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Study effects of plant extracts from *Conocarpus erectus* and *Mytrus communis* on the growth of some fungi isolated from different types of insects

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Abstract : This study was done to find the effects of two plants extracts *Monocarps erectus* and *Mytrus communis* against the growth of three types of fungi :*Aspergillusflavus* , *Alternaria alternata* and *Macrophomina phaseolina.* In comparison with three kinds of chemical antibiotics (cefuroxime axetil, fluconazole and metranazole) , the extraction was done by using two types of solvents acetone and ethyl acetate.The results showed a potential activity for both plants as antifungal agents. The inhibition zones of the *A. flavus* have showed 0.4, 0.2 and 0.1 cm with *C. erectus* , *M. communis* and *Fluconazole* respectively , both cefuroxime axetil and metranazole were not effective against *A. flavus*. While the inhibition zones of the *A. alternata* were 2.1 and 0.6 cm with *C. erectus* and *M. communis* respectively. All three types of chemical antibiotics were not effective against *A. alternata* , while for *M. phaseolina*, the inhibition zones were 3.2, 2.5 and 0.2 cm , with cefuroxime axetil, *M. communis* and *C. erectus* respectively, whereas both fluconazole and metranazole were not effective against *M. phaseolina*.
Keywords: *Conocarpus erectus*, *Mytrus communis*, insects, fungi.

Introduction

Insects are without a doubt the most dominant class and they have exceeded the number of other classes of the animal kingdom by far[1]. Insects are known for their important benefits include pollinating and provide important products such as honey and waxes, but it also can cause different disease and illness to humans, as well as has effects on the corps. The insects are known to carry one out six of the diseases in humans[2].

It is worth to mention that the mechanical transport of disease agent rely on the method of contact with host and feeding behavior[3]. Many insects can bear various bacteria and fungi that cause disease to humans, such as *Musca domestica L.*[4,5],*sarcophaga africa*[3,6], *calliphora vicina*[7,8], *Lucilia sericata*[9,10], *chrysomya albiceps*[11], monomorium ants and myriopoda .

Fungi have a great medical important and can lead to different diseases to humans, animals and plants. *Alternaria alternata* has been reported to produce variable toxins (alternariol, alternariol methyl ether and tenuazonic acid), also can occur different diseases such as Alternaria brown spot of tangerines, leaf spot in rough lemon and Alternaria black rot on citrus fruits[12], it also can cause esophageal cancer by producing MBzA (N-nitrosomethylbenzylamine)[13].

On the other hand ,*Macrophomina phaseolina* can cause a major losing in wild plants species more than 500 cultivated species by causing charcoal rot infects [14].

M. phaseolina is considered as the major disease with fungi in sunflower in Egypt [15]. *M. phaseolina* was found in the soil, plant seeds, and it can cause root inhibition by producing tuber or cushion shaped 1-8 diameter black sclerotia that serve as means of survival[16]. While *Aspergillusflavus* is known to construct alfatoxin , that can be responsible for big problems in agriculture[17], and can result in human invasive aspergillosis, as well as it can be responsible for the disease that attack corps such as peanuts, corn, mazie and wheat[18-19].

The inhibitory properties of plants extracts have been tested on some fungal activity, Al-Rahmah, *et al.*[20] ,He had used the extract of *Lantana camara, salvadora persica, Thymus vulgaris, Zingiber officinale* to suppress the growth of *pythiuma phanidermatum Rhizoctonia solani and Fusarium oxysporum*.

Amadi, *et al.*[21]Studied the effects of plant extracts (extracted with water, ethanol and acetone) from *psidium guajava* L. leaf and *Zingibar officinale* rhizome on the spore germination and sporulation of fungi that affect the stored melon seeds.

Tijjani, *et al.*[22] has studied the effects of plants extracts of (Neem seeds, moringa seed, garlic bulb and emulsified neem seed oil) on the growth of *A. flavus*, the results showed that garlic bulb and emulsified neem seed oil had the highest effects on the *A. flavus* growth.

Dawar, *et al.*[23] showed that *Datura alba* extracts had suppression action on the growth of *M. phaseolina* and *Rhizoctonia solani* completely.

The study of Regmi, *et al.*[24]showed that the extracts of all six plants extracts (*Jatropacurcas, Datura strumarium, Azadiracha rindica, Moringa oleifera, Calotropis gigantean* and *Morus alba) was able to inhibites the growth and sporation of A. alternata*.

This work is a simple effort to demonstrate new methods to suppress the growth of fungi to use the secondary metabolized materials (plants extracts).

Methods and materials

Insects' collection

The insects were collected from different places form Hilla province near the residential area, 7 types of insects were collected which include (housefly, flesh-fly, blue bottle fly, common green bottle fly, blow fly, ant and Myriapoda).

Fungi isolation

First of all, sample (left wing, right wing, insect body) were suspended in the 1 ml of distal water .Than From each sample (insect) take 0.5 ml and the spread on the following media :

- PDA Potato's Dextrose Agar
- SDA Sabroid Dextrose Agar

After that the sample have been kept in the incubator at temperature 25 c°, the colony were counted via (CFU) after 7 days, next the sample were purified and kept on PDA for further work[25], finally fungi identification was done according to[26]

Plants extracts preparation

Five grams of plant dry leaves have been socked in 25 ml of acetone, ethyl acetate in closed glass containers and kept in shaking incubator for proper time interval, then the solutions were filtrated under pressure , the resulted crude extract were concentrated by using the rotary evaporator to 5 ml, after that the plant extracts were but in the refrigerator[27].

Fungi inoculum

The method of Agar Well Diffusion was used[28] in which 20 ml of Emmons Sabour and Dextrose Agar (ESDA) in each dish with two replicates for each 11/ml) of plants extracts and control , the dishes were left To harden, 0.2 ml of the fungal inoculum were spread on each petridish with L shape class loop, after half hour, each dish was puncture by using cork piercer , and filled with plants extracts (0.2ml for each concentration), then the dishes were incubated in the incubator for 5-7 days at temperature (28-30^{o}C) , after that the inhibition zone was measured by ruler (two orthogonal Diameter).

Results and Discussion

At first we studied the frequency percentage % for fungi to determine the fungi abundance according to the following equation[29]:

$$frequency\ percentage\% = \frac{No.of\ single\ fungal\ isolates}{total\ No.of\ fungal\ isolates} \times 100 \qquad \ldots\ldots\ldots\ldots (1)$$

Table1: the frequency of each fungi in this study

Fungal Species	No. of fungal isolates		Frequency percentage %
	PDA media	SDA media	
A. flavus	17	5	34.37
A. alternata	20	11	48.43
M. phaseolina	8	3	17.18
Total No. of fungal isolates=64			

As showed in table 1 *A. alternate* was the most frequent fungal species followed by *A. flavus*, and the less frequent fungal species is *M..phaseolina*

For the extraction of the both plants we utilized two types of solvent (acetone) which is a non-polar solvent and (ethyl acetate) which is moderate polar solvent . The results showed that the *conocarpus erectus* extracts extracted by acetone (terpenoids) were effective against the fungi used in this study , while the extracted from the same plant leaves with ethyl acetate(phenols) had no effects on the study fungi ,whereas the extracts of *Myrtus communis* extracted with acetone (terpenoids) also were not effective against the fungi , while for the extracts from the same plant leaves extracted with ethyl acetate (phenols) were had activity against the fungi.These findings agree with the results of Mehrabani, *et al.*[30] which prove the effectiveness of phenolic extracts from *M. communis* .

The results showed that the extracts of these two plants used in this study were effective against the growth of *A. flavus* , the extracts of *C. erectus* have the most effective on the growth of *A. flavus* with inhibition zone radius 0.4cm (Fig.1), followed by the antibiotic fluconazole with inhibition zone radius 0.2 cm (Fig.2) , and the effects of the *M. communis* was the less effective with inhibition zone radius 0.1cm (Fig.3), and the results stated that the alternative plant extract of *C. erectus* was more effective than the regular antibiotic fluconazole, whereas both of antibiotics metranazole and cefuroxime axetil had no effect on the growth *A. flavus*.

The findings of our study were similar to that found by Yasir[31] which found that the extracts of *C. erectus* were highly effective against the growth of *Alternaria solani* and *Ulocladium botrytis*. The secondary metabolic compounds of C. erectus (tannin and phenolic compounds) could inhibit the growth of microorganisms and work as an antifungal when used with high concentration by coagulating the protoplasm of the microorganisms

The results of this study agree with findings of Mehrabani, *et al.*[30] which find the extracts from the leaves of *M. communis* effective against the growth of *M. canis , M. gypseum* and *T. mentagrophytes*, which state that leaves components are useful for antifungal uses, also that's can be due to the flavonoids find in the leaves of this plant.

Also, Nejad, *et al*.[32] found that the extracts of M. communis have active against the different types of fungi *Aspergillus*spp.

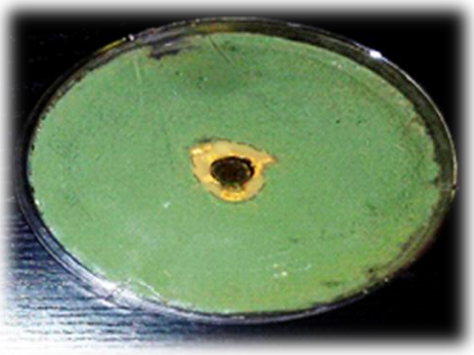

Figure 1: inhibition zone of *A. flavus* by *C.erectus* extract (extracted with acetone) , Inhibition zone Radius 0.4 cm

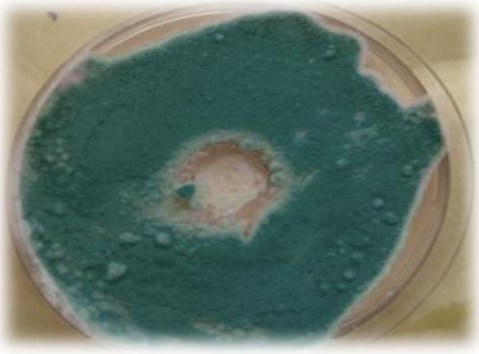

Figure 2: inhibition zone of *A. flavus* by fluconazole, Inhibition zone Radius 0.2 cm

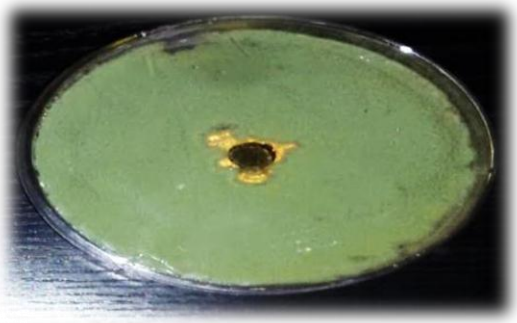

Figure 3: inhibition zone of *A. flavus* by *M. communis*extract (extract with ethyl acetate) with concentration 100mg/ml

The results showed that extracts of the *C. erectus* have highly effective in super pass the growth of the *A. alternata* with inhibition zone of 2.1cm (fig.4), while the extract of the *M. communis* were less effective against the growth of *A. alternata* with inhibition zone of 0.6cm (fig.5), all three of the antibiotic utilized in this current study had no effects on *A. alternata* , which state that the plant extracts used in this study act as antifungal agents better than the traditional medicinal antibiotic.

The findings of our study is similar to the result of Singh et al.[33] who studed the effects of six plants extracts against the growth of *A. alternata*, they find that the reduction in the growth of this fungi were the highest activity with *Oleander*, then *Parthenium*, *Ocimum*, *Lantana*, *Vernonia*and the least was with *Eucalyptus*, respectively.

The study of Bashir, et al.[34] found that alcoholic extracts of leaf, stem of C. erectus , fruit, were active against the three tested fungi (*Saccharomyces cerevisiae*, *Aspergillus niger* and *Penicillium. notatum*) with inhibition zones 14.3±0.58, 12.5±1.29 and 13.3± 0.58 mm respectively, which is similar to our findings, but with various type of fungi.

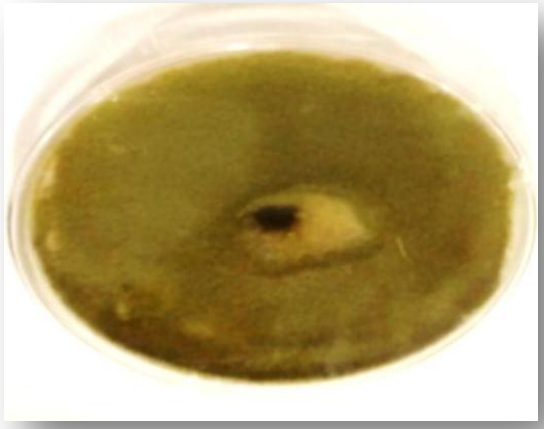

Figure 4: inhibition zone of *A. alternata* by *C. erectus* extract (extracted with acetone) , Inhibition zone Radius 2.1 cm

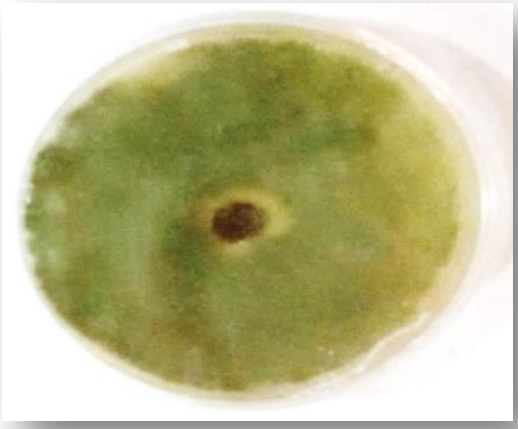

Figure 5: inhibition zone of *A. alternata* by *M. communis* extract (extract with ethyl acetate) , Inhibition zone Radius 0.6 cm

The results show that the antibiotic Cefuroxime was the most effective against the growth of *M. phaseolina* with inhibition zone of 3.2 cm (fig. 6), followed by the extracts of *M. communis* with inhibition zone radius 2.5 cm (fig.7) , and the least effect against this fungi was the extracts of C. erectus with inhibition zone radius 0.2 cm .

The results of our study is similar to the findings of Mert[35]which use the extracts of *M. communis* but against several types of bacteria , and they find that the different types of extracts (methanol, ethanol, ethyl acetate , and water extracts) of the *M. communis* were effective against these bacteria.

Also the study of Savaliya[36] found that garlic extracts was the most effective followed by onion extracts while ginger extracts was the least effective against the growth of *M. phaseolina* which was similar to the finding of our study .

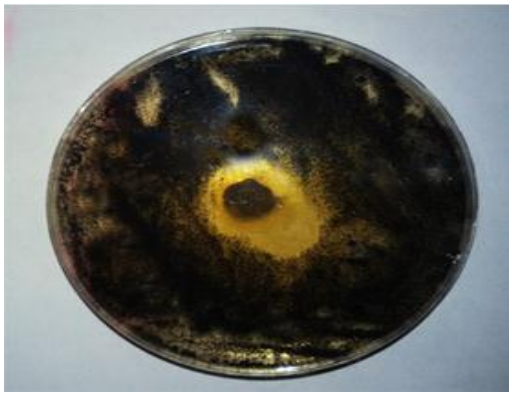

Figure 6: inhibition zone of *M. phaseolina* by cefuroxime , Inhibition zone Radius 3.2 cm

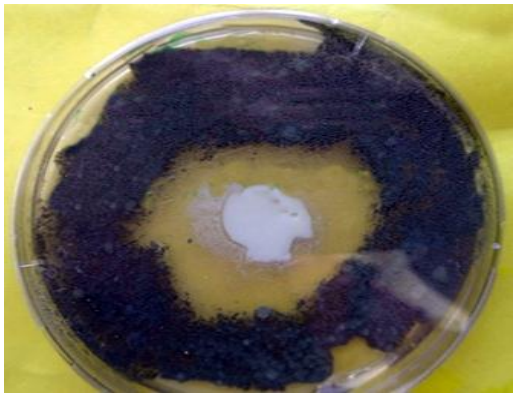

Figure 7: inhibition zone of *M. phaseolina* by *M. communis*extract (extracted with ethyl acetate) , Inhibition zone Radius 2.5 cm

Figure2: inhibition zone of *M. phaseolina* by *C. erectus* extract (extracted with acetone) , Inhibition zone Radius 0.2 cm

It's worth mention that for all types of plant extracts with three concentrations were used (60, 80, 100 mg/ml) respectively and the extracts were recorded effective only when used with concentration 100mg/ml.

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