et al.*¹⁸). The experiments were performed in triplicates.

Antagonism in media mimicking different environmental conditions

To evaluate the antagonistic impact of *A. giganteus* on the growth of *A. flavus* under different nutritional and environmental conditions, *in vitro* coculture experiments were conducted under different interacting conditions. This was carried out by changing the composition of basal media with variations in the carbon source, inorganic and organic nitrogen sources, under different pH, incubation temperature and water activities.

Different carbon sources such as dextrose, maltose and lactose were used to evaluate the competition between *A. giganteus* and *A. flavus* in a coculture. The carbon sources chosen were provided at their individual 2% levels by replacing the usual carbon source -sucrose in Czapek basal media as performed in a study by Durairaj¹⁹. Similarly, cocultures were carried out where, the sodium nitrate in Czapek basal media was replaced with 3% nitrogen source of each organic nitrogen source namely peptone, beef extract and urea. The inorganic nitrogen sources used were ammonium oxalate, ammonium sulphate, and ammonium dihydrogen orthophosphate¹⁹. The control and test plates were incubated at 28°C for 4-5 days after which the radial growth measurements of the mycelia were recorded. The percentage radial

growth inhibition (R) was calculated using the formula

$$R = \frac{C - T}{C} \times 100$$

where, C is the radial growth of *A. flavus* in control, T is the radial growth of *A. flavus* in co culture with *A. giganteus* (test). The experiments repeated five times for consistent inhibitions.

The responses of fungi to culture pH needs to be assessed in strongly buffered media as fungi tend to rapidly change the pH by selective uptake or exchange of ions. Therefore, cocultures of *A. flavus* with *A. giganteus* were performed, where the pH of Czapek basal media was adjusted to 6.0 and 8.0 from the normal pH of 7.0 in basal media²⁰. The control and test plates were incubated at 28°C for 4-5 days after which the radial growth measurements of the mycelia were recorded. The percentage inhibitions obtained were noted. Since *A. flavus* is capable of surviving at high temperatures of 35°C and remains dormant for long periods, cocultures were performed at different temperatures ranging from 25 to 40°C to test the inhibitory potential of *A. giganteus*.

By mimicking the vapour pressure conditions in field, the ability to inhibit *A. flavus* was also evaluated with four different water activities in Czapek media by the method of Dallyl and Fox²¹. The water activity of the basic medium was adjusted from 0.999 to 0.922, 0.901, 0.884 and 0.846 by the addition of known amounts of nonionic solute glycerol and cocultures were performed by placing the petriplates in polythene bags at an incubation temperature of 28°C.

The control and coculture plates for all these experiments were monitored for the radial growth measurements of the mycelia and the percentage radial growth inhibition was calculated for triplicate experiments.

Preparation of spore suspension

Spores of *A. giganteus* were prepared by growing the fungi on CYA plates for 7 days at 28°C. The plates were flooded with sterile distilled water and 0.02% Tween 20. The spores were dislodged with mild scraping and the number of spores were determined by using a haemocytometer²². Spore concentration of 1x10⁸ spores/ml was used as inoculum for experiments.

Conidial quantification of *A. flavus* on solid media at culture conditions favourable for antagonism

The conidia of *A. flavus* was quantified by a method of Spraker *et al.*²³, in order to determine the capacity of *A. giganteus* to inhibit *A. flavus* spore formation in the culture conditions favourable for antagonism. Inoculums containing 10^8 spores/ ml of *A. flavus* and *A. giganteus* were obtained from 7 day old cultures and point inoculated 3 cm apart in solid media containing sucrose as the carbon source, beef extract as the nitrogen source, at a pH of 6, a_w of 0.846 and incubated at 28°C in sealed polythene bags. The cocultures were allowed to grow for 9 days and three 1cm diameter cores were punched from the centers of *A. flavus* cultures. The fungal mass and agar was homogenized and 1 ml was removed from each sample for conidial quantification by using a haemocytometer. Two technical replicates were obtained from four separate plates and pooled. Conidia from control plates grown with axenic *A. flavus* cultures were also counted and compared in terms of fold reduction in conidia number.

Effect of *A. giganteus* inhibition on sclerotial number of *A. flavus*

Spore suspensions of concentration 10^5 spores/ ml of *A. flavus* and *A. giganteus* were prepared from 7 day old cultures. These were point inoculated 3 cm apart in solid media containing sucrose as the carbon source, beef extract as the nitrogen source, at a pH of 6.0, a_w of 0.846 and incubated at 28°C for 15 days. Plates were maintained in polythene bags to maintain water activity. Sclerotia were obtained adopting

a method described by Nesci *et al.*²⁴, by scraping the surface of the culture plate over a whatman four sieve during irrigation with water containing Triton- X 100 (0.1%) followed by rinsing in sterile water. Sclerotia were further cleaned in a beaker with repeated rinses and decanting, air dried and stored in a dessicator. Sclerotium shape was approximated to be prolate spheroid and the number of sclerotia were counted on each replicate plate.

Statistical Analysis

All the experiments were performed in triplicates and the values are represented as mean \pm standard error. The significance of the tests were analysed with t- test at a level of $p \leq 0.05$ with SPSS software (21.0)

RESULTS AND DISCUSSION

Antagonistic potential of *A. giganteus* in coculture against *A. flavus*

In the coculture assay, the growth of *A. flavus* was arrested by the overgrowth of *A. giganteus* which surrounded the *A. flavus* mycelia. According to Bell's ranking scale, the interaction is said to be class 1- if there is complete overgrowth of the pathogen by the antagonist, class 2- for 75% overgrowth of antagonist, class 3- for 50% overgrowth of antagonist, class 4- for growth inhibition at the line of contact and class 5- for pathogen overgrowing the antagonist. In the present study, the antagonism can be ranked as class 1, as there was complete overgrowth of *A. giganteus* over *A. flavus* with competition for space and nutrients (Fig. 1).



Fig. 1. Control plate shows axenic culture of *A. flavus* whereas in coculture, mycelial growth of pathogenic *A. flavus* is surrounded by the mycelia of *A. giganteus*. The interaction between the fungi was ranked as Class 1 based on Bell's Ranking scale as *A. giganteus* showed massive overgrowth over the pathogen.

A significantly higher percentage of growth inhibition of 86.1% was observed in coculture plates. Further, the peripheral region

between the fungi showed no intermingling of the hyphae suggesting that the antagonism was due to competition for space and nutrients (Fig. 2).

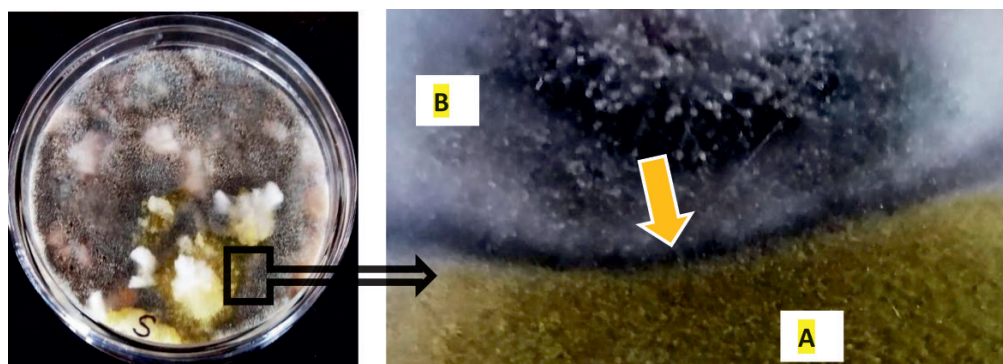


Fig. 2. Mycelial interaction between the pathogenic *A. flavus* (A) and *A. giganteus* (B) showed no intermingling of hyphae in the region between the two hyphae at the periphery as indicated (enlarged view)

A similar study conducted by Madbouly *et al.*²⁵ using bioassays such as dual culture proved that *Penicillium crustosum*, *Aspergillus giganteus*, *Fusarium verticillioides* and *Aspergillus fumigatus* isolates had promising antifungal activities against pathogenic fungi. This was due to the rapid growth of the antagonists competing for space and nutrients with the pathogenic fungi²⁶. Kucuk and Kyvanc²⁷, studied the *in vitro* interactions and established the antagonistic activity of several fungi from maize rhizosphere. The study involved several species of *Aspergillus*, *Penicillium*, *Fusarium* and *Trichoderma*, of which *Aspergillus ustus*, *A. versicolor* and *Gliocladium viride* were able to dominate all the fungi tested due to their rapid and invasive growth characteristic.

Antagonistic effect of *A. giganteus* in media mimicking different environmental conditions

There is a lag in the commercial use and application of biological control agents as the environmental conditions cause an impact in their performances in the field. Hence better understanding of the interactions between biological interactions and environmental factors such as substrate, temperature and water activity is a must to develop effective biocontrol strategies²⁸. Some metabolites produced by the biocontrol agents interfere with the pathogen growth but the effectiveness of these metabolites also depends on the carbon and nitrogen composition in the environment²⁹.

Growth inhibition in different carbon sources

Among the inhibitions seen for different carbon sources, the percentage inhibition by *A. giganteus* was found to be higher with 86.1% (Fig. 3, Fig.4), when sucrose was used as the carbon source. But the differences in inhibition levels were not very significant among the different carbon sources tested, and therefore proves that *A. giganteus* has significant inhibitory capacity of over 85% even when the carbon sources vary in the environment.

Simple sugars such as sucrose and glucose are associated with promoting high fungal growth and sporulation³⁰. Hence, evaluation of antagonism in different carbon sources is one of the primary steps in biocontrol development. Daryaei *et al.*³¹, performed a study by manipulating the culture conditions and nutritional requirements in a coculture assay to test the bioactivity of *Trichoderma atroviride* against *Rhizoctonia solani*, to help in optimum production of biocontrol agents. Similar binary competition assays on different carbon sources were done by Bodmer *et al.*³², in which *Candida subhashii* was identified as an effective antagonist exhibiting about 80% inhibition against filamentous plant pathogenic fungi. It is known that the antagonism of several fungi depend on the different mechanisms which include right from the production of antifungal metabolites to the competition for space and nutrients³³. Hence it can be said that *A. giganteus*

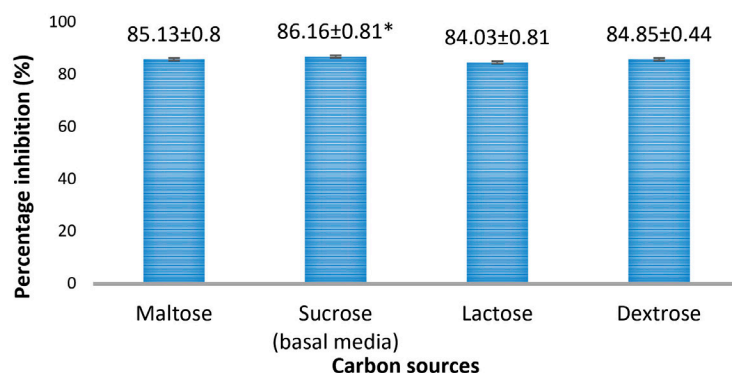


Fig. 3. Percentage inhibition of *A. flavus* growth in different carbon sources showing highest inhibition with sucrose as carbon source. Inhibition percentage = (Control- Test/ Control) x100. Values are represented as Mean ± SD (n=5). *Value is statistically significant for $P \leq 0.05$ in t-test.

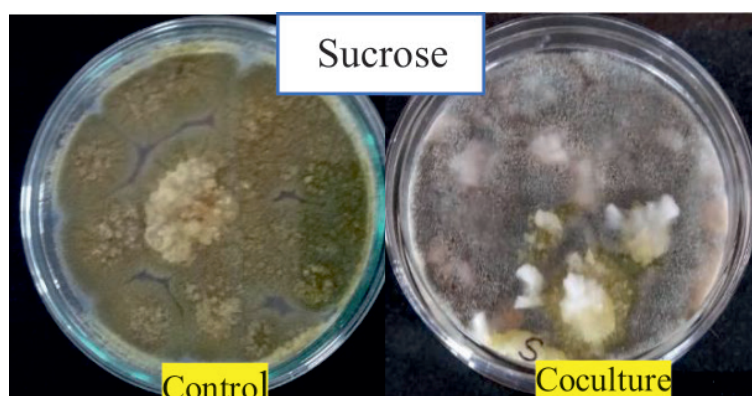


Fig. 4. Antagonistic effect of *A. giganteus* on pathogenic *A. flavus* during coculture in media with sucrose as carbon source

is capable of antagonizing the pathogenic *A. flavus* by competing for nutrients and space.

Effect of different organic and inorganic nitrogen sources on the inhibition of *A. flavus*

From Fig. 5 and 7, it can be seen that the highest percentage of inhibition was 90.3% when beef extract was used as nitrogen source. This inhibition was significantly higher than that obtained for sodium nitrate (86.1%), which was used in basal media. Also this did not vary much from the inhibitions obtained when different inorganic nitrogen sources were used (Fig. 6). Nitrogen sources play a role in the formation of developmental structures such as sclerotia in *Aspergillus flavus*. Further, studies have proved that nitrate forms of nitrogen source encourage sclerotia production³⁴. The results obtained in this study imply that *A. giganteus* is capable of utilizing both forms of nitrogen and exhibit a significantly

good antagonistic effect against pathogenic *A. flavus* though the highest inhibition was seen with beef extract.

A study by Daryaei *et al.*³⁵, showed that culture conditions and nutritional requirements in a coculture assay affect the biocontrol nature of *Trichoderma atroviride* against *Rhizoctonia solani* while infecting ryegrass. The *in vitro* antagonism of *Trichoderma harzianum* against *Mycosphaerella fijiensis* also used a mechanism of competition for space and nutrients where, invasion of the surface of the colony and colonization predominated³⁶. Competition for nutrients could lead to higher production of metabolites responsible for antagonism as it is considered as a stress factor by the antagonist fungi. This is due to the competition between the different forms of nitrogen such as the ammonium and nitrate forms between the phytopathogenic and antagonistic

fungi³⁷. Further, *A. giganteus* is already known to produce certain basic, cysteine rich antifungal proteins (AFPs) which have been used effectively against pathogens such as *Magnaporthe grisea*; and these AFPs might also be attributed to be responsible for the inhibitory effect against *A. flavus* in this study⁴⁷.

Influence of pH on the inhibition of *A. flavus* in co culture

The percentage inhibitions increased with decrease in pH, with a maximum inhibition of 89.75% observed at pH 6.0 (Fig.8 and 9). This was found to be higher when compared to the inhibition obtained when pH was 7.0 in basal media (86.1%). This indicates that *A. giganteus* has the ability to adapt to pH variations which will help to combat *A. flavus* in the field. Further

the soil pH for maize growing conditions is around 6.0 to 7.0. Hence, *A. giganteus* will be efficient in inhibiting the growth of pathogenic *A. flavus* in the environmental pH conditions which favour maize growth.

A similar study was conducted in *Candida parapsilosis* by Niknejad *et al.*³⁸, which showed significant reductions in mycelial growth of *Aspergillus* isolates at a range of pH variations. In a study by Armando *et al.*³⁹, growth inhibition of *Aspergillus parasiticus* by *Saccharomyces cerevisiae* strains at various environmental conditions was observed for proving it to be an effective biocontrol agent.

Inhibition of *A. flavus* at different temperatures

The percentage inhibition of *A. flavus* by *A. giganteus* was significant with 87.43% at

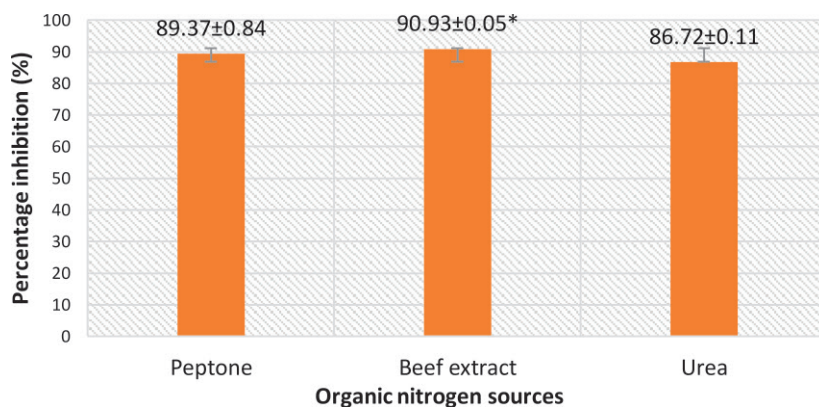


Fig 5. Percentage inhibition of *A. flavus* growth in different organic nitrogen sources with maximum inhibition obtained for Beef extract. Inhibition percentage = (Control- Test/ Control) x100. Values are represented as Mean ± SD (n=3). *Value is statistically significant at $P \leq 0.05$ in t- test

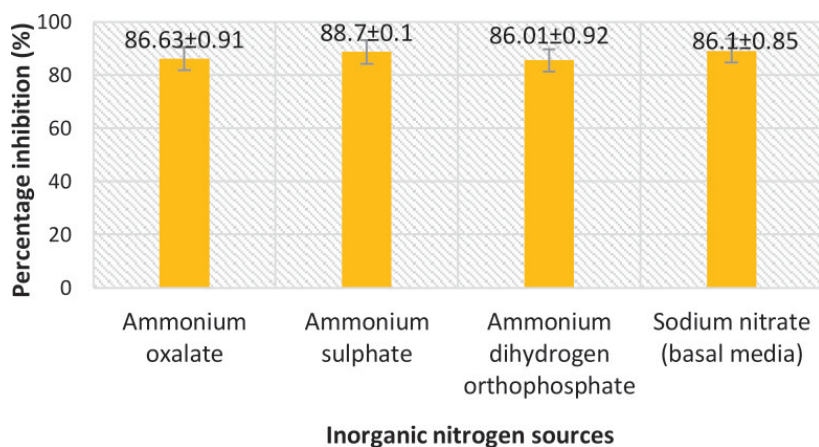


Fig. 6. Percentage inhibition of *A. flavus* growth in different inorganic nitrogen sources where the inhibitions are significantly less compared to those obtained for beef extract. Inhibition percentage = (Control- Test/ Control) x100. Values are represented as Mean ± SD (n=3)

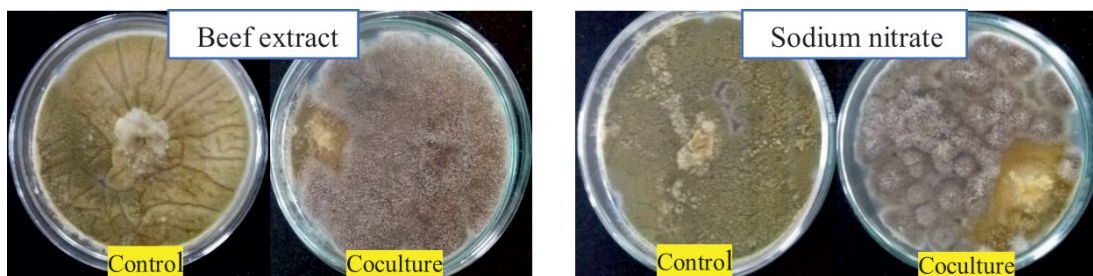


Fig. 7. Overgrowth of *A. giganteus* over pathogenic *A. flavus* during coculture in media with Beef extract as organic nitrogen source and sodium nitrate as inorganic nitrogen source

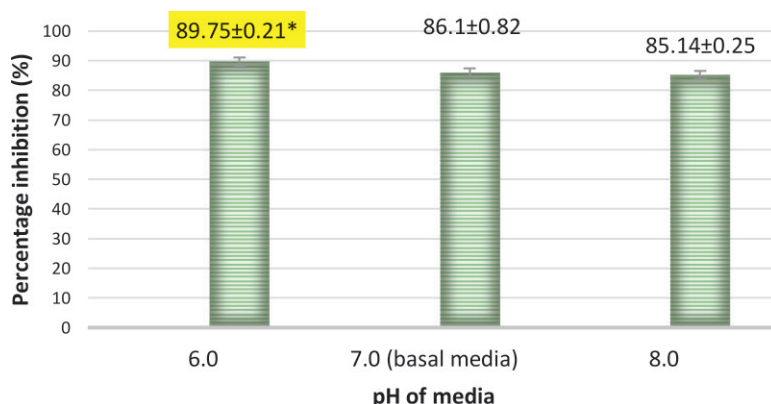


Fig. 8. Percentage inhibition of *A. flavus* growth at different pH on coculture with *A. giganteus* showing an increasing trend in inhibition as the pH decreased. Inhibition percentage = (ControlTest/ Control) x100. Values are represented as Mean ± SD (n=3). *Value is statistically significant at $P \leq 0.05$ compared to normal pH 7.0

30°C (Fig. 10 and 11) which is the predominant field temperature for maize growing regions. Though the inhibition at normal incubation temperature 28°C was about 86.1%, *A. giganteus* could also show an inhibition of 75.92% at higher temperatures of around 40°C suggesting that the organism will be capable of effectively controlling the pathogenic fungi thus confirming the ability of *A. giganteus* to combat *A. flavus* at extreme temperatures in the field.

A similar study by Niknejad *et al.*³⁸

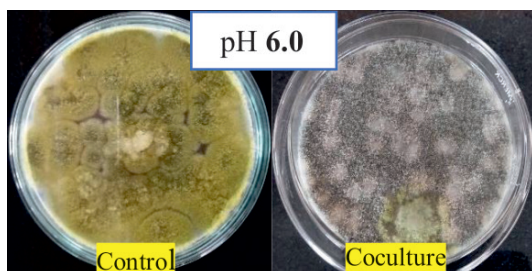


Fig. 9. Maximum antagonism of *A. giganteus* against pathogenic *A. flavus* (89.75%) at pH 6.0 showing arrested growth of pathogenic fungi

showed significant reductions in mycelial growth of *Aspergillus* isolates at a range of temperatures when cocultured with *Candida parapsilosis* having the other growth conditions optimised. In a study by Armando *et al.*⁴⁰, complete growth inhibition of *Fusarium graminearum* by *Saccharomyces cerevisiae* RC016 at a temperature range of 25-37°C was observed, considering it to be an effective biocontrol agent at different interacting environmental conditions.

Effect of various water activities on the inhibition of *A. flavus* in co culture

The percentage inhibition was found to increase gradually from 86.37 to 94.03% with decrease in water activity (Fig. 12 and 13). This suggests that as water activity decreases in the environment, *A. giganteus* growth is much aggressive with the capacity to inhibit *A. flavus* efficiently. This kind of tolerance to a range of water activity could provide high survival rates of the antagonist fungi when used in the fields, thus providing the capacity to compete and exclude

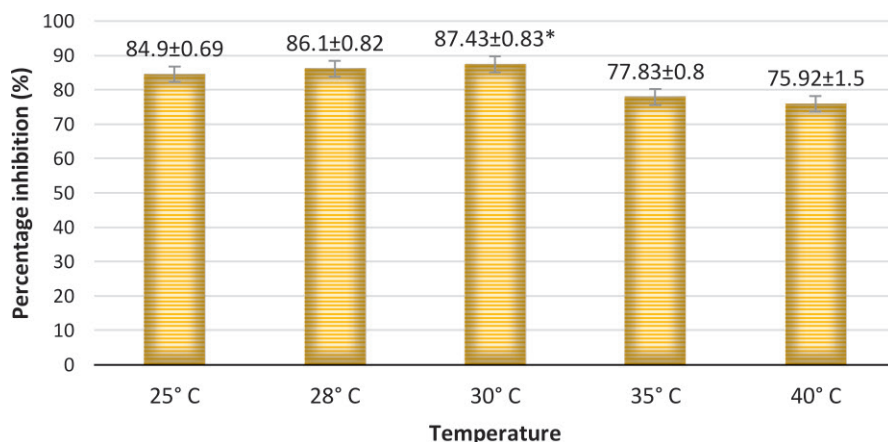


Fig. 10. Percentage inhibition of *A. flavus* at different temperatures where maximum inhibition was observed at a temperature of 30°C. Inhibition percentage = (Radius of Control- Radius of Test/ Radius of Control) x100. Values are represented as Mean ± SD (n=3). *Value is statistically significant at $P \leq 0.05$ in t- test

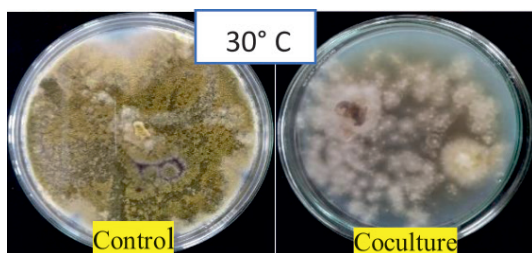


Fig. 11. Highest antagonism of *A. giganteus* observed at an incubation temperature of 30°C showing stunted growth of pathogenic *A. flavus*

toxigenic fungi from the ecological niche.

In vitro assays done by Etcheverry *et al.*⁴¹ have shown similar inhibitions by *M. oleovorans*, *B. amyloliquefaciens* and *Kluyveromyces sp* L16

acting as potent biocontrol strains against several *Fusarium* and *Aspergillus* strains. In another study by Nesci *et al.*²⁴, microbes such as *Amphibacillus xylanus*, *Bacillus subtilis* and *Sporolactobacillus inulinus* were able to inhibit the *Aspergillus* growth. This fact is also supported by a study by LaPenna and coworkers⁴², where the impact on the growth of *Aspergillus flavus* isolates at different water activities were observed when cocultured with *Kluyveromyces* species.

Conidial quantification under culture conditions favourable for antagonism

The conidia in coculture plates accounted to about $0.62 \pm 0.1 \times 10^8$ conidia/ml whereas it was $26.4 \pm 1 \times 10^8$ conidia/ml in control. Thus the

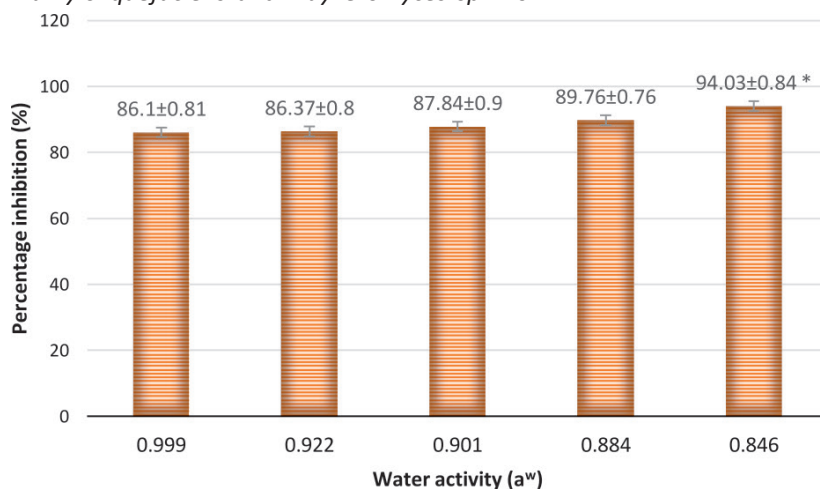


Fig. 12. Percentage inhibition of *A. flavus* at different water activities showing an increasing trend in inhibitions as water activity decreased. Inhibition percentage = (Radius of Control- Radius of Test/ Radius of Control) x100. Values are represented as Mean ± SD (n=3). *Value is statistically significant at $P \leq 0.05$ in t-test

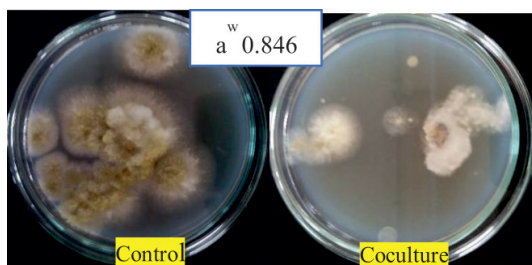


Fig. 13. Maximum antagonism of *A. giganteus* observed against pathogenic *A. flavus* at water activity of 0.846 where mycelia formation was stunted coculture plates had a highly significant reduction (at $P \leq 0.01$ confidence level) in conidial number by 42 fold when compared to the axenic *Aspergillus flavus* plates. This suggests that the sporulation process of *Aspergillus flavus* is inhibited by the antagonist *Aspergillus giganteus* which is a much required criteria for a potential biocontrol effect. Spraker *et al.*²³, studied the effect of volatiles of *Ralstonia solanacearum* against the aflatoxigenic *Aspergillus flavus* which were effective in reducing the conidiation of the latter. The conidiation and the fungal growth are said to be associated

factors in the metabolism of fungi and hence simultaneous reductions in conidia support reductions in mycelial growth⁴³.

Impact of *A. giganteus* on sclerotia production of *A. flavus*

Sclerotia are compact mass of hardened mycelium which survive under stressed conditions, thus giving the ability to the fungi to recoup in favourable conditions. In this study, the number of sclerotia in control plates were found to be 435 ± 7 whereas those found in the coculture plates were 6 ± 1 (Fig. 14). Thus, a significantly drastic reduction in the number of sclerotia was witnessed during the coculture which implies that the pathogen will not be allowed to form any sclerotia in the field conditions due to inhibition by *A. giganteus*. Also, the secondary metabolism of pathogenic fungi and the sclerotia formation is interlinked because they are triggered by a common gene cluster^{44,45}. Therefore inhibition of sclerotia production will definitely have an impact on the secondary metabolite production in the pathogenic fungi which is an advantage in biocontrol.

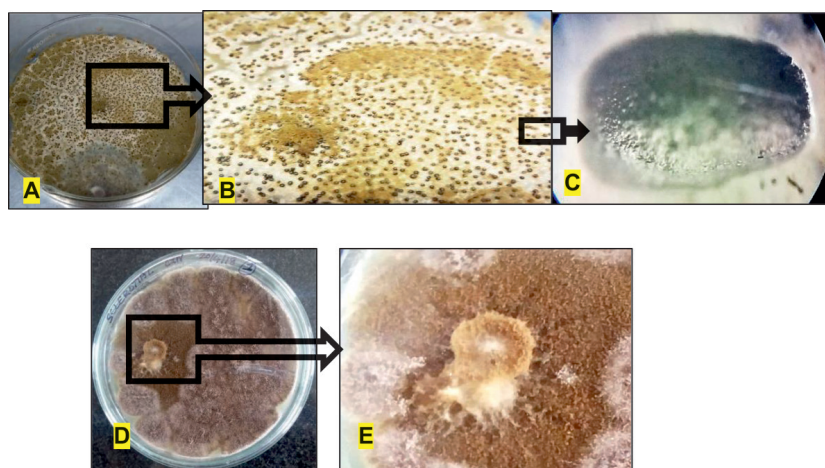


Fig. 14. Impact of *A. giganteus* on sclerotia production of *A. flavus*: A- Control petriplate showing growth of *A. flavus* with abundant sclerotia, B- Closer view of sclerotia in control plate, C- Zoomed picture of a single prolate spheroid sclerotia as viewed in a light microscope under 100X magnification, D- Coculture petriplate showing the reduced growth of *A. flavus*, E- Closer view of coculture plate showing no visible sclerotia formation.

CONCLUSION

The present study gives us a better understanding of the antagonistic behavior of *Aspergillus giganteus* providing a lead for the development of a successful biocontrol agent against aflatoxigenic *Aspergillus flavus*. Though the environmental conditions were mimicked *in*

vitro, the inhibitory activity of *A. giganteus* was remarkable implying that this strain will perform better in future field trials under extreme stress conditions. The inhibition of sporulation process of *A. flavus* by the culture filtrates of *A. giganteus* is an added advantage for field application as the compounds responsible for inhibition can be

assessed and used instead of releasing the whole strain for biocontrol. Thus with further focus on the application strategies, *A. giganteus* can be developed into a potent biocontrol agent against aflatoxigenic *A. flavus* to save maize crops.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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AUTHORS' CONTRIBUTIONS

RK and KD laid the work plan for the experiments. PRP and KD supervised the work and results. Experimental work, interpretation of results and manuscript preparation was carried out by R K.

DATA AVAILABILITY

All the data analysed during the course of this study are included in the manuscript.

ETHICS STATEMENT

This study did not involve any human subjects or animals.

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