Phytochemicals of Some Members of the Family Hyacinthaceae and their Significance in Plant Protection

Taye Temikotan, B. O. Akinyele, A. C. Odiyi and D. J. Arotupin

***Abstract* -- Members of the family Hyacinthaceae are found in all parts of Ondo State, Nigeria on rocky outcrops devoid of shady trees. They are known to contain phytochemicals which are useful for a number of purposes. The objective of this study is to investigate some members of the family commonly found in Ondo State, Nigeria with a view to examining the relevance of their phytochemicals to plant protection. Samples collected from several populations at different parts of the State were separated into four morphologically recognizable groups. Phytochemical screening and quantitative determination of the alkaloids, saponins, flavonoids, phytates, tannins, and cardiac glycosides were carried out to provide tools for assessing the relevance of the phytochemicals to plant protection. Antibacterial properties of the plants were investigated using the following bacteria, namely *Bacillus cereus, B. subtilis, Pseudomonas aureginosa, P. syringae* pv.*phaseolicola, Xanthomonas axonopodis* pv. *manihotis, X. axonopodis* pv. *vignicola* and *Staphylococcus aureus* while fungicidal properties were investigated using the following fungi, namely *Alternaria solani*, *Aspergillus niger, Colletotrichum lindemuthianum* and *Pythium aphanidermatum*. The study establishes that the plants contain biocides with antibacterial and fungicidal properties. Group A, identified as *Dipcadi taccazzeanum* is active against all the test organisms. Groups B and C, identified as representatives of *Urginea indica* Kunth complex show better biocidal properties than any of the other groups. Group D, identified as *Albuca nigritana*, has promising antibacterial properties but is not active against the fungi, especially *Alternaria solani*.**

***Index Terms*-- Chemical constituents, hyacinths, antibacterial, fungicidal, plant protection.**

I. INTRODUCTION

The family Hyacinthaceae comprises about 46 genera with 700-900 species found in North America, Eurasia and Africa. Members are found growing throughout Ondo State, Nigeria especially in areas devoid of shady trees. For the purpose of this study, four taxa that are preponderant have been chosen as experimental plant materials. They are generally rich in alkaloids and saponins (Davidson, 2009). These phytochemicals, along with tannins, phytates, cardiac glycosides and other phenolic compounds have pronounced biological and physiological consequences in microbes. It is, therefore, imperative to investigate the usefulness of the plants as biocides in plant protection. Hence, the study was set out to determine the phytochemical constituents, the antimicrobial and antifungal properties of the plants' extracts and assess their relevance to plant protection.

The leaves form a bulb which opens with lanceolate or linear-lanceolate blades. The leaves are of different shades of green, glabrous, with entire margin, sometimes spotted as in some members of the genus *Scilla* or with patches of whitish colour as in *Ledebouria* (Leisher, 2000; Manning *et al.,* 2002). Hyde and Werstent (2009) described some members as having linear leaves with smooth, undulate, ciliate or papillose margin.

The chemical constituents of plants have been of immense importance to human. Man's motivation to characterize plant secondary metabolites is often driven by commercial interest as they have been the source of valuable drugs, pesticides and chemicals important in the food industry (Lea and Leegood, 1999). Taiz and Zeigner (2002) gave several examples of the effects of these secondary metabolites on insects. Pyrethroids from *Cysanthenum species*, azadirachtin from neem tree (*Azadirachta indica*), and phytoecdysone from fern (*Polypodium vulgar*) were said to be toxic, repellent and anti-moulting agents. Many of the secondary metabolites have more than one application both in pests and disease control. For example, juglone from walnut (*Juglans regia*) has both fungicidal and phytotoxic properties. Isoflavonoids have become known for their phytoalexinic and antimicrobial properties and are synthesized by plants in response to bacterial and fungal infections (Taiz and Zeigner, 2002).

The use of plants extracts in the control of pests and diseases as well as in preservation of food is as old as history. Rojas *et al.* (2006) stated that ancient Egypt had developed the use of extracts from plants in the preservation of food, antimicrobial control and in embalming the dead. There is continuous rise of interest in the search for antimicrobial agents from plants (Ogunleye, 2000). According to Levy and Marshal (2004), two reasons are probably responsible for this: one is the increasing resistance of microbes to antibiotic therapy and two; the efficacies of these plant extracts have been confirmed in different situations in different parts of the world. Another problem associated with synthetic herbicides is the environmental hazards they cause. Margni *et al.* (2002) stated that pesticides cause harm to man and other living things. Tripathi and Shukla (2008) opined that they cause environmental pollution. This assertion is also upheld by the works of Fletcher *et al.* (2006) and Yemagachi and Fujimura

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(2005). Fabricant and Farnsworth (2001) discussed the value of plants in the discovery of drugs for medicinal purposes. The problem of resistance to antibiotics has necessitated the need to look for alternatives to antibiotics. According to Sosa (2007), the need is urgent since bacteria soon develop resistance to available drugs. According to Droby (2006), plant extracts are preferred to synthetic chemicals because they do not alter the quality of crops and have little side effects.

II. MATERIALS AND METHODS

Collection trips were made to parts of Ondo State, Nigeria to collect the plant materials. Several populations were encountered and five plants were sampled from each population. The samples collected were separated into four groups based on their morphological characteristics, put in bags and labeled appropriately. The bulbs were planted in 10 L buckets in the Screen House of the Department of Crop, Soil and Pest Management, The Federal University of Technology, Akure in a completely randomized design. Planting of the bulb of each plant group was replicated 20 times.

A. Phytochemical Screening:

Screening, using the methods of Adamu *et al*. (2007) was carried out for the presence of alkaloids, saponins, phlobatanins, cardiac glycosides, flavonoids, steroids, phytates, and tannins.

B. Quantitative Determination of Phytochemicals

The method used in determining the proportion of alkaloids was according to Harborne (1998) and adopted by Edeoga *et al*. (2007); saponin according to Obadoni and Ochuko (2001) and adopted by Faramola (2006) and Edeoga *et al.* (2007); tannin according to Van-Burden and Robinson (1981); flavonoid according to Bohn and Kocipal-Abyazan (1974); phytate according to Adamu *et al.* (2007); cyanide according to AOAC (1998); oxalate according to Day and Underwood (1991).

C. Antibacterial Properties

The procedure adopted was as described by Adamu *et al.* (2007). The test organisms employed were *Bacillus cereus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Pseudomonas syringae* pv *phaseolicola, Xanthomonas axonopodis* pv *vignicola, Xanthomonas axonopodis pv manihotis* and *Staphylococcus aureus.* All the bacteria isolates were collected from Germplasm Health Unit, International Institute for Tropical Agriculture (IITA), Ibadan, Nigeria.

D. Preparation of Extracts

Bulbs and leaves of each taxon were blended using an electrical blender. About 250g of each sample was soaked in each of the following extraction media: cold water, hot water, chloroform, petroleum ether, acetone and ethanol for 24 hours. The extracts were sieved through double sheet filter paper and concentrated in a rotary evaporator. The solid formed was serially diluted using Tween 20 or Dimethyl sulphur oxide (DMSO). The extracts were sterilized using a membrane filter.

E. Antibacterial Assay

Nutrient broth of each of the organisms was prepared about 24 hours before use. The nutrient agar was prepared at 28g/liter and autoclaved at 1.05 kg/cm^3 (15 psi) pressure and 121^{o}C for 15 minutes. After cooling to about 45^{o}C, the nutrient agar was poured on the test organisms, swirled gently in clockwise and anti-clockwise directions for even distribution of the inoculum within the media. It was then allowed to solidify at ambient temperature (28^{o}C). Sterile cork borer was used to make a well on the solidified agar. About 0.5ml of the sterilized extracts was aseptically introduced to each well separately. The plates were then incubated at 37^{o}C for 24 hours. Control experiment was carried out using streptomycin powder at 0.3mg/ml. The Plates were incubated at 37^{o}C for 24 hours after which the zones of inhibition were measured and recorded.

F. Collection and Preparation of Fungal Isolates

Alternaria solani, Aspergillus niger, Colletotrichum lindemuthianum and *Pythium aphanidermatum* were used as test organisms. Pure fungal isolates were collected from the Department of Microbiology, The Federal University of Technology, Akure, Nigeria and the International Institute for Tropical Agriculture, Ibadan, Nigeria. Each isolate was grown in the culture room of the Department of Crop, Soil and Pest Management, The Federal University of Technology, Akure, Nigeria.

G. Treatment

About 2ml of each extract was aseptically transferred into Petri dishes. About 20ml aliquot of molten potato dextrose agar (PDA) was poured into each Petri dish and swirled gently so that the extracts and the PDA will mix together properly to form a homogenous mixture. These were allowed to set at ambient temperature. A sterile 5mm diameter cork borer was used to cut and introduce the fungal isolate into the centre of the plate. Control experiments were carried out as follows:

1. "Standard antifungal agent 'Benlate' at 0.5mg/ml was used.
2. Each of the propellant (Tween 20 or DMSO) was used without adding the extracts.
3. The negative control was without any treatment, that is, none of the extracts were added.

The zone of mycelia growth was taken every 24 hours for 4-5 days depending on the rate of growth. All measurements taken were recorded. Percentage mycelial growth inhibition was determined using the formula:

$$\% \text{ mycelia growth} = \frac{\text{Zone of mycelia growth NTR - Zone of mycelia growth TR}}{\text{Zone of mycelia growth NTR}} \times 100\%$$

Where NTR = Non treated sample
TR = Treated sample.

Graphical representations were made using SPSS computer software statistical programme.

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III. RESULTS

The results of the various investigations carried out on the plants are as presented below:

A. Phytochemical Screening

The results of the phytochemical screening are as shown in Table I. The phytochemicals screened for are alkaloids, tannin, saponins, phytosterol, phlobatinins, terpenoids, cardiac glycosides, phytate and oxalate. As shown in the table, none of the four groups contains phlobatannins. Groups A and D lack phytosterols while the other phytochemicals are common to all the groups.

B. Quantitative Determination of Phytochemicals

Seven phytochemical groups, namely alkaloids, saponins, flavonoids, hydrogen cyanide, tannin, phytate and oxalate were quantitatively determined to know their proportions in each plant group. The results are shown in Table II.

C. Antibacterial Activities of the Extracts

Initial antibacterial screening was carried out using cold water, hot water, chloroform, petroleum ether, acetone and ethanol as media. Table III shows the results of the activities of the extracts against the test bacteria, namely *Bacillus subtilis, Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa.*

The result of further work on the extracts of the plants using ethanol is as shown in Table IV. Eight bacteria were used as test organisms. Two of the test organisms, *E. coli* and *S. aureus*, are of medical importance. *Bacillus cereus* and *B. subtilis* are implicated in both animal and plant diseases. However, the strains of the two pathogens used in this study are plant pathogens. The remaining four, *Pseudomonas aeruginosa*, *P. syringae* pv. *phaseolicola*, *Xanthomonas axonopodis* pv. *vignicola* and *X. axonopodis* pv. *Manihotis* are also plant pathogens.

D. Fungicidal Activities of Extracts of the Plants

The fungi used as test organisms to determine the fungicidal properties of the plants' extracts are *Aspergillus niger*, the causal organism of black mould in onion and several other crops, *Colletotrichum lindemuthianum*, the causal organism of cowpea anthracnose, *Pythium aphanidermatum*, the pathogen responsible for seedling damping off of several crop plants such as tomatoes, blight in cereals and rot in fruits such as water melon and *Alternaria solani*, the early blight fungus of tomatoes and other solanaceous plants. Acetone and ethanolic extracts of plant groups A, B and C cause 100% mycelia growth inhibition of all the test organisms except *Alternaria solani* while the extracts of plant group D show very low fungicidal activity against the test organisms. The fungicidal activities of the various extracts of the plants against the test organisms are presented in figures 1 – 4.

IV. DISCUSSION

Phytochemical screening shows that members of the four groups lack phlobatanin while members of groups A and D lack phytosterol. Members of the hyacinth family, especially the members of the subfamily Urginoideae are very poisonous due to the presence of the alkaloid bufotenin which is described as toxic even in small doses. They also cause hallucination when mistakenly ingested by grazing animals. This implies that, from members of the family, potential pesticides could be obtained which can be used as rodenticides, insecticides and molluscicides (Taiz and Zeigner, 2002). The presence of alkaloids in members of the family indicates their possible use in the control of bacterial and fungal diseases (Asl *et. al.,* 2008). The presence of saponin may signal the fact that these plants could become source of antifungal agents or chemicals (Yang and Zhang, 2006). The quantity of hydrogen cyanide in all the four groups implies that they are toxic since lethal dose of cyanide is put at 0.5 to 25mg/kg body weight. This lend credence to the fact that the hyacinths under discussion are a potential source of pesticides in the control of rodents as cyanides affect cardiac contraction causing death in animals (Kapitanyan, 2009).

The presence of phytate and other polyphenols in all members of these plants may be a pointer to their usefulness in plant disease control as well as in human health (Vucenik and Shamsuddin, 2003). The work of Weigertner *et al.* (2008) shows that plant sterol may result in serious damage in fish, even causing sex inversion and hermaphrodity, reduced fertility and feminization of male fish. As such, the presence of these compounds in these plants may indicate their usefulness as pesticides. In a nutshell, chemical composition of these plants points to their possible uses as pesticides, antibacterial agent and fungicide.

The chemical composition of the plants also presupposes that the plants have antibacterial activities. This investigation confirms that the plants have broad-spectrum antibacterial activities as they are active against both gram-positive and gram-negative bacteria. Their extracts are active against animal (human) pathogenic bacteria such as *Escherichia coli, Bacillus cereus, Bacillus subtilis* and *Staphylococcus aureus*. The extracts are also active against plant pathogenic bacteria such as *Pseudomonas aeruginosa, Pseudomonas syringae pv. phaseolicola, Xanthomonas axonopodis pv. manihotis, Xanthomonas axonopodis pv. vignicola* and plant pathogenic strains of *B. cereus* and *B. subtilis*. This indicates that the plants contain bioactive compounds that are useful in plant protection as well as have potential use in medicine.

Members of groups B and C show significantly better antibacterial activities than the other groups. Members of group D show promising antibacterial activity against the test bacteria.

The investigation also shows that the plants have fungicidal property that inhibits the growth of *Aspergillus niger, Colletotrichum lindemuthianum, Pythium aphanidermatum* and *Alternaria solani*. Test of different levels of concentration of the extracts against *Alternaria solani* showed that mycellial growth inhibition is not significantly different at 10mg/ml and 5mg/ml levels but different at 2.5mg/ml and 1.25mg/ml levels. Performance of the extracts of group D against the various fungi showed that they do not have promising fungicidal properties, especially against *Alternaria solani.*

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V. CONCLUSION

It has been established in this study that both *Dipcadi* and *Urginea* exhibit antibacterial and fungicidal properties while *Albuca species* exhibits antibacterial properties with little or no promising fungicidal activity, especially against *Alternaria solani*. Hence, the plants are of significance in plant protection. Environment friendly materials can be synthesized on a commercial scale from these botanicals to reduce dependence on synthetic materials and, therefore, their environmental consequences.

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TABLE I
PHYTOCHEMICAL CONSTITUENTS OF THE PLANTS

Group	Alkaloids	Tanins	Saponins	Steroids	Phlobatinins	Terpenoids	Flavonoids	Cardiac Glycosides	Phytates	Oxalates
A	+	+	+	□	□	+	+	+	+	+
B	+	+	+	+	□	+	+	+	+	+
C	+	+	+	+	□	+	+	+	+	+
D	+	+	+	□	□	+	+	+	+	+

Note: + = Present □ = Absent

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TABLE II
QUANTITATIVE ANALYSIS OF PHYTOCHEMICAL CONSTITUENTS OF THE PLANTS

	Alkaloids (%)	Saponins (%)	Flavonoids (%)	Hydrogen cyanide (m/kg)	Tannins (%)	Phytates (mg/g)	Oxalates (mg/g)
A	21.06 ±0.06b	22.78 ±0.04c	29.05 ±0.07b	1.36 ±0.04	5.14 ±0.04ab	7.04 ±0.08c	2.79 ±0.05b
B	22.82 ±0.07a	19.86 ±0.09d	27.56 ±0.07c	1.68 ±0.02	5.37 ± 0.17b	7.3 ±0.10c	2.94 ±0.03b
C	20.55 ±0.15b	28.07 ±0.05b	30.15 ±0.33a	1.53 ±0.01	4.50 ± 0.11c	9.16 ±0.07b	3.75 ±0.13a
D	11.71 ±0.33c	28.78 ±0.11a	18.34 ±0.08d	0.74 ±0.05	10.5 ± 0.41a	11.34 ±0.36a	3.90 ±0.11a

Note: Mean values in the same column followed by different letters are significantly different from one another ($p < 0.05$).

TABLE III
INITIAL ANTIBACTERIAL ACTIVITY OF THE PLANTS' EXTRACTS

Extraction medium →	Cold water										Hot water										Ethanol									
Bacteria ↓	A bulb	A leaf	B bulb	B leaf	C bulb	C leaf	D bulb	D leaf	A bulb	A leaf	B bulb	B leaf	C bulb	C leaf	D bulb	D leaf	A bulb	A leaf	B bulb	B leaf	C bulb	C leaf	D bulb	D leaf						
B. subtilis	–	–	–	8.5	–	–	–	–	–	–	6.0	–	–	–	–	–	–	–	–	–	–	–	–	–						
S. aureus	–	–	–	–	–	–	–	–	–	–	6.0	–	–	–	–	–	–	3.0	–	–	–	–	–	–						
E. coli	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–						
P. aeruginosa	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1.5	–	7.0	–	–	–	–						

TABLE IV
ANTIBACTERIAL ACTIVITIES OF THE EXTRACTS

Groups	*B. Cereus*	*B. Subtilis*	*E. Coli*	*P. Aeruginosa*	*Psp*	Xam	Xav	*Staphylococcus aureus*
A bulb	6.83 ± 0.44 a	9.33 ±0.67ab	2.67 ±0.67d	4.67 ±0.33c	3.33 0.33d	0.00 e	2.33 ± 0.33c	4.00 ±0.00 e
A leaf	3.00 ±0.00d	9.50 ±0.29ab	8.67.0 ±0.67cd	7.67 ±0.33b	2.00 ± 0.00e	2.33 ±0.33cd	2.67 ±0.33cd	8.33 ±0.33bc
B bulb	6.67 ± 0.33a	8.67 ±0.67b	7.00 ± 0.58c	7.00 ±0.58b	2.00 ± 0.00e	2.00 ±0.00d	3.67 ±0.33bc	7.67 ±0.33c
B leaf	4.83 ±0.44b	8.67 ±0.88b	9.33 ± 0.67a	7.33 ±0.33b	2.67 ±0.33de	3.00 ±0.58bc	2.50 ± 0.50c	10.17 ±0.44a
C bulb	2.68 ±0.33d	10.00 ±0.00ab	7.33 ±0.33bc	3.67 ±0.33b	4.33 ± 0.33c	3.67 ±0.33b	4.33 ± 0.33a	9.50 ±0.29ab
C leaf	4.33 ±0.33bc	10.00 ±0.58ab	9.67 ± 0.33a	9.67 ±0.17a	7.67 ±0.33b	6.33 ± 0.33a	0.00 d	10.33 ±0.33a
D bulb	7.68 ± 0.33a	10.67 ± 0.67a	8.67 ±0.33ab	9.33 ±0.33a	9.00 ± 0.00a	2.00 ±0.00d	4.00 ± 0.00a	8.33 ±0.33bc
D leaf	3.33 ±0.67cd	9.33 ±0.33ab	9.00 ± 0.00 a	9.33 ±0.67a	7.33 ±0.73b	0.00 e	4.33 ± 0.67a	8.83 ±0.17bc
Oil	-0.00 e	0.00 c	0.00 e	2.00d	0.00 f	0.00 0 e	0.0 0 d	0.00 f

Note: Mean values in the same column followed by different letters are significantly different from one another ($p < 0.05$).
Psp= *Pseudomonas syringae*pv.*phaseolicola*Xam = *Xanthomonasaxonopodis*pv. *manihotis*Xav = *X. axonopodis*pv. *vignicola.*

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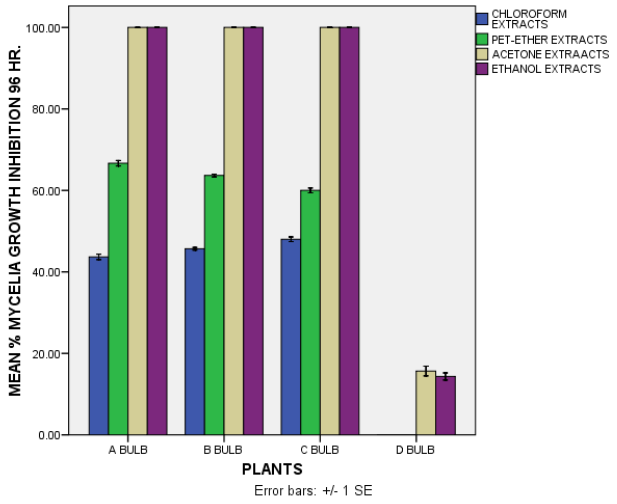

Fig 1: Effect of the different extracts on *A. niger* after 96 hours

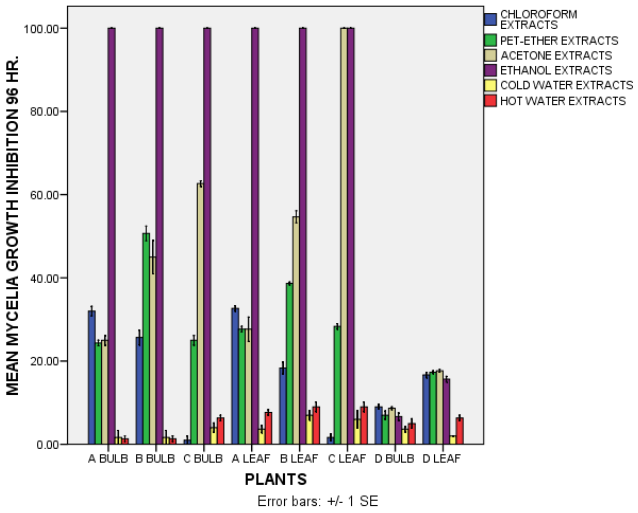

Fig 3: Effect of the different extracts on *P. aphanidermatum* after 96 hours

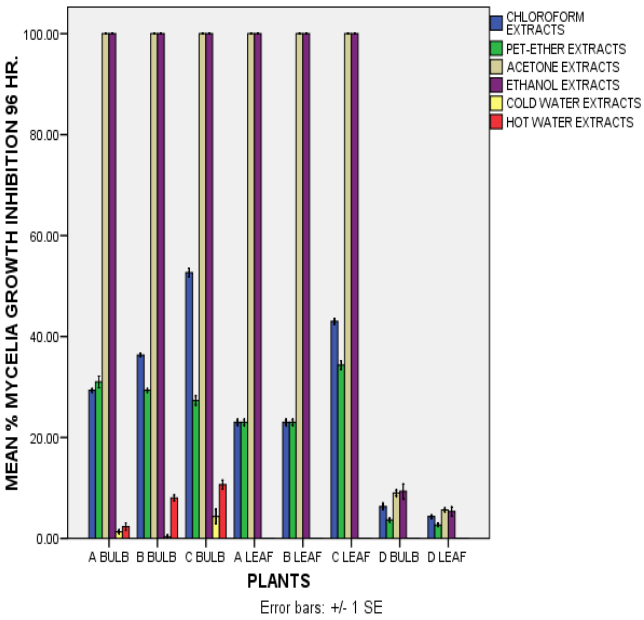

Fig 2: Effect of the different extracts on *C. lindemuthianum* after 96 hours

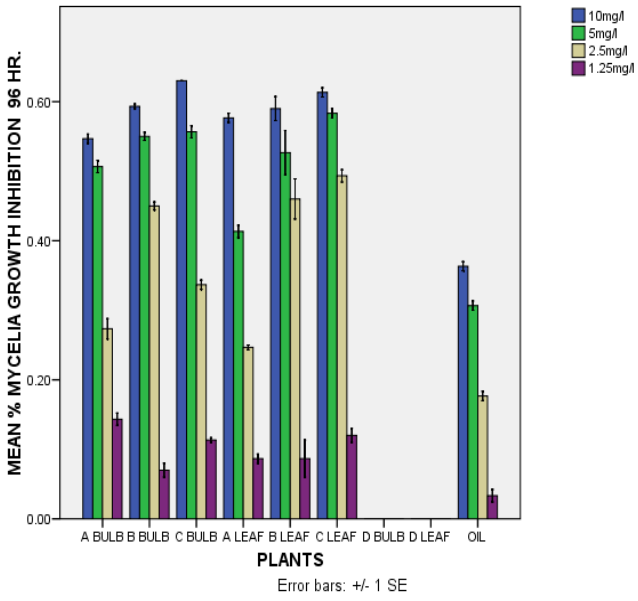

Fig 4: Effect of different concentrations of ethanolic extracts on *Alternaria solani* after 96 hours

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