Research Paper

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Effect of Biological Impact on 2,6Dimethyl Phenyl Phosphate Monoester

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KEYWORDS :

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ABSTRACT

It has been recognized much earlier that organic phosphates are biologically important, physiologically necessary and play an important role in human life. Generally organic phosphate esters have the tendency to polymerize or decompose before their boiling points and the product always contain a mixture of mono,di and tri- esters separated either by column chromatography or by reduced pressure distillation. The separation of these phosphate esters has been carried out by highly sophisticated vacuum pump at b10 or b15. Compounds belonging to the following system will be prepared by standard method P.Rudert Method. Some phosphate esters of C-O-P and C-N-P linkages play an important role in the maintenance of human life which is the result of reaction between some vital substances. They all play a very important and essential role in our daily activities. Phosphate esters of C-O-P and C-N-P linkages are useful as plant hormones, fungicides, defoliating good insecticide, germicide etc. Salts of mono and di-esters of few phosphates act as a good disinfectants .

Introduction Interaction between acids (organic or inorganic), hydroxyl and amino derivatives of aliphatic or aromatic compounds in presence of suitable catalyst and experimental conditions bring about esterification. Orthophosphoric acid being a tri basic acid will form a series of esters[1-4]. Aromatic hydroxyl compounds interact with the tri basic acid to form mono, di and tri- esters. These phosphate esters are used as fungicidal activity [5-6]. Fungus [7] had been great menace to the human as well as vegetable kingdom. Therefore, to minimize the human sufferings and to achieve maximum yield in agricultural products, suitable methods of controlling the growth of fungus [7] are of immense importance. A number of organic phosphate esters have been used as fungicide and insecticides [8-9]. The treatment of phosphate esters as fungicide not only reduces the occurrence of fungi and damage caused by them but also promote better growth [10] of the plants by incorporation of phosphate residue in the form of C-O-P[11-16] and C-N-P [17-19] linkage.

Preparation of the Compound- The method of preparation of the phosphate esters of 2,6 di methylphenol involves the direct reaction of phosphorous oxi-tri chloride ($POCl_3$) with respective phenol. Mono ester of the 2,6 di methylphenol have been prepared by the general method [20]

Biocidal Activity of Phosphate Esters- The organo phosphate esters play an important role in the maintenance of human life. The human life is result of reactions between some vital substances. They all play a very important and essential role in our daily activities.

Phosphate esters play a considerable role in the growth inhibition of microbes i.e fungi as well as bacteria. The following techniques[21-23] have been used to detect the biological activities of phosphate esters.

1)- Agar plate technique
2)- Broth serial dilution method.
3)- Filter paper disc method.

The above method have been chosen for the antimicrobial study. Amount of growth may be calculated by the Vincent method[24]. These techniques have been employed during the present investigations.

a)- Isolation of phosphate esters- The prepared phosphate esters were isolated, filtered, washed and dried in the oven. These phosphate esters were kept in sterilized vials in dry and cool place.

b**)- Preparation of culture media-** Separate culture medium were prepared for the growth of fungus and bacteria. In culture media there should be necessary nutrients and it should be free from contamination. The media were prepared in conical flask and closed by cotton plug. After that, media and petridishes were sterilized for about 20 minutes at 121^{0}C temperature and at 15 atmosphere pressure in autoclave.

c**)- Preparation of sample solution** –The uniform solution of phosphate esters have been prepared in there appropriate solvents (DMSO).

Antifungal activity by agar plate method. For the determination of antifungal activity of phosphate esters the two different sort of fungus has been taken i.e. *Alternaria triticina* and *Alternaria alternate*. The agar plate technique[25-27] is used for the determination of anti fungal activities. The solution of phosphate esters of different concentration viz 250 ppm, 502 ppm, 750 ppm and 1000 ppm have been prepared .

Almost 10-15 ml of Czapak-Dox media was poured in petridishes. Before pouring the media about 0.1 ml of solution of above concentration were taken in sterilized petridishes. After that the test fungi were placed in the petridish by the inoculation needle, the petridishes were than placed in incubator for about 4-5 days at 26^{0}C to 30^{0}C temperature. After 5th day the growth observation were taken and zone of inhibition[24] was calculated by measuring the diameter of fungal colony in control plate and in the test plate by the following expression

$$\% \text{ inhibition} = \frac{C - T}{C} \times 100$$

Where
C=Diameter of fungal colony in millimeter in control plate.
T= Diameter of fungal colony in millimeter in treated plate.

The result of fungal colony inhibition diameters in test plate as compared to control plate are given in table.

	Alternaria triticina				Alternaria alternator			
Phosphateesters	250ppm	500ppm	750ppm	1000ppm	250ppm	500ppm	750ppm	1000ppm
Monoester	+	+++	++++	++++	+	+++	++++	++++

The result shows that the antifungal activities increase with increasing the concentration of phosphate esters. Higher the concentration of phosphate ester increases the activitiy. Maximum growth inhibition was found at 750 ppm .

Antibacterial Activity by filter paper disc method_ The antibacterial activity of phosphate esters were observed by filter paper disc diffusion method[28]. In this technique, about 15 to 20 ml of culture media is poured in sterilized petridishes to give a uniform layer of agar about 3 to 5 mm. thick . Incubate these petridishes until the visible moisture present in media has been disappeared. The bacteria has been inoculated in these petridishes with the help of cotton swap. The filter paper disc moistened with test phosphate esters solution placed on the surface of media with the help of sterilized forceps very consciously. The plate are immediately placed in incubator at 37^0 C ± 2^0 C for about 24 hours. After the complete of incubation, the inhibitory effect are observed against the test organism by measuring the visible areas of inhibition of growth caused by the diffusion of test esters from the disc into it's surrounding medium. This method can yield a higher standard of reproducibility and it is of a reasonable degree of saccuracy. One advantage in this media is that many test esters can be investigated on a single plate against same bacterial colony. The size of zones varies with the molecular characterization of different compounds. The inhibition zone or efficiency of phosphate esters against the bacteria *E.coli*, *Salmonella typhi*, have been studied and results are shown in the given table.

	E.coli				*Salmonella.typhi*			
Phosphateesters	250ppm	500ppm	750ppm	1000ppm	250ppm	500ppm	750ppm	1000ppm
Monoester	+	+++	++++	++++	+	+++	++++	++++

Conclusion- The result shows that the antifungal and antibacterial activities increase with increasing the concentration of phosphate esters. Higher the concentration of esters increases the activity. Maximum growth inhibition was found at 750 ppm

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