JKAU: Mar. Sci., **Vol. 31** No. 2 pp: 55-65 (1442 A.H. / 2021 A.D.)

DOI :10.4197/Mar.31-2.5

Effect of Disinfectants on Sea Cucumber Juveniles (*Holothuria scabra*) in Farming Practices

Mohammed Broom*, Sathianeson Satheesh, Mamdouh Al-Harbi and **Mohamed H. Gabr**

Department of Marine Biology, Faculty of Marine Sciences, King Abdulaziz University, Jeddah, Saudi Arabia

* mbroom1982@kau.edu.sa

Abstract. The rapid development of the intensive sea cucumber industry poses the appearance of potential threats, which makes it necessary to develop safe concentrations of common disinfectants used in aquaculture for controlling pathogens or infestation of predators. In this study, the effect of dip immersion and prolonged exposure of disinfectants on sea cucumber was investigated in order to formulate the optimum treatment concentrations. Acetic acid, formalin-F™, copper sulphate, methylene blue, trichlorfon, potassium permanganate and dechlorinated freshwater were used as disinfectants. The effects such as evisceration, skin lesion and mortality were determined during exposure and post-exposure. The 1-min dip immersion of all chemicals used in the study was safe for disinfectant treatments and did not show any negative impact on sea cucumbers. The effect of prolonged exposure varied based on chemical toxicity, chemical concentration, and duration of exposure. Sea cucumber was less sensitive to acetic acid and trichlorfon for prolonged treatment and tolerates a wide range of safe concentrations up to 100 mg l^{-1} for acetic acid and 4 mg l^{-1} for trichlorfon. Sea cucumber was more sensitive to prolonged exposure to formalin, copper sulphate and methylene blue which caused severe effects. The upper-level concentrations which considered safe and did not show significant effect were 1 mg l^{-1}, 0.05 mg l^{-1} and 4 mg l^{-1} for formalin, copper sulphate and methylene blue respectively. Potassium permanganate at 2 mg l^{-1} was very toxic to the sea cucumber. Freshwater bath for 1 and 10 minutes considered safe treatments for sea cucumber and no negative impact was observed. The study concluded that short dip immersion is a potential treatment for sea cucumber and considered safe to control the external pathogens and predators. Prolonged treatment with freshwater, acetic acid and trichlorfon could also be positively considered for sea cucumber treatment due to low sensitivity. Even though some of the disinfectants used in this study were toxic, they can be used at lower concentrations.

Keywords: Aquaculture, Sea cucumbers, Echinoderms, Disease control, Pond management.

1. Introduction

The populations of sea cucumber (*Holothuria scabra*) are threatened by the depletion by artisanal fisheries due to the high price for human consumption and their precious nature (Choo, 2008). Therefore, since the first application of artificial sea cucumber breeding techniques in the 1980s, attempts have made to establish and refine the breeding protocols (Wang *et al*., 2004). The massive development and entrenchment of sea cucumber cultivation have resulted in the emergence of multiple diseases, causing significant economic losses for aquaculture and being one of the constraints of the industry's sustainable production (Michael and Kelly 2015). Furthermore, many parasites such as protozoans, turbellarians and gastropods besides to pathogenic bacteria and fungi emerge in the system when growing the sea cucumber in outdoor ponds and cause ulcers, skin erosion, and many diseases that contribute to high mortality, which can exceed 80 percentage of the yield (Jangoux, 1987, 1990;

Smith, 1984; Wang *et al*., 2004). Snails can attack sea cucumbers causing death with eviscerated animals. Depending on the severity of the injury, the healing process might take one to several weeks (Tresnati *et al*., 2019). Evisceration is a type of autotomy performed by many holothurian echinoderms which include ejection of the digestive tract and other internal organs (Emson and Wilkie 1980). Evisceration was found to be a response to artificial stimuli such as body injury, overcrowding or water pollution (Wilkie, 2001).

Many disinfectant compounds are used in fish culture ponds to reduce disease-causing parasites and pathogen bacteria (Jo and Andrew, 2007). In fish farming, formalin is used as disinfection to destroy parasites such as monogeneans and ciliated protozoans or it may be used for ammonia elimination and plankton killing at 25-40 ppm (Leal *et al*., 2018). On the other hand, potassium permanganate ($KMnO_4$) at 5 ppm is the most widely used inorganic compounds globally for the treatment of external parasites and bacterial disease which helps the healing of wounds (Duncan, 1978). Moreover, some compounds such as methylene blue, trichlorfon and potassium nitrate, etc., are also used in disinfection and cause considerable toxicity when their concentrations rise (Claude and Laurence, 1999; Tonguthai, 2000; Park *et al.*, 2019). The attack of predators and disease outbreaks were one of the main issues of sea cucumber *Holothuria scabra* production in the outdoor ponds (Tresnati *et al*., 2019).

Previous studies discussed the effect of different chemical disinfectants such as acetic acid (Forrest *et al*., 2007; Locke *et al*., 2009; Meira-Filho *et al*., 2017), copper sulphate (Bambang *et al*., 1995; Chen and Lin, 2001), formalin (Speare *et al*., 1997; Buchmann *et al*., 2004; Lamela *et al*., 2008), methylene blue (Turnipseed *et al*., 1997; Park *et al*., 2019), potassium permanganate (Duncan, 1978; Mischke *et al*., 2014) and trichlorfon (Meyer 1966; Flores-Nava and Vizcarra-Quiroz, 1988) on aquatic organisms. Therefore, the main objective of this study was to determine the safe concentration of different common disinfectant used in aquaculture on farmed sea cucumber *Holothuria scabra*.

2. Material and Methods

2.1 Animal Source and Acclimatization

The experiment was conducted in the fish farm at the Faculty of Marine Sciences, King Abdulaziz University, Jeddah, Saudi Arabia under lab-controlled conditions. Sea cucumber juveniles (average body weight: 35±5g) were obtained from the National Aquaculture Group, Saudi Arabia. Animals were transported in a 300L tub to the experimental site with filtered (10 microns) seawater (40 psu salinity, temperature 26°C) with oxygen supply. Animals were acclimatized in a fiberglass tank of 1.9 m diameter for one-week period (40 psu salinity, 7.9 pH and temperature 29°C). The bottom of the tank was covered with a substrate of 5 cm dune sand. Water exchange was carried out daily at 100% during the acclimatization period and animals were fed with a compound diet (14.5% protein, 4.3% lipid, 38% ash and 2395 kcal kg^{-1} energy) at the ratio of 2% of body weight.

2.2 Setup of the Experiment

The effect of seven treatments on sea cucumber was investigated. Each treatment was conducted in triplicates using a 250L fiberglass tank (Fig.1). All buckets were stocked with four sea cucumbers each with an average body weight of 35±5g. The treatments tested in the experiment were Acetic acid (CH_3COOH- 99.8%), Formalin-F™ (37%), Copper Sulphate ($CuSO_4.5H_2O$- 25%), Methylene blue ($C_{16}H_{18}ClN_3S.xH_2O$- 85%), Trichlorfon (2, 2, 2 trichloro-1-hydroxyethylphosphonate- 94%), Potassium permanganate ($KMnO_4$- 97%) and dechlorinated freshwater. Each chemical treatment was examined in dip immersion for a minute of exposure and prolonged exposure for 48 hours except the freshwater treatment which was conducted for a dip immersion of one and ten minutes. Animals were starved for 24 hours prior to conducting the treatments exposure. Upon the completion of each treatment, survived treated animals from both dip and prolonged exposure were washed and transferred to post-exposure observation tanks of 500L covered with 5cm dune sand for a period of 168 hours (7 days). Mortality, skin lesion and evisceration were observed during the exposure and post-exposure period. No feeding was given to animals during the exposure period whereas animals were fed in recovery post-exposure tanks at the rate of 2% after 24 hours of transfer. Observations in the post-exposure tanks were recorded every 24 hours and Dead animals were removed. The observation factors as mortality, skin lesion and evisceration were converted to percentage from the total number of animals in the tank. The selected chemicals are usually used in the aquaculture sector for pathogen removal at the concentrations reported by Noga (2010). However, higher chemical concentrations were tested in this study to determine the toxicity on sea cucumbers.

3. Results

Short dip immersion of studied treatments showed no mortality, skin lesion and evisceration on sea cucumber during exposure period or during the post-exposure period (Table 1). The results of prolonged chemical toxicity on sea cucumber showed various abnormal behaviours during exposure as a sign of stress such as rolling motion, size shrinking and at severely affected stage evisceration of intense and various levels of skin lesion (light to high) on the dorsal and ventral side of Sandfish were observed. Acetic acid did not cause any harmful effect on sea cucumber up to 75 mg l^{-1} whereas a noticeable increase of skin lesion was observed with a rise of concentrations at 100, 125 and 150 mg l^{-1} which recorded 10, 50, 100%, respectively. Mass mortality of 100% was noticed in sea cucumber when exposed to 150 mg l^{-1} acetic for 48 hours.

The formalin has shown varied toxicity effects on the sea cucumber. The formalin at 1 mg l^{-1} was very safe whereas 5 mg l^{-1} showed 100% skin lesion (12 animals) and 50% evisceration (6 animals). The concentration of 10 and 20 mg l^{-1} formalin was very toxic and recorded 100% (12 animals) mortality with 10 mg l^{-1} at 48 hours and with 20 mg l^{-1} at 24 hours of exposure. The high concentration of formalin showed skin burn after 8 hours of exposure (Fig. 2). Copper sulphate of 0.05 mg l^{-1} did not show any effect on sea cucumber whereas 0.06 mg l^{-1} resulted in 50% mortality during the post-exposure period. Effects on sea cucumber appeared to increase with a rise of copper sulphate concentration. Copper sulphate 0.08 mg l^{-1} showed 25% mortality at 48 hours of exposure and rose to 100% during the post-exposure period. The concentrations of 0.1 and 0.2 mg l^{-1} resulted in 100% mortality in sea cucumber at 24 and 48 hours, respectively.

Methylene blue at 2 mg l^{-1} was safe and did not show any effect on sea cucumber whereas 4 mg l^{-1} showed 25% evisceration at 48 hours of exposure without any mortality during exposure or post-exposure. The concentrations of 5 and 6 ppm copper sulphate were highly toxic and showed a severe effect of skin lesion and evisceration and resulted in 50% and 100% at 48 hours of exposure with 5 and 6 mg l^{-1}. Trichlorfon concentrations at 2, 3 and 4 mg l^{-1} did not record any mortality during treatment or post-exposure period. A slight effect was noticed in 4 mg l^{-1} resulted in 11% evisceration during the post-exposure period whereas potassium permanganate at 2 mg l^{-1} was very toxic and showed 100% mortality, skin lesion and evisceration at 48 hours exposure period. Further, the freshwater treatment bath in 1 and 10 minutes did not show any effect on sea cucumber health or survival during exposure or post- treatment.

4. Discussion

The global rapid growth of sea cucumber intensive farming necessitated to investigate about the toxicity of some common chemical disinfectants and to develop safe concentrations for use to control pathogens or infestation of predators. Previous studies reported the outbreak of diseases on sea cucumbers resulted in significant mortality such as viral diseases (Yin-Geng *et al*., 2005; Wang *et al*., 2007; Deng *et al*., 2008), bacterial diseases (Ma *et al*., 2006; Deng *et al*., 2009; Lu *et al*., 2015; Zhang *et al*., 2015), fungal diseases (Yin-Geng *et al*., 2005) predatory of copepods and snails (Liu *et al*., 2002; Tresnati *et al*., 2019).

Sea cucumber exposed to chemical disinfectants showed extreme mortality. However, the mortality occurred within the groups exposed to the high concentration and this could be due to the ability of acclimation reaction in sea cucumber under the chronic metal exposure (Li *et al*., 2016). It was reported by Tresnati *et al*. (2019) that the small skin lesions due to the effect of predators on sea cucumber *Holothuria scabra* can be healed in a week and larger could take longer period (2-3 weeks) for complete healing whereas eviscerated sea cucumber was unable to accumulate the energy needs for regenerating the internal organs and healing the body lesions. It was revealed that eviscerated sandfish *Holothuria scabra* can regenerate internal organs after viscera ejection and the development could take 30 days (Dolmatov *et al*., 2012).

In the current study, acetic acid for prolonged bath up to 125 mg l^{-1} for 48 hours exposure period was safe for treating the sea cucumber. Previous studies reported the use of acetic acid as anti-parasitic using different protocols of concentrations and exposure time. Harms (1996) recommend the concentration of 500 mg l^{-1} for 30 seconds for the elimination of protozoa. It was concluded by Meira-Filho (2017) that a bath of glacial acetic acid for one hour at 238 and 467 mg l^{-1} is effective against Ciliophora protozoans and the registered mortality in exposed fish juveniles *Mugil liza* was 0 and 5%, respectively. Another study reported that acute toxicity (96 hours) in sand shrimp showed no mortality up to the exposure of 50 mg l^{-1} acetic acid and 100% mortality was recorded at 100 mg l^{-1} whereas chronic toxicity (14 days) showed 15% and 100% mortality in sand shrimp when exposed to 100 mg l^{-1} and 320 mg l^{-1} acetic acid (Locke *et al*., 2009).

The result of the current study indicates that sea cucumber is sensitive to formalin. Although dip immersion at 250 mg l^{-1} for 10 min did not affect the sea cucumber, a high effect has appeared on sea cucumber when exposed to formalin for 48 hours resulting in 100% evisceration at exposure of 5 mg l^{-1} and 100% mortality at 10 mg l^{-1}. It was revealed that formalin is used in aquaculture for water treatment and to kill the infectious agents as prophylactic or with therapeutic measure and found to be highly successful against most protozoan parasites and monogenetic trematodes (FDA, 1995; Francis-Floyd, 1996; Shao, 2001; Leal *et al*., 2018). The recommended dose of formalin for short dip treatment is up to 250 mg l^{-1} for 1-hour bath (Scott, 1993; Francis Floyd, 1996; Shepherd and Bromage, 2001). Previous studies on olive flounder *Paralichthys olivaceus* fingerlings registered the lethal concentration (LC_{50}) of 182 mg l^{-1} at 48 hours and 141 mg l^{-1} at 96 hours (Jung and Kim 1998). The mean lethal concentration reported in adult shrimp *Streptocephalus seali* was between 15 and 25 mg l^{-1} (Moss 1978).

Copper sulphate is commonly used in shrimp ponds for the elimination of filamentous algae and reducing the abundance of phytoplankton, and in the fish, culture ponds, for treatment of protozoan ectoparasites (Chen and Lin, 2001; Noga, 2010). The dip immersion as well as the prolonged exposure at 0.05 mg l^{-1} for 48 hours of copper sulphate in the current study did not show any harmful effect on sea cucumber, whereas higher concentrations were very toxic to sea cucumbers and caused 25% and 100% mortality when exposed to for 48 hours at 0.08 and 0.1 mg l^{-1}, respectively. It is worth noting that to kill parasites, free copper ion levels must be held between 0.15 and 0.20 ppm (Noga, 2010). It was reported by Chen and Lin (2001) that copper sulphate has a greater lethal effect at lower salinity and the mean lethal LC_{50} at 48 hours exposure for shrimp juvenile *Penaeus monodon* was 6.92 and 13.15 mg l^{-1} at salinity 15 and 25 psu, respectively.

The methylene blue is applied in aquaculture to control protozoan parasites and could be used as antifungal and to reduce the incidence of bacterial and water mold infection (Turnipseed *et al*., 1997; Noga, 2010). The doses of dip immersion used in the current study were safe for sea cucumber. The 48 hours prolonged exposure up to 4 mg l^{-1} had no effect on sea cucumber while 50% and 100% mortality was recorded at the exposure of 5 and 6 mg l^{-1}, respectively. Noga (2010) recommend using 1-3 mg l^{-1} of methylene blue as prolonged immersion for treatment of ectoparasite. A previous study on the toxicity of methylene blue determined the 48 hours LC_{50} for rainbow trout was 16 ppm (Willford, 1967).

Trichlorfon is useful in eradicating ectoparasites such as trematodes, water lice and for the elimination of undesirable zooplanktonic crustaceans such as copepods (Flores-Nava and Vizcarra-Quiroz, 1988; Wang *et al*., 2005). The current study revealed that sea cucumber can tolerate trichlorfon as no mortality was detected in dip immersion as well as 48 hours of prolonged exposure up to 5 mg l^{-1}. The recommended dose of trichlorfon for dip immersion was between 2 and 5 mg l^{-1} and between 0.5 and 1 mg l^{-1} for marine organisms (Noga, 2010). The lethal toxicity of trichlorfon (48 hours LC_{50}) to fry of *Cichlasoma uwphthalmus* Giinther was 23.71 mg l^{-1} (Flores-Nava and Vizcarra-Quiroz, 1988).

Potassium permanganate is an effective external parasiticide and bactericide, particularly, for protozoan parasites and crustaceans. It is also effective for *Trichodina* or *Ambiphrya* infestations, as well as for external columnaris infections (Tucker and Robinson, 1990; Stoskopf, 1993; Noga, 2010). The current study indicated that dip immersion at 100 mg l^{-1} caused 10% mortality whereas 48 hours prolonged treatment at 2 mg l^{-1} resulted in 100% mortality. Concentration of potassium permanganate recommended is 100 mg l^{-1} for 5-10 minutes as dip immersion and 2 mg l^{-1} for prolonged treatment (Noga, 2010).

The freshwater treatment bath is used for marine ectoparasites and that can be useful with the therapy of clinical case of marine *Trichodina,* some crustaceans and other protozoans Monogenea (Langdon, 1992). The current study showed that freshwater dip had no negative effects on the sea cucumber. Further, previously Noga (2010) recommend the freshwater bath for 3-15 minutes.

Fig. 1. Setup of experimental tanks.

Fig. 2. Skin burn after 8 hours of 20 ppm formalin exposure.

Table 1. Toxicity of dip immersion (1 minute) of chemical treatments.

Treatment	Dosage (mg l^{-1})	Observation (%)	While exposure 1 minute	Post exposure 168 hours
Formalin -FTM	250	Mortality	0	0
		Skin lesion	0	0
		Evisceration	0	0
Copper Sulphate	2	Mortality	0	0
		Skin lesion	0	0
		Evisceration	0	0
	1	Mortality	0	0
		Skin lesion	0	0
		Evisceration	0	0
	0.5	Mortality	0	0
		Skin lesion	0	0
		Evisceration	0	0
Methylene blue	10	Mortality	0	0
		Skin lesion	0	0
		Evisceration	0	0
Trichlorfon	2	Mortality	0	0
		Skin lesion	0	0
		Evisceration	0	0
Potassium permanganate	100	Mortality	10	10
		Skin lesion	10	10
		Evisceration	0	0

Table 2. Toxicity of prolonged exposure (48 hours) of chemical treatments.

Treatment	Dosage (mg l^{-1})	Observation (%)	While exposure		Post exposure
			24 hours	48 hours	168 hours
Acetic acid	75	Mortality	0	0	0
		Skin lesion	0	0	0
		Evisceration	0	0	0
	100	Mortality	0	0	0
		Skin lesion	0	10	0
		Evisceration	0	0	0
	125	Mortality	0	0	0
		Skin lesion	0	50	0
		Evisceration	0	75	0
	150	Mortality	0	100	-
		Skin lesion	0	100	-
		Evisceration	50	75	-
Formalin -FTM	1	Mortality	0	0	0
		Skin lesion	0	0	0
		Evisceration	0	0	0
	5	Mortality	0	0	0
		Skin lesion	0	100	0
		Evisceration	0	50	0
	10	Mortality	0	100	-
		Skin lesion	0	50	-
		Evisceration	0	100	-
	20	Mortality	100	-	-
		Skin lesion	80	-	-
		Evisceration	100	-	-
Copper sulphate	0.05	Mortality	0	0	0
		Skin lesion	0	0	0
		Evisceration	0	0	0
	0.06	Mortality	0	0	50
		Skin lesion	0	50	0
		Evisceration	0	0	50
	0.08	Mortality	0	25	100
		Skin lesion	0	75	-
		Evisceration	0	25	-
	0.1	Mortality	60	100	-
		Skin lesion	30	70	-
		Evisceration	40	100	-
	0.2	Mortality	100	-	-
		Skin lesion	50	-	-
		Evisceration	100	-	-
Methylene blue	2	Mortality	0	0	0
		Skin lesion	0	0	0
		Evisceration	0	0	0
	4	Mortality	0	0	0
		Skin lesion	0	0	0
		Evisceration	0	25	0
	5	Mortality	25	50	0
		Skin lesion	0	0	25
		Evisceration	25	75	0
	6	Mortality	0	100	-
		Skin lesion	0	50	-
		Evisceration	25	100	-
Trichlorfon 92%	2	Mortality	0	0	0
		Skin lesion	0	0	0
		Evisceration	0	0	0
	3	Mortality	0	0	0
		Skin lesion	0	0	0
		Evisceration	0	0	0
	4	Mortality	0	0	0
		Skin lesion	0	0	0
		Evisceration	0	0	11
Potassium permanganate	2	Mortality	11	100	-
		Skin lesion	55	100	-
		Evisceration	66	100	-

5. Conclusion

Short dip immersion is a potential treatment for sea cucumber and considered safe to control the external pathogens and predators as no obvious negative impact was determined. Prolonged treatment with freshwater, acetic acid and trichlorfon could also be positively considered for sea cucumber treatment due to low sensitivity. Even though some of the disinfectants used in this study were toxic, they can be used at lower concentrations. It is recommended to develop higher concentrations for safe short dip immersion treatment and to determine the efficacy in eliminating the potential predators and pathogens.

Acknowledgements

The authors would like to express the gratitude to the Managing Director of National Aquaculture Group for the support extended. We also thank the Dean, Faculty of Marine Sciences, King Abdulaziz University, Jeddah for the facilities provided at KAU Fish Farm, Obhur.

References

Bambang, Y., Thuet, P., Charmantier-Daures, M., Trilles, J.P. and **Charmantier, G.** (1995) Effect of copper on survival and osmoregulation of various developmental stages of the shrimp *Penaeus japonicus* Bate Crustacea, Decapod. *Aquat. Toxicol*., **33**: 125–139. https://www.oieau.org/eaudoc/system/files/documents/7/36325/36325_doc.pdf

Buchmann, K., Bresciani, J. and **Jappe, C.** (2004) Effects of formalin treatment on epithelial structure and mucous cell densities in rainbow trout, *Oncorhynchus mykiss* (Walbaum), skin. *J. Fish Dis*., **27**: 99–104. https://doi.org/10.1111/j.1365-2761.2003.00519.x

Chen, J.C. and **Lin, C.H.** (2001) Toxicity of copper sulfate for survival, growth, molting and feeding of juveniles of the tiger shrimp, *Penaeus monodon*. *Aquaculture*, **192**:55-65. https://doi.org/10.1016/S0044-8486(00)00442-7

Choo, P.S. (2008) Population status, fisheries and trade of sea cucumbers in Asia. In V. Toral-Granda, A. Lovatelli and M. Vasconcellos (eds). Sea cucumbers. A global review of fisheries and trade. FAO Fish. and Aquac. Tech. Pap., 516: 81-118. https://hdl.handle.net/20.500.12348/1511

Claude, E.B. and Laurence Massaut (1999) Risks associated with the use of chemicals in pond aquaculture. *Aquac. Eng*., **20**: 113–132. https://doi.org/10.1016/S0144-8609(99)00010-2

Deng, H., He, C., Zhou, Z., Liu, C., Tan, K., Wang, N., Jiang, B., Gao, X. and **Liu, W.** (2009) Isolation and pathogenicity of pathogens from skin ulceration disease and viscera ejection syndrome of the sea cucumber *Apostichopus japonicus*. *Aquaculture* **287**: 18-27. https://doi.org/10.1016/j.aquaculture.2008.10.015

Deng, H., Zhou, Z.C. and **Han, J.B.** (2008) An observation on the pathogeny of stomach atrophy in sea cucumber *Apostichopus japonicus* (Selenka) larva by electron microscope. *J. Fish. China*, **2**: 023. https://en.cnki.com.cn/Article_en/CJFDTotal-SCKX200802023.htm

Dolmatov, I.Y., Khang, N.A. and **Kamenev, Y.O.** (2012) Asexual reproduction, evisceration, and regeneration in holothurians (Holothuroidea) from Nha Trang Bay of the South China Sea. *Russ. J. Mar. Biol*., **38**: 243-252. https://doi.org/10.1134/S1063074012030042

Duncan, T.O. (1978) The Use of Potassium Permanganate (KMnO4) in Fisheries: A literature review. National Technical Information Service, PB275397, 56. https://ntrl.ntis.gov/NTRL/dashboard/searchResults/titleDetail/PB275397.xhtml

Emson, R.H. and **Wilkie, I.C.** (1980) Fission and autotomy in echinoderms. Oceanogr. *Mar. Biol. Ann. Rev*., **18**: 155-250. http://pascal-francis.inist.fr/vibad/index.php?action=getRecordDetail&idt=PASCALZOOLINEINRA8110075348

FDA (1995*) Environmental Impact Assessment for the Use of Formalin in the Control of External Parasites on Fish. Environmental Assessments*, Food and Drug Administration, Washington, D.C.

Flores-Nava, A. and **Vizcarra-Quiroz, J.J.** (1988) Acute toxicity of trichlorfon (Dipterex) to fry of *Cichlasoma urophthalmus* Günther. *Aquacult. Fish. Manage*., **19**:341-345. https://doi.org/10.1111/j.1365-2109.1988.tb00583.x

Forrest, B.M., Hopkins, G.A., Dodgshun, T.J. and **Gardner, J.P.A.** (2007) *Efficacy of acetic acid treatments in the management of marine biofouling. Aquaculture,* 262:319-332. doi:10.1016/j.aquaculture.2006.11.006

Francis-Floyd, S.R. (1996) Use of formalin to control fish parasites. University of Florida Cooperative Extension Service, *Institute of Food and Agriculture Sciences, EDIS*, **5**: 77. https://agrilifecdn.tamu.edu/fisheries2/files/2013/09/Use-of-Formalin-to-Control-Fish-Parasites.pdf

Harms, C.A. (1996) Treatments for parasitic diseases of aquarium and ornamental fish. In Seminars. *J. Exot. Pet Med*., **2**: 54-63. https://10.1016/S1055-937X(96)80018-1

Jangoux, M. (1987) *Diseases of Echinodermata* (I). Dis. Aquat. Org., 2:147-162. http://www.int-res.com/articles/dao/2/d002p147.pdf

Jangoux, M. and **Kinne, O.** (1990) Diseases of Echinodermata. Dis. Aquat. Org., 3:439-567. https://difusion.ulb.ac.be/vufind/Record/ULB-DIPOT:oai:dipot.ulb.ac.be:2013/89815/Details

Jo, Sadler and **Andrew, Goodwin** (2007) *Disease Prevention on Fish Farms.* SRAC, No. 4703. https://freshwater-aquaculture.extension.org/wp-content/uploads/2019/08/Disease_prevention_on_fish_farms.pdf

Leal, J.F., Neves, M.G.P., Santos, E.B. and **Esteves, V.I.** (2018) Use of formalin in intensive aquaculture: properties, application and effects on fish and water quality. *Rev. Aquac*., **10**: 81-295. https://doi.org/10.1111/raq.12160

Jung, S.H. and **Kim, J.W.** (1998) The lethal concentration (LC50) and lethal time (LT50) of formalin on olive flounder fingerlings, *Paralichthy olivaceus*. *NFRDI*, 54:125–129.

Lamela, R.L., Quintana, Y.C., Silveira, C.R., Martínez, M. and **Herrate, N.G.** (2008) Effects of formalin on total haemocytes count and histopathological changes in the shrimp *Litopenaeus schmitti* (Pérez-Farfante & Kensley 1997). *Aquac. Res.*, **39**: 1316-1321. https://doi.org/10.1111/j.1365-2109.2008.01997.x

Langdon, J.S. (1992) Major protozoan and metazoan parasitic diseases of Australian finfish. In: *Fin Fish Workshop. Proceedings 182*, Post Graduate Committee in Veterinary Science, University of Sydney, Sydney, Australia, 1– 26.

Li, L., Tian, X., Yu, X. and **Dong, S.** (2016) Effects of acute and chronic heavy metal (Cu, Cd, and Zn) exposure on sea cucumbers, *Apostichopus japonicus*. *BioMed Res. Int*. https://doi.org/10.1155/2016/4532697

Liu, X., Gu, B.X. and **Zhang, X.L.** (2002) Several problems in seed-rearing of *Apostichopus japonicus* (Selenka) and corresponding activities. *Mod. Fish. Inf.*, **17**: 26-27.

Locke, A., Doe, K.G., Fairchild, W.L., Jackman, P.M. and **Reese, E.J.** (2009) Preliminary evaluation of effects of invasive tunicate management with acetic acid and calcium hydroxide on non-target marine organisms in Prince Edward Island, *Canada. Aquat. Invasions*, **4**: 221-236. https://doi: 10.3391/ai.2009.4.1.23

Lu, M., Zhang, P., Li, C., Zhang, W., Jin, C. and **Han, Q.** (2015) MiR-31 modulates coelomocytes ROS production via targeting p105 in *Vibrio splendidus* challenged sea cucumber *Apostichopus japonicus* in vitro and in vivo. *Fish Shellfish Immunol*., **45**: 293-299. https://doi.org/10.1016/j.fsi.2015.04.024

Ma, Y., Xu, G., Chang, Y., Zhang, E.P., Zhou, W. and **Song, L.S.** (2006) Bacterial pathogens of skin ulceration disease in cultured sea cucumber *Apostichopus japonicus* (Selenka) juveniles. *J. Dalian Fish Univ*., **21**:13-18.

Meira-Filho, M.R.C., Ramirez, J.R.B., Vianna, R.T. and **Júnior, J.P.** (2017) Efficacy of glacial acetic acid in the control of *Trichodina* sp. and *Apiosoma* sp. associated with Mugil liza. *Aquaculture*, **479**: 7-12. https://doi.org/10.1016/j.aquaculture.2017.05.014

Meyer, F.P. (1966) A new control for the anchor worm *Lernea cyprinacea*. *Progressive Fish-Culturist*, **28**:33-9. https://doi.org/10.1577/1548-8640(1966)28[33:ANCFTA]2.0.CO;2

Michael, J.S. and **Kelly S.B.** (2015) Diseases in marine invertebrates associated with mariculture and commercial fisheries. *J. Sea Res*., 16–32. https://doi.org/10.1016/j.seares.2016.06.001

Mischke, C.C., Griffin, M.J., Wise, D.J. and **Greenway, T.E.** (2014) Potassium permanganate is not an effective pond disinfectant to control *Dero digitata*. **J**. *World Aquac. Soc.***, 45**: 350-353. https://doi.org/10.1111/jwas.12118

Noga, Edward (2010). *Fish disease: diagnosis and treatment*. John Wiley & Sons.

Moss, J.L. (1978) Toxicity of selected chemicals to the fairy shrimp, *Streptocephalus seali*, under laboratory and field conditions. Progress. *Fish Cult*., **40**:158-160. https://doi.org/10.1577/1548-8659(1978)40[158:TOSCTT]2.0.CO;2

Park, I., Se-Won, B. and **Ki, H.M.** (2019) The Sterilization Effect of Methylene Blue, Formalin, and Iodine on Egg and Adult Stage of Marine Medaka, *Oryzias dancena*. *Dev. Reprod*., **23**:199-211. https://10.12717/DR.2019.23.3.199

Scott, P. (1993) Therapy in aquaculture. In: Brown L (ed.) *Aquaculture for Veterinarians: Fish Husb*. Med., pp. 447. https://agris.fao.org/agris-search/search.do?recordID=XF2016043746

Shao, Z.J. (2001) Aquaculture pharmaceuticals and biologicals: current perspectives and future possibilities. *Adv. Drug Deliv. Rev*., **50**: 229-243. https://doi.org/10.1016/S0169-409X(01)00159-4

Shepherd, J. and **Bromage, N.** (2001) *Intensive Fish Farming.* Blackwell Science Ltd, Oxford.

Smith, T. (1984) Ultrastructure and function of the proboscis in *Melanella alba* (Gastropoda: Eulimidae). J. Mar. Biolog. Assoc. U.K. *J Mar Biol. Assoc. Uk*., **64**: 503-512. https://doi.org/10.1017/S0025315400030198

Speare, D.J., Arsenault, G., MacNair, N. and **Powell. M.D.** (1997) Branchial lesions associated with intermittent formalin bath treatment of Atlantic salmon, *Salmo salar* L, and rainbow trout, *Oncorhynchus mykiss* (Walbaum). J. Fish Dis., 20: 27–33. https://doi.org/10.1046/j.1365-2761.1997.d01-103.x

Stoskopf, M.K. (1993) *Fish Medicine*. W. B. Saunders Company, Harcourt Brace Javanovich, Inc., Philadelphia, Pennsylvania. http://www.worldcat.org/oclc/925348013

Tonguthai, K. (2000) The use of chemicals in aquaculture in Thailand. In: J. R. Arthur, C. R. Lavilla-Pitogo, & R. P. Subasinghe (Eds.) Use of Chemicals in Aquaculture in Asia: *Proceedings of the Meeting on the Use of Chemicals in Aquaculture in Asia 20-22 May 1996, Tigbauan, Iloilo, Philippines* (pp. 207-220). Tigbauan, Iloilo, Philippines: Aquaculture Department, Southeast Asian Fisheries Development Center. http://hdl.handle.net/10862/597

Tresnati, J., Yasir, I., Aprianto, R., Yanti, A., Bestari, A.D. and **Tuwo, A.** (2019) Predators effects on mortality of sandfish *Holothuria scabra* cultured in multitrophic system. *J. Phys. Conf. Ser*., **1341**: 022-026. https://doi:10.1088/1742-6596/1341/2/022026

Tucker, C.C. and **Robinson, E.H.** (1990) *Channel catfish farming handbook*. Springer Sci. Busi. Media. https://www.springer.com/gp/book/9780412123313

Turnipseed, S.B., Roybal, J.E., Plakas, S.M., Pfenning, A.P., Hurlbut, J.A. and **Long, A.R.** (1997) Determination of Methylene Blue in Channel Catfish (*Ictalurus* *punctatus*) Tissue by Liquid Chromatography with Visible Detection. *J. AOAC Int*., **80**: 31-36. https://doi.org/10.1093/jaoac/80.1.31

Wang, Y.G., Zhang, C.Y., Rong, X.J., Chen J.J., Shi, C.Y., Sun, H.L. and **Yan, J.P.** (2004) Diseases of cultured sea cucumber (*Apostichopus japonicus*) in China. Advances in sea cucumber aquaculture and management. FAO Fish. Tech. Rep., 463:297-310. http://www.fao.org/3/y5501e/y5501e10.htm

Wang, P., Chang, Y., Yu, J., Li, C. and **Xu, G.** (2007) Acute peristome edema disease in juvenile and adult sea cucumbers *Apostichopus japonicus* (Selenka) reared in North China. *J. Invertebr. Pathol*., **96**:11-17. https://doi.org/10.1016/j.jip.2007.03.001

Wang, Y.G., Rong, X.J., Zhang, C.Y. and **Sun, S.F.** (2005) Main diseases of cultured *Apostichopus japonicus*: prevention and treatment. *Mar. Sci*., **29**: 1-7 (in Chinese with English Abstract).

Wilkie, I.C. (2001) Autotomy as a prelude to regeneration in echinoderms. *J. Microsc. Res. Tech*., **55**: 369-396. https://doi.org/10.1002/jemt.1185

Willford, W.A. (1967) Toxicity of 22 therapeutic compounds to six fishes. Invest. Fish. Control, Bur. Sport Fish. *Wildlife*, 3-10. http://www.nativefishlab.net/library/textpdf/13281.pdf

Yin-Geng, W., Chun-Yun, Z., Xiao-Jun, R., Jie-Jun, C., Cheng-Yin, S., Hui-ling, S. and **Jing-Ping, Y.** (2005) Diseases of cultured sea cucumber, *Apostichopus japonicus*, in China. FAO Fish. *Tech. Pap*., 297-310. http://www.fao.org/3/y5501e/y5501e10.htm

Zhang J., Cao, Z., Li, Z., Wang, L., Li, H., Wu, F., Jin, L., Li, X., Li, S. and **Xu, Y.** (2015) Effect of bacteriophages on Vibrio alginolyticus infection in the sea cucumber, *Apostichopus japonicus* (Selenka). *J. World Aquac. Soc*., **46**: 149-58. https://doi.org/10.1111/jwas.12177

تأثير المطهرات على زريعة خيار البحر (*Holothuria scabra*) في ممارسات الاستزراع

محمد بروم*، و**ساتيش سانتوسون**، و**ممدوح الحربي**، و**محمد جبر**

قسم الأحياء البحرية، كلية علوم البحار، جامعة الملك عبد العزيز، جدة، المملكة العربية السعودية

* mbroom1982@kau.edu.sa

المستخلص. يشكل التطور السريع لصناعة خيار البحر المكثف ظهور تهديدات محتملة، مما يجعل من الضروري تطوير تركيزات آمنة للمطهرات الشائعة المستخدمة في الاستزراع المائي للسيطرة على مسببات الأمراض أو لو أصابه المفترسات. في هذه الدراسة، تمت دراسة تأثير الغمر والتعرض المطول للمطهرات على خيار البحر لتكوين تركيزات المعالجة المثلى. تم استخدام حمض الخليك، والفورمالين– FTM، وكبريتات النحاس، والميثيلين الأزرق، والترايكلورفون، وبرمنجنات البوتاسيوم، والمياه العذبة منزوعة الكلور كمطهرات. تم تحديد التأثيرات مثل نزع الأحشاء وآفات الجلد والوفيات أثناء التعرض وبعد التعرض. كان الغمر لمدة دقيقة لجميع المواد الكيميائية المستخدمة في الدراسة آمنًا للمعالجات المطهرة، ولم يُظهر أي تأثير سلبي على خيار البحر. اختلف تأثير التعرض المطول بناءً على السمية الكيميائية والتركيز الكيميائي ومدة التعرض. كان خيار البحر أقل حساسية لحمض الأسيتيك والترايكلورفون للمعالجة المطولة، ويتحمل مجموعة كبيرة من التركيزات الآمنة تصل إلى ١٠٠ مجم لتر$^{-١}$ لحمض الأسيتيك و٤ مجم لتر$^{-١}$ للترايكلورفون. وكان خيار البحر أكثر حساسية للتعرض المطول للفورمالين، وكبريتات النحاس، والميثيلين الأزرق، مما تسبب في تأثيرات شديدة. وكانت تركيزات المستوى الأعلى التي تعتبر آمنة ولم تظهر تأثيرًا معنويًا هي: ١ مجم لتر$^{-١}$، و٠,٠٥ مجم لتر$^{-١}$، و٤ مجم لتر$^{-١}$ للفورمالين وكبريتات النحاس والميثيلين الأزرق على التوالي. وكانت برمنجنات البوتاسيوم عند تركيز ٢ ملجم لتر$^{-١}$ شديدة السمية لخيار البحر. ويعتبر حمام المياه العذبة لمدة ١ و١٠ دقائق أحد العلاجات الآمنة لخيار البحر، ولم يلاحظ أي تأثير سلبي. وخلصت الدراسة إلى أن الغمر بالغطس القصير هو علاج محتمل لخيار البحر، ويعتبر آمنًا للسيطرة على مسببات الأمراض الخارجية وإصابات الحيوانات المفترسة. ويمكن أيضًا النظر بشكل إيجابي في المعالجة المطولة بالمياه العذبة، وحمض الأسيتيك، والترايكلورفون، لمعالجة خيار البحر بسبب الحساسية المنخفضة. وعلى الرغم من أن بعض المطهرات المستخدمة في هذه الدراسة كانت سامة، إلا أنه يمكن استخدامها بتركيزات أقل.

الكلمات المفتاحية: تربية الأحياء المائية، خيار البحر، شوكيات الجلد، مكافحة الأمراض، إدارة الأحواض.