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Synthesis and fungicidal properties of 2,4-diaza-1,3,5-pentanetrione compounds

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The preparation of 2,4-diaza-1,3,5-pentanetrione compounds were described. The fungicidal effects of these compounds on the mycelial growth of the isolate, *Phoma eupyrena* were carried out by *in vitro* experiment. The results show that the response to treatment depended not only on the concentration of the compounds used and duration of inoculation but also on the chemical constitution. All the compounds tested were effective at concentrations greater than serial dilution 10^{-2}. At lower concentrations, they were not too effective. The compounds were very active against *P. eupyrena*.

Key words: Synthesis, fungicidal, 2,4-diaza-1,3,5-pentanetrione, *Phoma eupyrena*.

INTRODUCTION

The lethal action of a chemical depends upon the concentration of the active compound and the time of exposure. Species of fungi exhibit great variation in their ability to resist the action of certain fungicides (Bent et al., 1970; Fernandez-Ortuno et al., 2013; Pang et al., 2013). Basic fungi toxicity tests are designed mainly to measure the effect of test chemicals on germination, growth or respiration of fungi. The first two approaches have been widely used in the primary evaluation of chemicals for fungicidal activity. Erwin et al. (1975; Everett and Timudo-Torrevilla, 2007; Abdel-Kader et al., 2011) used linear growth rate per day to assess the fungicidal properties of tributyl (5-chloro-2-thienyl-methyl) phosphonium chloride (TTMP). Erwin et al. (1975) reported that when TTMP was added to potato dextrose agar at concentration of less than 500 µg/ml, there was no reduction in growth of *Verticillium albatrium*. At 1000 µg/ml, there was about 50% reduction in diameter of the colonies.

In an *in vitro* experiment to control blight, root rot and crown rot diseases of pepper (*Capsuim annum*), a non-systemic fungicide cis-N-(1,2,3-tetrachloroethyl-thio)-4-cyclohexane-1,2-dicarboximide (captafol) and a systemic fungicideN(2,6-dimethylphenyl)-N-(methoxyacetyl)alanine methylester administered to five isolates of *Phytophthora capssici* was studied in lima bean agar and broth. Captafol was more effective than metalacyl in reducing growth rate in solid and liquid media, inhibiting zoospore release from sporangia, stopping zoo pores mobility and inhibiting germination of sporangia and zoopores. At fungicide concentration from 2.5 to 10 µg/ml, metalacyl inhibited zoospores germination than captafol (Papavizas and Bowers, 1980). The toxicity of a compound can be determined by measuring the time required for the fungal colony to reach a particular diameter or the size of colony attained in a certain period of time (Rowelli, 1968; Kim et al., 2012).

Phoma eupyrena is a fungal plant pathogen, the causal agent of leaf blight in *Pistia stratiotes (L) Fam Araceae* (water lettuce), Phoma blight of fir and douglas-fir seedlings (Moham Babu et al., 2003; Kliejunas et al., 1985; Hansen and Hamm, 1988). Some urea compounds have been cited to possess antifungal and antibacterial properties (Obaleye et al., 1995, 2000). Some, which have been patented as herbicides and growth regulators includes diuron, linuron, benzuron and phenobenzuron (Obaleye et al., 1995). Therefore, in the light of the fore-

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going, this research work was aimed at the preparation of some potential fungicidal agents, 2,4-diaza-1,3,5-pentanetrione compounds from urea and investigation of their effect on growth rate of the isolate *P. eupyrena*.

MATERIALS AND METHODS

Materials and reagents

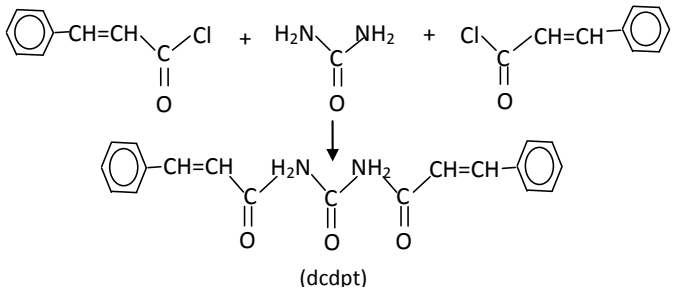
The reagents used for this research work were purchase from Aldrich chemical company. They were all of analytical grade and were used directly without further purification. The chemicals included cinnamoyl chloride, crotonoyl chloride, acetyl chloride, benzoyl chloride, urea and calcium chloride. The solvents were dried in molecular sieves and distilled to obtain the anhydrous solvents for refluxing. The solvents used included benzene, toluene, acetone, chloroform, ethanol, methanol, acetonitrile, petroleum ether, diethyl ether among others.

Synthesis

Preparation of 2,4-diaza-1,3,5-pentanetrione compounds

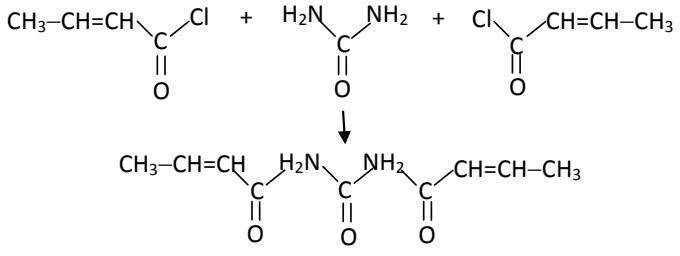
The ligands were prepared by employing the techniques of previously reported methods (Obaleye et al., 1995, 2000). This involves the refluxing of 7.6 g (100 mmoles) of urea with 16.8 g (100 mmoles) of cinnamoyl chloride in dry benzene for a period of 4 h until the evolution of HCl gas seizes. The solution was cooled in an ice bath over night and the white solid product formed was collected by filtration. The product was washed with petroleum ether, sodium bicarbonate solution and later recrystallized from aqueous ethanol. Similar reactions were performed using crotonyl chloride, acetyl chloride, benzoyl chloride, respectively, in the place of cinnamoyl chloride. The equations of the reactions are as follows:

i) 1,5-dicinnamyl-2,4-diaza-1,3,5-petanetrione (dcdpt)



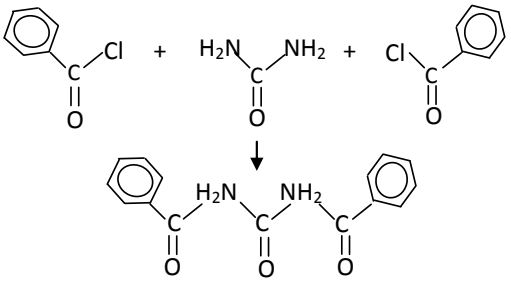
(dcdpt)

ii) 1,5-diallyl-2,4-diaza-1,3,5-petanetrione (dadpt)



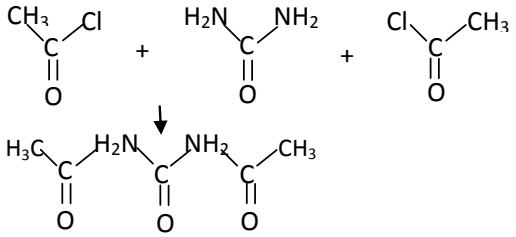
(dadpt)

iii) 1,5-dibenzyl-2,4-diaza-1,3,5-petanetrione (dbdpt)



(dbdpt)

iv) 1,5-dimethyl-2,4-diaza-1,3,5-pentanetrione (dmdpt)



(dmdpt)

Fungicidal studies

Collection of sample

A sample of the isolate, *P. eupyrena* was collected from the culture room, Biological Science Department, Igbinedion University, Okada, Edo State, Nigeria.

Sterilization method

Petri dishes and conical flasks were washed with detergents and allowed to dry. The Petri dishes were stacked in canisters and sterilized in the oven at a temperature of 180°C for 2 h. 95% alcohol was used for sterilization of laboratory benches. Inoculating loop was sterilized by heating into a red-hot burning flame light.

Preparation of media

Potato dextrose agar (PDA) was prepared through the following processes: 250 g of peeled Irish potatoes were cut into smaller units and boiled with water just covering the weighed potatoes. An extract was made from the cooked potatoes with the help of Mushin rag. Water was added to the extracted aliquot to make 1 L. 20 g of agar and 15 g of dextrose were added to the 1 L suspension. The mixture was placed on the hot plate to dissolve with an intermittent hand shaking of the mixture until they were all dissolved. The resultant liquid was dispensed into flasks and autoclaved at 121°C for 15 min. To an autoclaved medium, 30 ppm (μg/ml) streptomycin sulphate was added at about 39 to 43°C and shaken properly. The introduction of streptomycin sulphate was done aseptically. The remaining medium was kept in the refrigerator for subsequent use.

Preparation of serial dilution of the compound

The compound used for the fungicidal studies were coded as dcdpt,

Table 1. Physical and spectroscopic properties of the 2,4-diaza-1,3,5-pentanetrione compounds.

Physical and spectral property	Compound			
	Dcdpt	**Dbdpt**	**Dadpt**	**Dmdpt**
Colour	White	White	White	White
M.Pt (°C)	221	215	210	231
% yield	71	72	74	75
R_f-value	0.64	0.62	0.70	0.72
Infrared band (cm^{-1})				
ν(N-H)	3376 s	3360 s	3500 s	3360 s
	3200 s	3260 s	3200 s	3250 s
ν(C=O)	1711 s	1750 m	1751 s	1750 m
	1668 s	1700 s	1720 s	1710 s
	1650 s	1650 s	1660 s	1660 s
δ(N-H)	1620 s	1620 s	1625 s	1620 s
ν(N-C-N) + ν(C=C)	1510 s,	1480 s	1470 s	1480 s
	1368 s	1320 s	1330 s	1330 s
Ultraviolet bands (nm)				
$\pi\rightarrow\pi^*$, $n\rightarrow\pi^*$	220, 295	220, 285	220, 285	220, 284

dbdpt, dadpt and dmdpt. All the compounds were dissolved in warm water. Serial dilution method was used in the preparation of their solutions. This was done by dissolving 0.1 g of each compound in 10 ml sterile water in separate test tubes to produce the stock solution. For each test tube, 1 ml of the stock sample solution was measured using a graduated 1 ml pipette and introduced into other separate test tube, each containing 9 ml of sterile distilled water. Following this sample procedure, serial dilutions were prepared up to 10^{-5} for each sample (that is, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5}) where 10^{-5} is equal to 0 .01 μg/L. Control was set up using distilled water instead of the compound solution.

Radial growth measurement on the linear mycelial growth of the isolate *P. eupyrena*

Toxicity of all the synthesized compounds (dcdpt, dadpt, (dbdpt and dmdpt) to linear mycelia growth of the isolate, *P. eupyrena* was evaluated *in vitro* by incorporating various concentrations of the compound, in PDA medium, employing an earlier method of Farih et al. (1981) and Obaleye et al. (1995). About 8 to 12 ml of the agar medium fungicide mixture were poured into 9 to 12 diameter sterile Petri dishes. Using 4 mm sterile cork borer, 4 mm diameter was cut from the isolate, *P. eupyrena* and inoculated into the center of the solidified media-fungicide mixture in different Petri dishes. The cultures were then incubated at 27°C and linear growth was taken each day for 10 days. Control was set up using prepared 2 ml sterile water in 8 ml of distilled water. Without opening the Petri dishes, measurements was carried out in different concentration of fungicide in various petri dishes by turning upside down the Petri dishes and the radial growth in diameter (cm) were measured along all the four sides with ruled lines and average of these sides was taken to make the growth rate. Measurement was carried out by using a graduated ruler. Average radial growth rate for each treatment in two replicate were recorded daily for 10 days.

RESULTS AND DISCUSSION

Physical and spectroscopic studies

The physical and spectroscopic property of the compounds is presented in Table 1. The melting points of the ligands are sharp and quite distinct from that of the starting material. The melting point increased in the order cadpt →cbdpt→dcdpt→cmdpt which can be attributed to increase in carbon chainlength and measure of unsaturation. The selected infrared and ultraviolet bands for characterization are mainly due to stretching bands, ν(N-H), ν(C=C), ν(C=O), ν(C=N) and deformation bands δ(N-H). About three bands were observed for carbonyl stretching, ν(C=O) within the range 1751 to 1650cm^{-1} which is due to the presence of 1,3,5-tricarbonyl system in all the compounds. Also, the ultraviolet spectra gave two peaks around 220 and 294 to 284 nm in all the compounds which are assigned to $\pi\rightarrow\pi^*$ and $n\rightarrow\pi^*$ intra-ligand transition bands of the 1,3,5- tricarbonyl system in all the compound. The result of melting points, R_f-values, infrared and ultraviolet spectra fully established the formation of the new compounds.

Antifungal studies

The result of the antifungal studies is presented in Table 2 and this is presented graphically in Figures 1 and 2. The *in vitro* experiment showed that the chemicals had different comparative effects on the mycelia growth. It can be established that all the compounds possess antifungal properties against the test organisms when you consider their results with that of control. The magnitude of the antifungal properties depends not only on the concentration used and duration of inoculation but also on the chemical constitution. It could be observed that the antifungal properties of the compounds varied in the order: dcdpt > dbdpt > dadpt > cmdpt, which can be related to structure.

Table 2. Daily radial growth (cm) of the isolate, *P. eupyrena.*

Number of days	2	3	4	5	6	7	8	9	10
Control	0.50	0.73	0.88	1.00	1.50	1.65	1.80	2.0	2.02
Compounds									
dcdpt									
10^{-5}	0.23	0.25	0.29	0.32	0.35	0.35	0.40	0.42	0.57
10^{-4}	0.20	0.23	0.26	0.29	0.32	0.35	0.38	0.40	0.49
10^{-3}	0.15	0.19	0.22	0.26	0.31	0.33	0.35	0.40	0.45
10^{-2}	0.10	0.12	0.14	0.15	0.20	0.25	0.27	0.30	0.33
dbdpt									
10^{-5}	0.25	0.29	0.32	0.35	0.35	0.40	0.43	0.55	0.57
10^{-4}	0.23	0.26	0.29	0.32	0.35	0.38	0.42	0.48	0.54
10^{-3}	0.16	0.20	0.26	0.30	0.32	0.35	0.40	0.45	0.50
10^{-2}	0.12	0.14	0.15	0.20	0.25	0.28	0.30	0.40	0.42
dadpt									
10^{-5}	0.43	0.46	0.48	0.50	0.55	0.64	0.70	0.76	0.80
10^{-4}	0.40	0.43	0.45	0.47	0.50	0.60	0.68	0.70	0.75
10^{-3}	0.20	0.30	0.35	0.40	0.45	0.55	0.60	0.65	0.70
10^{-2}	0.18	0.23	0.29	0.34	0.35	0.40	0.45	0.50	0.58
dmdpt									
10^{-5}	0.55	0.60	0.65	0.70	0.80	0.90	1.00	1.10	1.20
10^{-4}	0.50	0.55	0.62	0.67	0.74	0.82	0.90	1.00	1.10
10^{-3}	0.40	0.50	0.60	0.65	0.70	0.76	0.80	0.90	0.98
10^{-2}	-	-	-	-	-	-	-	-	-

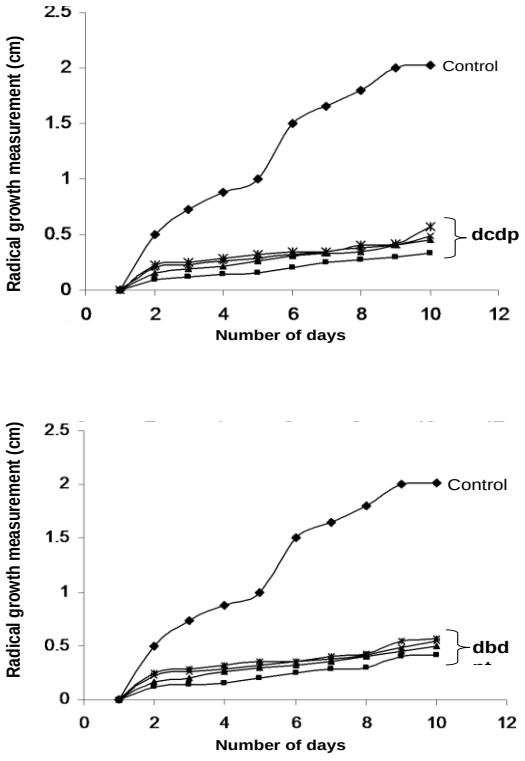


Figure 1. Radial mycelial growth of the isolate, Phoma eupyrena under the influence of (a) dcdpt; (b), dbdpt.

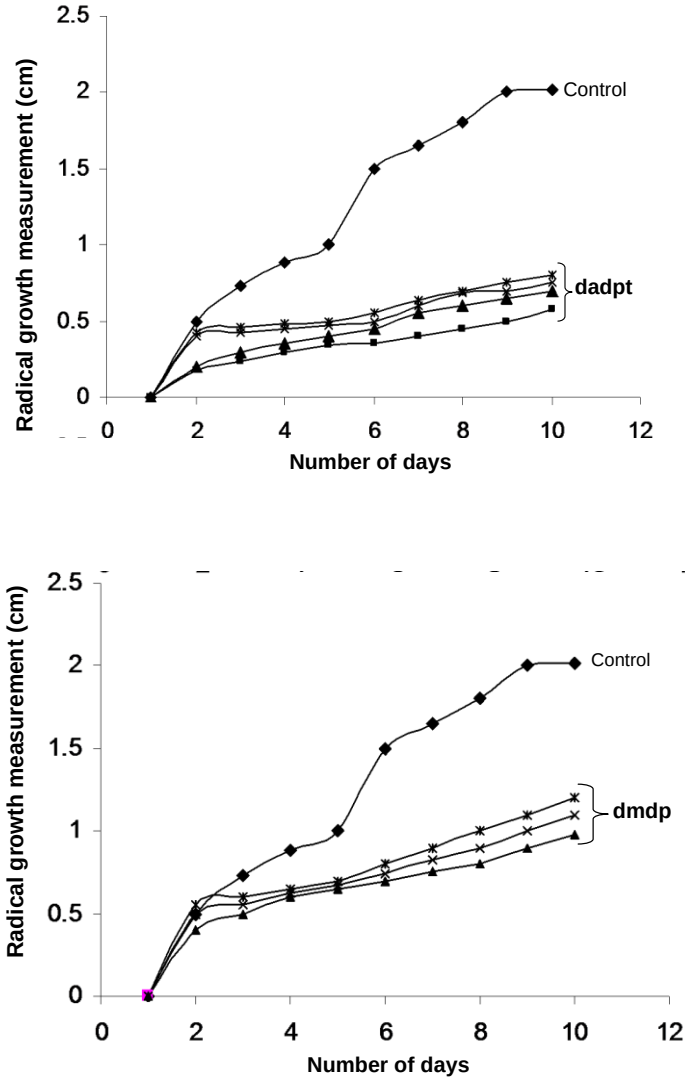


Figure 2. Radial mycelial growth of the isolate, Phoma eupyrea under the influence of (a) dadpt and (b), dmdpt.

Conclusion

The following deductions can be made from the results: 1, mycelial growth decreased with increase in concentration of the chemical compounds and increased with increase in number of days after inoculation; 2, it can be said that the fungal activity of the compounds is fungistatic since the compounds only help to regulate the rate of growth but does not obliterate the effect of the pathogen completely.

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