Contribution on some medicinal plants and drugs as antifungal against fungal fish diseases

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Abstract

This study was conducted to investigate the antifungal effect of some Medicinal plants were garlic (raw, powder and oil) and clove (grain, powder and oil) and the drugs Nystatin, Clotrimazole and Fluconazole were used in this study. The tested fungi were (Saprolegnia spp., Cladosporium sp., Chrysosporium sp., Scopulariopsis brevicaulis, Aspergillus versicolor sp., Aspergillus ochraceus, Aspergillus niger, Aspergillus glaucus, Aspergillus wentii, Rhodotorulla sp., Penicillium sp., Syncephalastrum sp., Aspergillus tamarii, Aspergillus flavus, Trichoderma sp., Acremonium sp., Rhizopus sp., Fusarium sp., Phoma sp., Absidia sp., Aspergillus candidus, Alternaria sp., Aspergillus terreus, Aspergillus fumigatus and Paecilomyces sp.), which previously isolated from diseased fish. Raw Garlic was efficient against Absidia sp., while Garlic powder had positive effect against Aspergillus flavus, Aspergillus ochraceus, Fusarium sp., Rhizopus sp. and Absidia sp. Clove grain had positive effect against Fusarium sp., Rhizopus sp. and Absidia sp. on the other hand, clove powder affected in Fusarium sp., Rhizopus sp. and Absidia sp. Clotrimazole, Nystatin and Fluconazole, had positive effect against all tested fungi.

Key word: antifungal, fungal fish diseases, medicinal plant, Garlic, Clove, Clotrimazole, Nystatin and Fluconazole.

Introduction

It is argued that fish when available, contain protein levels of 17-20% as well as minerals and vitamins. Moreover, aquaculture integrated into the existing farming system has been shown to improve both food and income security with little or no external input (Wetengere, 2010). Increase in aquaculture has been accompanied by
outbreaks of disease from an ever-increasing range of pathogens. Fungal infections are mainly secondary to environmental stressors exist in water environment and may result in economic losses to farming projects due to increased mortality, poor weight gain, besides low market value (Rajinikanth et al., 2010).

The different species of molds (Saprolegnia spp., Aspergillus spp., Penicillium sp., Mucor sp., Rhizopus sp., and Cladosporium sp.) which isolated from different localities in Egypt caused tail and fin rot syndrome and skin lesions in tilapia species and Nile catfish (Marzouk et al., 1990). Saprolegniasis was a worldwide serious mycotic winter fresh water disease often affects wild and cultured fishes (Osman et al., 2010). Hyphae of Saprolegnia sp. may invade deep tissues of fish and penetrate the vital organs even the central nervous system (Zaki et al., 2003). Saprolegnia delica and Dictyuchus carpophorus (the greatest fungal populations) were the most dominant isolated zoosporic fungal species where they were highly occurred especially at the hyper-polluted waters with the heavy metals (Ali, 2007).

(RefaI et al, 2010) Isolated moulds in Oreochromis species belonged to the following genera: Saprolegnia, Aspergillus, Fusarium, Mucor, Penicillium, Rhizopus, Scopulariopsis, Paeciliomyces and Curvularia.

The change in environmental temperature increases the rate of transmission of fungal disease between fish (Hunter et al., 1980). The effect of elevated ammonia and carbon dioxide levels combined with low dissolved oxygen concentration. They proved that increase in the mortality rate of Channel cat fish in an experimental infection with fungi under the above mentioned condition (Walters and Plumb, 1980). Overcrowding and bad handling result in significantly higher mortalities due to the flourishing fungal infection (Hosmer, 1980).

The medicinal plants are currently used in commercial aquaculture as growth promoting substances, nutrients and antimicrobial agents for prevention and control of fish diseases (Galina et al., 2009). Garlic (Allium sativum) had been agreed upon as an antibiotic for fungal associated diseases (Ghahfarokhirt et al., 2006 and Shalaby et al., 2006). Clove oil possesses antibacterial, antifungal and antiviral properties (Chaieb et al., 2007). Clove is considered an antifungal herb (Steven, 2010).
Nystatin was discovered as antifungal drug since that time the drug was used for treatment of yeast infections specially candidiasis (Brown and Hazen, 1949). Clotrimazole, a tritylimiazole derivative is a new drug with a wide spectrum of antifungal activity against dermatophytes, candida and other yeast species, Aspergilli, and many of the organisms responsible for subcutaneous and systemic mycotic infections and some Gram-positive bacteria (Clayton and Connor, 1973).

This study concerned with the control or prevention of fungal fish diseases using some drugs and medicinal plants in vitro.

Material and methods

Fungi;
(Aspergillus ochraceus, Aspergillus niger, Aspergillus flavus, Rhizopus sp., Fusarium sp. and Absidia sp.,) previously isolated from clinically diseased O. niloticus in the Fish Disease Department CLAR were used in this study.

Media;
Czapek’s Yeast Extract Agar (CYA), Malt Extract Agar (MEA), Oatmeal Agar (OA), Potato Dextrose Agar (PDA) and Sabouraud’s Dextrose Agar (SDA) were used for fungus growth and storage and Czapek’s Extract Agar medium for sensitivity test of tested fungi to medicinal plants and synthetic drugs.

Plants and drugs;
Medicinal plants which used in this study were garlic (Allium sativum) in three forms (raw (slices), powder (dissolved in distilled water) and oil (dissolved in Ethanol)) and clove (Syzygium aromaticum) also in three forms (grain (used as it is), powder dissolved in water and oil dissolved in Ethanol) which collected from the market. Ethanol was used as control. Antifungal drugs Nystatin 100.000 units/ml (Mycostatin, Squibb, Egypt), Clotrimazole 10 mg/ml, (Dermatin, Pharco Pharmaceuticals, Alexandria) and Fluconazole 150 mg/capsule (Fungican, Pfizer, Egypt) (Table 1) also were examined its antifungal effect against tested fungi.
Table (1) Concentration and symbol of the tested antimycotic drugs discs for testing the antimycotic susceptibility of the tested fungi.

<table>
<thead>
<tr>
<th>Disc</th>
<th>Diffusible amount of antibiotic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nystatin</td>
<td>100 I.U.</td>
</tr>
<tr>
<td>Clotrimazole</td>
<td>50 µg</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>50 µg</td>
</tr>
</tbody>
</table>

Preparation method of antifungal drugs

Fluconazole a fresh solution was prepared for antifungal examination by dissolving the substance in Dimethyl formamide (DMF). Nystatin and Clotrimazole dissolved in distilled water. 1 mg of antifungal of Clotrimazole was dissolved in 1 ml of distilled water. 2000 I.U. of Nystatin dissolved in 1 ml distilled water. 1 ml of Fluconazole dissolved in 50 ml dimethyl formmamide. dimethyl formamide was used as control.

Testing the antifungal susceptibility by disc diffusion technique

In this well-known procedure, agar plates are inoculated with standardized inoculums of the test microorganism \((0.4-5) \times 10^4\) cfu/ml (Heatley, 1944). Then, filter paper discs (about 6 mm in diameter) containing the tested medicinal plants and synthetic drugs at a desired concentration, are placed on the agar surface using disk diffusion method. The Petri dishes are incubated at \(30 \pm 2^\circ C\) for three days. Generally, antimicrobial agent diffuses into the agar and inhibits germination and growth of the test microorganism and then the diameters of inhibition growth zones were measured (mm).

Results and Discussion

1- Garlic (Allium sativum):

The results from table (2) showed the inhibition zones of garlic in three forms (raw, powder and oil) against tested fungi (photo, 2, 3 and 4). Raw garlic had antifungal effect only against Absidia sp and gave inhibition zone (20 mm). Garlic powder had antifungal effect against Aspergillus flavus, Aspergillus ochraceus, Fusarium sp., Rhizopus sp. and Absidia sp.. The highest effect of garlic powder was against Absidia sp. (30 mm) inhibition zone in diameter. Garlic powder gave the same diameters of inhibition zones with the other tested fungi Aspergillus flavus, Fusarium sp., Rhizopus sp. and Aspergillus Ochraceus (10 mm) and had no effect against As. niger. Garlic oil had
no effect against the all tested fungi. This result may match with results of other researchers used garlic extracts by adding it to grass of fishes but by improvement of immune system of fish to resist fungal infection. Some approaches suggested that garlic extract exerts its effect by the oxidation of thiol groups present in the essential proteins, causing inactivation of enzymes and subsequent microbial growth inhibition (Bokaeian et al., 2010, Belguith et al., 2010 and Elias and Abd El Ghany, 2008).

2- Clove

Clove in grain form had positive effect as antifungal against Absidia sp., Fusarium sp. and Rhizopus sp. The highest effect was against Fusarium sp. and Rhizopus sp. giving the same inhibition zone (30 mm) in diameter, followed by Absidia sp. (25 mm). Clove grains had no effect on the other tested fungi. Also clove powder had positive effect against Absidia sp., Fusarium sp. and Rhizopus sp. It gave high inhibition growth of Absidia sp. forming inhibition zone (35 mm) in diameter, and the same inhibition zone in diameter (30 mm) with Fusarium sp. and Rhizopus sp.. Clove oil had no effect against the all tested fungi. Also This result may match with results of other researchers used clove oil which used as a bath for short time for one hour with certain doses that was recorded by (Abd El-Ghany et al., 2009) by adding it to grass of fishes but by improvement of immune system of fish to resist fungal infection where clove contain Eugenol compound which have antifungal effect. This result matches with (Velisek et al., 2006) who recorded that Eugenol can be used as an antimycotic agent in warm water fish aquaculture. Also in case of treatment with clove oil, no clinical signs or mortalities were detected, and (Sulieman et al., 2007) found also that, the highest inhibitory effect of clove bud oil was found against Aspergillus niger.

3- Nystatin

From table (2) Nystatin had positive effect against all tested fungi by inhibiting the fungal growth. Nystatin gave the highest inhibition zone with Absidia sp. 17 mm in diameter, the Rhizopus ap. and Fusarium sp.15 mm of each. Nystatin had the same antifungal effect against Aspergillus flavus, Aspergillus ochraceus and Aspergillus niger (10 mm) in diameter.
4- **Clotrimazole**

Clotrimazole was highly effective against the tested fungi by inhibiting its growth giving inhibition zones ranged from 10 to 50 mm in diameters. The highest inhibition zone was 50 mm in diameter against *Aspergillus ochraceus*, after that 40 mm against *Absidia* sp.. Nystatine had inhibition zones 25, 15, 10 and 10 mm in diameters against *Aspergillus flavus*, *Aspergillus niger*, *Fusarium* sp and *Rhizopus* sp. respectively.

5- **Fluconazole**

Data from table (2) showed the positive effect of Fluconazole against all tested fungi. Fluconazole gave the highest inhibition zone 40 mm in diameter against *Fusarium* sp and *Rhizopus* sp. Fluconazole gave inhibition zones 20, 20, 19 and 20 mm in diameters against *Aspergillus flavus*, *Aspergillus ochraceus*, *Aspergillus niger* and *Absidia* sp. respectively.

Measurements of antifungal drugs included the curing effect; the size of inhibition zone about a disc saturated by the antifungal or well contains antifungal, failure of the fungus to grow in liquid or solid media or even in vivo experiments using mouse or golden hamster, as (Emmons *et al.*, 1979) recorded.

Our findings matched with that reported by (Kassem, 1982) who concerning molds, *Aspergillus flavus* was highly sensitive to Econazole followed by Clotrimazole, Trioconazole, Ketoconazole, Miconazole, Nystatin while amphotericin B had the lowest activity. As for *Fusarium* sp. Fluconazole showed highest inhibition zone diameter (40 mm) followed by Nystatin (15 mm) and Clotrimazole (10 mm). these results matches with that reported by (Kassem, 1982) who indicated that Econazole had the highest activity, followed by Miconazole, Ketoconazole, Triconazole, amphotericin B, Nystatin and Clotrimazole while the fungus was resistant to 5-Fc.

Generally the interpretation of results of susceptibility testing of antifungal drugs *in vitro* may present problems, one factor is that the development of standaralized procedures is still in early stage and at present each institution carrying out such tests uses its own preferred methods so that the comparisons of results is difficult. Another factor is the difference of each strain in its susceptibility to antifungal drugs.
Table (2) Antimycotic sensitivity test of saprophytic molds to synthetic drugs and medicinal plants.

<table>
<thead>
<tr>
<th>Tested Molds</th>
<th>Raw garlic</th>
<th>Garlic powder</th>
<th>Oil+ethanol</th>
<th>Ethanol</th>
<th>Grain clove</th>
<th>Clove powder</th>
<th>Oil+ethanol</th>
<th>Ethanol</th>
<th>Nystatin</th>
<th>Clotrimazole</th>
<th>Flu+DMF</th>
<th>DMF</th>
</tr>
</thead>
<tbody>
<tr>
<td>As. flavus</td>
<td>-</td>
<td>10</td>
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<tr>
<td>As. ochraceus</td>
<td>-</td>
<td>10</td>
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<tr>
<td>As. niger</td>
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<tr>
<td>Fusarium sp.</td>
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<td>10</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>30</td>
<td>30</td>
<td>-</td>
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<td>-</td>
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</tr>
<tr>
<td>Rhizopus sp.</td>
<td>-</td>
<td>10</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>30</td>
<td>30</td>
<td>-</td>
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<td>-</td>
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<td>-</td>
</tr>
<tr>
<td>Absidia sp.</td>
<td>20</td>
<td>30</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>25</td>
<td>35</td>
<td>-</td>
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</tr>
</tbody>
</table>
Fig. (1) Clotrimazole effect on *A. flavus*

Fig. (2) Fluconazole effect on *A. flavus*

Fig. (3) Nystatin effect on *A. flavus*

Fig. (4) Garlic powder effect on *A. flavus*

Fig. (5) Clotrimazole effect on *A. ochraceus*

Fig. (6) Fluconazole effect on *A. ochraceus*
**Fig. (7)** Garlic powder effect on *A. ochraceus*

**Fig. (8)** Negative effect of raw garlic, Garlic Oil, clove grain, clove powder and Clove oil on *A. ochraceus*

**Fig. (9)** Nystatin effect on *A. niger*

**Fig. (10)** Fluconazole effect on *Fusarium* sp.

**Fig. (11)** Clove grain effect on *Fusarium* sp.

**Fig. (12)** Clotrimazole effect on *Fusarium* sp.
Contribution on some medicinal plants and drugs as antifungal against fungal fish diseases

**Fig. (13)** Clotrimazole and garlic powder have the same effect on *Fusarium* sp.

**Fig. (14)** Fluconazole effect on *Rhizopus* sp.

**Fig. (15)** Nystatin effect on *Rhizopus* sp.

**Fig. (16)** Garlic powder effect on *Rhizopus* sp.

**Fig. (17)** Clotrimazole effect on *Absidia* sp.

**Fig. (18)** Clove powder effect on *Absidia* sp.
REFERENCE


الفطريات المرتبطة بالبلطي النيلي المجمع من مزارع سمك العباسة وحساسيتها تجاه النباتات والأدوية المضادة الفطرية

سمية محمد محمود عوض، محسن السيد إبراهيم، أمنى عبد المنعم الأعصر

1- المعمل المركزى لبحوث الثروة السمكية بالعباسة - شرقيه - مركز البحوث الزراعية - مصر
2- كلية العلوم - جامعة بورسعيد - مصر

الم länger العربي

تربية الأحياء المائية لا تزال واحدة من أسرع قطاعات إنتاج الأغذية نمواً، ومن المقرر أن تلعب دوراً رئيسياً في تلبية الطلب المتزايد على المنتجات السمكية التي تعتبر مصدر آخر للبروتين، فالبلطي النيلي من أسماء المياه العذبة الأكثر اقتصاداً في مزارع العباسة، وواحد من أهم مزارع الأسماك في مصر.

1) تم فحص 340 سمكة من أسماك البلطي النيلي بأوزان مختلفة تم تجميعها من ثلاث مزارع مختلفة تم تجميعها من ثلاث أشهر مختلفة لأسماك البلطي النيلي بالعباسة أثناء الفترة من أكتوبر 2014 إلى يوليو 2015. وقد خضعت هذه الأسماك لفحص الأكليينيكي والفطرى والهستوباثولوجي.

2) الفطرات المعزولة من الأسماك المختلفة أُثبتت على الأنواع التالية:
- سبروليجنيا والكلادوسبوريم والكريسوسبوريم والاسكوبوليوربويس بريفيكالس والأسرابجلس فيرسي كرجروبا والأسرابجلس اوكراشيس والأسرابجلس فيوميجاتس
- الأسبرجلس فلافي والأسرابجلس جلاكس والأسرابجلس كندايس والأسرابجلس كندايس الألتوناريا والأسرابجلس كندايس التيريس
- الرودوتورولا والبيسيليم والسينسيفاليسترم والأسرابجلس فلافي واسكربيولاريوبس بريفيكالس واسكوبوليوربويس بريفيكالس واسكربيولاريوبس بريفيكالس
- الباسيلوميس فارينوسز

3) أوضحت هذه الدراسة أن معدل الاصابة بالفطريات في الأسماك التي تم فحصها كان (4.2% متوسط) بين أسماك البلطي النيلي.

4) أوضحت الدراسة أن معدل الاصابة بالفطريات طوال العام كان (18.3%) و(21.2%) و(10.5%) و(9.1%) و(9.9%) للبيسيليم وال Bersapex الأسرابجلس فلافي والأسرابجلس تاميراي والروتوترولا والأسرابجلس فيرسي كرجروبا واسكربيولاريوبس بريفيكالس

5) بالنسبة لمناخ السنة المختلفة فقد أوضحت النتائج أن أعلى معدل للإصابة خلال فصل الصيف بمعدل (46.6%)، بينما أقل معدل للإصابة كان (4.5%) في فصل الربيع.
تمت المعالجة أو اختبار حساسية تلك الفطريات عملياً تجاه بعض من النباتات مثل الثوم والقرنفل في صورهم المختلفة من زيت أو حب أو مسحوق مجفف حيث كان الثوم فعال في حال الأبسيديا فقط وفي حالتين الثوم البودر كان فعال في حالة الآسبراجيلس فلافيس والأبسيديا أوكرشيس والفيوزاريوم والريزوبيس والأبسيديا بينما زيت الثوم لا يؤثر على كل الفطريات المختبرة. ولكن القرنفل الحب كان فعال في حالة الفيوزاريوم والريزوبيس والأبسيديا كذلك القرنفل البودر كان فعال في حالة الفيوزاريوم والريزوبيس والأبسيديا ولكن زيت القرنفل أعطى نتيجة سلبية في كل الفطريات المختبرة. وعند استخدام بعض العقاقير الطبية ذات المواد الفعالة ضد النمو الفطري مثل الكلوترايمزول والنيستاتين والفلوكونازول كان فعال تجاه كل الفطريات. وكان تأثيرات تلك النباتات وكذلك العقاقير على النمو الفطري ملحوظ على الفطريات المختبرة مكوناً منطقة شفافة أو عديمة اللون تعني منع نمو الفطر بها حيث كانت أكبر فاعليه للكلوترايمزول على الآسبراجيلس أوكرشيس. وكانت أكبر فاعليه للفلوكونازول على الفيوزاريوم والريزوبيس بنفس القدر، أما النباتات فكان تأثيره الأكبر على الأبسيديا.