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The Beneficial Effects of Using Some Natural Products for Prevention and Treatment of Saprolegnniosis in Grass Carp Eggs and Fingerlings

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ABSTRACT

Saprolegniosis is a major concern in freshwater aquaculture, particularly hatcheries since it causes high mortality and economic losses. Various chemical compounds are used to treat the disease, but they are not recommended due to their hazard effects on fish, humans, and ecosystems. The current study assessed the potential anti-oomycetes activity of crude and aqueous extracts of clove (*Syzygium aromaticum*) and thyme (*Thymus vulgaris*) against *Saprolegnia australis* (*S. australis*) and *Achylia bisexualis* (*A. bisexualis*) *in vitro* and *in vivo*. Both herb aqueous stock solutions were eventually prepared using the decoction method. The minimal inhibitory concentrations (MICs) of both herbs' aqueous extracts were determined using the agar plate diffusion method. The ability of both herb aqueous extracts to kill the selected water molds was tested at various time points of exposure. Based on *in vitro* results, two *in vivo* field trials were planned to assess the efficacy of crude clove and thyme, first on the fecundity and hatchability of fertilized grass carp eggs. The second trial was designed to assess the antifungal potential against fungal infection during artificial egg incubation. Additionally, two experiments were planned to determine the toxicity parameters of each crude herb in grass carp fingerlings. The second experiment assessed the anti-saprolegniosis activity of selected crude herbs in artificially infected fingerlings. The results showed that *S. australis* and *A. bisexualis* were significantly inhibited at a 10% concentration in clove (0.8g/ 100mL) and thyme (3.2g/ 100mL) aqueous solutions. However, both herb aqueous extracts had fungicidal properties and killed both fungi at a 50% concentration of their stock solutions (4 and 16g/ 100mL of clove and thyme, respectively). Incubated eggs exposed to concentrations of 2 and 8g/ L clove and thyme, respectively, exhibited high fecundity and hatchability rates. Furthermore, clove and thyme at concentrations of 2 and 4g/ L could protect incubated eggs and fry from attack by *S. australis* and *A. bisexualis*, respectively. Clove and thyme at concentrations of 0.2– 0.25 and 0.5– 0.8g/ L provided a safe indefinite bath, but at a concentration of 0.5 and 1g/ L caused anesthesia after 24 hours of exposure. To successfully treat the infected *Ctenopharyngodon idella* (*C. idella*) fingerlings, the most effective doses were 0.25-0.5g/ L for clove and 0.5g/ L for thyme. Finally, clove and thyme in both crude and aqueous forms could introduce safe, low-cost, environmentally friendly, and easy applications into freshwater hatcheries.

INTRODUCTION

In recent years, aquaculture has become increasingly as a fast-growing food sector worldwide. It could supply high nutritional value protein at a low cost; as a result, it plays

an important role in supplementing the global population **(OECD-FAO, 2018)**. As a matter of fact, the starting point of all aquacultures' rearing systems is fish hatcheries **(FAO, 2018)**. They are considered a cornerstone for fish seed production and enhancement of their survival through protective propagation **(O'Reilly & Doyle, 2007)**. Several fish species could be artificially propagated either in fresh or marine hatcheries. One of the most important species propagated in the Egyptian fish hatcheries is grass carp (*Ctenopharyngodon idella, C. idella*) **(Mur, 2014)**. *C. idella* is an essential component of fish farming, stocking both public and private fish farms and acting as a significant source of protein for human consumption **(Saleh, 2007; Chen *et al*., 2012; FAO, 2012)**. Furthermore, fish act as a biological control of aquatic vegetation, as adult individuals could consume large quantities of aquatic macrophytes daily rather than other carp species **(Zweerde, 1990; Bozkurt *et al*., 2017)**. To obtain promising propagation, numerous activities were performed periodically and/or seasonally in the Egyptian fish hatcheries, including the rearing of brood stocks, egg collection, egg incubation, and transmission of fry. These practical approaches act as stressors and may result in immune suppression, as well as increasing the possibility of disease incidence **(Becker *et al*., 2015; Skrzynska *et al*., 2018)**. Although there are variable pathogens causing infection in fish species, mycotic ones dramatically threaten hatchery productivity, particularly in the genus *Saprolegnia* (family Saprolegniaceae). Saprolegniosis is a common term for mycotic affection that is caused by members of the genus *Saprolegnia*, of which *Saprolegnia* species are the most incriminated etiological agents **(Ibrahim *et al*., 2022)**. In the genus, *Achlya* sp. could take part in the disease occurrence with no clinically significant differences **(Pickering & Willoughby, 1982; Noga, 2000; Fregeneda-Grandes *et al*., 2007; Hussein & Hatai, 2011)**. The disease causes severe economic losses and serves as a crisis when it affects eggs and fry patches in freshwater hatcheries due to the total loss of the seed crop yield **(Hussein & Hatai, 2002)**. For that reason, plenty of chemicals have been utilized inside hatcheries as routine disinfectants, such as formalin, malachite green, quadrated ammonium, and potassium permanganate, to tackle this issue **(Fitzpatrick *et al*., 1995; Kitancharoen *et al*., 1997; Zaki *et al*., 2008; Fuangsawat *et al*., 2011)**. Due to their high cost, carcinogenic and mutagenic effects, residues inside fish body and effects on ecosystem, these chemicals were limited or banned in some countries. As a result, a great interest in the replacement of chemical drugs with natural alternatives that are effective, available, easily applied, and act in harmony with nature and humans is required **(Akinpelu & Onakoya, 2006; Mousavi *et al*., 2009a)**. Natural plants and herbs harbor chemical compositions that have potential biological benefits. Apart from the long herbal list, clove (*Syzygium aromaticum*) and thyme (*Thymus vulgaris*) came as old as natural herbs used in traditional medicine. Clove belonging to the family Myrtaceae showed antioxidant, antibacterial, and antifungal activities **(Radünz *et al*., 2018; Ginting *et al*., 2021)**. However, few studies have been reported in the literatures as antimycotic agent against aquatic fungi **(Schmidt *et al*., 2007)**. Similarly, thyme is an aromatic plant that belongs to the family Lamiaceae and has strong antifungal **(Moghtader, 2012)**, antioxidant **(Sönmez *et al*., 2015; Ahmed *et al*., 2022)**, and antibacterial activities **(Kuebutornye & Abarike, 2020)**.

As saprolegniosis is the main problematic issue in freshwater hatcheries and cannot be controlled without using therapeutics, alternative therapy of natural origin comes as a solution. In recent years, some research reports indicated that essential oils of clove and

thyme have inhibitory activities against *Saprolegnia* spp. **(Mousavi *et al*., 2009b; Gormez & Diler, 2014; Hoskonen *et al*., 2015; Shah *et al*., 2021)**; however, infrequent studies demonstrated the inhibitory effect of crude clove and thyme or their aqueous extracts.

The current study was designed to evaluate the *in-vitro* antimycotic activities of clove and thyme aqueous extracts against selected water molds, *Saprolegnia australis* (*S. australis*) and *Achylia bisexualis* (*A. bisexualis*). A field application trial was also arranged in the Abo-Saleh fish hatchery, Beni-Suef, Egypt, to investigate the efficacy of crude clove and thyme applications as antifungal flushing agents during the incubation of *C. idella* eggs, with an emphasis on their role in the fecundity and hatchability rates. Additionally, an experiment was conducted to evaluate the therapeutic activities of the selected crude herbs in *C. idella* fingerlings artificially infected with the selected water molds.

MATERIALS AND METHODS

1. Ethical committee

All the experiments were approved by the BSU-IACUC (Beni-Suef Institutional Animal Care and Use Committee, number 022-288) at the Faculty of Veterinary Medicine, Beni-Suef University, Egypt. Moreover, all methods were performed in accordance with the relevant guidelines and regulations. The study was reported in accordance with ARRIVE guidelines.

2. Natural herbs

Clove and thyme buds were purchased from Sigma-Aldrich (Chemie GmbH, Steinheim, Germany). Experimental research and field studies on plants (either cultivated or wild), including the collection of plant material, complied with relevant institutional, national, and international guidelines and legislation.

3. Fungal strains

Two members belonging to the genus *Saprolegneacae* were selected for assessment in the presented work based on their pathogenicity and disease outbreak incidence. The selected molds were *S. australis* and *A. bisexualis*, which had a recent significant outbreak history and caused economic losses at the Abo-Saleh fish hatchery in Beni-Suef, Egypt, in 2019. Both fungal species were isolated morphologically and molecularly identified, then submitted to GenBank under accession numbers of MT075633 and MT075632, respectively. Both selected molds were preserved in hemp seed culture according to the methodology described earlier **(Johnson, 1956; Hatai & Egusa, 1979; Fadaeifard *et al*., 2011)** and kept in the Fish Diseases and Management Department, Beni-Suef University, Egypt. Prior to using the fungi, both molds were sub-cultured on sterilized glucose yeast agar (GYA) and incubated for 24- 48 hours at 20 and 25^{o}C for *S. australis* and *A. bisexualis*, respectively **(Hussein & Hatai, 2002)**.

4. Experimental design

4.1. In vitro antimycotic activities of selected herbs aqueous extracts

The antimycotic activities of clove and thyme were investigated against *S. australis* and *A. bisexualis* fungal strains. Stock aqueous solutions of both herbs were prepared following the decoction methodology of **Al-Saidy *et al*. (2014)** and **Soltani *et al*. (2020)**, with few modifications. Briefly, 8 and 32g of freshly pulverized crude clove and thyme, respectively, were separately soaked in 100ml of sterilized distillated warm water (50^{o} C, SDW). Then, the socked herb bottles were left in a dark place overnight at room temperature (20^{o}C) to obtain the stock aqueous solution at concentrations of 80 and 320mg/ ml of both herbs, respectively. After elapsing extraction time, both crude herbs aqueous extracts were purified through series of sieving using gauze (100µ, diameter), sterilized filter papers (Double rings® 102) and syringe filters (0.45µl, Adev®, Japan). Then, the obtained stock solutions were collected separately in dark sealed bottles and subjected freshly for antimycotic assays **(Soltani *et al*., 2020)**. At all tested levels, the prepared stock solutions are treated as original solutions (100%).

The fungistatic activity assays of aqueous solution of the herbs against both tested fungal strains were performed through the determination of their minimum inhibitory concentrations (MICs) **(Aboubakr *et al*., 2016; Gedikoğlu *et al*., 2019)**. Initially, the freshly prepared original stock solutions (100%) of both herbs were double fold diluted with SDW to obtain a serial concentration of 100, 50, 25, 12.5, 6.3, 3.2, 1.6, 0.8, and 0.4% of the original one **(Shin *et al*., 2017)**. Then, 1mL of each double-fold dilution (for each herb's aqueous solution) was incorporated with 9mL of molten GYA plates with a continuous shaking on a flat surface to ensure proper mixing **(Stueland *et al*., 2005b)**. The prepared plates were centrally inoculated with each fungal strain's circular agar blocks that are 0.7cm in diameter using a sterilized cork-borer and kept at a temperature of 20 and 25°C for *S. australis* and *A. bisexualis*, respectively **(Hu *et al*., 2013)**. Triplicate plate sets were prepared for each tested dilution of both aqueous herb extracts. Control plate sets were also included, containing sterilized distilled water (SDW) instead of aqueous herb extracts in each plate patch. The mycelial growth of each fungal strain was measured after 24, 48, 72, 96, and 120 hours. With a vernier caliper along two radii at right angles to each other, and then the MICs were expressed as the calculated arithmetic mean **(Hussein *et al*., 2000; Emara *et al*., 2020)**.

Concerning the fungicidal activity evaluation of the selected herbs aqueous solutions against selected fungal strains, the same aforementioned dilutions used in the determination of MICs were employed in the assays with different approaches **(Tampieri, *et al*., 2003; Gormez & Diler, 2014)**. Briefly, using standard cork-borer agar blocks (0.7cm diameter) from peripheral highly growing mycelia of each fungus were cut, transferred, and then soaked into each prepared aqueous dilution. Then, soaked agar blocks were left for the proposed time of exposure. Five time points of exposure were tested starting at 30, 60, 120, 240min, and 24 hours. After elapsing exposure time points, inocula were subsequently removed and dried on sterilized filter papers before being mounted onto freshly prepared GYA plates **(Alderman, 1982; Hussein *et al*., 2000; Emara *et al*., 2020)**. Replicates were included in each tested dilution. Control inoculums treated with STW instead of each tested aqueous dilution were also included. Inoculated

plates were incubated at an appropriate temperature for each tested fungus. Measuring hyphal growth within different exposure times in a perpendicular manner using vernier calipers was recorded up to the full growth of the control groups **(Udomkusonsri, 2007; Fuangsawat *et al*., 2011)**. The growth inhibition rate (IR; %) was calculated from mean values as IR = 100 × [(A − B)/ (A − C)], where A is a mycelial growth in the control; B is a mycelial growth in the sample, and C is the average diameter of the fungal blocks **(Hu *et al*., 2013)**.

4.2. Preparation of fungal inocula for experimental infection

Hyphal mates of both fungal stains were prepared representing the source of the artificial infection of both eggs and fingerlings of *C. idella*. The hyphal mates were prepared as described earlier in the study of **Hussein and Hatai (2002)**. Briefly, agar blocks of 1 x 1cm^2, with actively growing mycelia of each fungus, were obtained from freshly cultured GYA plates of *S. australis* and *A. bisexualis*. Then, 3~ 4 blocks of each fungus were separately inoculated in petri dishes (90mm in diameter and 20mm in depth) containing 20mL glucose yeast broth (GYB). Inoculated plates were kept at 20°C for *S. australis* and 25 °C for *A. bisexualis* for 24hrs. The growing mycelia were cut and washed three times in sterilized tap water (STW) before being transported into sterilized gauze bags. Gauze bags of both fungal strains were either placed inside a loosed screw-capped test tube filled with 10mL STW for transportation to the field trial site **(Kitancharoen & Hatai, 1996; Zahran *et al*., 2016)** or used directly in fingerlings artificial infection at the Fish Diseases and Management Department Laboratory.

4.3. Field trials at Abo-Saleh fish hatchery

Abo-Saleh fish hatchery is a governmental fish hatchery belonging to the General Authority for Fisheries Development, Ministry of Agriculture (http: //www. gafrd.org). The hatchery is located in Beni-Suef Governorate, Egypt, and is employed as a carp and tilapia seeds supplier for almost all of the northern Upper Egypt regions. For its importance, it was chosen as a site for performing field trial applications through the represented study.

Two field trials were designed and applied inside the hatchery. The first trial was designed as a preliminary investigation to estimate the effects of different concentrations of both crude herbs on the rate of fecundity, hatchability, and toxicity in *C. idella* fertilized eggs and/or hatched fries during artificial incubation in Zug-jar units **(Khan *et al*., 2004; Dada & Ejete-Iroh, 2016)**. Briefly, one Zug-jar unit (*Ca.* 8 jars) was designed for the experiment, 4 jars for each herb's designated concentrations either crude clove or thyme. The other 2 jars served as control negative ones and received only fertilized eggs without applying any of the tested herb concentrations. Prior to applying each herb concentrations, 20mL (15– 20 eggs/ ml) of fertilized *C. idella* egg suspension in water was eventually distributed into each jar of the Zug-jar unit. Then, coursed crude of each herb at concentrations of 0.25, 0.5, 1, 2 and 1, 2, 4, and 8g/ L for clove and thyme, respectively, was bagged in sterile gauze bags. At all tested levels, both course crude herb bags were independently dispensed in corporation with different jars of fertilized eggs. Duplicated Zug-jar units with their control jars were set as replicates for the experimental one, including the same concentrations of each herb and control jars. During the

incubation time (36hrs, 24± 1°C), the running water current exchange was adjusted at a rate of 5L/ min, and then reduced to 2L/ min, when the eyed egg stage was observed **(Korwin-Kossakowski, 2012)**. After elapsing the incubation time, the number of hatched fry in each jar within both experimental and replicates were recorded, and their viability was also monitored. The percentage rate of the fecundity as well as hatchability was calculated following the formula of **Rashid *et al*. (2014)**. Additionally, hatchlings fry were collected separately, counted, and monitored for 7 days, and their percentage rate of survival (PRS) was calculated according to the formula of **Yeasmin *et al*. (2014)**.

Fecundity rate (%) = No. of live eyed eggs × 100/ total number of fertilized eggs

Hatching rate (%) = No. of hatchlings ×100/ total number of fertilized eggs

$$\text{PRS of hatchling fry} = 1 - \frac{\text{No.of alive hatchlings up to 7th days}}{\text{Total number of hatched eggs}} \times 100$$

The second trial was planned depending partially on the results obtained from the first one. Within the trial, both crude herbs were used as antifungal agents in their crude form for the control of infection with the selected water molds during *C. idella* fertilized eggs incubation. The trial was designed following the outlines figured out earlier by **Metın *et al*. (2015)**, with little modifications. Briefly, 6 Zug-jar units (8 jars in each) were used for performing the experiment. Two Zug-jar units served as experimental ones, and 2 units were used as replicates for each herb trial experiment. The first unit was used for applying crude clove as an antifungal agent, 3 jars against artificially infected *C. idella* eggs with *S. australis* hyphal mates, and 3 jars against infected *C. idella* eggs with *A. bisexualis* hyphal mates. The rest 2 jars of the unit were used separately as positive controls for each selected fungus, without herb application. Prior to applying different clove concentrations as antifungal agents, fertilized eggs were mounted (20mL/ Jar) together with submerging upload of the selected hyphal mates into the 8 jars of the Zug-jars unit. Subsequently, double sets of sterile gauze bags (3 bags in each) containing coursed crude clove at concentrations of 1, 0.5, and 0.25g/ bag were submerged into Zug-jars containing egg patches subjected to each fungal strain infection. Control jars (2 jars) separately received fungal mates of the selected fungus without any clove application. Meanwhile, the running water change rate of the Zug-jars unit was adjusted at a rate of 5L/ min and then reduced to 2L/ min when the eyed egg stage was observed **(Korwin-Kossakowski, 2012)**. Similarly, the antifungal activities of the crude thyme were evaluated at the same investigation levels performed with crude clove using the other 3 Zug-jars units employed with exception. The exception was that the thyme concentrations were 4, 2, and 1g/ bag. After the elapse of egg incubation time (36hrs at 24± 1°C), fish fry started to hatch, and therefore, hatchability was monitored and recoded for each jar of the experimental Zug-jar units and their three replicates. The hatchability rate was calculated as aforementioned in the research work of **Yeasmin *et al*. (2014)**. The antifungal potentials of different clove and thyme concentrations against both fungal species were also recorded and experienced as the percentage of infected to non-infected eggs in comparison with the control ones. Patches of un-hatched dead eggs representing different experimental jars and/or their replicates were sampled in sterile screwed test tubes and transported in an ice box (10°C) for further laboratory investigations at the

laboratory of Fish Diseases and Management Department. The collected egg samples were immediately subjected to microscopical examinations for abnormalities and fungal growth **(Mousavi *et al*., 2009a)**. Egg samples that showed abnormal lesions were then photographed. To prove Koch's postulates, a few eggs were also spread onto freshly prepared GYA plates for the re-isolation of incriminated fungal strains of concern. The re-isolated fungi were subjected to morphological characterization and taxonomy conformation **(Seymour, 1970; Diler & Timur, 1995; Bruno & Woo, 1999; Fadaeifard *et al*., 2011)**, followed by purification procedures **(Özçelik *et al*., 2020)**.

4.4. Experimental fish

A total of 750 apparently healthy *C. idella* fingerlings, with an average body weight of 8± 4g and an average body length of 10± 2cm, were collected alive from the Abo-Saleh fish hatchery. The collected fingerlings were safely transported to the wet laboratory of the Fish Diseases and Management Department. After 2 weeks of acclimation, the fingerlings were kept in 3 fiberglass tanks (capacity: 500L/ tank) supplied with chlorine-free tape water, with continuous aeration and filtration. Fingerlings were left for a conditioning period of 2 weeks and fed 4 times/ day at a feeding rate of 3% of their body weight. The light was adjusted as 12/ 12hrs, light/ dim. Feeding of fingerlings was stopped 3 days prior to the start of any experiment.

4.5. Toxicity estimation of the selected crude herbs and their potential control activities against experimentally infected C. idella fingerlings with selected fungal strains

For the evaluation of clove or thyme toxic activities on the *C. idella* fingerlings, 240 fingerling individuals were randomly divided into 4 groups for each herb (clove or thyme), distributed in 12 aquaria (10 fingerlings in each), and designed as experimental with 3 replicates **(Emara *et al*., 2020)**. All experimental aquaria (76 x 31 x 25cm) were filled with 60L chlorin free tap water and supplemented with air stones and a filtration system. Four concentrations of each herb were employed for the experiment. The tested concentrations were 0.2, 0.25, 0.5, 1 and 0.5, 0.8, 1, 2g/ L for clove and thyme, respectively. All of the used concentrations were separately packed in gauze bags and submerged in their corresponding aquaria **(Zahran *et al*., 2016)**. At all tested levels, both herbs were applied as an indefinite bath for up to 1 week with daily recording of *C. idella* fingerlings mortality.

For the evaluation of both selected herbs as potential antimycotic control agents, 390 fingerlings were divided into 3 group sets, namely A, B, and C. Group set A was designated for the treatment of experimentally infected fingerlings with clove as an antifungal agent against both molds (*S. australis* and *A. bisexualis*). In this group, there were 2 subgroups, namely As and Aa, representing infection with *S. australis* and *A. bisexualis*, respectively, and consisting of 9 aquaria in each. Subgroup As was designated for 3 different concentrations of crude clove including 0.2, 0.25, and 0.5g/ L, with 2 replicates for each concentration, and was experimentally infected with *S. australis*. The second subgroup (Aa), the same concentrations were used but the infection was performed with *A. bisexualis*. Group set B was treated as group A with exception; crude thyme at concentrations of 0.5, 0.8, and 1g/ L was used instead of clove as an antimycotic against tested fungal strains. Group set C (controls) consisted of 3 aquaria (60L, 10 fish

individuals in each) and designated as 2 aquaria as control positive infected with *S. australis* and *A. bisexualis* and 1 aquarium as control negative for ami-momi treatment only **(Hussein & Hatai, 2002; Hussein *et al*., 2013; Özçelik *et al*., 2020)**. To induce infection, all fingerlings' groups, including the negative control, were exposed to ami-momi (net-shaking) treatment before being challenged with *S. australis* and/or *A. bisexualis*' hyphal gauzes. In this technique, fish were shaken in the air in a fan-shaped scoop net for 2min, then rapidly washed before being returned to their aquaria **(Hussein & Hatai, 2002; Hussein *et al*., 2013)**. The hyphal gauze bags of both fungal species were submersed immediately after return of fingerlings into their aquaria to enhance zoospores release and induction of infection.

Twenty-four hours post infection, bags containing the different concentrations were separately submerged into the corresponding designated aquaria. Fingerlings were monitored, and abnormalities were recorded. Numbers of infected fish, those that fell into anesthesia, moribund, dead, and/or recovered ones were recorded every 24hrs up to one week. Cumulative mortality was recorded, and the relative percentage of survival (RPS) was calculated according to **Amend (1981)** using the formula of RPS = 1 − (% of mortality in treated groups / % of mortality in control group) ×100.

In fact, both applied herbs (Clove and thyme crude buds) obtain an anesthetic effect on fish **(Akinrotimi *et al*., 2015; Hoseini *et al*., 2019; Mphande *et al*., 2023)**. Therefore, a full chapter of our master's thesis discussed the various stages of anesthesia and euthanasia during the experiment. However, it has not yet been published as a stand-alone part.

5. Statistical analysis

All treatments were achieved in 3 replicates. Statistical analyses of all data were obtained by advanced models 16.0 software of one-way ANOVA (SPSS, Tokyo, Japan). Post hock test, LSD choice, together with optional choices of descriptive and/or homogeneity test(s) were applied. $P< 0.05$ was considered statistically significant.

RESULTS

1. Fungistatic and fungicidal activities of the selected herbs aqueous extracts

The antifungal activities of clove and thyme aqueous extracts against *S. australis* and *A. bisexualis* were assessed by the determination of their MICs (Table 1). The growth of *S. australis* and *A. bisexualis* was entirely inhibited when cultured on GYA media, incorporated with 10% of aqueous clove original stock solution. This inhibition was evident for 24, 48, and 72hrs of incubation at corresponding temperatures for each fungal strain (Fig. 1A, E). Although the clove aqueous solution of 5% showed no significant growth inhibition zones at the tested times of incubation, the tested fungi displayed about half growth values in comparison with controls (Fig. 1B, F). On the other hand, subsequent decreased serial concentrations represented no or weak inhibitory activities against both tested fungi. There were no significant differences in growth zones between low concentrations starting at 3.2% and controls (8.5cm in diameter). While, MICs of

thyme aqueous solution against tested fungi exhibited weaker inhibition activities to a lesser extent at all its concentrations levels tested (Fig. 1C, G). Oppositely, the control groups of both fungi showed a full growth (Fig. 1D, H).

Table 1. Fungistatic activity of clove and thyme aqueous extracts on selected Oomycetes

Fugal strain	Conc. (%)	Growth zone*					
		Clove			Thyme		
		24hrs.	48hrs.	72hrs.	24 hrs.	48 hrs.	72 hrs.
Saprolegnia australis **	100	*0.00±0.00 [a]	0.00±0.00 [a]	0.00±0.00 [a]	2.10±0.05 [a]	3.56±0.08 [a]	3.66±0.08 [a]
	50	0.00±0.00 [a]	2.10±0.05 [a]	4.16±0.08 [a]	2.80± 0.05 [a]	4.10±0.05 [a]	4.33±0.08 [a]
	25	1.60±0.05 [a]	3.76±0.08 [a]	6.33±0.08 [a]	3.33 ±0.08 [a]	4.50±0.05 [a]	4.60±0.05 [a]
	Control	3.00±0.05 [c]	6.53±0.08 [c]	8.50±0.00 [c]	4.43±0.08 [c]	8.50±0.00 [c]	8.50±0.00 [c]
Achylia bisexualis **	100	0.00±0.00 [a]	0.00±0.00 [a]	0.00±0.00 [a]	1.36±0.08 [a]	2.76±0.08 [a]	2.86±0.08 [a]
	50	1.10±0.05 [a]	1.16±0.08 [a]	1.40±0.05 [a]	1.53±0.08 [a]	3.26±0.08 [a]	3.33±0.08 [a]
	25	1.33±0.08 [b]	1.90±0.05 [a]	2.80±0.05 [a]	1.80±0.05 [a]	3.76±0.08 [a]	3.80±0.05 [a]
	Control	1.73±0.08 [c]	2.83±0.08 [c]	4.53±0.08 [c]	2.53±0.08 [c]	5.03±0.08 [c]	5.03±0.08 [c]

[(*)] Means ± SD, [(**)] Three replicates of each fungal strain, [(a)] Highly significant ($P \leq 0.05$), [(b)] Less significant ($P \leq 0.05$), [(c)] Control (no significance).

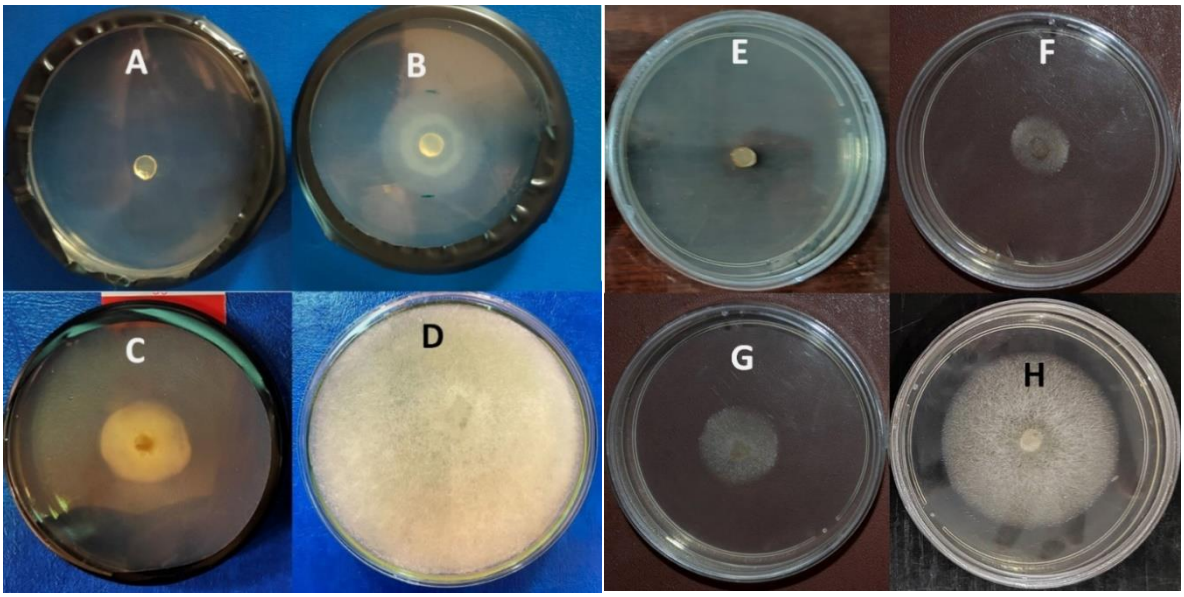

Fig. 1. The fungistatic activities of clove and thyme aqueous extracts against *S. australis* MT075633 (A~D) and *A. bisexualis* MT075632 (E~H) showing: **A & E:** No fungal growth at concentration of 10% (0.8g/ 100ml) clove aqueous extracts. **B & F:** Growth inhibition zone at concentration of 5% (0.4g/ 100mL) clove. **C & G:** Zones of growth at concentration of 10% (3.2g/ mL) of thyme aqueous extract. **D & H:** Full growth of *S. australis* and *A. bisexualis* on control plates after 72hrs of incubation

Concerning the fungicidal activities of both herbs aqueous solutions against the tested fungal strains, no considerable different results were obtained (Table 2). After only 24 hours of exposure, both the studied fungal strains were destroyed by the 50% concentrations of clove and thyme's fungicidal properties. At the first four points of exposure examined, however, the identical concentration (50%) of both herbal aqueous solutions failed to eradicate the chosen fungi.

2. Field study at Abo-Saleh fish hatchery

For the effects of crude selected herbs on the fecundity and hatchability percentages of *C. idella* eggs, clove showed more potential effects than those of thyme. Fertilized eggs (fertility %, 87.33± 1.45) incubated with clove at a concentration of 2g/ L expressed 94.3 and 79.6% for the fecundity and hatchability, respectively. The percentages of fecundity and hatchability exhibited lower levels at concentrations of 1 and 0.5g/ L of clove, expressing percentages of 94.33± 2.33, 86.00± 2.08, and 70.00± 2.30, 64.66± 0.88%, respectively. As a result, both the fecundity and hatchability percentages at the lowest (0.25g/ L) clove concentration used showed a transient difference compared with the controls (Table 3). On the other hand, incubated fertilized eggs exposed to a concentration of 8g/ L thyme showed the fecundity and hatchability percentages of 90.33± 2.60 and 70.66± 1.76, respectively, (Table 3). At the same behavior of clove, incubated fertilized eggs experienced 60.00± 3.46, 50.00± 2.88, and 84.33± 2.33, 80.33± 1.45 fecundity and hatchability percentages when exposed to thyme at concentrations of 4 and 2g/ L, respectively. Meanwhile, eggs in controls and the lowest

concentration (1g/ L) of thyme showed the same percentage levels of fecundity and hatchability.

Table 2. Fungicidal activity of clove and thyme aqueous extract on selected oomycetes

Fugal strain	Conc.** (%)	Exposure time (hour)					
		Clove			Thyme		
		2	4	24	2	4	24
Saprolegnia australis ***	100	*1.10±0.05 [a]	1.03±0.08 [a]	1.96±0.08 [a]	4.60±0.05 [a]	2.33±0.08 [a]	0.00±0.00 [a]
	50	3.93±0.08 [a]	3.60±0.05 [a]	3.60±0.05 [a]	5.03±0.08 [a]	4.66±0.08 [a]	4.50±0.05 [a]
	25	4.50±0.05 [a]	4.26±0.08 [a]	3.76±0.08 [a]	6.90±0.05 [b]	5.50±0.05 [a]	5.40±0.05 [a]
	Control	8.50±0.00 [c]	8.50±0.00 [c]	8.50±0.00 [c]	8.50±0.00 [c]	8.50±0.00 [c]	8.50±0.00 [c]
Achylia bisexualis ***	100	0.00±0.00 [a]	0.00±0.00 [a]	0.00±0.00 [a]	0.00±0.00 [a]	0.00±0.00 [a]	0.00±0.00 [a]
	50	3.83±0.00 [a]	3.60±0.05 [a]	0.00±0.00 [a]	4.03±0.08 [a]	3.83±0.08 [a]	3.16±0.08 [a]
	25	4.23±0.08 [a]	3.93±0.08 [a]	3.10±0.05 [a]	4.33±0.08 [b]	4.33±0.08 [b]	3.50±0.05 [a]
	Control	6.00±0.00 [c]	6.00±0.00 [c]	6.00±0.00 [c]	6.00±0.00 [c]	6.00±0.00 [c]	6.00±0.00 [c]

[(*)] Means ± SD, [(**)] Stock solution of clove aqueous extract was 8g/ 100mL and stock solution for thyme aqueous extract was 32g/ 100mL, [(***)] Three replicates of each fungal strain, [(a)] Highly significant ($P \leq 0.05$), [(b)] Less significant ($P \leq 0.05$), [(c)] Control (no significance).

Table 3. Impact of clove and thyme on hatchability of *C. idella* eggs

	Doses of selected herb (gram/ l)									
	Clove					Thyme				
	2	1	0.5	0.25	control	8	4	2	1	control
Fund. % **	*94.33 ±2.33 [a]	86.00± 2.08 [a]	79.66± 2.60 [a]	70.00±4 .04 [b]	49.33±2 .90 [c]	90.33± 2.60 [a]	84.33± 2.33 [b]	80.33± 1.45 [b]	75.00± 2.88 [b]	70.33 ±2.60 [c]
No. of hatch. eggs **	240.33 ±2.60 [a]	210.33 ±2.60 [a]	195.00 ±2.88 [a]	179.67± 2.02 [b]	120.67± 2.33 [c]	211.00 ±3.21 [a]	180.67 ±1.20 [a]	150.00 ±2.88 [a]	135.00 ±2.88 [b]	120.00±2.88 [c]
No. of non-hatch. eggs **	59.66 ±2.60 [a]	87.00± 1.52 [b]	135.00 ±32.53 [b]	120.33± 2.02 [b]	179.33± 2.33 [c]	91.00± 3.21 [a]	119.33 ±1.20 [a]	150.00 ±2.88 [a]	16500 ±2.88 [b]	180.00±2.88 [c]
Hatchability % **	79.66 ±2.60 [a]	70.00± 2.30 [a]	64.66± 0.88 [a]	59.66±3 .17 [b]	40.66±1 .76 [c]	70.66± 1.76 [a]	60.00± 3.46 [b]	50.00± 2.88 [b]	45.00± 2.88 [b]	40.00 ±2.88 [c]

[(*)] Means ± SD, [(**)] Three replicates of each herb, [(a)] Highly significant ($P \leq 0.05$), [(b)] Less significant ($P \leq 0.05$), [(c)] Control (no significance).

The antifungal activities of both crude herbs investigated against *C. idella* eggs exposed to experimental infection with the selected fungal species showed considerably various values (Table 4). As a result, clove at concentrations of 1 and 0.5g/ L significantly decreased the numbers of infected eggs ($P< 0.05$) exposed to both fungal species mates in comparison with controls. Additionally, clove at a concentration of 0.25g/ L was not as strong as the two previous ones, but it reduced the infection incidence when compared with eggs in control jars. Eggs in control jars showed degrees of infection regarding the exposure to *S. australis* or *A. bisexualis* fungal mates, representing a massive infection with the former and a lesser infection with the latter (Fig. 2). Out of the 3 thyme tested concentrations, thyme at a concentration of 4g/ L exhibited high significant ($P< 0.05$) antifungal activities against both fungi, with the lowest existence of infected eggs. On the contrary, thyme at concentrations of 2 and 1g/ L, incorporating the same jars with exposed eggs to mycelia mates of both fungi, expressed degrees of lower significates as antimycotic effective doses. Meanwhile, eggs habituated controls and those of the later thyme concentration (1g/ L) jars exhibited similar levels of infection, which was clearly obvious with both selected fungi (Fig. 2).

Table 4. Antifungal activities of crude clove and thyme against *S. australis* and *A. bisexualis* in experimentally infected *C. idella* eggs

Fungal spp.	**Crude herb dose (g/ L)**															
	Clove								**Thyme**							
	1		**0.5**		**0.25**		**control**		**4**		**2**		**1**		**Control**	
	N	I	N	I	N	I	N	I	N	I	N	I	N	I	N	I
*S. australis***	*250.33	49.66	220.33	79.66	151.67	148.33	100.00	200.00	211.00	89.00	180.33	119.67	90.33	176.33	50.33	249.67
	±3.17[a]	±3.17[a]	±2.02[a]	±2.02[a]	±2.72[b]	±2.72[b]	±2.30[c]	±2.30[c]	±2.08[a]	±2.08[a]	±2.02[a]	±2.02[a]	±3.17[b]	±33.31[b]	±3.17[c]	±3.17[c]
*A.bisexualis***	259.00±2.08[a]	41.00±2.08[a]	231.67±2.72[a]	68.33±2.72[a]	169.33±3.48b	130.67±3.48[b]	149.33±2.90[c]	150.67±2.90[c]	220.00 ±3.46[a]	80.00±3.46[a]	170.67±3.48[a]	129.33±3.48[a]	120.33±4.33[b]	179.67±4.33[b]	69.66±2.02[c]	230.33±2.02[c]

(*) Means ± SD, (**) Three replicates of each fungal strain, (a) Highly significant ($P\leq 0.05$), (b) Less significant ($P\leq 0.05$), (c) Control (no significance), N= No. of non-infected eggs. I= No. of infected eggs.

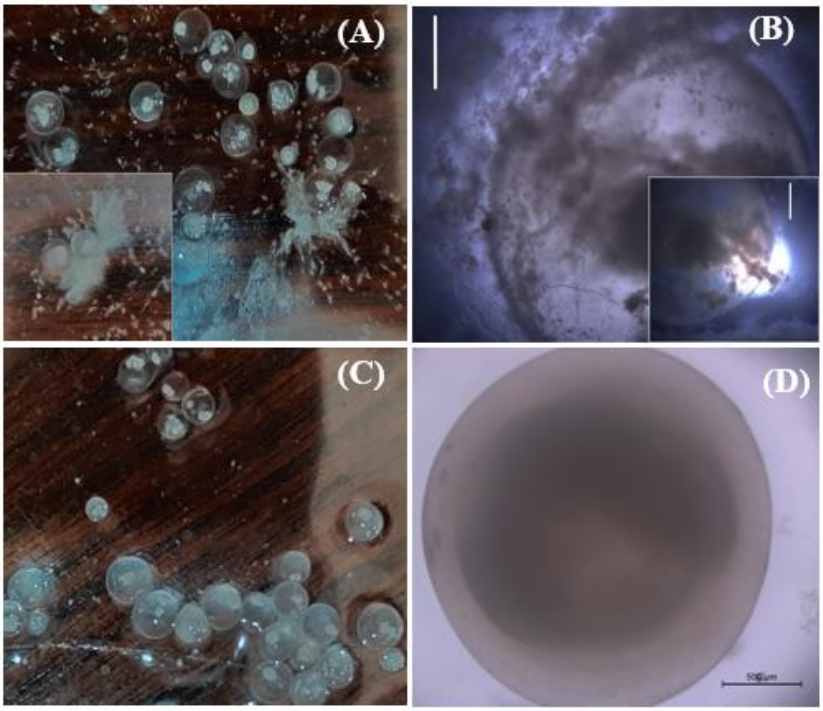

Fig. 2. Saprolegniosis induction and treatment by clove and thyme. **A.** Aggregated fugal hyphae around the infected eggs and transmitted into another healthy one. **B.** Variable degrees of fungal infection during microscopic examination. **C.** Fertilized eggs protected by herbal application. **D.** Close examination of treated eggs with no hyphal mates on the external surface

3. Crude clove and thyme toxicity estimations and their potential control activities against experimentally infected *C. idella* fingerlings with *S. australis* and *A. bisexualis*

With respect to the toxic effects related to exposure to different clove concentrations, *C. idella* fingerlings showed no abnormal behavioral alterations and/or mortality at concentrations of 0.2 or 0.25g/ L until the end of the experimental period (7 days). *C. idella* fingerlings exposed to 0.5 and 1g/ L of clove showed different degrees of anesthesia corresponding to clove exposure doses, followed by 20 and 80% of cumulative mortality existence at the first day. Cumulative mortality extended to 100% on the second day with exposure to both clove concentrations tested (Fig. 3). On the other hand, no mortality occurred in *C. idella* fingerlings groups exposed to 0.5g/ L thyme until the end of the experimental period (7 days). Thyme concentration of 0.8g/ L showed signs of lethargies, followed by cumulative mortality of 13.33± 3.33, 20.00± 5.77, 33.33± 6.66, 40.00± 0.00, 56.66± 6.66, 70.00± 10.00, and 83.33 ±8.81% at the 1st, 2nd, 3rd, 4th, 5th, 6^{th,} and 7th days, respectively, (Fig. 3). Thyme concentrations of both 1 and 2g/ L caused anesthesia to exposed *C. idella* fingerlings on the first day of exposure, followed by cumulative mortality of 30.00± 5.77 and 100%, respectively, and reached up to 100% on the second day only for the former concentration (1g/ L).

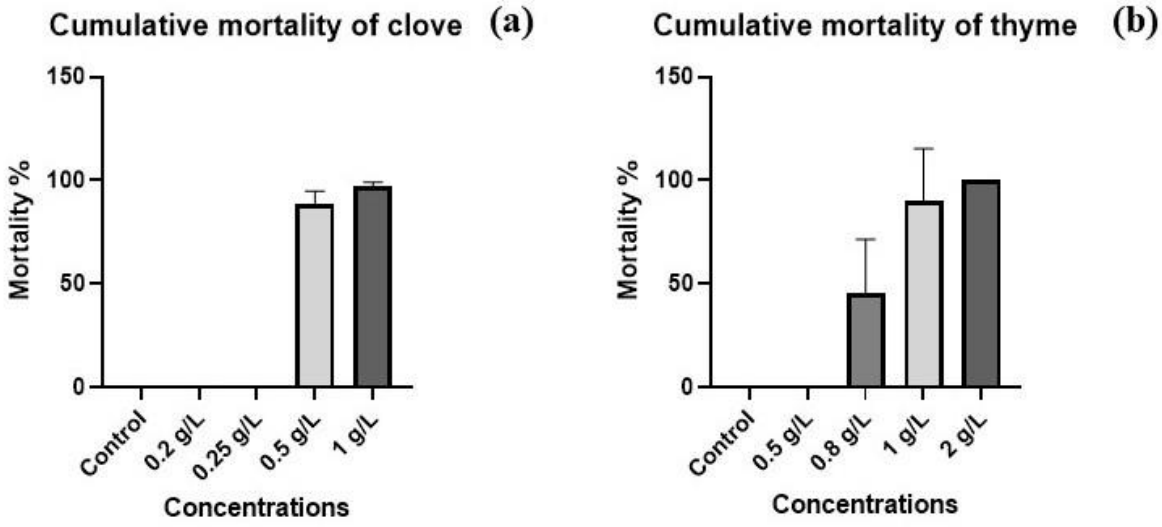

Fig. 3. Tolerance availability of *C. idella* fingerlings against different doses of clove and thyme showing: **(a)** No mortality at concentrations of 0.2 and 0.25g/ L clove, while at concentrations of 0.5 and 1g/ L cumulative mortality increased dramatically up to 80 and 100%, respectively. **(b)** A significant tolerance at 0.5g/ L of thyme, while the subsequent higher doses associated with potential increase resulted in cumulative mortality up to 2g/ L

C. idella fingerling groups treated with "ami momi" and followed by a separate experimental infection with both selected fungi showed fungal vesicles on their body surface after 24hrs, indicating saprolegnosis occurrence (Fig. 4). The capability of crude clove and thyme to control experimentally induced saprolegnosis revealed various results (Table 5). Experimentally infected *C. idella* fingerling groups, exposed to crude clove at concentrations of 0.25 and 0.5g/ L for 24 hrs, showed 70 and 90% healing degrees with cumulative mortality of 30 and 0.0%, respectively. Meanwhile, experimentally infected *C. idella* fingerlings in control positive groups and those indefinitely exposed to 0.2 clove showed a progression of the infection associated with a cumulative mortality, reaching 100 and 90%, respectively. On the other hand, experimentally infected *C. idella* fingerlings groups could cope with the infections when indefinitely exposed to 0.5g/ L of thyme. Within those groups, the recorded healing and cumulative mortality percentages were 85 and 15%. Experimentally infected *C. idella* fingerlings groups indefinitely exposed to 0.8g/ L clinically experienced light saprolegniosis patterns together with gradual cumulative mortality up to 90% by the end of the experiment. Experimentally infected *C. idella* fingerlings groups were indefinitely exposed to 1g/ L under deep anesthesia, and subsequent euthanasia started after 24hrs of exposure. Whereas, control negative *C. idella* fingerlings groups with only "ami momi" treatment neither showed infection nor mortality. At all tested level, considerable various degrees of healing and cumulative mortality percentages are illustrated in (Table or Chart) regarding the fungal strains and crude herbs used.

Fig. 4. The development of saprolegniosis in fingerlings and treatment with clove and thyme displaying: **A.** Removal of the dorsum and tail scales in fingerling exposed to ami-momi technique. **B.** Underwater view of infected fingerling with fluffy mycelia with massive destruction of the skin and the underling tissues. **C.** Hyphal aggregates almost disappeared with little remnants after treatment with 0.5g/ L clove. **D.** A higher number of hyphal remnants by applying 0.5g/ L thyme treatment

Table 5. Treatment of *C. idella* fingerlings with different doses of clove and thyme

Fungal strain	Exp. fingerling	Concentration (g/L)							
		Clove				Thyme			
		0.2	0.25	0.5	Control	0.5	0.8	1	Control
*S. australis***	Treated	*10.00 ±5.77	70.00±5.77 [b]	90.00±5.77 [a]	0.00±0.00 [c]	80.00±5.77 [a]	10.00±5.77	0.00±0.00	0.00±0.00 [c]
	Anaesth.	0.00± 0.00	0.00±0.00	80.00±5.77 [a]	0.00±0.00 [c]	0.00±0.00	0.00±0.00	90.00±5.77 [a]	0.00±0.00 [c]
	Cumul. mortal.	90.00 ±5.77	30.00±5.77 [a]	0.00±5.77 [a]	100.00±0.00 [c]	3.33±3.33 [a]	90.00±5.77 [b]	0.00±0.00 [a]	100.00±0.00 [c]
*A. bisexualis***	Treated.	6.66± 3.33	73.33±3.33 [b]	93.33±3.33 [a]	0.00±0.00 [c]	83.33±3.33 [a]	10.00±5.77 [b]	3.33±3.33 [a]	0.00±0.00 [c]
	Anaesth.	0.00± 0.00	0.00±0.00	80.00±5.77 [a]	0.00±0.00 [c]	0.00±0.00	0.00±0.00	66.66±8.81 [a]	0.00±0.00 [c]
	Cumul. mortal.	93.33 ±3.33 [b]	23.33±3.33 [a]	0.00±0.00 [a]	100.00±0.00 [c]	6.66±3.33 [a]	86.66±6.66 [b]	83.33±3.33 [b]	100.00±0.00 [c]

(*) Means ± SD, (**) Three replicates of fungal strain, (a) Highly significant ($P \leq 0.05$), (b) Less significant ($P \leq 0.05$), (c) Control (no significance).

DISCUSSION

In the aquaculture sector, water molds have become a substantial concern, particularly in freshwater fish hatcheries **(Jalilpoor *et al.*, 2006; Fregeneda-Grandes *et al.*, 2007; Ali, 2009)**. Additionally, they are responsible for the noticed cumulative mortality-associated economic losses in cultured and wild fish species worldwide **(Ke *et al.*, 2009)**. Outbreaks of such infections in freshwater hatcheries represented crises that dramatically threatened fish seeds productivity. Members of the family Saprolegniaceae, in particular those of the genera *Saprolegnia, Achlya,* and *Aphanomyces*, are considered the main issues in concern with water mold outbreaks **(Noga, 1993; Ke *et al.*, 2009; Shahbazian *et al.*, 2010; Hussein *et al.*, 2013)**. To cope with such outbreaks, chemicals are usually used as prescribed solutions. Most of these chemical substances were reported as effective tools for controlling infection despite of their hazardous impacts on the environmental ecosystem and humans as well. For this reason, finding natural antimycotic alternatives became valuable. In this respect, clove and thyme, as natural herbs, were selected in the current investigation. Based on research papers, the selected two herbs were emphasized to obtain antifungal potentials for their extracts and essential oils **(Omidbeygi *et al.*, 2007; Najafi & Zamini, 2013)**.

In the current study, *in vitro* MICs of clove and thyme aqueous solutions exhibited that the highest concentrations (10%) of both herbs had the best fungistatic activities against selected water mold strains compared to the subsequent ones. These results indicate that each of fungal strains is more sensitive to high aqueous concentrations of both herbs used, with different sensitivity degrees to the lower ones. At all tested levels, aqueous-extracted clove had more potential fungistatic activities than those of thyme. In terms of the fungicidal activities of both investigated herb aqueous extracts, the best fungicidal effects were obtained at concentrations of 50% of both herb stock solutions. Such activities were gradually decreased at concentrations of 30% stock solutions of both herbal aqueous extracts. Fungal growth at 20% stock herbal solutions was not significantly different ($P > 0.05$) than those for all other subsequent dilutions and looked similarly like the controls. These findings elucidated that the 20% aqueous extracts of both herbs had no significant fungicidal effect against *S. australis* and *A. bisexualis*. Interestingly, the obtained results assessed that the quantities of 0.04 and 0.16g/ mL of crude clove and thyme, respectively, were suitable enough to prepare stock solutions with fungicidal activities against both *S. australis* and *A. bisexualis*. Moreover, the aqueous solution of clove experienced antimycotic activities superior to those of thyme. These results are comparable with those reported by **Hussein *et al.* (2000)**, who concluded that the antimycotic activities against water molds tested were mainly related to eugenol, which is the main component of clove. Similarly, **Huang *et al.* (2015)** selected clove extract out of 30 investigated Chinese herbs as a potent anti-saprolegnia ferax agent of herbal origin.

The antifungal activities of both herbs were evoked by complex mixtures of low molecular weight compounds called essential oils (EOs) that were obtained during the aqueous extraction of both herbs. These oils contain secondary metabolites belonging to a variety of chemical classes **(Carson & Hammer, 2010)**. Terpenoids and phenylpropanoids form the major constituents of these EOs, including carvacrol, thymol, and eugenol (the phenolic components), which are known to possess fungicidal characteristics **(Manohar *et al.*, 2001; Gayoso *et al.*, 2005)**. Clove bud EO mainly

comprises eugenol and lesser extents of eugenyl acetate and β-caryophyllene **(Jirovetz *et al.*, 2006)**, while thyme oil contains phenols, mainly thymol, carvacrol, and terpenoids **(Ernst, 2003)**. These compounds radically expressed their antifungal actions on the cellular membrane with morphological damage and cellular deformities **(Cox *et al.*, 2001; Chami *et al.*, 2005)**. Through this mechanism, the fungal cell wall permeability was altered and, therefore, a loss of cellular macromolecules eventually occurred **(Šegvić Klarić *et al.*, 2007)**. Regarding the noticeable difference between clove and thyme, the possible explanations may be related to the eugenol concentration represented in clove. Moreover, impurities included in crude thyme may hinder its antifungal efficacy **(Hussein *et al.*, 2000; Tampieri *et al.*, 2003)**.

Generally, the incidence of aquatic mold infections becomes more problematic during artificial spawning, particularly at the time of fertilized eggs incubation. The infections started when swimming secondary fungal zoospores colonized sporadic dead eggs within the incubated egg patches. Consequently, the infection spread to infect the nearby live eggs, causing their suffocation and death **(Pottinger & Day, 1999)**. Saprolegniaceae-infected eggs are easily recognized by the fluffy, cotton-like, and white to greyish patches surrounding the eggs **(Stueland *et al.*, 2005a)**. At all experimental levels, the mycelial mates of the selected oomycetes were utilized to simulate the natural incidence of the infection as they undergo to contain ripped zoosporangia bearing the zoospores. Releasing of the infected stage (secondary fungal zoospores) originated from hyphal mates usually represented on diseased fish or even dead eggs **(Hussein & Hatai, 2002)**.

The *in vivo* trials in *C. idella* artificially infected eggs pointed out that both herbs had antifungal potential against selected water molds. These results agree with those of **Mousavi *et al.* (2009b)**. The highest protection levels against fungal infection were obtained with the applications of clove at concentrations of 2, 1, and 4g/ L of thyme. No significant differences were obtained with the applications of other lower concentrations of both herbs and those of the controls. However, statistical analysis of both herb results revealed that clove at all concentrations used had a higher potential significance for eggs survival than those of the thyme. Additionally, clove and thyme at concentrations of 2 and 8g/ L showed positive effects on fecundity and hatchability, with a superior efficacy of clove. These findings coincide with those of **Holcomb *et al.* (2004)** who elucidated that, clove has no significant harmful effect on the developmental stages of fertilized steelhead and white sturgeon eggs. Moreover, these findings concur with those of **Usta *et al.* (2002)**, **Mousavi *et al.* (2009b)**, **Gormez and Diler (2015)** and **Hoskonen *et al.* (2015)** who proved that, using clove and thyme EOs succeeded in controlling saprolegniosis in fish and eggs associated with an increase in hatching rate. Furthermore, the sanitary actions resulted from continuous irrigation of the incubated eggs with either crude clove or thyme clearly washed other pathogens off egg surfaces and hatched fry, as well as limiting other infections. Such limiting sanitary activities of both herbs shared their anti-saprolegniosis ones and relatively increased the fry yield. From a supporting point of view, several studies have postulated the antimicrobial activities of clove and thyme and/or their EOs **(Starliper *et al.*, 2015; Kačániová *et al.*, 2017; Yildirim & Türker, 2018; Pathirana *et al.*, 2019; Dawood *et al.*, 2021)**. The anti-saprolegniosis (antifungal) activities of clove and thyme EOs relied on different aspects related to their

components. For instance, eugenol, the major component of clove, is a lipophilic compound that has the capability to penetrate membranes and may act as a mitochondrial uncoupling agent **(Manohar *et al.*, 2001; Usta *et al.*, 2002)**. Moreover, **Jian-Guo *et al.* (2016)** reported that eugenol could destroy microorganism cell walls, penetrate the cytoplasmic membranes, and inhibit the normal synthesis of DNA and proteins. On the other hand, thymol and carvacrol, the main components of thyme, are known to possess a wide range spectrum of antifungal characteristics including activities against fungi isolated from onychomycosis **(Ernst, 2003; Huang *et al.*, 2015)**. Such activities are mainly attributed with the disturbance of cell membranes, disrupting the proton motive force, electron flow, active transport, and coagulation of intracellular contents **(Denyer & Hugo, 1991; Burt, 2004)**.

Preliminary tolerance experiments carried out on *C. idella* fingerlings were performed to determine the effectiveness, exposure time, and safety margins of the designated crude clove and thyme doses (concentrations) that could be used as treatments for experimentally induced saprolegniosis by selected molds. The obtained results showed toxic levels of clove at concentrations of 1 and 0.5g/ L after 24 and 48hrs of prolonged bath, respectively. The picture patterns did not look different with thyme at concentrations of 2 and 1g/ L after elapsing the same time of exposure. The toxic effect of clove at those concentrations may originate from the anesthetic activities on the exposed fish. These results may be explained by what were noted in the reports of **Javahery *et al.* (2012)** who declared that, clove EO is a highly lipophilic substance and therefore rapidly penetrates into the gill epithelium, and once it reaches the blood circulation, it is absorbed by body tissues such as those of fat and brain. They added that, with prolonged exposure, the light anesthetic effect is gradually converted into a deep one, and finally euthanasia occur. Concerning mortality occurred by using both high and low doses of thyme, the possible explanation may be related to the excess foam formation on the water surface of experimental aquaria in parallel with thyme anesthetic action. These results were clear when thyme was used at concentrations of 0.5 and, and to a lesser extent, at 0.8g/ L. Additionally, it was reported that, foam could be originated from the chemical impurities included in the herb's crude form such as saponin **(Makkar *et al.*, 2007; Fayad *et al.*, 2013)**. Saponins have been reported to be highly toxic to fish at high doses and/or prolonged exposure time because of their damaging effect on the gill's epithelia **(Roy & Munshi, 1989; Roy *et al.*, 1990)**.

Given the generated results of tolerance experiments of herbs, doses and exposure time periods were determined for conducting *in vivo* saprolegniosis-controlling experiments. As a result, both 0.25 and 0.5g/ L of crude clove possessed satisfactory control levels against artificial infection by both selected water molds, with an exception. The exception was that the former dose could be safely applied until the experimental period ended, while the later one was applied only for less than 24hrs, to avoid falling fingerlings in drawbacks of anesthesia. The potential effects of the clove aqueous extract could have emerged from eugenol and eugenol acetate, which represented the most effective antimycotic constituents of clove extract **(Hussein *et al.*, 2000; Hu *et al.*, 2013)**. In contrast, clove at 0.2g/ L was not potentially enough to combat the infections with both selected fungi. On the other hand, the application of thyme selected doses concentrations showed that 0.5g/ L succeeded in controlling both fungal infections, while high doses

concentrations accused degrees of cumulative mortality, particularly with prolonged exposure periods. Toxic impurities represented in thyme crude are the possible explanation incriminated in destruction of gill's tissues and significant mortality. Therefore, using thyme at 0.5g/ L could be effective for treating *S. australis* and *A. bisexualis* infected fingerlings with prolonged exposure to a low threat of impurities toxicity. It could be noticed that, the concentration of 0.5g/ L clove had a better curable effect in comparison with that of the thyme. The antifungal activities of clove against *A. bisexualis* were stronger than those recorded on *S. australis*, which could be related to different virulence factors of *S. australis* **(Shahbazian *et al.*, 2010; Vega-Ramírez *et al.*, 2013)**. Additionally, the inferior effect of thyme could be a result of the presence of a lesser amount of eugenol **(Committee on Herbal Medicinal Products, 2013; Hina *et al.*, 2013; Taheri *et al.*, 2014)** and a high concentration of chemical impurities that hinder thymol and/or carvacrol from being in an active form and acting effectively against both fungal strains **(Shyamapada & Manisha, 2016)**.

CONCLUSION

The present research paper identified the possible antifungal efficacies of clove and thyme, either in their crude forms or aqueous extracts against *S. australis* and *A. bisexualis*, nominating clove as a strong one. The herbs notably demonstrated a potential for susceptibility as an alternative to chemotherapeutics in the management of saprolegniosis. For the preservation of *C. idella* eggs during artificial incubation, the recommended crude herb doses are 1 and 4g/ L for clove and thyme, respectively, and 2 and 8g/ L for better fecundity and hatchability percentages. While, the recommended doses for the treatment of *C. idella* fingerlings are 0.25– 0.5g/ L for crude clove and 0.5g/ L for thyme extracts.

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