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"SYNTHESIS AND STUDY OF IMIDAZOLE DERIVATIVES OF ARYL SUBSTITUTED 1,3-THIAZINES AND THEIR NANOPARTICLES WITH SPECIAL REFERENCE TO PLANT PATHOGENS OF SOME VEGETABLE CROPS"

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ABSTRACT

The synthesis, spectral analysis and biological activities of 4-(2'-hydroxy-3',5'-dichlorophenyl)-6-(4"-nitrophenyl)-2-[4-(2-hydroxy-3,5-dichlorophenyl)-2-mercapto-imidazolo]-3-6-dihydro-1,3-thiazine(A") have been carried out. In this case 4-(2'-hydroxy-3',5'-dichlorophenyl)-6-(4"-nitrophenyl)-2-imino-3,6-dihydro-1,3- thiazine (A), 4-(2'-hydroxy-3',5'-dichlorophenyl)-6-(4"-nitrophenyl)-2-[(2-hydroxy-3,5-dichlorophenyl)ethanonylamino]-3-6-dihydro-1,3-thiazine (A') & 4-(2'-hydroxy-3',5'-dichlorophenyl)-6-(4"-nitrophenyl)-2-[4-(2-hydroxy-3,5-dichlorophenyl)-2-mercapto-imidazolo]-3-6-dihydro-1,3-thiazine (A'') have been screened. The compounds A was synthesized from 2'-hydroxy-3,5-dichlorophenyl-4-(4"-nitrophenyl) chalcone (a) by the action of thiourea, while (A'') was synthesized from (A) by reaction with α-bromo,2-hydroxy-3,5 dichloroacetophenone to get 4-(2'-hydroxy-3',5'-dichlorophenyl)-6-(4"-nitrophenyl)-2-[(2-hydroxy-3,5-dichlorophenyl) ethanonylamino]-3-6-dihydro-1,3-thiazine (A'). Further (A') on treatment with KSCN was dissolved in acetic acid gave (A''). The compound (a) was synthesized from 2'-hydroxy-3',5'-dichloroacetophenone by the action of p-nitrobenzaldehyde in ethanol and 40% NaOH. The nanoparticles of the compounds A, A'and A'' have been prepared by using ultrasonic technique. The titled compounds and their nanoparticles were assayed for antipathogenic impact against some common crop pathogens viz - *Aspergillus niger, Pseudomonas lachrymans, Fusarium oxysporum* and *Fusarium solani.*

KEYWORDS: Chalcone, thiazine, thiourea, α-bromo, 2-hydroxy-3,5 dichloroacetophenone, KSCN was dissolved in acetic, antipathogenic activities.

INTRODUCTION

Thiazine is a six membered ring system, which contains two hetero atoms [N and S] placed in a heterocyclic ring at 1, 3 positions. Many workers have synthesized different 1,3-thiazines. The researchers have reported the synthesis of several thiazines[1-6] and also their potent biological activities such as blood platelet aggregation inhibitors[7], antibacterial[8-9] antiallergic[10], anticholesterenic[11] and antifungal[12] Moreover thiazine nucleus is a pharmacophore of cephalosporin that occupy a very important place in the field, of antibiotics and drug chemistry. Chalcones and their analogues having α, β-unsaturated carbonyl system are very versatile substrates for the evolution of various reactions and physiologically active compounds. The reaction of thiourea with α, β-unsaturated ketones also results in the formation of 1,3-thiazines.

Plant Pathology or Phytopathology deals with the cause, etiology, resulting losses and control or management of the plant diseases The chlorosubstituted thiazines with amino group at position 2 in the ring exhibit promising biological activities[13-16], antimicrobial[17], antibacterial activity against gram positive & gram negative bacteria[18], biological activity[19], herbicidal activity.[20]

Nanotechnology has the potential to change the entire scenario of the current agricultural and food industry with the help of new tools developed for the treatment of plant diseases, rapid detection of pathogens using nanobased kits, improving the ability of plants to absorb nutrients etc. Nanobiosensors and other smart delivery systems will also help the agricultural industry to fight against different crop pathogens.

Previous studies confirmed that metal nanoparticles are effective against pathogens, insects and pests.

Nanoparticles can be used in the preparation of new formulations like nanomedicines as anticancer drugs like drugs for human breast cancer[21] & liver cancer.[22]

In the present study, the chlorosubstituted 1,3-thiazole and its derivatives (A, A' and A") have been prepared along with their nanoparticles and were assayed for antipathogenic impact against some common crop pathogens viz - *Aspergillus niger, Pseudomonas lachrymans, Fusarium oxysporum* and *Fusarium solani.*

EXPERIMENTAL

All the glassware's used in the present work were of pyrex quality. Melting points were determined in hot paraffin bath and are uncorrected.The purity of compounds was monitored on silica gel coated TLC plate. IR spectra were recorded on Perkin-Elmer spectrophotometer in KBr pelletes, H^1 NMR spectra on spectrophotometer in $CDCl_3$ with TMS as internal standard. UV spectra were recorded in nujol medium. The analytical data of the titled compounds was highly satisfactory. All the chemicals used were of analytical grade. All the solvents used were purified by standard methods. Physical characterisation data of all the compounds is given in Table 1.

Table 1: Characterisation data of newly synthesized compounds.

Comp -ounds	Molecular formula	M.P. in ^{0}C	% of yield	% of element				
				C	H	N	S	Cl
	$C_8H_6O_2Cl_2$	54	80	47.90/48	2.95/3			34.15/34.58
a	$C_{15}H_9O_4NCl_2$	250	70	53.10/53.25	2.40/2.66	3.98/4.18		21/21.77
A	$C_{16}H_{11}O_3N_3Cl_2S$	120	70	48.50/48.60	2.35/2.53	10.40/10.63	8/8.10	17/17.92
A'	$C_{25}H_{15}O_6N_3Cl_4S$	128	70	56.80/56.25	2.98/2.92	8.25/8.20	6.77/6.82	28/28.73
A"	$C_{26}H_{18}O_5Cl_4N_4S_2$	165	70	46.80/46.42	2.70/2.67	8.40/8.33	9.56/9.52	21.60/21.13

2'-Hydroxy 3',5'-dichloroacetophenone

2'-Hydroxy-5-chloroacetophenone (3g) was dissolved in acetic acid (5 ml), and mixed with sodium acetate (3g). To this reaction mixture chlorine in acetic acid reagent (40 ml; 7.5 w/v) was added dropwise with stirring. The temperature of the reaction mixture was maintained below 20^{o}C. The mixture was allowed to stand for 30 minutes and then poured into water. A pale yellow solid thus obtained was filtered, dried and crystallized from ethanol to yield the compound.

Preparation of 2'-hydroxy-3,5-dichlorophenyl-4-(4"-nitrophenyl)-chalcone (a)

2'-Hydroxy-3',5'-dichloroacetophenone (0.1 mol) was dissolved in ethanol (50 ml) and p-nitrobenzaldehyde (0.1 mol) was added gradually to the solution and the mixture was heated to boiling. Then aquous sodium hydroxide solution [40%; 40 ml] was added dropwise with constant stirring. The mixture was stirred mechanically at room temperature for about half an hour and kept for overnight. It was then acidified by hydrochloric acid (10%) solution. The solid product thus separated, was filtered and washed with sodium bicarbonate (10%) followed by water. Finally it was crystallized from ethanol acetic acid mixture to get the compound (a).

Preparation of 4-(2'-hydroxy-3',5'-dichlorophenyl)-6-(4"-nitrophenyl)-2-imino-3,6-dihydro-1,3-thiazine (A)

2'-Hydroxy-3,5-dichlorophenyl-4-(4"-nitrophenyl)-chalcone (a) (0.01 mol) and thiourea (0.02 mol) were dissolved in ethanol (30 ml). To this aquous KOH solution (0.02 mol) was added,. The reaction mixture was refluxed for three hours, cooled and diluted with water then acidified with 1:1 HCl. The product thus obtained was crystallized from ethanol to get the compound (A).

Preparation of 4-(2'-hydroxy-3',5'-dichlorophenyl)-6-(4"-nitrophenyl)-2-[(2-hydroxy-3,5-dichlorophenyl) ethanonylamino]-3-6-dihydro-1,3-thiazine (A')

A stoichiometeric mixture of 4-(2'-hydroxy-3'-5'-dichlorophenyl)-6-(4"- nitrophenyl)-2-imino-3-6-dihydro-1,3- thiazine (A) and α-bromo,2-hydroxy-3,5 dichloroacetophenone was dissolved in ethanol and refluxed for one hour. It was then cooled, diluted with water and crystallized from ethanol to get the compound (A').

Preparation of 4-(2'-hydroxy-3',5'-dichlorophenyl)-6-(4"-nitrophenyl)-2-[4-(2-hydroxy-3,5-dichlorophenyl)-2-mercapto-imidazolo]-3-6-dihydro-1,3-thiazine (A'')

A stoichiometeric mixture of 4-(2'-hydroxy-3',5'-dichlorophenyl)-6-(4"-nitrophenyl)-2-[(2-hydroxy-3,5-dichlorophenyl)ethanonylamino]-3-6-dihydro-1,3-thiazine (A') and KSCN was dissolved in acetic acid and refluxed for 4.5 hours, cooled and diluted with water. The product thus separated was crystallized from ethanol to get the compound (A'')**.**

The newly synthesized compounds were characterised on the basis of elemental analysis, molecular determination, UV, IR, NMR. spectral data.

The UV, IR, and NMR spectral data

Compound (A)

UV: Spectrum No. 1.

The UV-Vis spectrum of the compound A reported in dioxane showed λ_{max} value 495 nm corresponding to $n \rightarrow \pi^*$ transition.

IR (KBr):- Spectrum No. 2
3365.34 cm^{-1} (-OH phenolic), 2925.2cm^{-1} (aliphatic -C-H stretching), 3068.24cm^{-1} (aromatic -C-H stretching), 3017.30 cm^{-1} (-N-H stretching), 1648.7 cm^{-1} (-C=N stretching), 1342 cm^{-1} [(C-N) (C-NO_2) stretching], 738.13 cm^{-1} (C-Cl stretching in aliphatic), 1177.7 cm^{-1} (C-Cl) stretching in aromatic).

PMR:- Spectrum No. 3
∂ 1.2 (s, 1H, -C-**H**); ∂ 2.7 (s, 1H, =N -**H**) ; ∂ 3.6 (s, 1H, =N-**H**) ; ∂ 3.7 (s, 1H, =C-**H**) ; ∂ 7.6 to 8.1 (m, 6H, Ar-**H**) ; ∂ 12.6 (s, 1H, O-**H**)

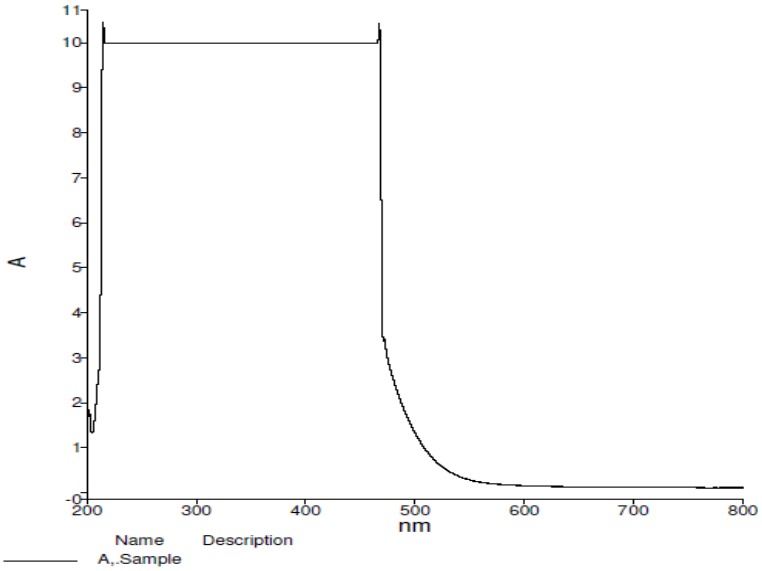
Compound (A'')
UV: Spectrum No. 4

The UV-Vis spectrum of the compound A" reported in dioxane showed λ_{max} value 492 nm corresponding to n→π* transition.

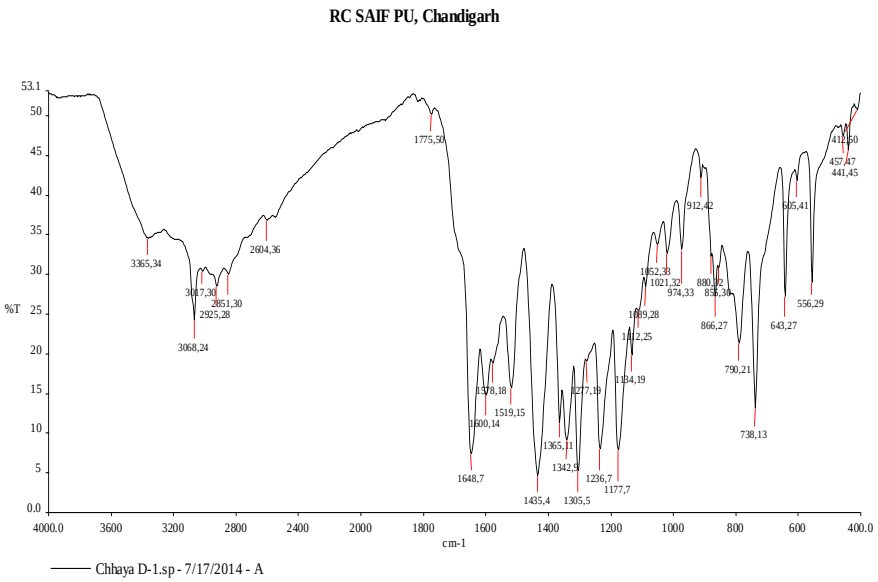
IR (KBr):- Spectrum No. 5
1605.23 cm^{-1} (=C=O stretching), 3391.39 cm^{-1} (-OH phenolic), 2925.36cm^{-1} (aliphatic -C-H stretching), 3068.24cm^{-1} (aromatic -C-H stretching), 1435 cm^{-1} (-C=N stretching), 1365.14 cm^{-1} [(C-N) (C-NO_2) stretching], 738.15 cm^{-1} (C-Cl stretching in aliphatic), 2547.43 cm^{-1} (-S-H stretching),1605.23 cm^{-1} (=C=O stretching),

PMR:- Spectrum No. 6.

∂ 7.7 to 7.9 (m, 8H, Ar-**H**) ; ∂ 12.5 (s, 1H, O-**H**)



Spectrum No. 01



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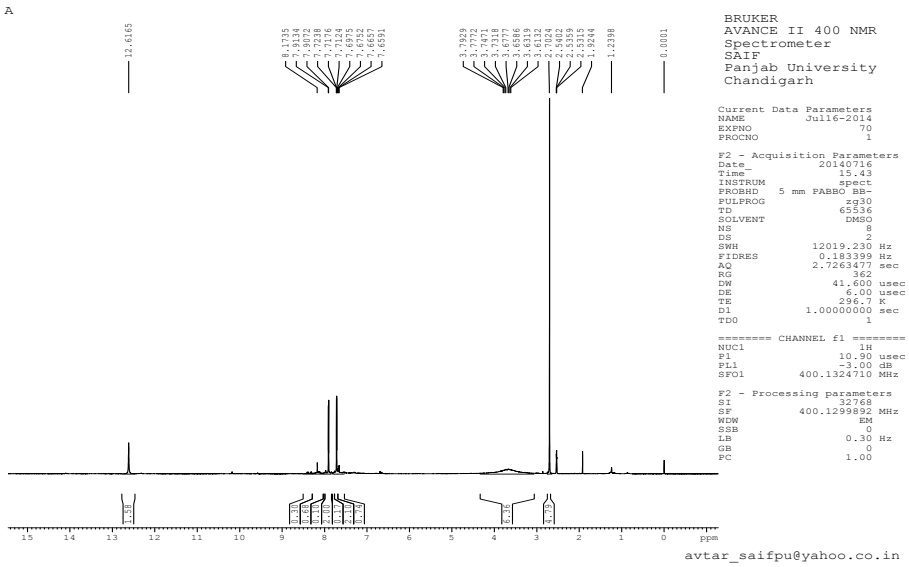

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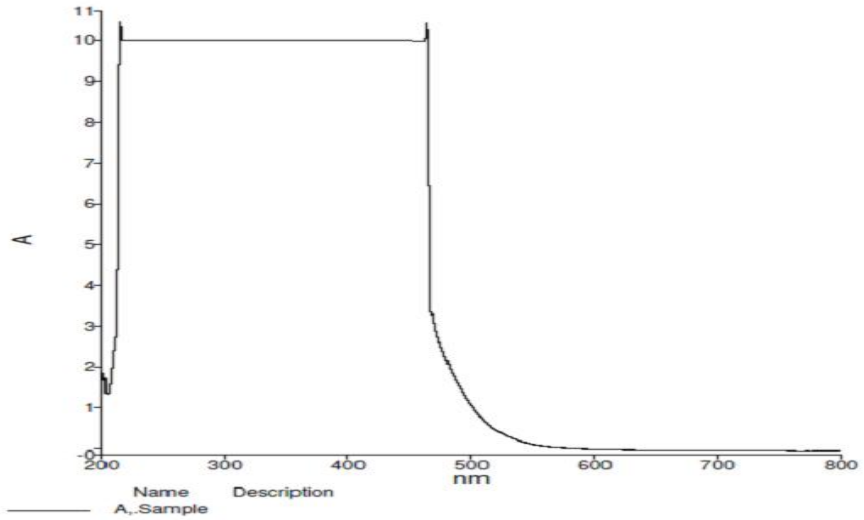

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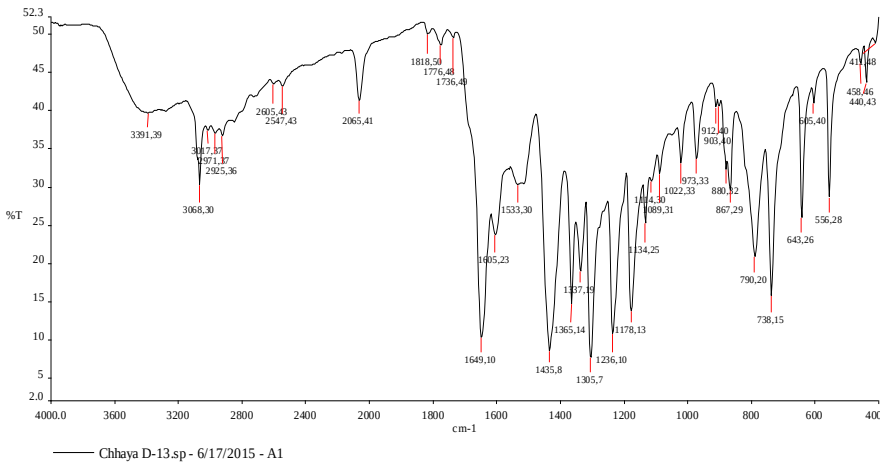
RC SAIF PU, Chandigarh



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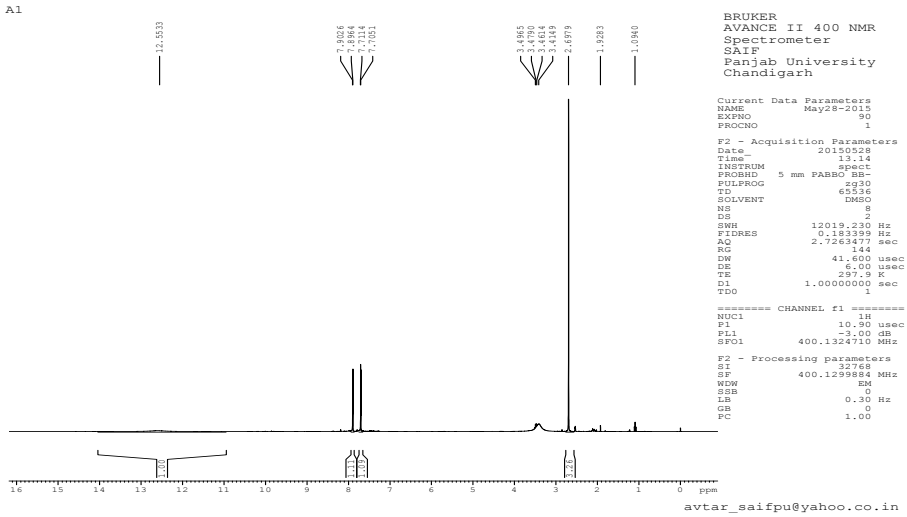
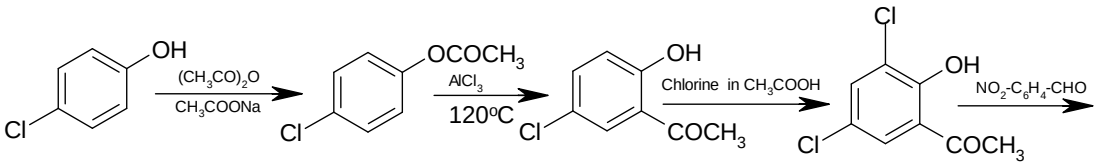
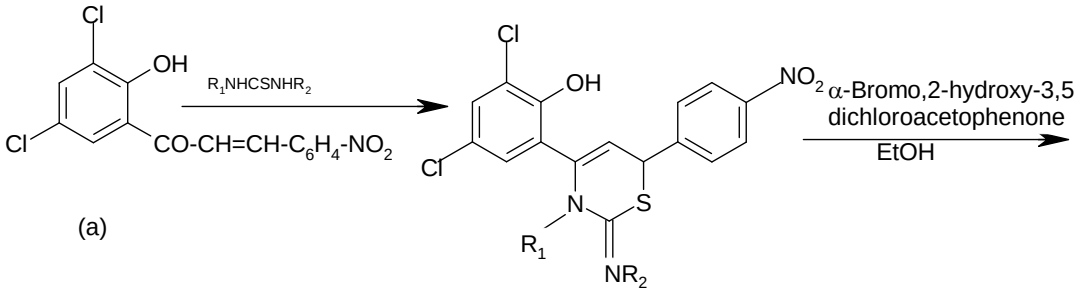


Spectrum No. 06

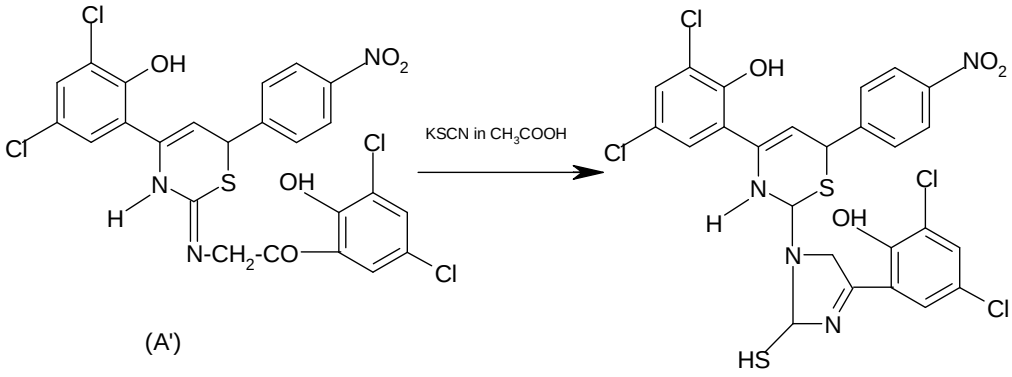
Scheme





(a)

(A)



(A')

(A")

Where

1) R_1 = -H, -C_6H_5
2) R_2 = -H, -C_6H_5

EXPERIMENTAL DETAILS AND DISCUSSION OF RESULTS

The newly synthesized thiazines (A, A'& A") and their nanoparticles in the study were tested against some common pathogens for their antifungal and antibacterial activities, using disc diffusion method. The vegetable crop pathogens namely *Aspergillus niger, Pseudomonas lachrymans, Fusarium oxysporum, Fusarium solani* were procured from Department of Plant Pathology, Punjabrao Deshmukh Agriculture Krishi Vidyapeeth, Akola.

The punch discs of 6.25 mm diameter of whatman filter paper No. 1 were prepared and dispensed in the batches of 100 inch in screw capped bottles. These were sterilized by dry heat at 140^{o}C for 60 minutes. The solutions of 0.01 mole dilution of the nanoparticles of test compounds mentioned in the part V of the study were prepared in dioxane solvent. The discs were soaked assuming that each discs will contain approximately 0.01 ml of the test solution.

The culture media for pathogens was prepared by using the following composition for one litre distilled water.

Composition of nutrient agar-agar

Peptone : 5.0 g/litre
Sodium chloride : 5.0 g/litre
Beef extract : 1.5 g/litre
Yeast extract : 1.5 g/litre
Agar : 15.0 g/litre
pH (approximately) : 7.4 $\pm$ 0.2

The culture medium prepared was sterilized in an autoclave at 15 lbs/inch pressure at 121^{o}C temperature for 15 minutes. After sterilization it was cooled down to about 50^{o}C and poured into presterilized petriplates of 8.5 cm in diameter each and allowed to solidify the nutrient agar medium of about 14 m depth. The petriplates were kept with nutrient broath at 37^{o}C for 4 hours in an incubator.

The cultures of pathogens were innoculated separately in petriplates on the surface nutrient agar broath uniformly with all a septic precautions. The plates were dried again for 30 minutes and without further delay the discs soaked in the test compounds were applied at adequate spacing 2 cm or more apart to the surface medium with the help of sterilized forceps. The discs were pressed gently to ensure their full contacts with the medium. The control was run using plane dioxane solvent for aseptic conditions. The plates were kept in incubator at 37^{o}C for about 18 to 24 hours. Soon after the incubation period is over the degree of sensitivity to test the compounds were determined by measuring the visible clear area of growth free zones [zone of inhibition] produced by diffusion of the antibiotics into media from the discs by calipers in mm. The results are tabulated as.

Vegetable Crop Pathogens
Zones of Inhibition (mm)
Vegetable Crop Pathogens

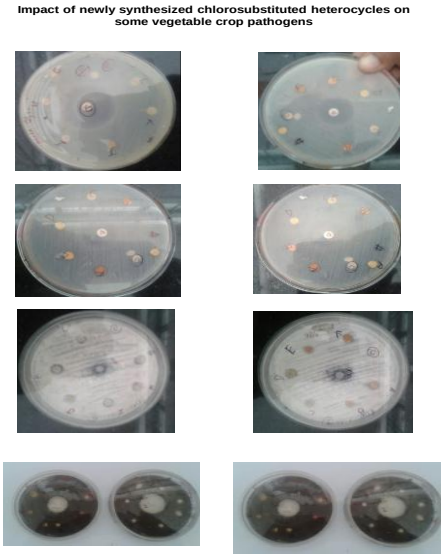
Sr.No.	Aspergillus niger	Pseudomonas lachrymans	Fusarium oxysporum	Fusarium solani
(1) A	0.5 mm	2.5 mm	2.0 mm	0.5 mm
(2) A'	2 mm	1 mm	0.5 mm	2 mm
(3) A"	0.5 mm	2.5 mm	2.5 mm	2.5 mm
(4) Control	-	-	-	-
(5)Antibacterial agent	-	11 mm	11 mm	-
(6)Antifungal agent	8 mm	-	8 mm	-

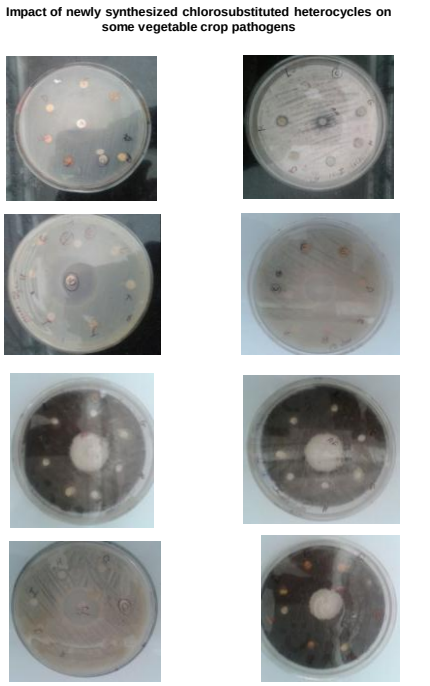
Zero mm : Non active
0 – 2 mm : Weakly active
3 – 5 mm : Moderately active
6 – 8 mm : Active
9 – 11 mm : Strongly active
12 – 14 mm : Very strongly active

RESULT AND DISCUSSION

The nanoparticles of test compounds when screened *in vitro* against test vegetable crop pathogens viz. *Aspergillus niger, Pseudomonas lachrymans, Fusarium oxysporum, Fusarium solani* then it was noticed that most of these compounds (A, A' & A") showed remarkable inhibitory activity against all the test organisms.

Compound A" showed remarkable inhibitory activity against vegetable crop pathogens *Pseudomonas lachrymans, Fusarium oxysporum, Fusarium solani.*





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