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*Research Article*

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# Allelopathic impact of Cyanobacteria on pathogenic fungi

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## ABSTRACT

*Cyanobacterial allelopathy can be regarded as one of the significant factors influencing their dominance in diverse habitats. They are unique producers of a variety of allelochemicals, which can be utilized as eco-friendly biocontrol agents. The present work describes the allelopathic impact of cyanobacteria against plant pathogenic fungi Aspergillus niger and Alternaria solani. Cyanobacterial extracts had excellent acumen to inhibit the growth of A.niger and A. solani as evidenced by experimental findings. Three cyanobacterial strains S. elongates, S. platensis, and A. variabilis were explored for the allelopathic activity. While all the strains inhibited the growth of plant pathogenic fungi, S.platensis showed more allelopathic activity compared to other cyanobacterial strains Anabaena variabilis and Synechococcus elongates. Phenol and Polysaccharide concentration was also observed maximum in Spirulina platensis as compared to A. variabilis and S.elongates. Aspergillus niger showed high inhibition of growth by cyanobacterial extracts as compared to the other strain i.e. Alternaria solani. Allelopathic potential of cyanobacteria need to be further investigated as these microbes can offer promising solutions in biocontrol against pathogenic microorganisms.*

***Keywords**: Allelopathy, Cyanobacteria, Blooms, Antifungal activity, Pathogenic fungi.*

## INTRODUCTION

Allelopathy is a unique adaptation to achieve a competitive advantage over other organism inhabiting the same microbial community. Cyanobacteria are known to produce intracellular and extracellular metabolites with diverse biological activities such as antialgal, antibacterial, antifungal and antiviral activity[17]. They have received little attention as potential biocontrol agents of plant diseases. The antimicrobial substances involved may target various kinds of microorganisms. Secondary metabolites influence other organisms in the vicinity and are thought to be of phylogenetically important. Cyanobacteria produce a large number of antibacterial and antifungal products. Cyanobacteria from local habitats seem to be a source of potential new active substances that could contribute to reduction of the number of bacteria, fungi, viruses and other microorganisms. Cyanobacteria produce a potential source of biological active secondary metabolites, which are compounds that are not essential for cell metabolism[9]. Many of these compounds have been reported to possess antibiotic and pharmacological effects such as toxicity for eukaryotic organisms and antibacterial[5], antifungal, antiviral, and enzyme-inhibiting activities. Kulik[15] stated that for a number of reasons, cyanobacteria and algae are suitable candidates for exploitation as biocontrol agents of plant pathogenic bacteria and fungi. Cyanobacteria and algae produce a large number of antibacterial and antifungal products, many can grow in quantity in mass culture and they are not a threat to the environment. Most of cyanobacterial metabolites are accumulated in the cyanobacterial biomass. Moreover, cyanobacteria excrete various organic compounds, the hepatotoxic microcystins and nodularins or the neurotoxic anatoxins and saxitoxins, into their environment. Cyanobacteria have been identified as a new and rich source of bioactive compounds[1,23,2,6]. Most of the bioactive compounds isolated from cyanobacteria tend to be lipopeptides, i.e. they consist of an amino acid fragment linked to a fatty acid portion. Screening of cyanobacteria for antibiotics has opened a new horizon for discovering new drugs. Exploring allelopathic efficacy of cyanobacteria to control plant pathogenic fungi can prove to be an excellent option as they are easy to grow with minimum nutrients, cost effective, no side effects and environment friendly.

Many plant pathogenic agents are fungi. *Alternaria solani* is a fungal pathogen that produces a disease in tomato and potato plants called early blight. The pathogen produces distinctive "bullseye" patterned leaf spots and can also cause stem lesions and fruit rot on tomato and tuber blight on potato. Despite the name "early," foliar symptoms usually occur on older leaves, uncontrolled, early blight can cause significant yield reductions. *A.niger* causes a disease called black mold on certain fruits and vegetables such as grapes, onions, and peanuts, and is a common contaminant of food**.**

The occurrence of phenolic compounds in cyanobacteria is less documented than that in all plants. Algal phenolic compounds were reported to be a potential antioxidant to combat free radicals, which are harmful to our body and food systems. Exopolysaccharides (EPS) have been reported to play a significant role in providing protection to the cell as a boundary layer[5], contributing to soil aggregation dye to its gluing properties and binding heavy metals due to the presence several active functional groups onto it[12,22]. Role of phenols and polysaccharides content as antifungal agents, the relationship between the antifungal activity of the cyanobacteria and their polysaccharides and phenol contents were determined by estimating their contents in the tested cyanobacterial species[17].

This work describes the alleopathic effect of cyanobacteria against disease causing plant pathogenic fungi.

## MATERIALS AND METHODS

### Biological agents

Cyanobacteria were isolated and purified by solid liquid transfer technique and purified strains were grown in BG-11 media[19].

Fungal strains were isolated from diseased plants and purified strains were cultivated on potato dextrose agar media.

### Preparation of Extracts

Depending upon the type of solvent used, the extracts were subjected to different treatments followed by evaporation and cold treatment. The extracts were evaporated by keeping them at a temperature of 60-80$^{o}$C. Cold treatment was given by keeping the cells overnight at a temperature of 4$^{o}$C. Extracts with single solvents contained Methanol (60%, 80%, and 100%), Ethanol, Water, Acetic acid, Acetone and Butanol. Extracts with different solvents consisted of Methanol: acetone (1:1), Butanol: acetone (1:1), Ethanol: acetic acid (2:1).

### Estimation of Phenol content

Phenols were determined by Folin ciocalteu reagent[16].

### Estimation of polysaccharide content

The polysaccharide content was determined using Phenol-sulfuric –acid method[11].

## RESULTS

### Allelopathic activity

The allelopathic activity of cyanobacteria was tested against two fungal strains of *Aspergillus niger* and *Alternaria solani*. It was observed that there was high visibility of inhibition zone formation with butanol, methanol (80%) and methanol (100%) as compared to the other extracts. *Aspergillus niger* showed greater inhibition in growth by cyanobacterial extracts as compared to the other strain i.e. *Alternaria solani*. High inhibition activity was observed in *Spirulina platensis* as compared to the other strains i.e., *Anabaena variabilis* and *Synechococcus elongates*. The efficiency of butanol extract was much more compared to methanol and ethanol extracts.

### Phenol and Polysaccharide estimation

Phenol and Polysaccharide concentration was observed maximum in *Spirulina platensis* as compared to *A. variabilis and S.elongates.* The phenol and polysaccharide content in *S.platensis* was 33.4mg/g and

150µg respectively whereas in *S.elongates* it was 25.35mg/g and 120µg. It was very low in *A.variabilis* i.e, 25.12mg/g and 80µg. The antifungal activity of cyanobacteria could be attributed to their phenol content and/or polysaccharides content. This interpretation based on the results concerning the content of these substances in the tested cyanobacteria where their antifungal effect were increased as their polysaccharides and/or phenol content increased. In agreement with our explanation, there are a number of reports by authors on the antifungal activity of phenolic substance e.g., Phenolic compounds in extracts from cells of *N. muscurum* significantly inhibited the growth of *Candida albicans* and *Staphylococcus aureus*[7].

## DISCUSSION

Cyanobacteria are known to produce metabolites with diverse biological activities such as antibacterial, antifungal, antiviral, anticancer, antiplasmodial, algicide, antiplatelet aggregation and immunosuppressive activities. Several reports have shown that the extracts of *Nostoc* species significantly inhibited the growth of phytopathogenic fungi[3,26,24]. Cyanobacteria produce biologically active compounds that have antibiotic and toxic activity against plant pathogens[4,14]. Kim and Kim[13] reported inhibition of *F. oxysporum* f. sp. *lycopersici* by extracts of *N. commune* FA-103. Biological control of *Fusarium oxysporum* f. sp. *lycopersici* (FOL) causing wilt disease of tomato was studied *in vitro* as well as under pot conditions and was concluded that *N. linkia,* can be utilised for the biological control of wilt disease of tomato which may help to obtain a higher yield and good health in agriculture[10]. Mohamed *et al.*[17] investigated the suppression effect of cyanobacterial species- *Nostoc endophytum* and *Nostoc muscurum* against, the causal agent of soyabean root rot *Rhizoctonia solani.*

Cyanobacterial production of extra cellular polymers, mainly EPS is well documented[8]. EPS have been reported to play a significant role in providing protection to the cell as a boundary layer[5] contributing to soil aggregation due to its gluing properties. Samapundo *et al.*[20] observed that the phenolic compounds e.g., vanillic and caffeic acid treatments caused reduction in *F. verticillioides* and *F. proliferatum* growth. Sekine *et al.*[21] detected that phenolic hydroxyl compounds have antifungal activity against white- and brown-rot fungi. Mohamed *et al.*[17] have also concluded that the antifungal activity induced by cyanobacteria could be attributed to phenol and polysaccharides content.

Cyanobacteria showed significant allelopathic activity in this study. This kind of investigation creates a much generalized view that cyanobacteria are capable of antagonistic activity against fungal strains. The degree of efficiency is subjected to the kind of biological treatment being imparted. This kind of investigation, although, creates very general view of cyanobacterial possibility to produce biologically active compounds but certainly points out the necessity of exploring cyanobacteria as potentially excellent sources of these substances and reveals the most prospective strains for further investigations. The action and the potency of different cyanobacterial extracts is strain dependent. This work majorly focuses on the ability of cyanobacteria to be used as an allelopathic biocontrol agent, since a long time the antifungal drugs are chemical based, they are not only having problems of being hazardous when overused but also has led to the increase in the resistant power of many disease causing fungi.

**Table 1: Allelopathic activity of *Synechococcus elongates***

| Cyanobacterial extracts | *Aspergillus niger* | *Alternaria solani* |
|---|---|---|
| **Methanol 60%** | 8 mm | 6 mm |
| **Methanol 80%** | 15.5 mm | |
| **Methanol 100%** | 11.5 mm | |
| **Ethanol** | 4 mm | 2.5 mm |
| **Water extract** | 2 mm | - |
| **Acetone** | 9 mm | 12.5 mm |
| **Acetic acid** | - | 8 mm |
| **Butanol** | 19 mm | 17 mm |
| **Methanol: acetone(1:1)** | 5 mm | - |
| **Butanol: methanol(1:1)** | 6 mm | 4 mm |
| **Ethanol: acetic acid(2:1)** | 4 mm | - |
| **Control (Chloramphenicol)** | 20 mm | 18 mm |

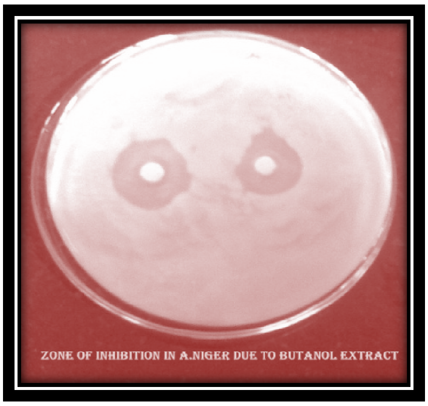
**Table 2: Allelopathic activity of *Spirulina platensis***

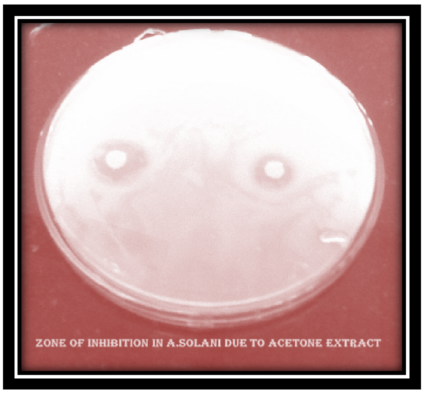
| Cyanobacterial extracts | *Aspergillus niger* | *Alternaria solani* |
|---|---|---|
| **Methanol 60%** | 20 mm | - |
| **Methanol 80%** | 24 mm | 21 mm |
| **Ethanol** | 25 mm | 32 mm |
| **Butanol** | 14 mm | - |
| **Control** | 34 mm | 34 mm |

**Table 3: Allelopathic activity of *Anabaena variabilis***

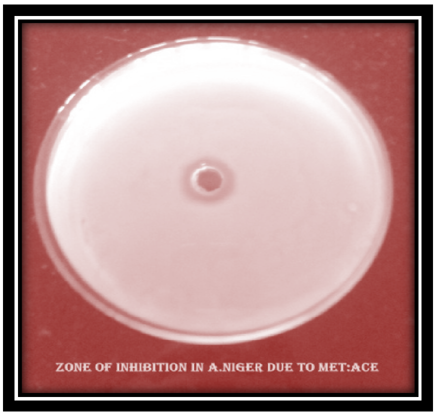
| Cyanobacterial extracts | *Aspergillus niger* | *Alternaria solani* |
|---|---|---|
| **Methanol 60%** | 18 mm | 19 mm |
| **Methanol 80%** | - | 22 mm |
| **Butanol** | 22 mm | - |
| **Control** | 26 mm | 26 mm |

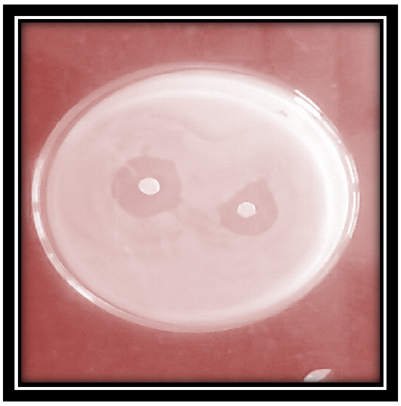
**Fig. 1: Zone of inhibition in fungi due to *Synechococcus elongates***







Zone of inhibition due to test (Chloramphenicol)

**Figure 2: Zone of inhibition in fungi due to *S.platensis***

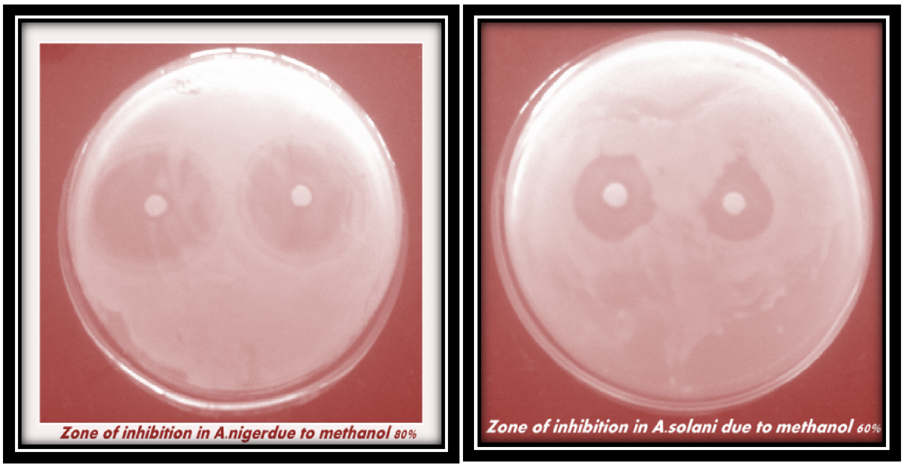
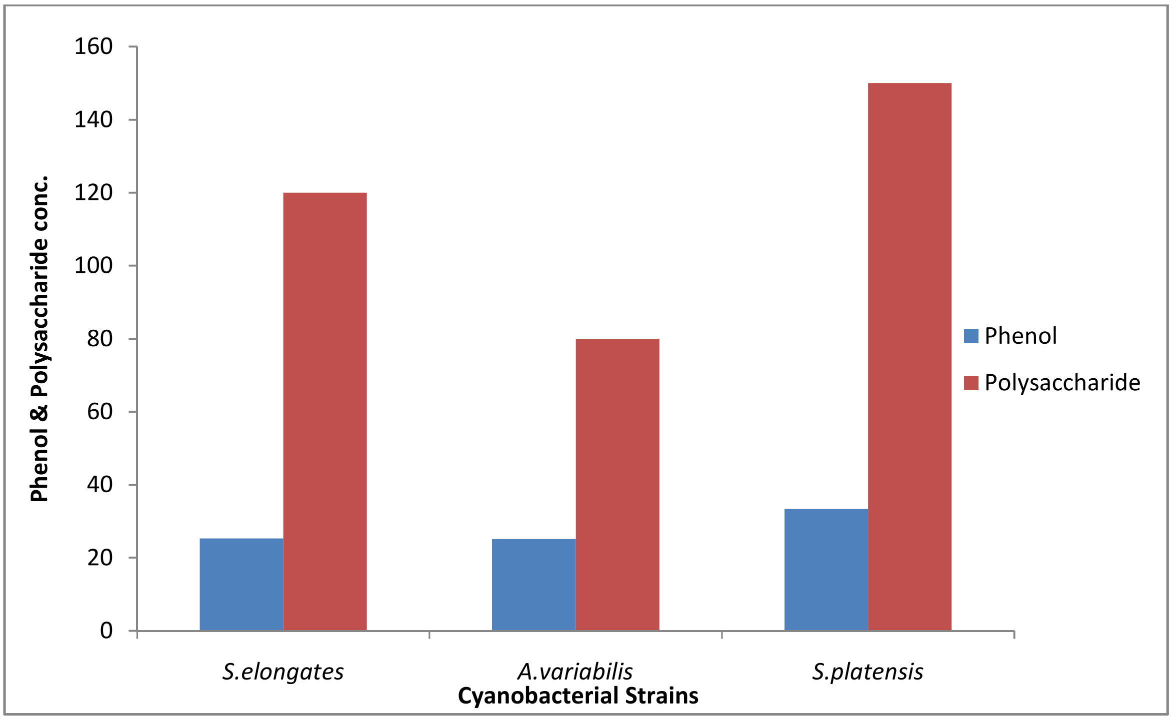
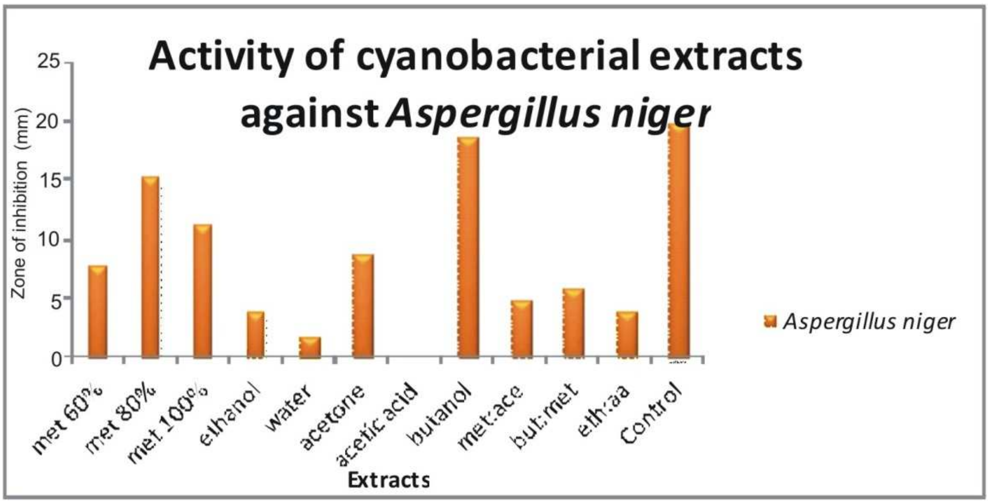



**Figure 3: Zone of inhibition in fungi due to *A. variabilis***

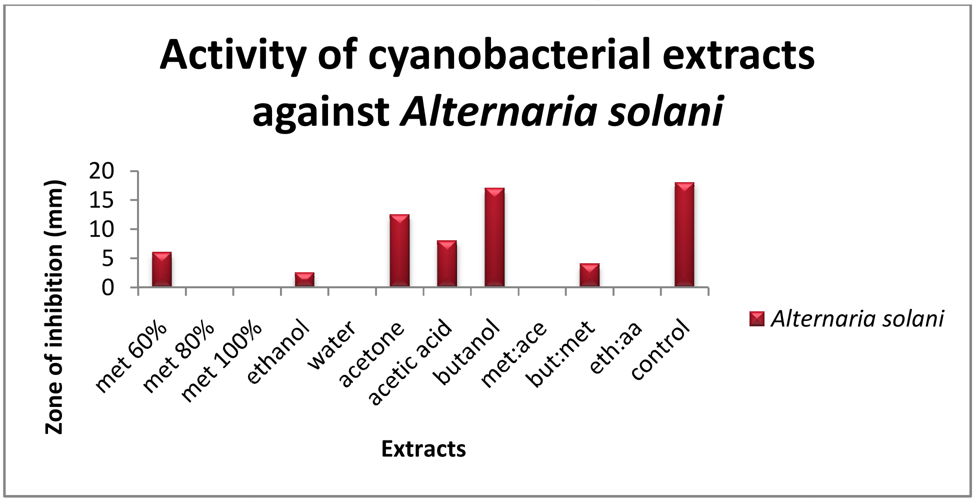

**Graph 1: Phenol and Polysaccharide content in Cyanobacterial strains**



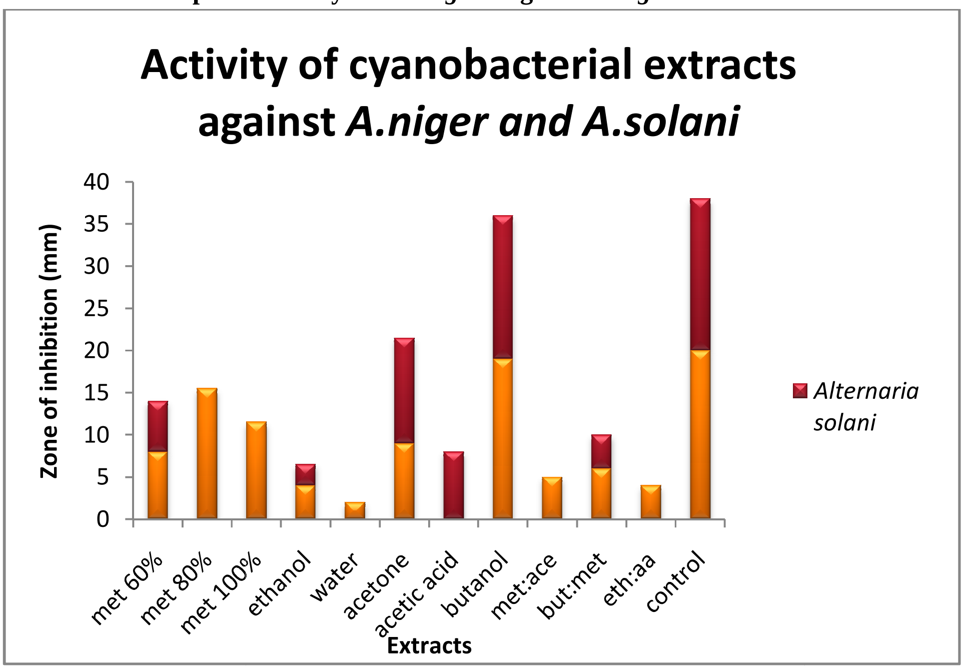
**Graph 2: Activity of *S.elongates* against *A.niger***



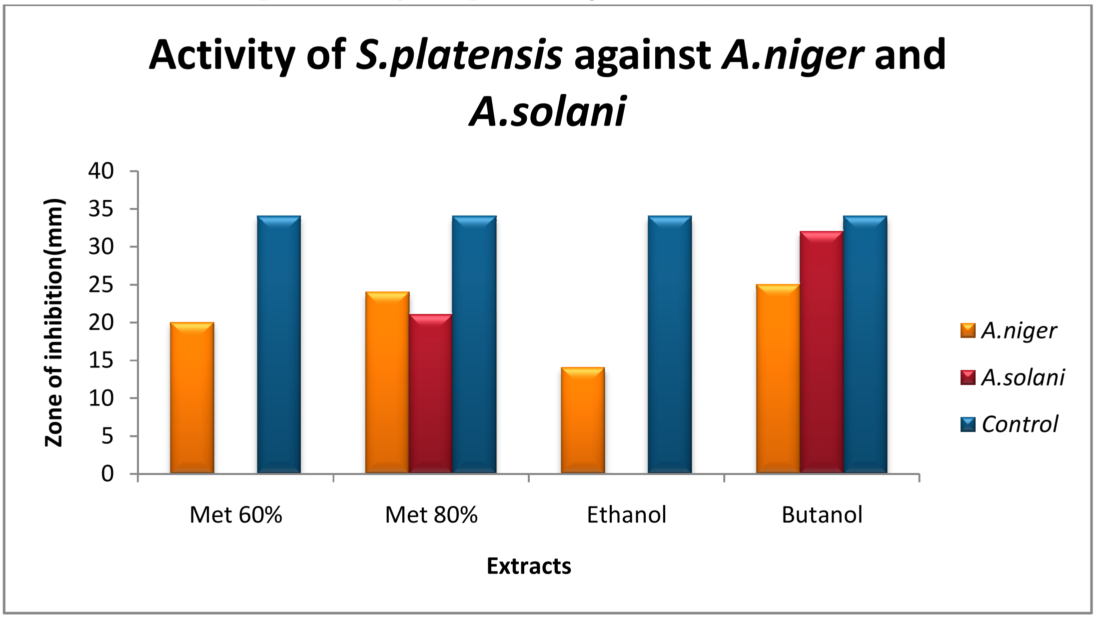

**Graph 3: Activity of *S.elongates* against *A.solani***



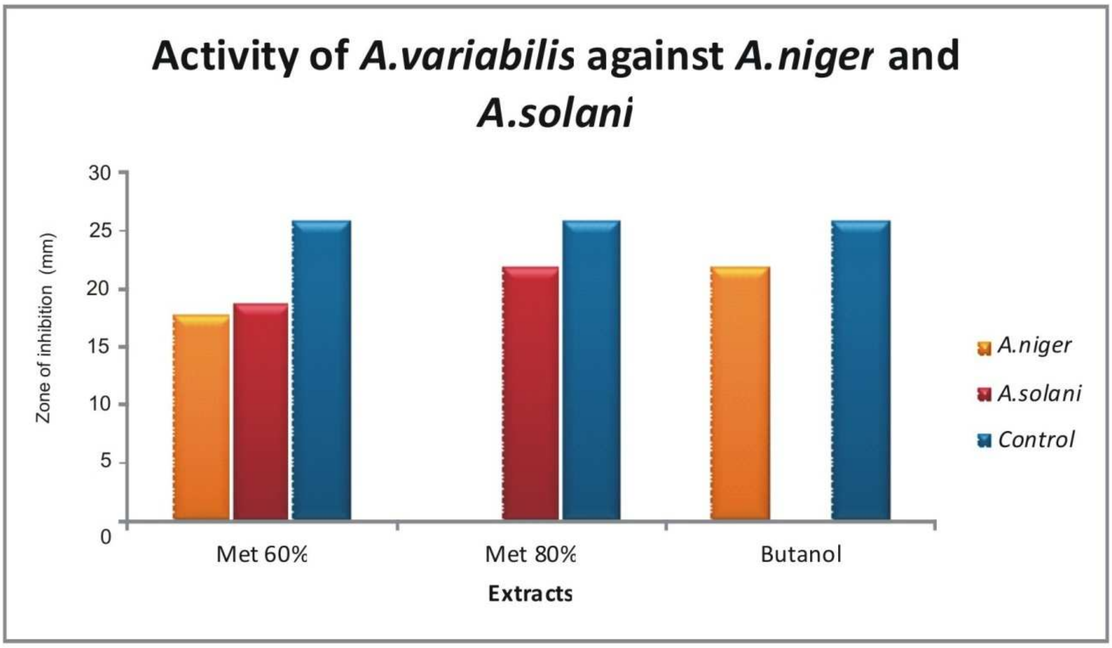

**Graph 4: Activity of *S.elongates* against *A.niger* and *A.solani***



**Graph 5: Activity of *S. platensis* against *A.niger* and *A.solani***




**Graph 6: Activity of *A.variabilis* against *A.niger* and *A.solani***

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