I N T R O D U C T I O N

Man in his fight against insects to save himself, his animals and crops, has been used various substances, the insecticides. Plants at all stages of their growth, and later as harvested products in storage, are susceptible to attack by insects. All through the centuries and to days man has succeeded in developing chemicals whose insecticidal properties are phenomenally powerful. The use of insecticides in controlling agricultural pests has attracted all attentions since the end of the 2nd , world War. The discovery of benzene hexachloride (BHC) and the discovery of the insecticide action of dichloro-diphenyl - trichloro - ethane (DDT) that the plant protection work received a great impetus (Mukundan, 1964). Thus the effect of plant protection by using chemicals (insecticides, fungicides and herbicides) on the plant and soil micro-organisms attracted the attention to very important subjects. Early in the begining Appleman and Sears (1946), studied the effect of (DDT) on bacterial nodulation of legumes. When grown in sand, the hight of the legumes was diminished in all Jars containing (DDT) at a rate equivalent to 100 Ib/acre, while nodulation was inhibited in Jars receiving 1000 Ib/acre.

plants grown in cultivated soil did not develop symptoms of injury to the same degree as those grown in sand, but nodulation was adversely affected. Smith and Winzel, (1947) applied insecticides as antifungal and antibacterial agents and found that, after the application of (DDT, BHC) and chlordan, the number of bacteria, fungi, actinomycetes and protozoa were not affected by (DDT) applied at rates up to 400 Ib/acre under field, green house or laboratory conditions. Benzen-hexa-chloride (BHC) which containing 10-12 % of the γ -isomer, greatly reduced the nitrifying bacteria at rates of 100 and 500 Ib/acre with or without addition of cotton seed meal. On other hand chlordan was not as toxic, Benzen-hexa-chloride to those groups, and some recovery of nitrifies was noted within three months.

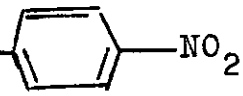
A chlorinated camphene stimulated the development of bacteria and fungi with or without the addition of cotton seed meal to the soil, which suggests that it was utilized as food by these organisms. Wilson and choudhri (1948) reported that Benzene-hexa-chloride-epsilon - isomer showed slightly toxic effect on algae and also to several micro-organisms such as Rhizoctonia solani, actinomycetes urea-decomposing bacteria and nocardia.

Simkover and Shenefell (1951) indicated that γ - Benzene-hexa-chloride (γ -BHC) greatly reduced damping off in conifer seedling caused by Rhizoctonia, while chlordan had no effect on this micro-organism. Sancheze (1954), studied the effects of chlordan, Toxaphene and Benzene-hexa-chloride (BHC) on soil ammonification and nitrification as well as nodule formation in two legumes. He found that chlordan stimulated ammonification and reduced nitrification especially at the highest doses, Benzene-hexa-chloride (BHC) effect was less pronounced and in the opposite direction, however Toxaphene promoted both ammonification and nitrification. Furthermore, both Toxaphene and (BHC) markedly reduced nodulation on roots of beans and Cratalaria, but the effect of chlordan was not marked in this respect.

Bollen et al. (1954) recorded that mold counts of several soil types were inhibited by the application of (BHC) and (DDT), while Toxaphene increased significantly molds and percentage of Penicillium in a Peat soil; chlordan showed no definite toxic effects. Eno and Everett (1958) indicated the influence of soil application of 10 chlorinated hydrocarbon insecticides on soil micro-organisms. Microbiological results after one month application of these

insecticides, at rates of 12.5,50 and 100 p.p.m, showed that they had no effect on the number of soil bacteria with the exception of Dieldrum which increased the number of fungi. After sixteen months application of the insecticides, no significant changes observed in fungal or bacterial numbers. Grossman and Steckhan (1960) studied the effect of insecticides lindane, chlordan and Aldrin in laboratory and green house experiments with respect to their effect on the development of Pythium sp., Ophiobolus graminis, Rhizoctonia solani and Rhizoctonia crocorum in vitro on the attack of beets, wheat, cauliflower and carrot respectively, by these fungi and on the growth of the host plants. They found that all the insecticides had a toxic effect on examined fungi. Baranowski (1962) indicated that some systemic insecticides, when applied topically, were effective in controlling a serpentine leaf miner (Liroimyza archboldi), the effect was more pronounced if these compounds were added to the irrigation water. Jones (1965) found that the black scurf (Rhizoctonia solani) in potatoes was affected by Aldrin.

Ruschel and Da Costa (1966) investigated the effect of Gesaral 33, Carunchol 50, Malagran, Phostoxin, Arasan 75 and Uspulun on nodulation of bean seed which

X is - F, - CN, or  (Adrain etal; 1947 and Mackworth and webb, 1948; Metcalf and March, 1949, and Toy 1948 and 1950). Examples of these groups of insecticides are parothion (0,0 diethyl O-P- nitropheyl phosphorothioate), Malothion (0,0- dimethyl - S 1,2 - bis carbethoxy-ethyl phosphorodithioote); Dipterex (0,0 - dimethyl - 2,2,2 - trichloro -1- hydroxyethyl phosphonate); Dimethoate (0,0 - Dimethyl S (N-methyl acetamide) phosphorodithioate); Leptophos (O- 4- bromo 2,5 dichlorophenyl) - O - methyl phenyl phosphonothioate) and cyolane (phospholan) (2- diethoxy - phosphinyl - imino) 1,3 - dithiolane) and many others.

The effect of four organo-phosphorus insecticides (Bayer 37289, Diazinon, Dursban and Zinophos) on soil micro-organisms have been studied by Tu (1970), concentrations of 10 and 100 p.p.m were applied to sand loam soil, and a toxic effect on bacteria and fungi was observed for the first and second week of incubation. Furthermore many workers studied the effect of insecticide Dipterex (O-O dimethyl - 2,2,2- trichloro-1- hydroxyethyl phosphonate,) on the growth and activity of soil micro-organisms as well as its degradation by bacteria and fungi (El-Zawahry, 1972; Adam 1973; Salama etal,

1973, 1974, 1975). The concentrations of insecticide up to 3×10^{-3}, 4×10^{-13} and 5×10^{-3} reflected a toxic effect on the growth of Rhizobium leguminosarum and Rhizobium trifolii and shifted the optimum temperature from 30 to 25 ^{o}C, and the optimum pH from 7 to 6.5 (El-Zawahry 1972 and salama et al. 1973). They found that the inhibition of nodulation in broad bean was evident when ammonium sulphate was used as fertilizers, while clover plants revealed equal nodulation under the various manurial treatments and no inhibition of nodulation was observed in presence of sodium nitrate (100 p.p.m), but was evident when ammonium sulphate (p.p.m) was applied. On other hand Adam (1973) studied the effect of Dipterex on rhizospheric fungi and found higher concentrations of insecticides were suppressive for growth, the lowest dose was generally initiative, but no complete inhibition of growth was observed with any of the concentrations used. The enzymatic degradation of ^{14}C -Labelled Dipterex showed that mono-methyl phosphete dimethyl phosphate, monomethyl. Dipterex and unknown substance were the catabolic products of Dipterex by the examined fungi and bacteria (El-Zawahry, 1972 ; Adam, 1973; and salama etal, 1975). The same authors observed the formation of $^{14}CO_2$ from bacterial and fungal cultures containing radioactive

Dipterex, and they suggested that some of the liberated methanol groups (during breakdown of Dipterex are oxidative by the micro-organisms.

Afifi and Abdulla (1977) studied the effect of insecticide thiolane on the spore germination potentialities of Artemisia vulgaris phyllospheric fungi. They found the percentage of spore germination of Alternaria alternata, Aspergillus niger and Penicillum frequentans increased in presence of the combination of thiolane and Artemisia emanations than in presence of each singly. The contrary was observed in case of Aspergillus flavus and Fusarium species The same authors added that spore germination of Helminthosporium sp. was inhibited completely under the influence of thiolane, emanations and combination of both.

Draughon and Ayres (1979, 1981) reported the inhibition and the stimulation effect by various insecticides on growth of some fungal species. They found significant inhibitions in growth of Aspergillus parasiticus cultures resulted when cultures were treated with dichlorvos (28.9% inhibition), Landrin (18.9%), Sevin (15.7%), and noled (100%). Stimulation in growth has been also observed in cultures of Aspergillus parasiticus

and Penicillium utricae treated with Diazinon. The same investigators suggested that the increase in mycelial weight or cellular mass could be response to the stress caused by Diazinon and on attempt to increase survival by increasing assimilatory materials.

Mahmoud et al. (1982) investigated pesticide sensitivity in the sporogenous yeasts Hanseniaspora gwllier-mondii and Hansenula anomala, together with the asporogenous yeast Rhodotorula rubra. They indicated that the akaricide Takel exerted the highest toxicity over both of the organophosphorus insecticide lebaycid and the carbamate fungicide Antrocol.

Several workers investigated the effect of pesticides on the growth and metabolic activity of microorganisms (Nagiub, 1968, Wu and Ayers 1974, Heikel 1976, Berisford and Ayres, 1976, Widstrom, 1976, Draughon and Ayers, 1978, 1979 Abd-El-Razak, 1979; Hassanien 1980, Rosas et al. 1980 and Draughan and Ayers 1981). Nagiub (1968) found that concentration of sevin insecticide up to 125 p.p.m reduced the rate of respiration and growth of Rhizoctonia solani coupled with low sugar and phosphorus absorption. Concurrent with such observations were low carbohydrate and protein

building accompanied by low organic phosphorus , keto acid and sulphydryl content. It has been indicated also that the production of mycotoxin by different fungal species significantly reduced by the addition of insecticides to the culture medium (Wu and Ayers, 1974, Draughan and Ayers, 1978, 1979, 1981). Draughon and Ayers (1981) found that moled completely inhibited production of aflotoxin B_1, B_2, G_1 and G_2 by Aspergillus parasiticus at a 100 p.p.m concentration. The insecticide dichlorvos, landrin, pyrethrum, sevin malotbion and Diazinon significantly inhibited production of aflatoxins at a 100 p.p.m concentration. They indicated also that the insecticides may selectively inhibit aflatoxin biosynthesis Furthermore, Rosas et al. (1980) studied the effect of insecticides on the fatty acid and phospholipid composition of Escherichia coli. They reported that parathion increased the concentration of all phospolipid species without changes in their polar head groups, (DDT) decreased the proportion of neutral serine-derived phosphatides and dieldrin decreased the proportion of negatively charged phospholipids. The saturated, unsaturated plus cyclopropane fatty acid ratio was increased in all cases. The changes suggested that cell adapted their membrane to compensate for the presence of insecticide in environment.

Heikal (1976) studied the effect of four herbicides (dimethyl phenyl urea derivatives) on the growth and metabolic activity of Fusarium oxysporum and found various effect of different herbicides on the respiration of tested fungus. The results showed also that increasing the concentration of four herbicides attenuated sucrose inversion and uptake. The same author reported that the four herbicides lowered both protein soluble and non - protein nitrogen of the mature mats.

Hassanein (1980) studied the effect of herbicides (gesagrad, linuron afalon and trifluralin) on the metabolism of certain rhizospheric fungi namely Alternaria humicola, Penicillium martensii and Sepedonium chrysospermum. She found that total lipids of Alternaria humicola were reduced by trifluralin at a rate of 500 p.p.m, and increased in the Sepedonium chrysospermum by herbicidal treatment. The total lipids of Penicillium martensii were not affected by any of the three tested herbicides. The treatment with herbicides showed qualitative and quantative changes in the amino acids of rhizospheric fungi, and high concentrations of herbicides caused a reduction or absence of organic acids from the mycelium of tested fungi. The same author reported that the sugars of Alternaria humicola and Penicillium martensii

were not affected by the herbicides, however sugars of Sepedonium chrysopermum especially sucrose increased by the herbicides .

Abd-El-Razak (1979) reported that tested fungi Cladosporium cladosporiodes and Cunninghamella echinulata were unable to utilize the fungicide cycloheximide as carbon and nitrogen source. The growth of two fungi was retarted and Cladosporium cladosporiodes appeared more sensitive than Cunninghamella echinulata to the effect of cycloheximide. He reported also that cycloheximide induced a reduction in the uptake of nitrate, sugars, amino acids by the growing fungal mycelia. Treatment with cycloheximide led to marked changes in the total amount and in the relative composition of the nitrogen and carbohydrate pools in the fungal mycelial. The same author (Abd-El-Razak, 1979) discussed the previous observations in relation to the rates of growth and the expected changes in respiration rates.

Cyolane or (Phospholan), (2- (diethoxyphosphinyl imino -) - 1,3 - dithiolane); is a systemic organophosphorus insecticide widely used for the control number of insects. The application of cyolane for the control of the cotton leaf worm Spodoptera littoralis was indicated by

several investigators. They reported that cyolane controls the different stages of Spodoptera littoralis Vermes, 1967, Kamel and Moustafa 1968, Zeid et al. 1968; quoted from lindley and Seval, 1973 -; Vassilaina Alexopoulou et al. 1970, Serghion, 1971; Khalil et al. 1972, and Shoeib et al. 1973. Furthermore cyolane has been successfully used for the control of a number of insects e.g. Thrips tabaci on anions (Damiano, 1967 a), the cotton melon aphid Aphis gossypii on citrus trees (Damiano 1967 b)' Plutella xylostella (Sanchez et al. 1969, house fly breeding Yew 1970, the tobacco looper Plusia argentifera (Cunningham, 1971), the stone leek Leaf miner Phytobra cepae on spring onions (Chang, 1972,) the bean - Fly Melanogromyza phaseoli (El-Kifl et al. 1973), the pea aphid Acyrothosiphon pisna on cotton (Marei et al. 1974 a,b) and the larva of Agrotis orthogonia Morrison in winter wheat Depow, 1975).

Recent study have been done in Egypt by several workers to investigate the effect of cyolane on biochemical constituents of some animals as well as the metabolism of cyolane by insects, animals, plants and micro-organisms (Abd-El-Messih, 1977, Wafa 1981, Zayed et al. 1981,

Mostafa et al. 1982 and Bahig et al. 1982). Mostafa et al. (1982) and Bahig et al. (1982) investigated the metabolism of cyolane in soil fungi Rhizoctonia solani, Penicillium notatum, Penicillium chrysogenum, Aspergillus flavus and Trichoderma viride as well as in soil bacteria Rhizobium leguminosarum and Rhizobium trifolii, by using ^{14}C- cyolane labelled in the imino - carb on position or in diethoxy position. At least 9 metabolites could be separated in addition to the parent compound . Mostafa et al. (1982) reported that about 18,34,29,14 and 23% of the applied dose of ^{14}C- diethoxy labelled cyolane was metabolized during incubation period with soil micro-organisms Rhizoctonia solani, Penicillium notatum, Aspergillus flavus, Rhizobium leguminosarum and Rhizo-bium trifollii respectively. On other hand Bahig et al. (1982) found that Penicillium chrysogenum was the most active fungns in the degradation of ^{14}C- cyolane labelled in the imino-carbon position, while Rhizoctonia solani was found to be the least active organism.

Aim of the Investigation

During last three decades organophosphorus compounds have attracted the attention towards their use as insecticides. From (1967) the organophosphorus systemic insecticide cyolane (Phospholan) (2- (diethoxy phosphinyl imino) - 1-3 - dithiolane) has been used in tremendous amounts in controlling cotton leaf worm (Spodoptera littoralis) and other insects. The insecticide get into the soil during the spray application to the arial parts of plants, thus excess solution may drip from the leaves to the soil surface. Moreover, the insecticide can get into the soil during other treatments. There is no doubt that the extensive use of these compounds year by year has a profound effect on plants as wall as in soil micro-organisms. Since the soil fertility depends on equilibrium of micro-organisms that inhabit the soil, the contamination of the soil by insecticides could show a serious effect in this equilibrium which will reflect in soil fertility.

The Objective of the present work was to investigate the effect of different concentrations of cyolane on the growth of some common or phytopathogenic soil fungi which isolated from Zagazig areas. The effect of varions incubation temperature, pH values and using different

nitrogen sources on the toxicity of cyolane were indicated. Moreover the possible influence of cyolane in protein carbohydrate and lipids accumulation in fungal mycelium were studied.

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of sterile distilled water in sterilized Erlenmayer conical flask (500 ml capacity). The flasks were shaken for 30 minutes. Serial dilution were made in sterilized containers using sterile distilled water. The roots of infected cotton seedlings were washed several times with suitable amount of sterile distilled water. The washings were collected in a sterile container shaken, diluted with sterile distilled water.

One ml from each diluted suspensions were transferred into sterilized petri-dishes (15 cm. diameter). Then, each of these dishes was supplied with 20 ml of sterile melted (at 45 ^{o}C) Czapek's agar (Dox) medium of the following composition:

Agar	15.00	gm.
Sucrose	30.00	gm.
$NaNO_3$	350	p.p.m as nitrogen
KH_2PO_4	1.00	gm.
Kcl	0.50	gm.
$FeSO_4 \cdot 7H_{20}$	0.01	gm.
$MgSO_4 \cdot 7H_{20}$	0.50	gm.
Distilled water to	1000.00	ml.

Five plates were used for each dilution. Then, after 4 to 7 days incubation period at 28-30 ^{o}C the

total fungal colonies per gram of soil were determined.

b) Direct inoculation method: (Warcup, 1950).

In this method small amount of soil samples after mixing in the sterile mortar or small pices of the infected cotton roots, were transfered to sterile petri - dishes, and a little sterile distilled water was added. Then melted Czapek's agar medium (at 45 °C) was poured into the dish (20 ml.) After solidification the plates were incubated at 29 ± 1 °C for 4-7 days and used for isolation of fungi.

c) Fungal identification:

Fungal colonies arising on the plates were microscopicaly examined to help in the indication of similar colonies. The most common occuring forms were isolated at the highest dilution on Dox's agar medium. The common isolated specimens were identified to species and genera levels by using the following guides:

- Gilman (1957), for soil fungi ingeneral.
- Raper and Thom (1949), for penicillium species and penicillium related genera.
- Barnett (1960), for the genera of Imperfect fungi.
- Raper and Fennel (1965), for Aspergillus species.

The average of total fungal colonies per gram of tested soil was 3×10^4. The isolated fungi from the control soils were also detected from the zones of healthy and infected plants with the exception of Rhizoctonia solani.

Rhizoctonia solani was isolated from samples of infected roots of cotton seedlings.

The common fungal isolates were identified as: Rhizoctonia solani, Aspergillus flavus Aspergillus niger , Aspergillus fumigatus Fusarium sp.

Soil Analysis:

The physical and chemical characters of the soil were examined according to the methods by Piper (1950).

a) **Soil texture:**

Studies of texture character of examined soil indicated the absence of gravel, while the percentage of coarse sand, fine sand, silt and clay were:- 00.7, 12.8, 20.9 and 65.6 % respectively.

b) Moisture content:

The moisture content of the soil was determined gravimetrically on oven dry basis and calculated as gram percentage . oven dry weight (Osman 1973). Five replicates were used in the present experiment. The result showed that the soil moisture and water hold capacity were 60.41 and 84.3 % respectively.

c) Total soluble salts:

Suspension of soil distilled water (1 : 10) was filtered. The filterate was washed with 50-100 ml. of distilled water and made up to 500 ml. with distilled water (Osman 1973). A hundred ml. sample was then concentrated over a water bath to about 5 ml., then dried in an oven at 105 °C. The weight of the total soluble salts in the original solution was calculated as gram percentage oven - dry soil (Russel 1956). The results indicated that the amount of the total soluble salts was 1.56%.

d) Organic carbon:

The organic carbon was determined using Walkely and Block's rapid titration method (Osman 1973). In an Erlenmeyer flask(250 ml capacity) 10 ml. of 1 N potassium dichromate solution were added to not more than

10 gram of the oven - dry soil followed by 20 ml of concentrated sulphuric acid. After shaking, the flask was left to stand for 30 minutes on asbestos. Then 20 ml. of concentrated O- phosphoric acid and 1 ml. of diphenyl amine indicator were added. Titration was carried out against 1 N. ferrous sulphate (Piper 1947). The results obtained that the organic carbon was 0.31 % of oven - dried soil.

e) pH value:

The pH value were determined using a Beckman meter with glass electrode. The result showed that the tested soil is moderate alkaline and its pH value was 8.4.

Applied Insecticide:

The tested insecticide in present work is a 25% emulsifiable concentrate formulation cyolane insecticide (2- (diethoxy - phosphinyl imino) - 1,3 - dithiolane) which was produced by American cyanamid company and applied for control cotton leaf worm Spodoptera littoralis at rates between 750 and 900 gram active ingredient per hectar. The rates of insecticide concentration (in p.p.m.) in the present investigation were determined as active ingredient (a.i).