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Synthesis, Characterization and Screening of Substituted 4-Biphenyl acétamides against *Curvalaria lunata*

Abstract

A new series of Substituted-4-Biphenyl-acetamides have been synthesized by condensation of 4-Biphenyl acetic acid with different primary amines (aromatic and aliphatic). 4-Biphenyl Acetic Acid was first treated with Thionyl Chloride in dry benzene to prepare substituted-4-biphenyl acetyl chloride, which is then treated with different aliphatic or aromatic amines to synthesize various substituted-4-biphenyl Acid-amide derivatives. The Structure of newly synthesized compounds has been established by analytical and spectral methods. These all six biphenyl compounds were screened for antimicrobial activity. All compounds at different concentrations were analyzed for antifungal activity via disc diffusion method. The antimicrobial activity was screened against fungus *Curvalaria lunata.* All six compounds showed moderate to good activity against selected fungal strain. These synthesized compounds have shown Anti-fungal properties when they tested against *Curvalaria lunata.*

Keywords: Synthesis, 4-Biphenyl Acetic Acid (4-BPAA), Substituted-4-Biphenyl acetamide, Spectral studies, antimicrobial activity, Anti-Fungal Properties.

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1. Introduction

The two aromatic nuclei are attached to each other at only one point. Thus, biphenyls with independent benzene rings have been categorized in the Class of Polyphenyl Compounds or isolated Polynuclear hydrocarbons. Biphenyls are the Polynuclear Aromatic Hydrocarbons (PAHs) having more than one aromatic nucleus. Biphenyl acetic acid itself and its derivatives have been found to be effective against many therapeutic diseases. *Curvalaria* is a dematiaceous fungus present in the environment worldwide, mainly in tropical and subtropical regions. The genus Curvalaria comprises several species. Of these, mostly three ubiquitous species are known to cause several types of infection in both immunocompetent and immune compromised hosts: C. lunata, C. pallescens and C. geniculate, C. lunata being the most commonly reported in human infections. We report a case of *Curvalaria lunata* infection in an immunocompetent male with an initial diagnosis of suspected left side allergic fungal rhino sinusitis (AFRS) treated surgically. He had a relapse of nasal polypsis and underwent a surgical revision under local anaesthesia with endoscopic nasal polypectomy. The histological examination of the surgical specimen showed an inflammatory property of the par nasal sinuses, with eosinophil and lymphocyte infiltration, but without evidence of fungi. However, *Curvalaria spp*. fungus grew in cultures of nasal sinus drainage and bioptical specimens. The fungus

was identified by DNA sequencing as C. lunata. C. lunata is most commonly associated with allergic fungal sinusitis. Therefore there's need to discover novel antifungal agents targeting *Curvalaria lunata* infection. Biphenyl derivatives have considerable attention due to their wide range of antifungal activities. Biphenyl acetamide analogs have been reported as important pharmacological molecules having significant antifungal properties against *Curvalaria lunata*. The present research deals with the investigation of six synthesized biphenyl compounds on fungal species *Curvalaria lunata*. All the six compounds of 4-Biphenyl acetamide derivatives namely N-phenyl-4-Biphenyl acetamide, N-Para Toluene-4-Biphenyl acetamide, N-2-pyridine-4-Biphenyl acetamide, N-Para-benzoic acid-4-Biphenyl acetamide, N-2-Chloro-4-Biphenyl acetamide and N-Para-bromo-phenyl-4-Biphenyl acetamide showed antifungal activities against *Curvalaria lunata*. Literature findings have also been shown its various therapeutic uses, such as: anti-inflammatory agent[1], as analgesic[2], antipyretic[3], antiarthritis[4], antirheumatoid[5], antihypertensive[2] and a binder to human blood plasma-prealbuen etc. 4-Biphenyl acetic acid itself has been reported to possess many effective pharmacological activities, such as anti-inflammatory, analgesic, antibacterial[6] and topical steroidal anti-inflammatory activity[7]. The Ointment Containing-4-biphenyl acetic acid work very effectively as anti-inflammatory as well as analgesic agents[8]. 4-Biphenyl acetic acid cyclodextrin inclusion compounds are reported to show effective mono-nuclearrogenic anti-inflammatory properties and its phenyl alkanamide derivatives have shown agro horticultural bactericidal activity[9]. Substituted biphenyls can also be used as anti-allergic drugs[10]. Biphenyl compounds have stronger analgesic activity along with anti-allergic and anti-inflammatory activity[7]. Substituted biphenyl-4-acetamides have therapeutic use in the treatment of cancer[11].The title compound of biphenyl is also used as an antitumor agent[12]. (Felbinac an active metabolite of 4-BPAA) patch shows anti-inflammatory and analgesic activities and also used in the treatment of adjuvant-arthritis[2]. Biphenyl-3-acetamide, 2-amino-thiazole shows anti-tumour activity also used in the treatment of cancer, Alzheimer disease, viral infection, auto-immune disease or neurodegenerative disorder[13]. 2-Biphenyl-acetic acid and 2-biphenyl acetamide used as agrochemical antifungal agent[14-18]. Biphenyl containing compounds possesses anti-psychotic and anxiolytic activity[19]. Some of the Biphenyl hydrazide- hydrazone are known to exhibit very good anti-microbial activity[20,21]. Some of the compounds having biphenyl moiety possess valuable medicinal properties like anti-hypertensive and calcium channel blockers [22,23]. Tetrazole are very well known to possess antimicrobial properties[24]. PCB's are proved to cause reproductive, endocrine and neurological disorders, thyroid dysfunction, cognitive and motor deficits. Prenatal exposures are known to cause increased susceptibility to infectious diseases in earlychildhood[25]. PCB's has influenced the diffused oxygen from plants in soil promoting growth of aerobic microbes. Soil aeration is also improved by formation of air channels when roots die, decay and by direct root oxygen release[26]. Molecules containing biaryl moieties are relatively common within natural products. For their preparation, nature has developed an ample array of biosynthetic strategies[27]. A number of these biaryl natural products belong to the biogenetic class of lignans.[28] During the last years, we have been investigating a range of analogs of natural products[29]. These products and many derivatives both of natural and synthetic origin, have been reported to display antioxidative, anti-inflammatory, anti-tumor, antidiabetic, anti-microbial, anti-neurodegenerative, anti-depressant, pain control, gastrointestinal, cardiovascular and liver protective properties, among others [30,31]. Until 1971, 61% of PCB's were used in closed electrical systems, 13% were used in nominally closed systems, and 26% were used in open-end applications. After 1971, almost 100% of all PCB's produced were used in closed electrical systems [32]. In the present work we describe the synthesis of the first representatives of previously unknown imidazoles, benzimidazoles containing a 4-biphenyl group at position 2, and different substituents at the nitrogen atom N. The presence of not one but two "privileged" fragments in the molecules of these compounds, namely, imidazoles, benzimidazoles and biphenyl, should promote multitarget action. We also collected and analyzed the data on the activity of the synthesized biphenyl compounds with respect to a number of biological targets important for the therapy of diabetes mellitus and its vascular complications[33-36]. In view of these observations and in continuation of our research work on biologically active biphenyl derivatives, it is proposed to synthesize substituted biphenyl acid-amide derivatives derived by the condensation of 4-biphenyl acetic acid precursor first with Thionyl chloride and then with different primary amines (aliphatic as aromatic).

2. Methods

Preparation of media

Czepeck's media was prepared for the growth of fungus *Curvalaria lunata*, dissolved the contents of Czepeck's media as described – **Czepeck's Media Requirements (For 500ml. solution)**

Agar – Agar: -	7.5 gm	**KH_2Po_4: -**	500 mg.
$MgSo_4.2H_2O$: -	250 mg.	**KCI: -**	500mg.
$FeSo_4$: -	Traces	**Yeast Powder:-**	500mg.
$NaNo_3$: -	1.0 gm	**Dextrose: -**	05.0 gm
Distilled Water: -	500ml.		

The media was subjected to sterilization by autoclaving at 15 psi for 15 minutes. Cool down to room temperature before inoculation.

Fungal culture preparation

Small volume of fungal culture was inoculated in autoclaved media and subjected to incubation at 37°C for 4-5 days. Spreading of fungal culture to solidified Czepeck's agar (15ml.) plates was done with the help of spreader. The culture was allowed to grow at 30°C for 4-5 days of incubation. After sufficient growth, 3 mm pieces of fungal cultures were cut with the help of cork borer and placed on another Czepeck's agar plate already having 1ml. solution of the compound to be tested.

Preparation of Biphenyl compounds

All six biphenyl derivatives were dissolved in absolute ethyl alcohol to make 1000 ppm solution which were further diluted to make 1000 ppm, 500 ppm and 250 ppm solution in 3 replicates. One control was taken having absolute ethanol only. All these preparations were inoculated in 12 plates, 3 plates for each concentration including control in one experiment.

Identification of antifungal properties of six biphenyl derivatives on *Curvalaria lunata*

Efficacy of all six compounds was tested by measuring zone of inhibition using disc diffusion method. One compound of volume 1ml one at a time at the concentration of 250 ppm, 500 ppm, 1000 ppm and one as control was added to 12 plates containing 15 ml of Czepeck's media respectively. The set of 4 Petri plates and 3 replicates were used having 12 plates in one experiment. Small pieces of size 3mm was cut from already grown fungal culture and placed on these 12 plates to check antifungal nature of the compound.

3. Results and Discussions

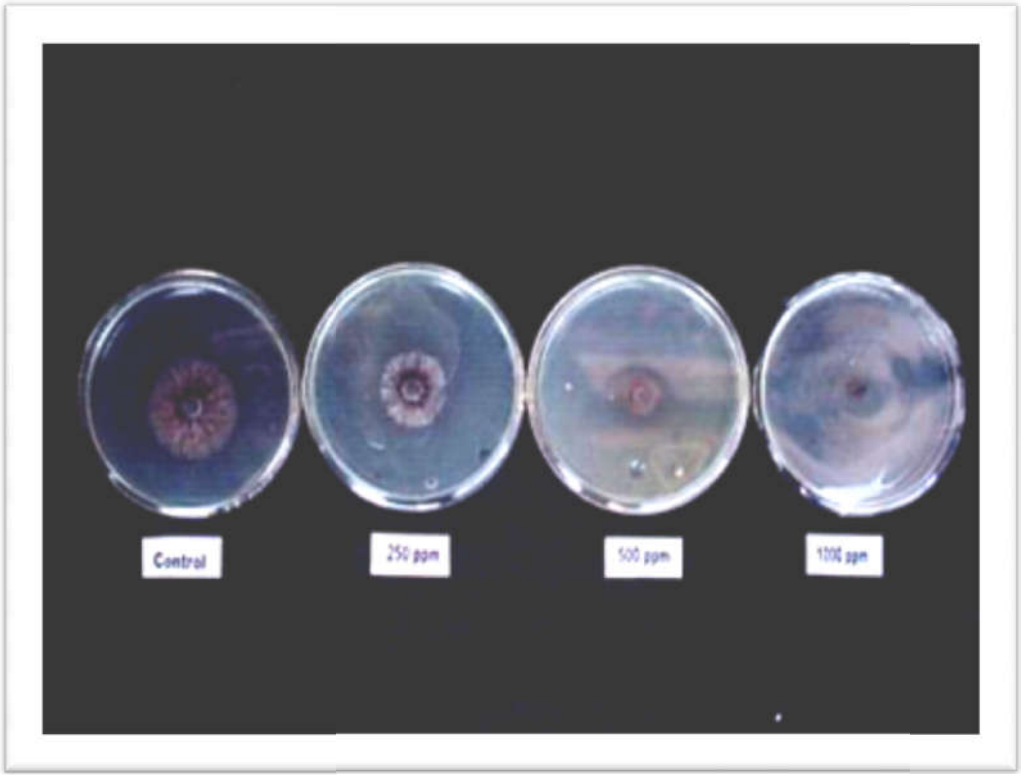
All the six compounds showed positive results and resist the growth of fungus *Curvalaria lunata.* The disc diffusion method was used for the identification of antifungal properties of all six biphenyl acetamide derivatives such as:

A. N-Phenyl -4-Biphenyl acetamide - 2A.
B. N-Para-Toluene -4-Biphenyl acetamide - 4A.
C. N-2-Pyridine -4-Biphenyl acetamide - 9A.
D. N-Para-benzoic acid -4-Biphenyl acetamide - 10A.
E. N-2-Chlorophenyl -4-Biphenyl acetamide - 12A.
F. N-Para-Bromo-phenyl -4-Biphenyl acetamide - 15A.

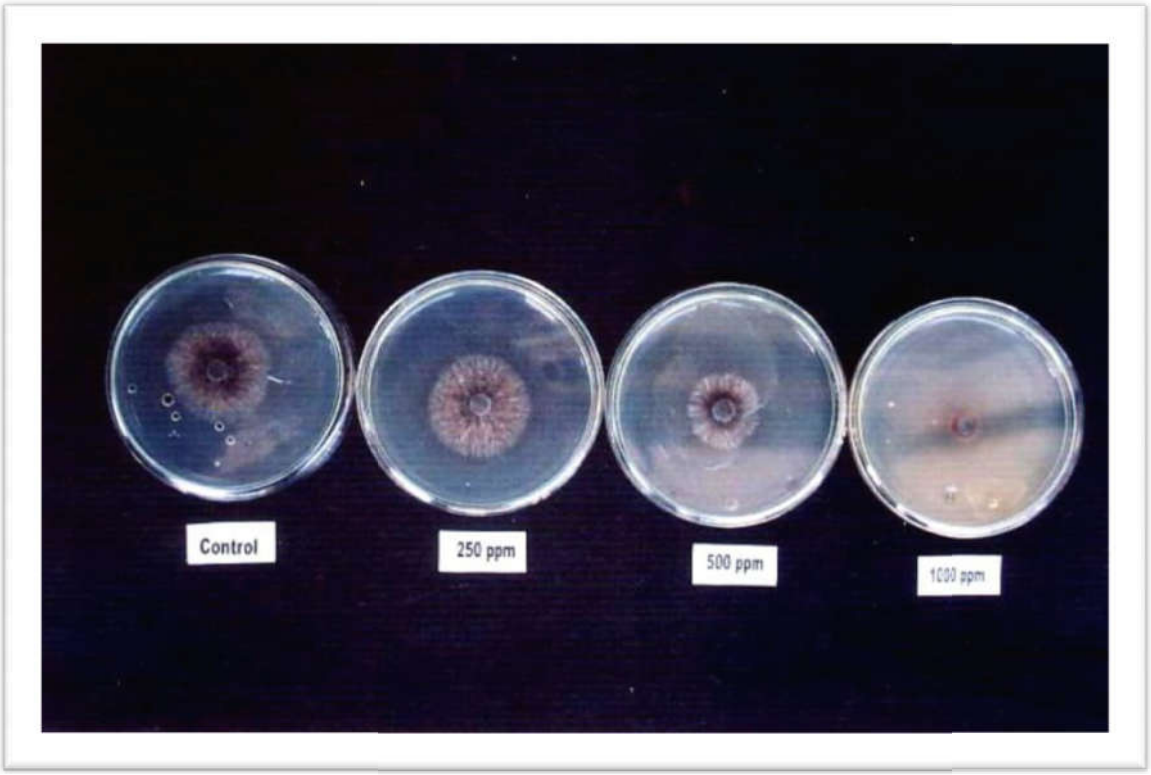
Size of Block of fungus used: - 3 mm
Solvent used: - Absolute Ethyl alcohol

Table 1: The identification of anti-fungal Property of 4-Biphenyl acetamide derivatives on *Curvalaria lunata*

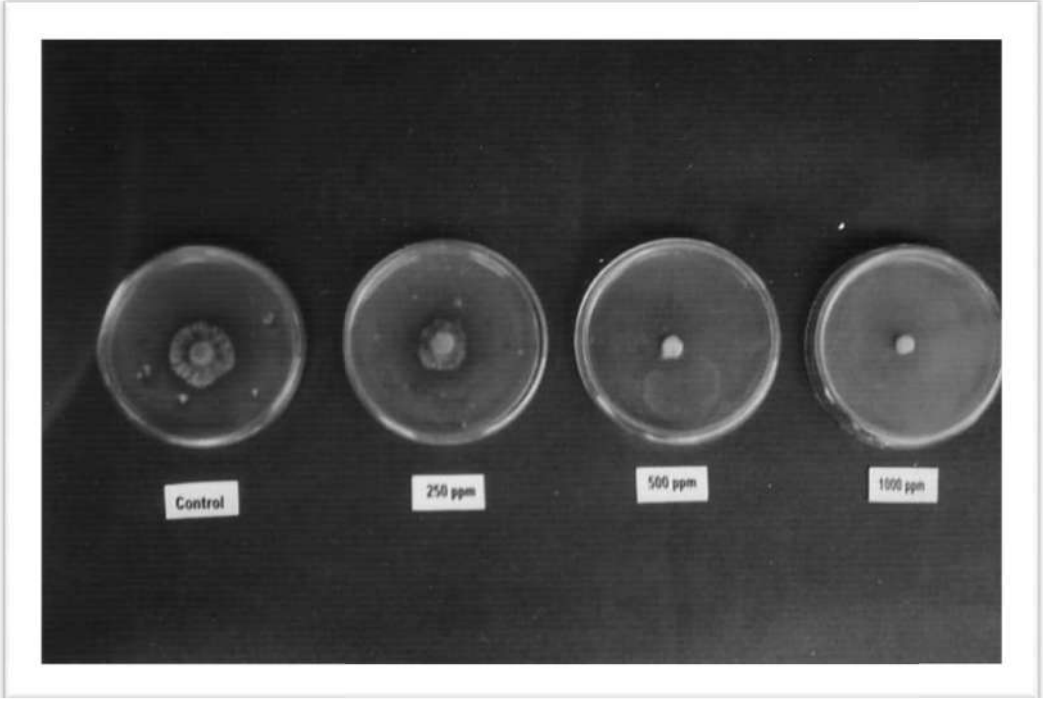
Sr. No.	Code	Time	Temperature	Control (Ethyl alcohol)	250 ppm	500 ppm	1000 ppm
1.	2A	144 hrs.	12±3°C	18mm	13mm	6mm	0mm
2.	4A	144 hrs.	12±3°C	20mm	17mm	8mm	2mm
3.	9A	200 hrs.	10±2°C	15mm	11mm	3mm	1mm
4.	10A	140 hrs.	12±3°C	22mm	19mm	10mm	2mm
5.	12A	144 hrs.	13±2°C	15mm	11mm	8mm	2mm
6.	15A	120 hrs.	13±2°C	11mm	8mm	5mm	1mm



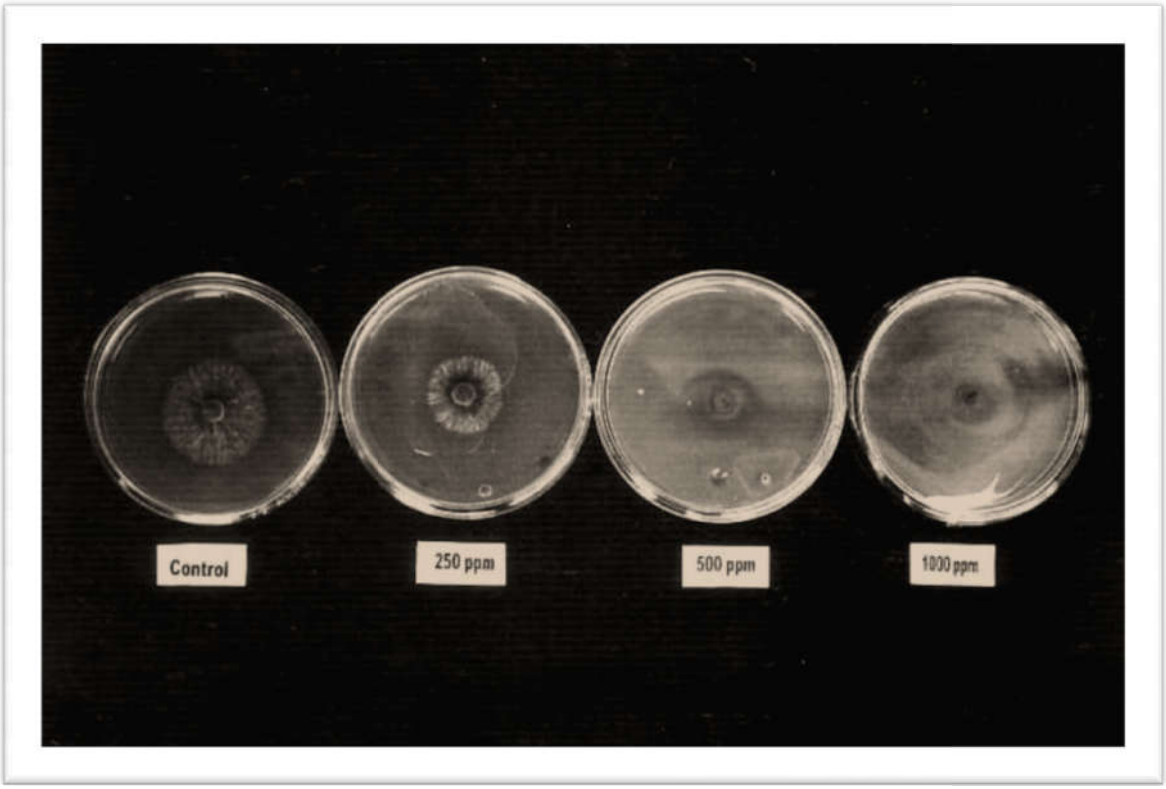
Photographes 1: Shows the inhibition of growth of fungus due the effect of concentration of compound Antifungal property shown by N-p Toluene-4-Biphenyl acetamide (2A) on *Curvalaria lunata*



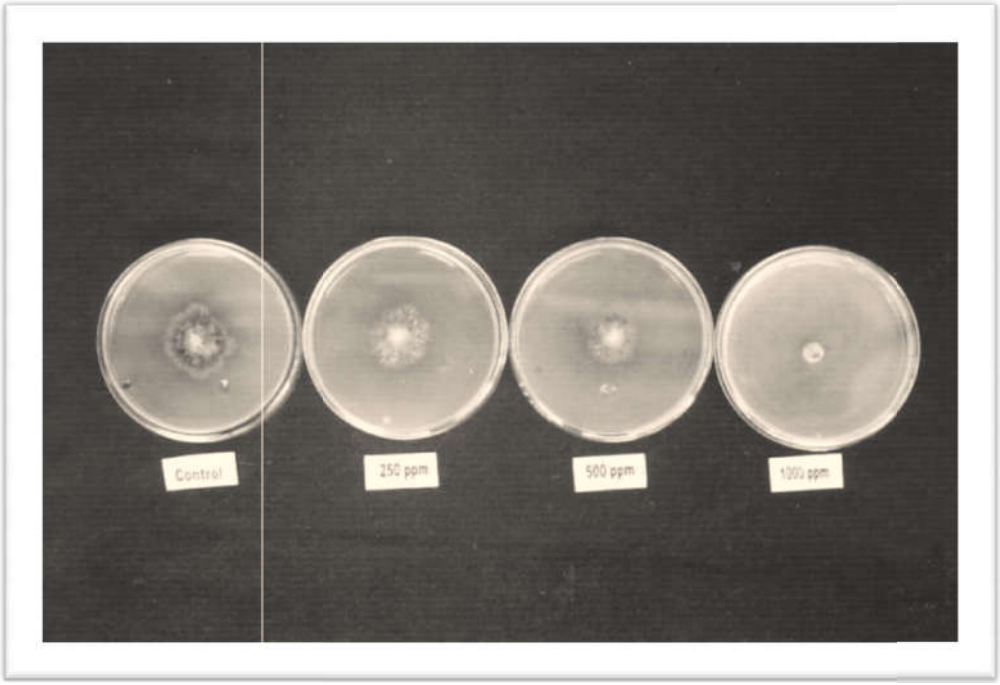
Photographes 2: Antifungal property shown by N-p Toluene-4-Biphenyl acetamide (4A) on *Curvalaria lunata*



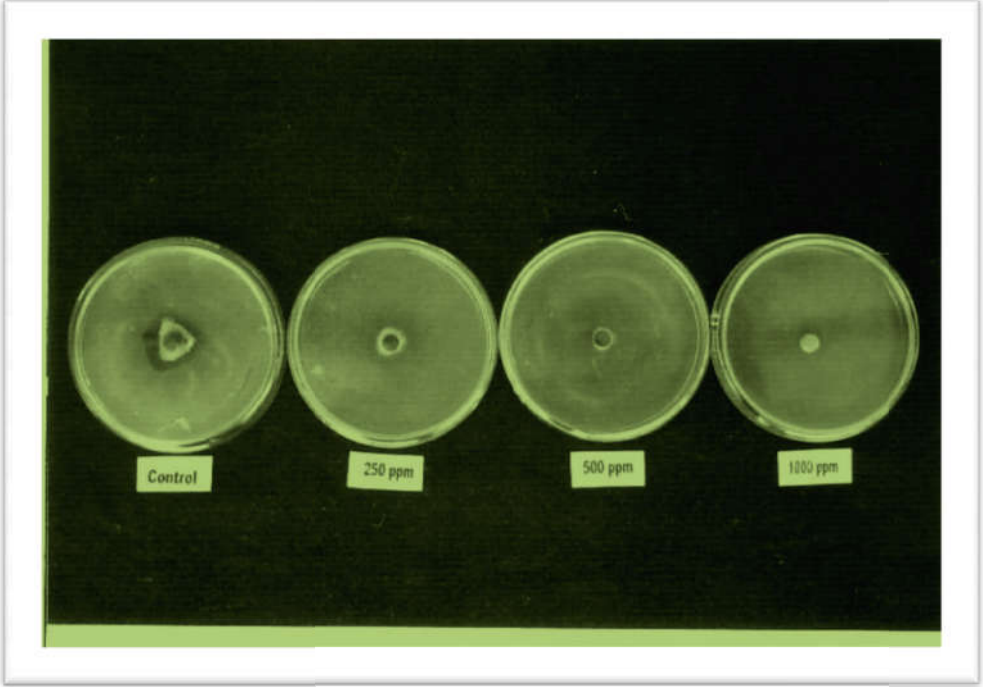
Photographes 3: Antifungal property shown by N-2-Pyridine -4-Biphenyl acetamide (9A) on *Curvalaria lunata*



Photographes 4: Antifungal property shown by N-p-benzoic acid -4-Biphenyl acetamide (10A) on *Curvalaria lunata*



Photographes 5: Antifungal property shown by N-2-Chlorophenyl -4-Biphenyl acetamide (12A) on *Curvalaria lunata*



Photographes 6: Antifungal property shown by N-p-bromo-phenyl-4-Biphenyl acetamide (15A) on *Curvalaria lunata*

4. Conclusion

Commercially available 4-biphenyl acetic acid (white crystalline solid) was used for the preparation of Substituted-4-biphenyl acetamide derivatives. 4-Biphenyl acetic acid was treated was treated with Thionyl chloride in dry benzene on water bath for 2-3 hrs at 70-80^{0}c. After 1 hr., the colour of reaction mixture becomes change from pale yellow to orange and then on completion of reaction, it changes from orange to brown. This oily mass of 4-biphenyl acetyl chloride is then treated with different types of aliphatic and aromatic amines in Pyridine or 4 N-NaOH to synthesis a series of amides. These synthesized-4-biphenyl acetamide derivatives were treated with n-hexane for crystallization. Pure compounds were then analyzed with the help of M.P., TLC, NMR, IR and Elemental analysis techniques.

Biological activities: In the present study six compounds namely-: N-Phenyl -4-Biphenyl acetamide – (2A), N-Para-Toluene -4-Biphenyl acetamide- (4A), N-2-Pyridine -4-Biphenyl acetamide – (9A), N-Para-benzoic acid -4-Biphenyl acetamide – (10A), N-2-Chlorophenyl -4-Biphenyl acetamide – (12A), N-Para-Bromo-phenyl -4-Biphenyl acetamide – (15A) were tested for antimicrobial activity against fungus *Curvalaria lunata*. All these six compounds were found to show antifungal activity against the fungus and these compounds can be used as therapeutic agents for the treatment of plant infections by *Curvalaria lunata*. The culture of each species was incubated at 12 ± 3 ^{o}C and the zone of the inhibition was measured after 120-200 hrs. All compounds with concentration 250 ppm showed minimum growth while compounds with concentration 1000 ppm showed maximum growth when observed via disc diffusion method while the compounds with 500 ppm showed intermediate level of growth. These results justify further research to be done to clarify the mechanism of action of these compounds.

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Conflict of Interest

The author declared that there is no conflict of interests regarding the publication of this article.

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