Contents

Co-management Feasible in Achieving Fisheries Sustainability? A Case of Oman's Lobster Fishery

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هل الادارة التشاركية مجدية لتحقيق استدامة المصايد: دراسة حالة لمصايد الشارخة في سلطنة عمان

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ABSTRACT. This study assesses the feasibility of co-management arrangements in managing scalloped spiny lobster (*Panulirus homarus*) fishery in the Sultanate of Oman. The appraisal is conducted using various indicators involving pre-implementation phase and institutional characteristics and substantiated by findings of the review of relevant local and global literature and the results of a structured questionnaire survey conducted during 9-28 June, 2013 with 536 randomly selected fishers from three Governorates namely Al-Sharqiyah South, Al-Wusta, and Dhofar. The former method helps identifying suitable assessment criteria and provides indication of readiness while the later method helps determining primary stakeholders' perceived views on various aspects of regulatory measures and co-management arrangements. While findings related to fishers' commitments, willingness to support government decisions, willingness to participate in co-operative research, and preference for co-operative decision-making are promising, institutional weaknesses identified under the category of 'interactive organization', 'resource ownership and control', 'existence of planned process', and 'diversity' should be of major concerns for policy-makers in moving towards co-management arrangements. Based on the findings some policy implications are also discussed.

KEYWORDS: co-management; fisheries sustainability; lobster fishery; Oman.

المستخلص: تقيّم هذه الدراسة جدوى الإدارة التشاركية في إدارة مصايد شارخة الصخور (*Panulirus homarus*) في سلطنة عمان. تم إجراء التقييم باستخدام العديد من المؤشرات شملت مرحلة ما قبل التنفيذ وخصائص المؤسسة مدّعمة بمخرجات الدراسات المرجعية المحلية والعالمية ذات الصلة ونتائج استبيان أجري خلال الفترة من ٩ الى ٢٨ يونيو ٢٠١٣ مع ٥٣٦ صياداً تم اختيارهم عشوائياً من ثلاث محافظات هي: جنوب الشرقية، والوسطى، وظفار. ساعدت المنهجية الاولى في تحديد معايير التقييم المناسبة كما وفرت مؤشرًا على مدى الاستعداد، بينما ساعدت المنهجية اللاحقة في تحديد وجهات نظر أصحاب المصلحة الرئيسيين حول الجوانب المختلفة للتدابير التنظيمية وترتيبات الإدارة التشاركية. وعلى الرغم من أن النتائج المتعلقة بتقيّد الصيادين، والرغبة في دعم القرارات الحكومية، والرغبة في المشاركة في البحوث التعاونية، وتفضيل اتخاذ القرارات بصورة تعاونية هي قضايا واعدة، الا ان الضعف المؤسسي المحدد تحت فئة «المؤسسة المتفاعلة»، و «ملكية الموارد والتحكم بها «، و «وجود خطة للعمليات» و «التنوع» يجب ان تكون أكبر اهتمامات صانعي السياسات في الاتجاه نحو ترتيبات الإدارة التشاركية. في ضوء المخرجات تم ايضاً مناقشة بعض الآثار المترتبة على السياسات.

الكلمات المفتاحية: الإدارة التشاركية، استدامة المصايد، مصايد الشارخة، عمان

Introduction

The 2018 Food and Agriculture Organization of the United Nations (FAO) report on the state of world fisheries and aquaculture highlighted the critical importance of fisheries and aquaculture sector to the global economy and well-being of coastal communities through providing employment, food and nutri-

tional security, and income and livelihoods (FAO, 2018). It is also well-recognized that small-scale fisheries (SSF) that predominate in developing countries constitute a significant part of the global fisheries sector and are critical for socio-economic well-being and food security of the world's most vulnerable communities (Schuhbauer and Sumaila, 2016; Jentoft, 2014; Teh et al. 2011). For instance, Teh & Sumaila (2013) estimated that SSF employ about 22 million fishers (i.e. about 44% of all fishers) engaged in harvesting sector. In a consultative meeting organized by FAO in co-operation with the Ministry of Agriculture and Fisheries (hereafter, MAF) held in Muscat, Oman, March 26-28, 2012, attainment of sustainability in small-scale fisheries in the Near East and North Africa region was highlighted with the purpose of bringing together responsible fisheries and social devel-

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opment (FAO, 2012).

However, SSF in developing countries including the Sultanate of Oman (hereafter, Oman) are usually characterized by the problems of overfishing, overcapacity, non-compliance, weak enforcement, economic inefficiency (Al Siyabi & Bose 2018; FAO 2017; Pomeroy, 2012; Salas et al. 2007). In Oman, the socio-economic significance of the fisheries sector in the country's economic diversification policies and sustainable development drive is well-echoed in the national policy agenda (MNE, 2007a; MNE, 2007b; Bose et al. 2010). For instance, to enhance achievement of economic diversification policy objectives. The fisheries sector has been identified as one of the five promising sectors in the 9th Five-Year Plan (2016-2020) and the sector is expected to achieve an average growth rate of 6.5% (in constant prices) and the share in GDP is expected to rise from 0.5% to 0.6% (SCP, 2016).

Accordingly, considerable efforts have been directed by government agencies in Oman to ensure sustainable utilization of fisheries resources and to maximize socio-economic benefits from the sector (MNE, 2007b). Despite such efforts, some challenges such as overfishing of high value commercial species such as kingfish (Al-Oufi et al. 2004; Al-Balushi et al. 2016) and lobster (Al-Haddabi, 2010), inefficiency in fishing operations (Al-Siyabi and Bose, 2018), weak enforcement and the presence of non-compliance (Al-Subhi et al., 2013; Bose et al., 2017) etc. are still confronting the sector and adversely impacting on the progress towards sustainability in fisheries.

 To address these challenges the implementation of co-management approach to effectively manage SSF has been advocated since the late 1980s by the scholars in the field (Jentoft, 1989; Pomeroy, 1995; Sen and Nielsen, 1996; McCay & Jentoft, 1996; Pomeroy & Berkes, 1997; Jentoft, et al. 1998; Jentoft, 2004; Al-Habsi, 2012; Kalikoski & Franz, 2014; to name a few). It is noted that routinely ascribed characteristics such as undemocratic, unfair, and ineffective to the top-down-management approach to fisheries management (i.e. command-and-control approach) form the basis for joint action or co-management (Fiorino, 1990; Berkes, 2009).

Co-management has been touted in fisheries management with the expectation to: 1) encourage democratic participation of resource user-groups in regulatory decision-making that helps advancing equity, legitimacy and efficiency (Nielsen et al. 2004; Al-Balushi et al., 2016), 2) improve rule compliance and hence regulatory effectiveness (Pomeroy & Berkes, 1997; Al-Subhi et al. 2013), 3) empower local community (Nielsen et al. 2004) and boost fishers' competence by upgrading their skills through training and through the formation of social capital (for example, fishers' social networks) (Rydin & Pennington, 2000; Schusler, et al. 2003; Nenadovic & Epstein, 2016), 4) foster mutual respect and hence cooperation (Bose & Cress-Morris, 2009), 5) minimize

transaction costs (Abdullah et al. 1998), 6) provide basis for the integration of scientific knowledge with fishers' knowledge and hence improved management (Mackinson, 2001; Bergmann et al. 2004; Hartley & Robertson, 2009), and 7) help broaden decision makers' understanding of user-groups' perceptions and attitudes that improves regulatory effectiveness and the successful implementation of policies (Mackinson, 2001; Jentoft & McCay 1995).

Recognizing these potentials, Al-Oufi (2002), Al-Habsi (2012), Al-Subhi et al. (2013), Al-Balushi et al. (2016) and Bose et al. (2017) advocated the approach to local fisheries management. The encouragement and development of fisheries co-management approach in managing fisheries resources in Oman is also echoed in the development of long-term strategic direction and policy recommendations for the sector by international experts (WB & MAF, 2015). Although, community-based management approach in the form of traditional committee (locally termed as 'Sunat Al-Bahar' Committee) has existed in Oman, the functioning of such committee has diminished over time.(Al-Oufi et al. (2000); Al-Subhi et al.2013). The top-down management approach is more evident in Oman and fishers' participation in decision-making is not well-articulated. While, research on co-management in fisheries was conducted with geographical intent (Al-Oufi, 2002, Al-Habsi, 2012), the feasibility of such management approach has not received adequate attention.

Given this background and considering the strategic importance of the fisheries sector, the main objective of this case study was to assess the feasibility and desirability of co-management approach to the management of the scalloped spiny lobster (*Panulirus homarus*) fishery in Oman. The main reasons for considering lobster fishery are two-fold: first, the fishery has been experiencing a prolonged period of high fishing pressure (Fig. 2). The decline in total landings has caused legitimate concern among policy makers, scientists, and fishers about its possible socio-economic consequences. Second, lobster is one of the high-valued species in Oman and the fishery provides significant employment and income opportunities to the traditional fishing communities.

This research has significance in terms of academic, industry and policy perspectives. While various country-specific studies have been conducted on this approach, however, research on this topic in Oman is scarce, and to this end, this paper not only fills the existing knowledge gap in local research but also complements the existing global literature by adding country-specific case study. The documentation and dissemination of country specific experience have been encouraged by Nielsen et al. (2004) to guide others. Furthermore, by presenting barriers and opportunities for moving towards partnership approach this study contributes to the practical realm as well, as it provides policy makers and fishery managers of local and global

origins with knowledge that has the potential to assist in designing effective strategies.

Co-management: pros and cons

Co-management was initially considered as a power-sharing arrangement between the management authority and a community of resource users and the concept has been evolved over time with the experience from the field (Sen & Nielsen, 1996; Pomeroy & Berkes, 1997). For instance, Berkes (2009) branded co-management as a process/mechanism of determining power sharing, building institutions, building trust and social capital, solving problems, and as people-centered governance approach.

The emerging interest in the co-management of natural resources has a sound theoretical basis as it has deep roots in public-choice and social-choice theories (Sen, 1995; Ostrom, 1998; Jentoft, et al. 1998). The contrast between these two theoretical postulates is that the former is based on individual rationality where - as 'homo economicus'—individual behaviour is based on self-interests, while the later promotes an integrated social preference from diverse individual preferences (Sen, 1995). Fisheries managers and policy makers are already aware of the problems caused by biological and economic overfishing, stock depletion, and user-group conflicts associated with open access fisheries (Gordon, 1954; Seabright, 1993). However, with regard to the effective management of common property resources like fisheries (Gordon, 1954) a dichotomy of opinion prevails in the existing literature. One group advocated government ownership or privatization to sustain commons. For instance, to sustain commons Hardin (1968) suggested for some sort of mutually agreed upon coercion involving the government to solve 'the tragedy of commons'. Linked to this proposition, economists argue that adoption of private property rights would results in conservation and economically efficient use of common property resources as the internal benefits and costs accrue to the owner (Schlager & Ostrom, 1992; Ostorm, 2003).

However, opponents (mostly anthropologists and social scientists) argued that this proposition is not cross-culturally valid and have promoted effective local institution by resource users as a way to avoid the 'tragedy' (Acheson, 1989; Ostrom, 1990; Agarwal, 2001). By drawing a distinction between 'common-property' and 'communally owned resources' and citing a number of cases, Acheson (1989) pointed out that communally owned property is not necessarily subject to overexploitation as property owned communally does have an owner after all. Here lies the idea of collective action or co-management as local level management system can co-exist with the centralized institution involving government and avoid 'tragedy' of commons. It is argued that under some circumstances local-level communities can generate institutions and rules to manage their resources called 'collective' or 'public good' (Ostrom, 1990). Interested readers are referred to Plummer & Fitzgibbon (2004) for further details on a conceptual framework developed through blending theory and practice on co-management.

Despite these potential benefits, some limitations of co-management approach are also documented in the literature. It is argued that no self-interested individuals would join a collective effort when there are incentives generated by externally enforced rules (address free-rider problem) (Ostorm, 2000). Another is the dilemma for legitimacy when representatives have fixed mandates and a system based on personal representation that is, fishers' cooperatives may represent only members' interests (Jentoft, 2000). Next is the accountability and motivation, which are related to human dimension (Grafton, 2007). It is worth mentioning that in reviewing fisheries governance mechanisms involving countries from South East Asia and Southern Africa, Nielsen et al. (2004) provided evidence of mixed results of co-management approach. Similar view of mixed results is also recognized in Cinner et al. (2012).

Case study background

In Oman, the lobster fishery is located in three Governorates namely Al-Sharqiyah South, Al-Wusta, and Dhofar (see Figure 1). The governorate Dhofar was dominant in terms of landings (67.8%) followed by Al-Wusta (25.8%) and Al-Sharqiyah South (6.4%) (MAF, 2012).
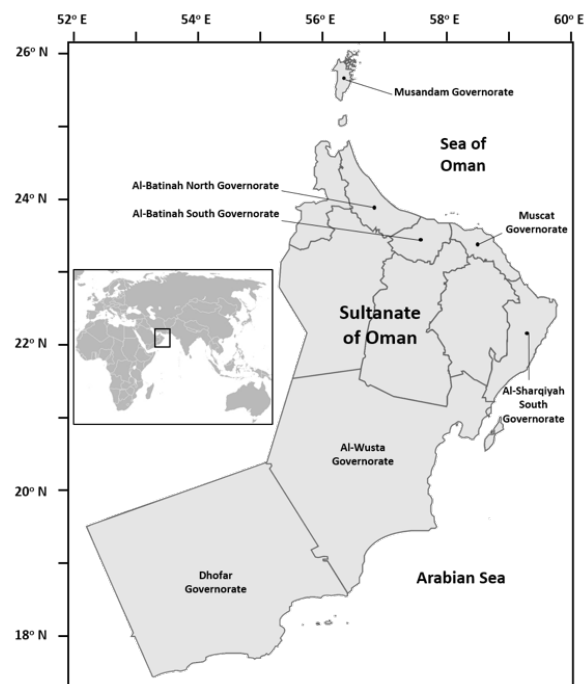
Figure 1. Map of the Sultanate of Oman with study sites
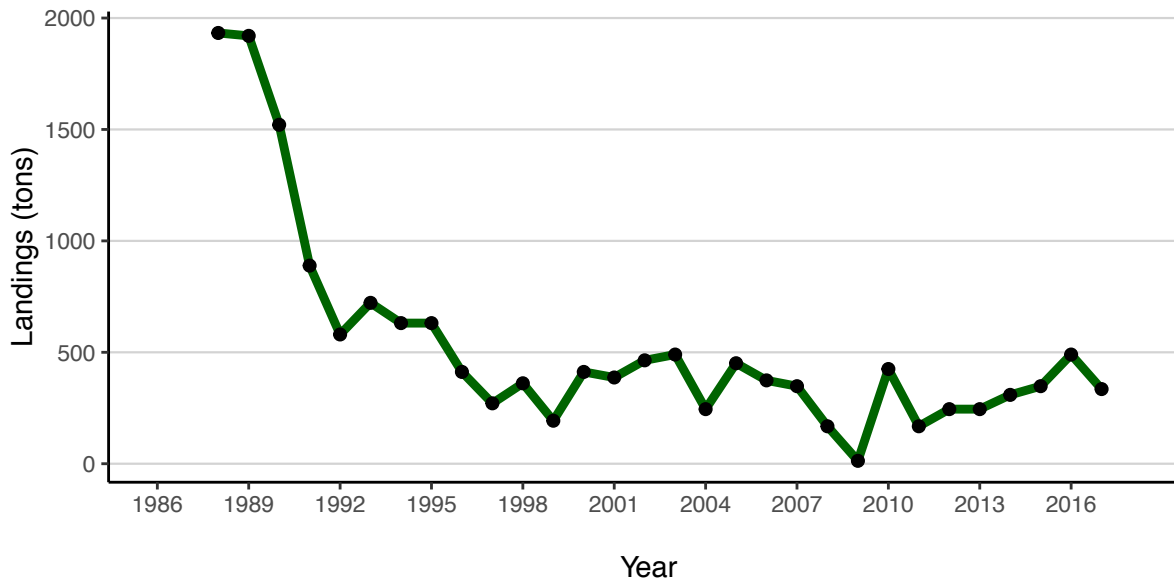
Figure 2. Total landings of lobster (mt) from 1988 to 2017.
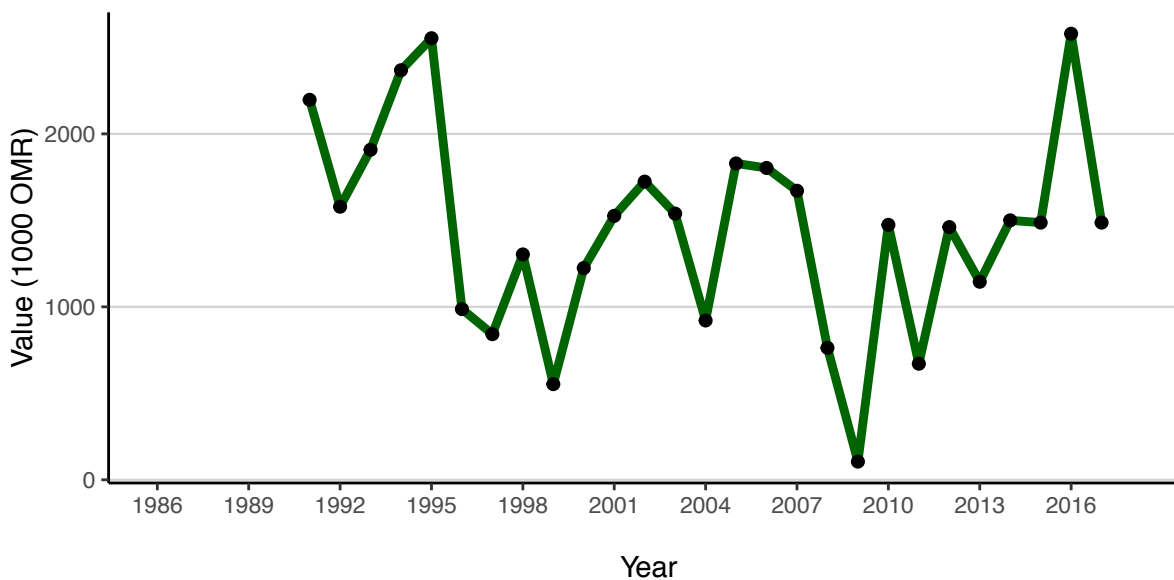
Figure 3. Gross value ('000 OMR) of lobster landings during 1991-2017.

The fishery is regulated under the Marine Fishing and Living Aquatic Resources Protection Law and its Executive Regulations. Besides other relevant articles, Article 14 of the Marine Fishing and Living Aquatic Resources Protection Law that is specific to the lobster fishery includes the following elements: 1) prohibition of lobster catching during the breeding and reproduction season, 2) harvesting lobster shall be by lobster traps with specifications determined by the competent authority, 3) prohibition of catching or handling egg bearing lobster, and lobster with carapace length less than 80 mm, 4) prohibition of processing or dealing in lobster during the breeding and reproductive seasons, and 5) amount

of lobsters in possession must be registered at the end of each season with the competent authority.

Since 1986, a number of Ministerial Decisions were issued related to the fishing season in the lobster fishery (Al-Haddabi, 2010). Non-compliance with the regulations includes penalties such as fine (fixed amount and doubled with recurrence of offense), imprisonment, confiscation of catch, gear, boat and license (for a period or permanently) based on court's decisions (Al-Haddabi, 2010).

Artisanal fishers have been using lobster trap, gill-nets and tangle nets to harvest lobster with nets being the dominant fishing gear. This suggests that the regula-
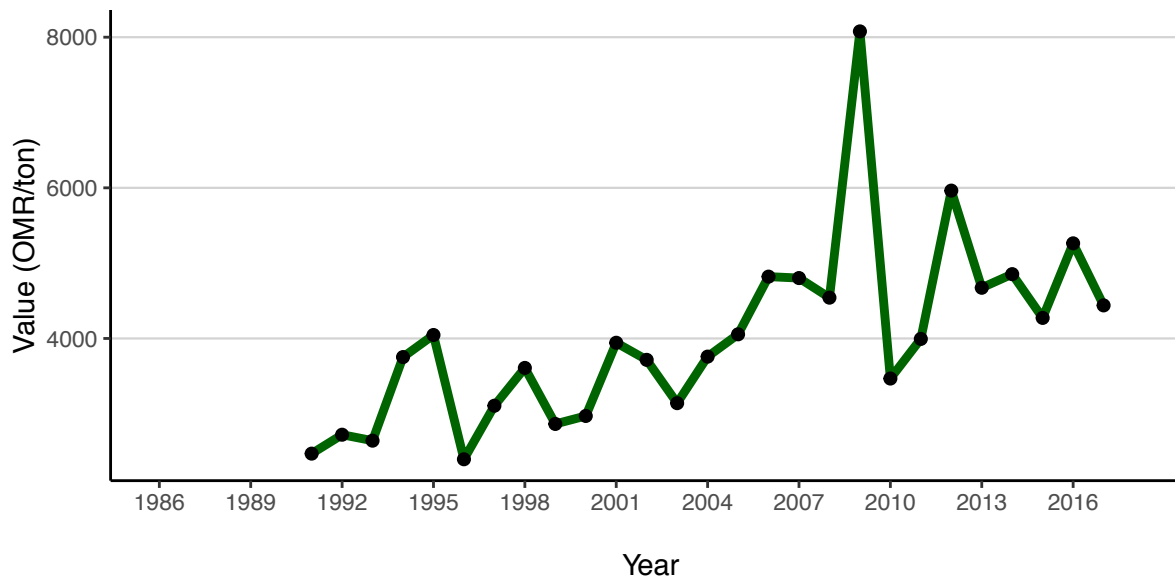
Figure 4. Gross value (OMR/mt) of lobster landings during 1991-2017.

tory measure involving fishing gears as stipulated in the *Marine Fishing and Living Aquatic Resources Protection Law* as '*Harvesting lobster shall be by lobster traps with specifications determined by the competent authority*' has not been enforced. In addition, there is no special license requirement for harvesting lobster. The data from the present study suggests that a considerable number of part-time fishers are involved in the lobster fishery.

According to data on landings collected by the MAF, the lobster landings dropped from 1936 mt in 1988 to 339 mt in 2017, an average growth rate of -5.83% during the period of 1988-2017 (Fig. 2) with most of the drop during in the first decade. The gross value of lobster catch has shown the similar pattern (but to a lesser extent), an average growth rate of -1.54% during 1991-2017 (Fig. 3). However, it is interesting to note that the unit value of lobster has shown a positive growth (2.12%) during the 1991-2017 (see Figure 4). This indicates an economic potential that can be achieved if the sustainable utilization of the resources was adhered to.

As shown in Figure 2, the fishery has experienced a considerable decline in total landings. During the period

Table 1. Assessment criteria with explanatory note for co-management feasibility.

Criteria	Explanatory note
Pre-implementation phase	
Initiator of the idea of co-management	How and by whom the idea of co-management is conceived?
Conditions and driving force	Issues and conditions (i.e. drivers) in fisheries that led to the idea of co-management
Community willingness to support the idea	Extent of fishers willingness to support the idea of co-management
Communicative process comprising participation, preparation, reflection and adaptation	Existence of mechanisms for regular exchange of management information with fishers, fishers' access and willingness to take part in management meetings, active participation of fishers in the preparation of co-management plan
Institutional characteristics	
Interactive organization	This promotes institutional flexibility not only in terms of space and time but also in terms of process/functions, and structure. It also embraces the presence of advisory organization that represents fishers' interests. It also points out institutional fitness to accommodate effectively geographically oriented fishing communities.
Resource ownership and control	This includes access and withdrawal rights, collective choice rights (management rights, exclusion rights, enforcement rights, and transfer rights).
Existence of planned process	Existence of the process of preparing co-management plans with clear objectives, and functions and responsibilities of each institutional actor.
Diversity	Comprises equity (i.e. fairness in the representation of the range of interests, and participatory democracy), fisheries contribution to economic development, and fisheries sustainability.

Source: Noble (2000), Chuenpagdee & Jentoft (2007), Cinner et al. (2012).

Table 2. Respondent's profile.

Item	Al-Sharqiyah South (N= 68) *		Al-Wusta (N= 106) *		Dhofar (N=362) *	
	No.	%	No.	%	No.	%
Boat Owner	67	98.5	82	96.5	298	83.5
Crew	1	1.5	3	3.5	59	16.5
Participation						
Full-time	63	92.6	25	23.8	225	62.2
Part-time	5	7.4	79	75.2	137	37.8
Experience in fishing						
≤ 10 years	6	8.0	16	15.2	78	21.8
Above 10 years	61	91.0	89	84.7	279	78.2
Educational Status						
≤ Elementary & Preparatory	61	92.4	64	62.7	91	25.4
Above Elementary & Preparatory	5	7.6	38	37.3	267	74.6
Is (was) your father a fisher?	68	100.0	105	100.0	235	65.1
Community dependency on fisheries	68	100.0	67	63.2	295	81.5
Is (was) your father a fisher?	68	100.0	105	100.0	235	65.1
Age of respondent (average years)	44.3		31.2		37.2	
Proportion of annual income from fishing (%)	90		51		46	

* For each question the percentage figure is calculated based on the total number of responses rather than the overall sample size (N). All figures have been rounded to nearest decimal place.

1988-2017, the fishery experienced changes in management measures related to fishing season including its duration and timing through the issuance of a number of Ministerial Decisions. For instance, a two months period (December-February) fishing season was first introduced in 1992 and subsequently changed to the period October 15-December 15 in 2002. Prior to 1992, fishing season was six months period (October-March). In 2009, the fishery was technically inoperative due to a decision on the shift in fishing season (from 15th October -15th December to 1st March- last day of April). The indicators of severity of exploitation of lobster resources are identified in various studies as follows 1) the catch is dominated disproportionately by younger size group (Mehanna et al. 2012; MAF, 2012; Rogers, 2002), and 2) the dramatic decline in catch over the period (Fig. 2). In addition, a large proportion of egg bearing females in the catch, as pointed out by Mehanna et al. (2012) and Rogers (2002) harms the reproductive potential of the population. There is also a substantial indication of fish-

ers' non-compliance with the regulations specific to the lobster fishery as stated above (Roger 2002, Al-Haddabi, 2010; Mehanna et al. 2012; MAF, 2012) which undermines the effectiveness and legitimacy of regulations.

Materials and Methods

Selection of criteria for assessment

Prior to addressing the research objective and to provide specificity to the subject-matter at hand, some relevant criteria were selected from the literature for evaluative purpose. Table 1 presents the selected criteria with brief explanation.

In appraising the pre-implementation stage of co-management, Chuenpagdee & Jentoft (2007) have identified some key criteria that include: i) how and by whom the idea of co-management is conceived, ii) conditions and driving force that led to the idea, iii) community willingness, iv) communicative process, v) participa-

Table 3. Crude estimates of average catch, average price, and gross earnings.

Governorates	Average Catch (kg)/ Boat	Average Price (OMR)/ kg	Average Gross Earning (OMR)/ Boat/ Trip	Average Operating Costs (OMR)/ Trip*
Al-Sharqiyah South	7	7.4	51.80	*7.00**
Al-Wusta	**36.56**	5.5	201.08	*30.32***
Dhofar	21.03	4.8	100.94	*67.60 (for trap)*** 38.15 (for net)***

* The average operating costs per fishing trip for the fiberglass boats engaged in harvesting kingfish was estimated to be OMR 6.87 (Al-Oufi et al., 2004).
** excluding labor cost.
*** including labor cost.

tion and preparation, and vi) reflection and adaptation. Similar thematic criteria were also used by Cinner et al. (2012) in analyzing transitions towards co-management involving three African countries. From an institutional perspective, Noble (2000) has added some institutional criteria (along with sub-criteria) that are prerequisites to effective co-management namely: i) the extent of interactiveness in organization, ii) the extent of resource ownership and control by community, iii) level of community support, iv) presence of planned process, and v) diversity. Furthermore, the following important observations are made from a brief review of literature. First, driving forces that led to the introduction of co-management initiatives were not common across countries that provide justification for the case study at hand. For instance, conflicts over fisheries resources that popularized co-management in South East Asia (Nielsen et al. 2004), is relatively uncommon in Oman. Second, while the factors of influence differ in their extent across countries, the overall objective of resource sustainability had been the main attraction to co-management approach. Third, the deliberation of co-management approach helps create a bridge between the two theoretical postulates namely public and social choice theories.

To facilitate the process of assessment, this paper draws evidence from studies on co-management of local and global origins, and elicits fishers' views on various aspects such as, socio-economic, technical, and regulatory aspects of the fishery, awareness of co-management requirements, willingness to participate in management decision-making, monitoring and research, and sharing management responsibilities, etc. A field survey based on face-to-face interviews with a structured question-

naire was conducted during June 9-28, 2013 with randomly selected 536 lobster fishers from three Governorates namely Al-Sharqiyah South South, Al-Wusta, and Dhofar. Prior to the design of the questionnaire for the field surveys, a pilot field visit was made to Al-Sharqiyah South (March 17, 2013) and discussions were held with local officials and fishers to gain a clear understanding of the key issues and challenges faced by the fishery. Following the field visit and based on the review of existing literature, a questionnaire was developed. In the absence of any specific register for lobster fishers to draw samples from, the interview was carried out by asking whether the respondent has involvement in lobster fishing. The sampling approach relied on a stratified sampling based on the number of boats involving 11 coastal villages from Al-Sharqiyah South, Al-Wusta, and Dhofar Governorates. The participation in the survey was voluntary and to avoid any inappropriate use of survey information the respondents were kept anonymous according to the code of conduct of the Ministry of Agriculture and Fisheries (MAF). On average it took about 40 minutes to complete the questionnaire in the field. Survey data were processed using Excel and SPSS. A workshop comprising the data collectors was held on June 5, 2013 to reduce potential 'interviewer bias', and to discuss the interview protocol and the structured interview questions. During the workshop data collectors were given opportunity to review the questionnaire and to clarify any question if arises. It can be noted from Table 2-9 that survey questions are both binary and scale-based in nature. The scale-based questions were measured on a five-point scale.

Results

This section presents respondents' background profile, some economic fundamentals, and the assessment of the feasibility of co-management arrangements in the lobster fishery under the selected criteria presented in Table 1.

Respondent's profile and some economic fundamentals

Table 2 presents respondent's profile. Majority of the respondents in all study sites were boat owners and had more than 10 years of fishing experience. The number of full-time fishers in Al-Sharqiyah South was comparative higher than that of the other study sites. Overall, a majority of the respondents had low educational attainment with the average age profile ranged from 31.2 years to 44.3 years. It was perceived by the respondents that fisheries are an important part of community livelihoods and the average proportion of fishing income (generated from all fishing activities) out of overall household income ranged from 46% to 90%.

The crude estimates of average catch, average price, average gross earnings, and average operating costs per

Table 4. Effect of charbohydrate type and concentration on solanine % (mg.g^{-1} DW) in microshoots, callus and cell suspension of in vitro grown *S.nigrum*, in addition to wild (in green house) grown *S. nigrum*.

Item	Al-Sharqiyah South (N=68)***		Al-Wusta (N=106)***		Dhofar (N=362)***		
	No.	%	No.	%	No.	%	
Compliance with fisheries regulations							
≤ 50%	34	50.0	68	64.1	236	65.6	
Greater than 50%	34	50.0	38	35.8	124	34.4	
Violation of lobster fishery rules							
≤ 50%	42	61.8	72	67.9	238	67.0	
Greater than 50%	26	38.2	34	32.1	117	32.9	
Enforcement							

Item (Measuring scale used: 1=Strongly agree and 5=strongly disagree)	Al-Sharqiyah South (N=68)		Al-Wusta (N=106)		Dhofar (N=362)		K-S Test (df =2)
	Mean	HFS*	Mean	HFS	Mean	HFS	
Adequacy of enforcement at sea	2.87	4	1.66	2	2.67	2	53.78**
Adequacy of enforcement at landing sites	2.76	4	2.44	2	2.52	2	2.65
Self-enforcement	3.63	5	2.50	2	3.46	5	36.32**

* HFS represents highest frequency score.
**The Kruskal-Wallis (K-S) test results indicate that the results are significant at the 1% level (Critical value is 9.21).
*** For each question the percentage figure is calculated based on the total number of responses rather than the overall sample size (N). All figures have been rounded to nearest decimal place.

trip for the boats engaged in lobster fishing are provided in Table 3. The price (OMR/kg) of lobster during the 2013 season ranged from 4.8 OMR in Dhofar to 7.4 OMR (Al-Sharqiyah South). In Al-Sharqiyah South and Al-Wusta, lobster is sold predominantly to retailers and truckers respectively, while retailers, truckers, and companies are engaged in buying lobster in Dhofar Governorate. The average (gross) earning of the respondents from Al-Wusta was about four and two times higher than that of the respondents from Al-Sharqiyah South and Dhofar respectively. During the pilot visit fishers and the local officials reported that lobster catch had always been high during the earlier part of the season and declined gradually towards the end of the season during which fishers divert their fishing efforts to catch other species. In Al-Sharqiyah South and Al-Wusta nets were used for harvesting lobster, however, in Dhofar, both traps and nets were used. On average, the crew members per boat ranged from 2 to 3. The trip duration per day ranged from 5 hours (Dhofar) to 11 hours (Al-Sharqiyah South), and trip numbers ranged from 5 to 6 times per week.

Criteria-based assessment of co-management feasibility

Initiators of the idea of co-management

It was evident from a number of local studies that the present-day concept of co-management was embedded in the currently operated Sunat Al-Bahar Committee at coastal *wilayat* level headed by the *Wali* and that includes member from fishing community and the MAF. The 'Sunat Al-Bahar' (meaning the 'code of the sea') historically encompassed rules and person with extensive knowledge. The task of the committee is to study fisheries related decisions of the Ministry, help in solving conflicting issues, and communicate fisheries issues with fishers and the management authority. (Al Oufi et al. 2000; Al-Balushi et al. 2016; Al-Subhi et al. 2013).

However, local studies also pointed out the diminishing role of the traditional committee (Al Oufi et al. 2000, Al-Subhi et al. 2013) and recommended the revitalization of the function of traditional institution to enhance community attitudes to resource use and rule compliance (Al-Balushi et al. 2016; Al-Subhi et al. 2013). Recently, the 2040 strategy developed for the sector by the World Bank (WB) has placed a greater emphasis on

Table 5. Views on management regulations.

Item (Measuring scale used: 1=Strongly agree and 5=strongly disagree)	Al-Sharqiyah South (N= 68)		Al-Wusta (N= 106)		Dhofar (N=362)		K-S Test (df =2)
	Mean	HFS	Mean	HFS	Mean	HFS	
Which of the following general tools of fisheries management do you feel are most effective in reducing fishing pressure?							
Size limit	3.13	4	2.26	1	2.84	4	18.92*
Closed season	3.03	4	2.82	1	2.49	1	9.05**
Market control	1.84	2	1.97	1	1.59	1	35.96*
Gear restrictions	2.01	2	2.02	2	1.61	1	41.52*
Closed area	3.07	4	3.36	4	3.15	5	2.08

*The Kruskal-Wallis (K-S) test results indicate that the results are significant at the 1% level (Critical value is 9.21).
** The Kruskal-Wallis (K-S) test results indicate that the results are significant at the 5% level (Critical value is 5.99).

consultation with stakeholders to improve communication necessary for effective fisheries management (WB & MAF, 2015). Therefore, it appears that local and international experts have been the initiator of the idea of co-management.

Conditions and driving force

The primary driver of the recent co-management appeal resulted from overfishing of certain high valued species (Al-Balushi et al. 2016), extent of non-compliance (Al-Subhi et al. 2013), weak enforcement (Bose et al. 2017) in general and the catch of undersized lobster in particular (Bose et al. 2017). To address this situation, local researchers recommended the implementation of partnership approach as a tool to revitalize the role of traditional committee, and to enhance awareness, mutual trust and legitimacy (Bose et al. 2017; Al-Subhi et al. 2013).

With regard to the case in hand, inadequacy of enforcement and non-compliance with regulations (Table 4), lack of fair market price, decline in stocks (Table 9), etc. pose threats to both biological and economic viability of the fishery which have been major concerns to both fishers and fishery managers. The decline in lobster stock (Fig. 2), and lack of enforcement and compliance were of common concerns to respondents. A majority of the respondents from Al-Sharqiyah South in particular viewed that fisheries enforcement at sea and at landing sites was inadequate. The extent of compliance with fisheries regulations in general and lobster regulations in particular perceived by respondents is consistent with enforcement inadequacy (see Table 4). It is noted that 'self-enforcement' is not a practical option at the current stage and there was a significant difference in views across the Governorates. Co-operative decision-makings involving fishers and the management authority were preferred by a majority of the respondents across

all Governorates and the non-parametric test results did not show any significant difference in their views (Table 6).

Community willingness and support

With regard to community willingness and support, the results are positive as respondents acknowledged the resource threats and a relatively high proportion of respondents from Al-Sharqiyah South and Dhofar expressed their high level of commitment to co-management. Furthermore, respondents' were willing to: 1) participate in decision-making, 2) provide fishery-related information, 3) take part in monitoring and enforcement activities, and 4) take part in cooperative research. Respondents were prepared to assist the authority by supporting decisions (Table 7). *When asked if they would like to form a local committee*, a majority of respondents agreed to form a local committee involving relevant stakeholders for effective management of the lobster fishery. It is also noted that a relatively high proportion of respondents from Al-Wusta and Dhofar showed low level of willingness to take full management responsibility (Table 7). This is, perhaps, because they felt they would be unable to manage the fishery on their own and hence co-operative decision-making was preferred by a majority of the respondents across all Governorates as mentioned above. While a generational transfer of fishing profession was observed in case of Al-Sharqiyah South and Al-Wusta Governorates, involvement of new generation fishers (34.9%) was noted in case of Dhofar (Table 2). When asked 'whether they would encourage their next generation to choose fishing as profession' 85% and 43.9% of the respondents from Al-Wusta and Dhofar respectively responded negatively.

Communicative process

To the best of authors' knowledge no planned process exists at present in connection with the preparation of co-management plan, and therefore, the contemplation of the criteria such as 'participation and preparation' and 'reflection and adaptation' mentioned by Chuenpagdee & Jentoft (2007) was not possible. Furthermore, mechanism for exchange of management information with fishers is unclear and information exchange is irregular in nature.

Interactive organization

The low level of fishers' participation in traditional institution (i.e. Sunat Al-Bahar committee) undermines its continued existence, operational effectiveness and the advisory role of traditional organization in fisheries management (Table 7). Furthermore, it weakens the representative voice of fishing communities. Responses with regard to the occurrences and level of awareness of consultation meeting, participation in ministry meetings in case of Al-Sharqiyah South and Dhofar, proper acknowledgement of fishers' views raised, extent of fishers' involvement in traditional committee, and level of fishers' representation (particularly in Al-Sharqiyah South), undermine the basic characteristics of interactive organization (Table 7).

The level of awareness of the 'terms and conditions'

of effective co-management that involves, among others, concept clarity, capacities of fishers (i.e. technical expertise, administrative skills, and social cohesion), provision of protection from outsiders, provision of legislative support, credible commitments and accountability from both parties, mutual trust, refraining from opportunistic behaviour etc. (Charles, 2008; Singleton, 2000) was found to be low among respondents across the sampled areas.

Resource ownership and control

While fishers having valid fishing and boat licenses have the legal right to access the fishery and harvest lobster, they do not possess collective choice rights that include management rights, exclusion rights, enforcement rights, and transfer rights. Furthermore, private property rights for individual fishers or communities in the form of individual quota (i.e. individual/community harvest rights) do not exist in the fishery. There is no clear evidence of power delegation or transfer (i.e. decentralization) of management functions to the traditional community organization to manage and control lobster resources. Furthermore, the scope of delegation and decentralization is diminished by the absence of fishers' technical capacities and lack of awareness about the essentials of co-management approach. The diminishing role of traditional institution within fishing communi-

Table 6. Views on management regulations.

Questions	Al-Sharqiyah South (N= 68) **		Al-Wusta (N= 106) **		Dhofar (N=362) **		χ^2 test (df=6)
	Yes	%	Yes	%	Yes	%	
Do you agree with having a lobster closed-season?	27	39.7	32	30.2	284	78.5	108.11*
Do you agree with the minimum size restrictions?	52	76.5	44	41.5	306	84.5	88.80*
Do you agree with the restrictions on gear that can be used?	49	72.1	82	77.4	338	93.4	46.05*
Do you agree with the restrictions on taking lobster with eggs?	53	77.9	91	85.8	348	96.1	41.43*
Do you respect lobster closed-season?	67	98.5	92	86.8	342	94.5	11.76
Do you catch lobster during closed season?	3	4.4	16	15.1	13	3.6	53.86*
Do you get lobster as bycatch during closed season?	18	26.5	60	56.6	213	58.8	33.42*
Should the Ministry, the fishermen or both make decisions on managing the lobster fishery?							χ^2 test (df=4)
Ministry	1	1.5	6	5.8	9	2.5	5.48
Fishermen	5	7.4	2	1.9	5	1.4	
Both	62	91.2	95	92.2	348	96.1	

The results are significant at the 1% level (Critical values for 6 and 4 dfs at 1% level are 16.81 and 13.28 respectively.
** *For each question the percentage figure is calculated based on the total number of responses rather than the overall sample size (N). All figures have been rounded to nearest decimal place.*

Table 7. Awareness, participation, membership and representation.

Item	Al-Sharqiyah South (N= 68) **		Al-Wusta (N= 106) **		Dhofar (N=362) **	
	Yes (%)	No (%)	Yes (%)	No (%)	Yes (%)	No (%)
Awareness of consultation meeting for making regulations	13.2	85.3	34.0	63.2	47.8	51.7
Participation in Ministry meetings (2011-2013)	11.8	88.2	50.9	45.3	37.3	62.7
Recognition of views raised in meetings (if participated)	51.5 (N=47)	17.6	16.0 (N=38)	26.4	19.3 (N=201)	36.2
Membership in Sunat Al-Bahar Committee	7.4	91.2	12.3	84.9	3.9	96.1
Do you feel that Sunat Al-Bahar Committee represents you?	41.2	58.8	81.1	16.0	62.7	36.2

Way of Helping the Authority	Al-Sharqiyah South (N= 68) **		Al-Wusta (N= 106) **		Dhofar (N=362) **		K-S Test (df =2)
	Yes (%)	No (%)	Yes (%)	No (%)	Yes (%)	No (%)	
Supporting decisions	76.5	17.6	52.8	46.2	94.5	4.1	119.18*
Participating in decision-making	79.4	14.7	72.6	26.4	92.0	4.1	46.02*
Sharing management responsibilities	45.6	48.5	58.5	39.6	71.3	21.3	26.02*
Providing information	61.8	32.4	71.7	26.4	84.5	13.5	19.10*
Monitoring and enforcement	86.8	7.4	58.5	39.6	92.8	5.5	81.93*
Participating in research project	61.8	32.4	30.2	67.0	76.2	15.5	97.14*
Taking full management responsibility	42.6	52.5	21.7	76.4	49.4	39.8	32.64*
Formation of a local stakeholder committee to better manage	67.6	32.4	75.5	22.6	82.0	16.3	9.73*

The Kruskal-Wallis (K-S) test results indicate that the results are significant at the 1% level (Critical value is 9.21).
** For each question the percentage figure is calculated based on the total number of responses rather than the overall sample size (N). All figures have been rounded to nearest decimal place.

ties, perhaps, influenced by the ever-changing nature of fisheries issues involving resources and user-group (Nielsen et al. 2004). Across all Governorates, a relatively high proportion of respondents (>50%) raise fisheries issues through the Sunat Al-Bahar Committee (Table 9), however, the traditional committee have no legal rights to impose any regulatory measures.

As discussed earlier the lobster fishery is managed through the implementation of regulatory measures such as closed season, gear restrictions, and size limits, by the management authority. Although the respondents respect the measure of closed season, less than 50% of the respondents from Al-Sharqiyah South and Al-Wusta agreed with the measures. With regard to 'minimum size' a similar pattern was observed in case of Al-Sharqiyah South. A significant difference in the views of the respondents on their agreement with the regulatory measures was observed across the sampled areas (Table 5). While prohibition of catching egg bearing females, market-based control, and gear restrictions were viewed as effective management measures by a majority of the respondents across the sampling areas, the extent of disagreement reflected through the perceived views on 'closed season' and 'minimum size' indicate the site-specific diversity that require specific attention from the authority (Table 6). Both functional and territorial characteristics should also be taken into consideration to promote equity through participatory democracy.

Existence of planned process

There is no evidence of common priority-setting efforts with participation from both sides. Lack of efforts in this regard reflects organizational inertia undercutting the progress to improve the situation. Based on the findings it can, perhaps, be suggested that interviewed fishers may not wish to maintain the status quo as the

Table 8. Factors of co-management.

Factors	Al-Sharqiyah South (N= 68)		Al-Wusta (N= 106)		Dhofar (N=362)		K-S Test (df =2)
	Mean	HFS[2]	Mean	HFS	Mean	HFS	
Awareness of 'terms & conditions' of Co-manage-ment[1]	1.2	1.0	2.1	1	1.9	1	28.33*
Willingness to take responsibility	3.1	5.0	2.6	3	2.1	1	32.95*
Ability to Manage lobster resource	3.2	5.0	2.7	3	2.2	1	29.53*
Level of commitment	4.1	5.0	2.8	3	3.1	5	32.60*

1'terms and conditions' of effective co-management involves, among others, concept clarity, capacities of fishers (i.e. technical expertise, administrative skills, and social cohesion), provision of protection from outsiders, provision of legislative support, credible commitments and accountability from both parties, mutual trust, refraining from opportunistic behaviour etc. (Charles, 2001; Singleton, 2000).
2 HFS denotes 'highest frequency score'.
* The Kruskal-Wallis (K-S) test results indicate that the results are significant at the 1% level (Critical value is 9.21).

current bio-economic situation (such as stock status, fishing costs, lack of local market outlets, competition etc.) affecting the livelihood of fishers (see Tables 4 and 5). Majority viewed that creating awareness and market opportunity through government initiative would help mitigating such concerns. This signal is conducive to the introduction of partnership arrangements. It is also noted that the existing institutional environments are not unfavorable either as there has been a long tradition of community organization (i.e. Sunat Al-Bahar Committee) and partnership in the sector. However, as noted from the responses (see Table 8), the delegation of responsibilities that suits the capacity of the local committee should be decided upon carefully.

Al-Balushi et al. (2016) argued that the authority could take essential steps to reform the traditional institution by establishing clearly defined roles for and responsibilities of the committee in relation to fisheries management. This action will not only provide an opportunity for the management authority to raise community awareness of fisheries issues, but also help reduce any communication gaps between the authority and the primary stakeholders. It is important to note that such approach focuses on disseminating conservation ethics through awareness programs and helps reduce local opposition to regulations. Of course, the effective solutions to the prevailing issues are likely to emerge over time through a learning process, however, inclusion and transparency in decision-making and, persistent dialogue with community leaders and members could help gain support from the local community.

Diversity

There is no evidence of the existence of well-articulated process that supports equity in relation to fishers' participation in decision-making. With regard to fisheries contribution to economic development, the existing

conditions identified above will not only weaken the sector's contribution to national food security and community well-being but also fail to ensure access of new generation of fishers to lobster resource. In addition, this study identified some key challenges for the fishery including overfishing, decline in economic returns, market inefficiency, ineffective management measures, lack of enforcement and compliance, amongst others that need to be addressed to protect stock and achieve long-term sustainability.

Discussion and policy implications

The results associated with boat-ownership, average age profile and the proportion of income from fishing indicate that the livelihoods of those owner operators and younger fishers are linked to the long-term sustainability of the fishery. The results with regard to the dependency on fishing income are consistent with the proportion of part-time fisher in the respective Governorate. The lowest proportion of annual income generated from fishing in case of Dhofar is consistent with Al-Marshudi & Kotagama (2006) who reported that fishers from Dhofar have other jobs along with fishing. The low level of educational attainment particularly in Al-Sharqiyah South and Al-Wusta not only limits fishers' choice of profession as pointed out by Bose et al. (2013), but also fishers' ability to contribute to co-management arrangements. Furthermore, if fishers' unwillingness to encourage their next generation to take fishing as profession is true it may inspire short-term interests of fishers which is not conducive to long term sustainability.

It should be noted that the existing regulatory measure of closed-season was not agreed upon by the majority of the respondents from Al-Wusta and Al-Sharqiyah South. Consequently, the existing management approach will be ineffective and, therefore, will not be able to deliver the desired results. The disagreement

Table 9. Raising issues, fishery concerns, and government support.

Item*	Al-Sharqiyah South (N= 68)*		Al-Wusta (N= 106)*		Dhofar (N=362)*	
	No.	(%)	No.	(%)	No.	(%)
Approach to raise fisheries concerns:						
Local Fisheries Office	10	14.7	2	1.9	44	12.2
Sunat Al-Bahar Committee	62	91.2	86	81.1	268	74.0
Wali's Office	4	5.9	48	45.3	142	39.2
Peers	20	29.4	50	47.2	98	27.1
List of major concerns about the lobster fishery:						
Fishing Season	Nil	0.0	Nil	0.0	174	48.1
Decrease in Stock	51	75.0	26	24.5	250	69.1
Non-Compliance	37	54.4	50	47.2	216	59.7
Market Price	39	57.4	11	10.4	1	0.3
Lack of Enforcement	32	47.1	82	77.4	236	65.2
Government Support: (Yes)	67	98.5	65	61.3	341	94.2
Type of support from the Government						
Management	21	30.9	5	4.7	166	45.9
Administration	53	77.9	0	0.0	230	63.5
Training	39	57.4	8	7.5	145	40.1
Research	18	26.5	1	0.9	141	39.0
Creating Market Opportunity	48	70.6	34	32.1	196	54.1
Creating Awareness	58	85.3	4	44.3	229	63.3

* For each question in the table, the percentage figure was calculated based on the total number of responses rather than the overall sample size (N). All figures have been rounded to nearest decimal place.

with regard to the existing closed-seasons may be influenced by the genetically different stock structures of scalloped spiny lobster (*Panulirus homarus*) as observed by Al-Breiki et al. (2018). Roger (2002) pointed out that if the priority is resource conservation then it is important to determine the reasons behind the non-preference of the existing closed-seasons by initiating a cooperative research. Although a majority of the respondents agree with the gear restrictions, the findings indicate that the use of traps as required by the regulation was not popular among fishers and consequently not followed by the majority of the respondents from Al-Sharqiyah South and Al-Wusta. Presence of significant diversity between Governorates with regard to views and perceptions of fishers should be taken into consideration and evaluated appropriately prior to developing partnership arrangements. The 'one-size-fits-all' approach would not be conducive as the findings indicated significant differences in respondents' preference structures with regard to the extent of participation in co-management.

The driving force behind the move towards co-management for the case in hand was found to be from local scientists and international experts which is in contrast with the findings from Cinner et al. (2012) where move towards co-management in case of Kenya, Tanzania, and Madagascar was mainly driven by donor ideology and subsequent support. However with regard to conditions that led to the development of co-management idea, the present finding is in line with the case of SSF of Lake Chiuta where circumstances such as environmental degradation and overexploitation of fish stocks led to the government initiated development of co-management in the early 1990s (Donda, 2017). Yang & Pomeroy (2017) mentioned that to prevent the increasing rate of deterioration of SSF resources in the Philippines and due to poor performance of the other management system government actively promoted the development of community-based fisheries management (CBFM). They argued that the CBFM approach improved equity in the fishing community with regard to community participation in fisheries management, control of resource use pattern, the level of bargaining power over decision-making, fair allocation of operational rights and family income.

Respondents' unwillingness to take full management responsibility should not hinder the possibility of co-management approach. Noble (2000) argued that even if fishers are not capable or not willing to take full responsibility of resource management, they can active-

ly participate in planning and decision-making through properly planned co-management arrangements. In addition, Noble (2000) pointed out that a full institutional restructuring is not always necessary to make partnership arrangements possible.

Despite respondents' unwillingness to take full responsibility of resource management (particularly in Dhofar and Al-Wusta) their high level of commitment (Al-Sharqiyah South and Dhofar) and full support for the co-operative approach signify the fact that the approach is demand (fishers)-driven. The management authority should take advantage of this signal and initiate a plan and capacity building efforts through the design of co-operative research projects which may provide opportunities for both parties to collectively arrive at more effective solutions to overfishing and non-compliance problems. Tightly constrained fishery budget make it all the more crucial to reduce management and enforcement costs. Furthermore, the finding with regard to fishers' willingness to take part in cooperative research provides support to design a knowledge-based planning process through the integration of socio-cultural and economic characteristics of local community and ecological knowledge of fishers.

Non-compliance has also been viewed as one of the key factors responsible for overfishing of lobster stock which is in line with the observation by Rogers (2002). The capacity to detect non-compliance is of utmost importance, not only to gain better information for stock assessment purpose but to protect stock and ensure the integrity of management measures and sustainability of lobster resources. As the majority of the respondents are ready and willing to support the authority (see Table 7), local officials and fishers can join together under a partnership arrangement to become stronger and more effective in enforcement monitoring and of rules and regulations. To cite an example, Cinner et al. (2012) pointed out that the enforcement of regulation banning beach seine nets was effective through the establishment of Beach Management Unit (BMU) – a formal organization of fishers and other stakeholders in Kenya. For the present case, the establishment of an integrated compliance program will be critical to the effectiveness of fisheries management. Donda (2017) examined the impacts of co-management arrangements in case of Lake Chiuta, Malawi and observed that such arrangements benefited government in terms of reduced enforcement costs and creating positive image for establishing a sustainable fishery through fostering user participation, and benefited fishers in terms of getting government protection from outsiders, increased income, employment and improved livelihoods. The recent report by the World Bank and MAF mentioned that the commitment to increase stakeholder input and participation in the fisheries sector is an important step toward maximizing voluntary compliance (WB & MAF, 2015).

With regard to respondents' low level of educational attainment and lack of awareness about the 'terms and conditions' of co-management indicate that intervention to augment fishers' capacity through the development of technical and administrative skills and strengthening traditional institutions through appropriate awareness program may prove beneficial in the long-run and may be the key for resolving local level fisheries issues. In stressing the gender issue in traditional fisheries in Oman and low educational attainment of fisherwomen involved in shellfish fisheries, Bose et al. (2013) also recommended a 'target group'- based approach to skill development programs to equip fisherwomen with generic skills that are easily transferable to other occupations. The 'Fisheries Extension Department' should play a significant role not only in delivering various extension services but also promoting awareness among fishers about their roles and responsibilities in achieving sustainability in the fishery. Fisheries extension program must strive to educate fishers on the association between overfishing and its impact on the resources on which many of the fishers depend for their livelihoods. Capacity building efforts, if they are to be sustained, must include provisions of long-term support from the authority. However, the ultimate success of any co-management initiatives would depend upon their ability to influence fishers' attitudes towards resource sustainability. However, extension officers should receive appropriate training to deliver intended services to fishers effectively. The details of such a partnership arrangement with respect to its type, selection of members, functions, responsibilities etc. need to be worked out.

Considering the fishery situation, an arrangement of participatory management should be considered involving the key stakeholder groups and strived for broad consensus on the management measures to ensure the protection and conservation of the lobster resources. This approach will enhance trust, legitimacy and encourage voluntary compliance and reduce enforcement expenditures. The evolution of co-management institution for Pacific Northwest salmon fisheries as reported by Singleton (2000) illustrates the fact effective partnership approach is possible despite the historical adversarial relations between the state and the American Indian tribes as they were engaged collectively in collecting and analyzing data and implementing other initiatives. In a Canadian case Davis (2008) found that incentive to participate, consensus decision-making and independent facilitation were essential to ensure fairness and effectiveness of the planning process. The process can be developed through the issuance of Ministerial Decision that will not only provide the legal mandate but also help attracting community and industry attention to the fishery. Examples of such decisions can be found in Al-Balushi et al. (2016). The study by Al-Balushi et al. (2016) exemplifies that a co-operative management plan could be developed and differences in interests could be accommodated with impressive rating on the compre-

hensiveness of such plan.

Development practitioners argue that property rights institutions should be designed to be beneficial, flexible, socio-culturally sensitive, politically encouraged, legally and socially recognized, enforceable by a legitimate authority, and conducive to local conditions in order to effectively conserve resources and protect community interests (Acheson, 1989; Seabright, 1993; Agarwal, 1994; Ostrom, 2003).

Conclusion

This case study focuses on the feasibility of co-management in the lobster fishery of Oman and highlights some strengths and weaknesses related to the feasibility and desirability of such management approach. While findings related to fishers' commitments, willingness to support government decisions, willingness to participate in co-operative research, and preference for co-operative decision-making are promising, institutional weaknesses identified under the category of 'interactive organization', 'resource ownership and control', 'existence of planned process', and 'diversity' should be of major concerns for policy-makers in moving towards co-management arrangements.

Although the results are dependent on the limited indicators used in the feasibility assessment, it is believed that the incorporation of additional indicators in the assessment process would not alter the basic thrusts of the conclusion. If continuity and long-term sustainability of the fishery is a priority, then fishers' commitment in running an economically viable and environmentally sustainable fishing should be secured through a partnership approach. Therefore, without successfully addressing these impediments, any co-management initiatives will not live up to their full potential. The real challenge is to create mechanisms that facilitate coordination between both parties. Genuine attention from the management authority to the subject matter would not only demonstrate the 'sense of moral duty', but would also be in harmony with the national policy direction on the inclusion of key stakeholders in socioeconomic development programs.

Acknowledgement

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RESEARCH PAPER

Gillnet selectivity of the Indian oil sardine *Sardinella longiceps* fished in the Sea of Oman

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إنتقائية الشباك الخيشومية لأسماك السردين الزيتي الهندي (Sardinella longiceps) المصطادة في بحر عمان

أنيش جوفيندر* وحمد العوفي**

ABSTRACT. The size selectivity of drift gill-nets for *Sardinella longiceps* was investigated in the Sea of Oman using a range of 5 nets between 3.3 and 5.7 cm stretched mesh size. All nets had a hanging ratio of 0.5. A total of 1211 Indian oil sardines were caught in the 5 experimental nets, the majority of which were retained by the 3.3, 3.8 and 4.5 cm mesh nets. Peak selectivity was reached at 14.9 cm FL for the 3.3 cm mesh size increasing to 16.9 cm for the 3.8 cm mesh size. The estimated and observed length frequency distributions were unimodal. A log-normal selection function indicated that the highest selectivity was obtained when the length class to mesh size (*l:m*) ratio was 4.4. *Sardinella longiceps's* relative abundance was estimated to be the highest at 16.4 cm FL, with numbers decreasing beyond this peak. This would indicate that selectivity by gilling is optimal with a mesh size of 3.8 cm with nets having a hanging ratio of 0.5. This mesh size appears to satisfy two criteria: high catch efficiency and protection of juveniles.

KEYWORDS: Sardine; gillnet selectivity; model; Sea of Oman.

المستخلص: تم البحث في إنتقائية حجم الشباك الخيشومية العائمة لأسماك السردين (Sardinella longiceps) في بحر عمان باستخدام ٥ شباك يتراوح حجم فتحات عيوبها بين ٣٫٣ و ٥٫٧ سم، وبإستخدام معامل تعليق واحد (٥٫٠). تم إصطياد ما مجموعه ١٢١١ سمكة من أسماك السردين الزيتي الهندي في الشباك التجريبية الخمسة، حيث أصطيدت غالبيتها من خلال الشباك ذات الفتحات ٣٫٣ و ٣٫٨ و ٤٫٥ سم. وتم الوصول إلى أفضل إنتقائية عند طول شوكي ١٤٫٩ سم بالشبكة ذات حجم ٣٫٣ سم والتي إرتفعت إلى ١٦٫٩ سم بالشبكة ذات حجم ٣٫٨ سم. ووجد أن توزيعات تردد الأطوال المقدرة والملاحظة كانت أحادية الوسائط. وأشارت دالة التحديد اللوغاريتمي المعتدل إلى أنه تم الحصول على أعلى انتقائية عندما كانت نسبة فئة الطول إلى حجم الشبكة تساوي ٤٫٤. وتم تقدير الوفرة النسبية الأعلى لأسماك السردين الزيتي الهندي (Sardinella longiceps) عند ١٦٫٤ سم، مع انخفاض الأرقام إلى ما بعد هذه الذروة. وقد يشير هذا إلى أن الانتقائية الخيشومية مثالية مع الشبكة ذات حجم ٣٫٨ سم ومعامل تعليق ٥٫٠. حيث يبدو ان هذا الحجم الشبكي يلبي معيارين وهما: كفاءة الصيد العالية وحماية صغار الأسماك.

الكلمات المفتاحية: السردين، إنتقائية الشباك الخيشومية، نموذج، بحر عمان

Introduction

Gillnet selectivity is the quantitative measure of the amount of fish caught within a size range that is retained by the net and it is a function of the probability of the fish encountering the net, the chance of being caught and the intensity of the fishing. Generally, one length class will be sampled most efficiently with efficiency decreasing symmetrically to zero both above and below this length class. Because gillnets are highly selective for fish of certain sizes, knowledge of the selection is necessary for both population assessment and management of the fishery (Carlson and Cortés, 2003). Selection curves can be used to set a mesh size that can restrict the size of fish captured and hence,

limit the catch of immature fish or reinforce minimum landing size regulations. In this study we describe the selection curve of the Indian oil sardine (*Sardinella longiceps*) harvested in the Sea of Oman.

The Indian oil sardine is found in the north and west Indian Ocean ranging from the horn of Africa, along the Arabian Sea, Sea of Oman and Aden (excluding the Red Sea and Arabian Gulf) and then eastward to the southern coasts of India and Sri Lanka (Al-Abdessalaam, 1995). Small pelagic fish, including *S. longiceps*, occur in abundance in Omani waters.

This species forms an important component of the marine food web because it comprises the bulk of the forage for large fish and other predators (Al-Barwani et al., 1989). The Indian oil sardine and other sardines also contribute significantly to the Omani marine fishery. In 2013 about 52544 tonnes of sardines were landed along the coast of Oman, of which 8320 (16%) tonnes were *S. longiceps* (Anonymous, 2013).

This species, as well as other sardines, is exploited

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Table 1. Estimates and their 95% confidence intervals for the parameters of the selection equation for three mesh sizes (3.3, 3.8 and 4.5 cm stretched mesh) with a hanging ratio = 0.5. The negative log-likelihood (log *L*) = -4345.944.

Parameter	Value	95% Confidence Interval
k	4.4	4.3 – 4.5
σ	0.124	0.117 – 0.133

primarily by cast nets, beach seines and gillnets. As they require little investment in manpower and equipment, and are efficient in catching pelagic fish, gillnets are widely used by traditional Omani fishermen. Because of the decline in large pelagics (e.g. *Scomberomorus commerson* (Govender et al., 2006), human consumption of sardines has increased, resulting in increased market prices. These issues will likely result in over-exploitation of sardines which may lead to a decrease in abundance in the future. Based on late 1980s data, (Dorr, 1991) already observed that sardines were caught at near optimum levels. Although fluctuations in sardine abundance are largely regulated by the environment (Kawasaki, 1991), intensive fishing in coastal waters may reduce fish productivity.

Seeing as the Indian oil sardine is considered one of the key species in the Oman fishery, selectivity estimates are potentially of great value in management and research. Therefore, the main objective of this study was to determine the gillnet selection parameters of the Indian oil sardine gillnet using nets of different mesh sizes fished simultaneously on the same population.

Materials and Methods

Sampling was carried out monthly between September 2001 and July 2003 on board the *RV Al-Jamiah*, a 17.5 m (overall length) multipurpose fishing boat. Green polyamide (nylon) gillnets of similar thickness (PA 210D × 6 plays) and of mesh sizes 3.3, 3.8, 4.5, 5.0 and 5.7 cm (stretched mesh) were used. For each mesh size a gillnet was constructed with 0.5 hanging ratio; thus 5 experimental nets of similar fishing power were constructed and joined together as a gang. For all nets, a similar hanging ratio was used for the float line and the lead line to restrict fish being tangled. All nets were made to the same finished length of 25 m, giving a total length of 125 m and a fishing height of 3 m. The nets were rigged with a 6 mm PE (polyethylene) float line fitted with several PVC floats (113 g buoyancy) regularly distributed to give a float line buoyancy of around 135 g/m, while the sinking force employed for the lead lines had a force of 50 g/m. The order of mesh panels was randomized at the beginning of the experiment. The nets were joined together with a 2 m gap between adjacent nets. This design was chosen to avoid the effect of larger fish being led by smaller mesh-sized nets into neighboring nets of a more suitable mesh size (Hovgård, 1996; Madsen et al., 1999). Each net was coded to enable identification on hauling.

Fishing stations were selected in shallow waters in the Muscat (23° 37' N, 58° 35 E') area. One end of the net series remained attached to the boat by a rope and both the net and boat was set to drift with the current. Fishing was carried out a few hours before sunrise and the net series was hauled-in after sunrise giving an average fishing time of 4 hours. The total number of valid sets was 34.

Catches were sorted by species and fork length (FL) was measured to the nearest cm and the weight to the nearest gram. Girth measurements at the snout, behind the head and at maximum girth (before the dorsal fin) were also recorded for each fish. Although girth measurements were taken for all fish, fork length was used to construct the selectivity curves because length is closely related to mesh size as is girth (Reis and Pawson, 1999). Gilled or wedged fish were used in the analysis while tangled fish were excluded from the analysis as tangling of fish is not a function of net selectivity.

Selectivity modeling

Hamley (1975) provided a review of the approaches to estimate gillnet selection curves and more recently Hovgård and Lassen (2000) provided an updated review of all methods, including new statistical approaches that are based on general models for the selection process.

$$\hat{C}_{l,m} = (qN_lS_{l,m}) + error \qquad \text{Eq. 1}$$

The parameters for the selection curves in this study were obtained using maximum likelihood (ML) (Kirkwood and Walker, 1987; Millar, 2000). Assuming that fishing effort and fishing power are constant, the selection equation (Hovgård and Lassen, 2000) is written as

$$S_{l,m} = exp\left[-0.5\left(\ln\left(\frac{l}{m}\right) - \ln k\right)^2/\sigma^2\right] \quad \text{Eq. 2}$$

where-?-is the predicted catch in length class l for mesh size m, q is the catchabilty co-efficient (assumed constant for each l and each m), N is the number of available fish and S is the selectivity function. We assumed that the selectivity function followed a log-normal distribution i.e.

$$error = \left[\frac{\left(\hat{C}_{l,m}\right)^{C_{l,m}}}{C_{l,m}!} exp\left(\hat{C}_{l,m}\right)\right] \qquad \text{Eq. 3}$$

and that the *error* was Poisson distributed i.e. where *C* is the observed catch, k is the predicted ratio of *(l/m)* that has the maximum selectivity and σ is the dispersion factor around *k*.
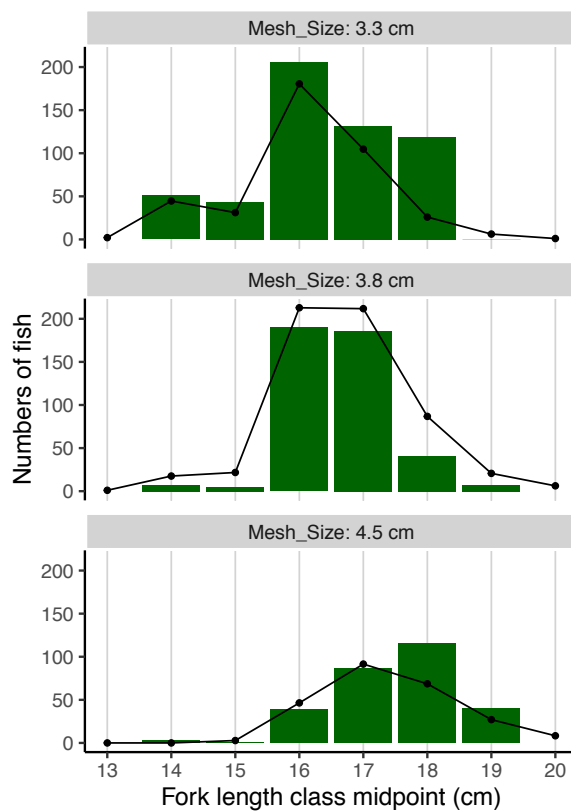
Figure 1. Observed (bars) and predicted (lines) numbers of *Sardinella longiceps* caught with gillnets of varying mesh sizes.

$$-\log(L) = \sum_l \sum_m \left[C_{l,m} \ln\left(\hat{C}_{l,m}\right) - \hat{C}_{l,m} \right] \quad \text{Eq. 4}$$

The estimates of the parameters k and σ were obtained by minimizing the negative of the log-likelihood (log *L*), with constant values removed.

The 95% confidence intervals for the parameters k and σ were obtained using the likelihood ratio test (Lebreton et al., 1992).

Results

A total of 1211 Indian oil sardines were caught in the 5 experimental gillnets, the majority of which were retained by the 3.3 (454), 3.8 (438) and 4.5 cm (301) nets. Because catches obtained with the 5.0 (17) and the 5.7 cm (1) meshes were small, these data were not included in the analysis. Almost all *S. longiceps* caught (gilled or wedged) were observed to have meshes stretched tight across their bodies in the region behind the gill cover to the maximum girth. Some were observed to have twine caught in their mouth (snagged) and some others were

entangled. Only the first category was included in the analysis, although the other two categories do not appear to affect their size selection as length distributions were similar for all three categories of entrapment in the nets.

As expected, the mode of the length frequency distribution of *S. longiceps* for different mesh sizes increased with mesh size (Fig. 1). The observed peak length for the 3.3, 3.8 and the 4.5 cm mesh gillnets were 16.1, 16.5 and 18.0 cm, respectively. The size range of fish caught by the 3.3 cm mesh size was 13-18 cm, compared with 14-19 cm for the 3.8 cm and 14-20 cm for the 4.5 cm mesh size (Fig. 1). The length distributions were unimodal except for the 3.3 cm mesh size which showed a second minor mode at 14 cm FL (Fig. 1)

Gill net selectivity

The estimated selectivity curves for *S. longiceps* appeared bell-shaped (Fig. 2), which is to be expected for fish that are gilled or wedged, as was confirmed during fishing trials. Peak selectivity was reached at 14.9 cm FL for the 3.3 cm mesh size, increasing to 16.9 cm for the 3.8 cm mesh size. The results indicated that the 3.3 and 3.8 cm mesh size had an equal selectivity for fish of 15.8 cm in length, whereas the 3.8 and the 4.5 cm mesh sizes had an equal selectivity for fish of 18.3 cm length (Fig. 2). The estimated parameter values and their 95% confidence intervals are given in Table 1. Maximum selectivity was achieved at an *l:m* ratio of 4.4 (Table 1). The small confidence intervals indicate that the parameters are well estimated (Table 1).

Discussion

There has been no previous attempts to estimate gillnet selectivity parameters for the Indian oil sardine and hence, comparisons cannot be made. The unimodal nature of the selection curve is linked to the catching process, namely gilling or wedging, whereas maxillae enmeshing or entangling, which would have resulted in either a sigmoid-shaped or a bimodal curve, was shown to have been of little importance. Since the Indian oil sardine has no spines or any other projections on its body which could result in entanglement the shape of the selection curve is therefore influenced by fish girth alone. Girth being directly proportional to fish length justifies the latter being used as a proxy for girth since fish length is easier and quicker to measure. These findings are in agreement with those of Reis and Pawson (1999) and Santos et al. (1998), although both these studies were investigating other fish species; they had similar body shapes to *S. longiceps*. In contrast, several studies have used bimodal selection curves with two distinct modes that are linked to two different catching processes, wedging and entangling (Madsen et al., 1999). Millar and Fryer (1999) used a bimodal selection curve to describe plaice catch data in a trammel net experi-
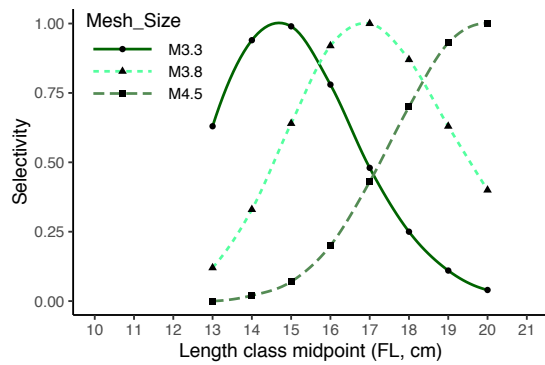
Figure 2. Selectivity curves for various mesh sizes for S*ardinella longiceps*. The plotted lines have been smoothed.

ment while Madsen et al. (1999) used it for sole, plaice and cod and Hovgård (1996) used it for cod catch data. While our data did showed a minor mode for the 3.3 cm mesh net we regarded it as negligible for the model fitting process.

Previous studies have indicated that *S. longiceps* reaches first maturity at 15.93 cm total length in the Sea of Oman (Siddeek et al., 1994). This total length was transformed to a fork length using the equation:

$$TL = 1.075FL + 0.72 \qquad \text{Eq. 5}$$

$R^2 = 0.96$ (H. Al-Oufi, unpubl. data).

Length at first maturity for this population is therefore equal to 14.1 cm FL. The length at first maturity is slightly lower than the optimal catch size computed for the 3.3 cm mesh size. In catches of the 3.3 cm gillnet, individuals under the length at first maturity made up slightly more than 11% of the catch, whereas this was around 2% for the 3.8 cm gillnet and was virtually zero for the 4.5 cm mesh. Although, a minimum landing size has not yet been set for *S. longiceps* in the Omani fishery, the results of this study show that in the interests for sustainable fishing of *S. longiceps* it can be suggested that gillnets with stretched mesh of 3.8 cm (with a hanging ratio = 0.5) should be used. This would certainly increase the average size caught when compared to the 3.3 cm net. Catches obtained with the 4.5 cm gillnet were always small, confirming that the large mesh size targeted only the larger individuals of the Indian oil sardine population in the Sea of Oman. The 3.8 cm mesh size gillnet appears to satisfy two criteria: high catch efficiency and protection of juveniles.

The use of smaller mesh sizes in this experiment may have resulted in a better match between the overall selectivity of the gang of nets and the relative Indian oil sardine population abundance. However, it was considered more important to include those mesh sizes that have direct applicability to gillnets used by local fishers. Construction details of nets used in this study, such as twine diameter and hanging ratio, is similar to that used by the local fishers (Stengel and Al Harthy, 2002).

The shape of the selectivity curve depends on several

characteristics of the fish and the net. Gray et al. (2005) reported an overall reduction in mean catch associated with an increased hanging ratio, although the results were not significant. Of particular importance are the physical properties of the twines used, such as extensibility and elasticity (Klust, 1982). Low extensibility and elasticity result in the loss of captured fish which ultimately will influence the estimated selectivity. The twines used to construct the experimental gillnets were polyamide continuous filaments, which has high extensibility coupled with high elasticity. Reported elasticity was above 96% (Klust, 1982) which means that these twines are almost completely elastic, a property that is needed to hold the gilled fish securely while the gear is in the catching process and during hauling (Klust, 1982). Other factors, such as sex, ripeness and amount of food in the gut, would also influence the girth of the fish and hence the shape of the selectivity curves. To minimize variation caused by reproduction and gut contents, (Salvanes, 1991) proposed pooling samples for months when food availability and temperature are the lowest. This could not be achieved in this study as this would have reduced sample sizes to unacceptably low numbers. Márquez-Farias et al. (2005) added oceanographic currents as a possible factor affecting selectivity; since strong currents can distort the shape of the meshes and therefore select for fish sizes not captured under conditions of a mild current. This effect was considered negligible in this study.

There are limitations to this study. The study was limited to the Muscat area (for logistic reasons) and since sardines generally school and migrate in similar age/ length classes it is quite possible that smaller/younger or larger/older classes were not available for capture. This research need should be accounted for in future research.

Acknowledgement

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RESEARCH PAPER

Evaluation of energy use efficiency for greenhouse cucumber production in Barka, Oman

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تحليل كفاءة إستخدام الطاقة لإنتاج محصول الخيار في بركاء، عُمان

نوال المزيني وعبدالرحيم الإسماعيلي* وسعيد تبوك

ABSTRACT. Sustainable agricultural production can be assessed through energy-use efficiency (EUE). This paper aims to evaluate the EUE for cucumber greenhouse production in Oman. Data were obtained through face to face interview with farmers. Results indicated that total energy inputs (e.g. electricity, water, fertilizers and chemicals) and total energy output (cucumber yield) were 1171.637 GJ ha^{-1} and 89.943 GJ ha^{-1}, respectively. The highest energy input in the greenhouse production was electricity, consuming 88% of total energy input. Electricity had the highest impact in cucumber greenhouse production and 99% of this electricity goes to cooling the greenhouse. When all energy inputs were classified into their different forms; direct (D) and indirect (ID), and renewable (R) and non-renewable (NR), the highest portion of total energy forms in greenhouse cucumber production was for D and NR energy. The EUE and energy productivity (EP) were found to be 0.08 and 0.10 ton GJ^{-1}, respectively. The energy use in greenhouse cucumber production was found to be inefficient and solar energy needs to be considered to improve the sustainable cucumber greenhouse production in Oman.

KEYWORDS: Energy-use-efficiency; cucumber; greenhouse; energy productivity; energy forms.

المستخلص: يمكن تقييم الإنتاج المستدام للزراعات المحمية من خلال تحليل كفاءة إستخدام الطاقة، تهدف هذه الدراسة إلى تقييم كفاءة إستخدام الطاقة في البيوت المحمية المزروعة بمحصول الخيار في سلطنة عمان، تم الحصول على البيانات عن طريق إجراء مقابلات مع المزارعين (وجها لوجه)، وأوضحت النتائج إن الكمية الإجمالية للطاقة المستخدمة والناتجة من البيوت المحمية وصلت الى 1171.637 و 89.943 (جيجاجول/هكتار)على التوالي، وتعد الكهرباء أكبر مدخلات البيوت المحمية المستهلكة للطاقة حيث يقدر إستهلاكها للطاقة بمعدل 88% من إجمالي الطاقة المستخدمة، لذلك تعد الكهرباء أكبر العوامل المستهلكة للطاقة وقد وجد أن معظم الكهرباء يتم إستهلاكها في تبريد البيوت المحمية، وعندما تم تقسيم الطاقة إلى أنواعها الأربعة وهي الطاقة المباشرة والغير مباشرة والمتجددة والغير متجددة، وجد أن الطاقة المباشرة والغير متجددة هي أكبر أنواع الطاقة المستخدمة في البيوت المحمية، كما توصلت النتائج إلى أن كفاءة استخدام الطاقة وانتاج الطاقة من البيوت المحمية وصلت الى0.08 و 0.01 (طن/ جيجاجول)على التوالي، وفي الختام فإن كفاءة استخدام الطاقة في البيوت المحمية غير فعالة، ومن أجل تحقيق الإستدامة في الزراعة المحمية، فإنه ينصح بإستخدام الطاقة الشمسية لإنتاج الطاقة التي يحتاجها البيت المحمي.

الكلمات المفتاحية: كفاءة إستخدام الطاقة، الخيار، البيت المحمي، إنتاجية الطاقة، أنواع الطاقة.

Introduction

Greenhouses provide higher production and more efficient water consumption in comparison to open-field cropping (Abdel-Rahman and Abdel-Magid 1993; MAF 2009). As a result, the number of greenhouses in Oman was increased 5 times between 2003-2015 (Al-Ismaili et al. 2017). Cucumber (*Cucumis sativus* L.) is the most commonly grown greenhouse vegetable worldwide (Mohammadi and Omid 2010; Omid et al. 2011) and this is also the case in Oman where more than 90% of greenhouses are used for cucumber cultivation (MAF 2013). This could be attributed to its fast growth, and good nutritional and economic value (Al-Kiyoomi 2006; Al-Sadi et al. 2010). Although greenhouses provide high yield, they also require high

energy consumption to achieve this yield (Bolandnazar et al. 2014; Heidari and Omid 2011).

Energy-use efficiency (EUE) is considered one of the most important factors contributing to agricultural sustainability (Firoozi et al. 2014). Such energy analysis helps in managing greenhouse production system by identifying the wasteful energy parameters and proposing management approaches (Bolandnazar et al. 2014). EUE in greenhouse production has been used by many researchers to assess greenhouse cropping systems (Hamedani et al. 2011; Taki et al. 2013). For instance, Mohammadi and Omid (2010) investigated the EUE of greenhouse cucumber production. Results revealed that cucumber production consumed 148.836 GJ ha^{-1} of the total energy input and diesel fuel was found to be the main energy consuming factor. In another study, Heidari and Omid (2011) investigated the EUE (output-input ratio) for cucumber and tomato greenhouse production. Results indicated that the EUE was 1.48 and 0.69 for

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Table 1. The energy equivalents correspond to each inputs and output use in greenhouse cucumber production.

Inputs and output (unit)	Energy equivalent (MJ Unit^{-1})	Reference
Inputs:		
Human labour (h)	1.96	(Taki et al., 2013)
Machinery (h)	13.06	(Mohammadi and Omid 2010; Ozkan et al. 2007)
Chemical fertilizers (kg):		
Nitrogen (N)	66.14	(Mohammadi and Omid 2010; Shrestha 1998)
Phosphate (P_2O_5)	12.44	(Mohammadi and Omid 2010; Shrestha 1998)
Potassium (K_2O)	11.15	(Mohammadi and Omid 2010; Shrestha 1998)
Calcium	1.12	(Mohammadi and Omid 2010)
Micro	120.00	(Banaeian et al. 2011; Mohammadi et al. 2008)
Manure (kg)	0.3	(Mohammadi and Omid 2010)
Chemicals (kg)	120.00	(Mohammadi and Omid 2010)
Water (m^3)	1.02	(Mohammadi and Omid 2010; Mousavi-Avval et al. 2011)
Electricity (kWh)	11.93	(Hatirli et al. 2005; Mousavi-Avval et al. 2011)
Seeds (kg)	1.00	(Mohammadi and Omid 2010)
Outputs:		
Cucumber (kg)	0.8	(Mohammadi and Omid 2010)

tomato and cucumber, respectively which reflected an inefficient energy use in cucumber greenhouse production. Diesel fuel was also the major contributing factors for both cucumber and tomato production (Mohammadi and Omid 2010). In strawberry greenhouses a total energy of 805.380 GJ ha^{-1} were consumed (Banaeian et al. 2011) and diesel fuel was the major energy depleting factor with 78% of the total energy. It appears thus that diesel fuel is the major variable influencing the EUE of greenhouse production.

In these studies, diesel fuel was mainly used to power heaters to provide temperature suitable growing environment. In Oman, where weather conditions are generaly hot, heaters are not necessary but cooling systems (generally fan-pad evaporative cooling systems) are used instead. In such conditions, EUE of cooled greenhouses needs to be evaluated. Therefore, this study aimed to investigate the EUE of evaporative-cooled greenhouse systems in Oman using major energy performance indicators for instance EUE, energy productivity (EP) and net energy (NE).

Table 2. The statistical summary of energy inputs (GJ ha^{-1}) and output (ton ha^{-1}) for cucumber production.

Inputs (unit)	Mean	SD	Min	Max
A. Inputs (GJ ha^{-1})				
Human labour	7.110	3.653	1.372	14.112
Machinery	1.221	0.496	0.560	1.959
Chemical fertilizers	62.582	55.535	18.793	180.802
Manure	0.008	0.004	0.000	0.015
Chemicals	5.775	3.370	1.714	10.971
Water	6.323	2.499	2.463	9.748
Electricity	1025.352	360.885	557.157	1550.185
Seeds	0.001	0.000	0.001	0.001
B. Output				
Cucumber (ton ha^{-1})	98.937	28.321	58.583	149.349

Table 3. Amount of inputs and output in cucumber production.

Inputs (unit)	Quantity per unit area (ha)	Total energy equivalent (GJ ha^{-1})	Percentage (%) of total energy	SD*
A. Inputs				
Human labour (h)	3627.454	7.110	0.607	3.653
Machinery (h)	93.464	1.221	0.104	0.496
Chemical fertilizers (kg):	2035.497	59.847	5.108	38.179
Nitrogen	609.533	40.315	3.441	
Phosphate (P$_2$O5)	610.364	7.593	0.648	
Potassium (K$_2$O)	752.171	8.387	0.716	
Calcium	34.143	0.038	0.003	
Micro	29.286	3.514	0.300	
Manure (kg)	20535.714	6.161	0.526	3.239
Chemicals (kg)	48.129	5.775	0.493	3.370
Water (m^3)	6199.506	6.323	0.540	2.499
Electricity (kWh)	85947.357	1025.352	87.514	360.885
Seeds (kg)	0.857	0.001		0.000
Total energy input (GJ ha^{-1})		1171.637	100.000	
B. Output				
Cucumber (ton ha^{-1})	112.429	89.943	100.000	32.180
Total energy output (GJ ha^{-1})		89.943		

Represents the standard deviation of energy inputs (GJ ha^{-1}) and energy output (ton ha^{-1})

Materials and Methods

Data were collected through a survey of 8 cucumber greenhouses farmers in Barka region, Sultanate of Oman who agreed to participate in the survey. Cucumber is the most cultivated crops in greenhouses.

Data were obtained through face to face interviews of the farmers which encompassed questions related to all expenses related to the greenhouses for three months (seeding-to-harvesting) during the cropping season (summer 2017).

The greenhouses were Quonset with polyethylene covering and fan-pad evaporative cooling system (Fig. 1). All greenhouses were of similar type, covering material and cooling system. The standard dimensions of greenhouses were 0.035 ha. The average row and plant spacing were 133 cm and 50 cm, respectively. Cucumber plants were trained to grow vertically in the greenhouse (Fig. 2). The average ambient temperature was around 29°C during the study period.

The 8 inputs considered in this study were human labor, machinery, chemical fertilizers chemicals, manure, electricity, water for irrigation and seeds and the single output was yield. The selection of the inputs was based similar studies from which we omitted diesel fuel which is not used in the greenhouses surveyed (Mousavi-Avval et al. ,2011; Mohammadi and Omid 2010 ; Omid et al. 2011).

For energy performance analysis, all inputs and output were converted into single energy unit, with conversion values obtained from different sources (Table 1). EUE, EP and NE were calculated using Equations 1-3, respectively (Mohammadi et al. 2008; Ozkan et al. 2011).

EUE = Energy Output (GJ ha^{-1})/Energy Input (GJ ha^{-1}) (Eq. 1)

EP = Cucumber Output (kg ha^{-1})/Energy Input (GJ ha^{-1}) (Eq. 2)

NE = Energy Output (GJ ha^{-1}) − Energy Input (GJ ha^{-1}) (Eq. 3)

In general, the energy inputs used in agricultural production systems are classified into direct (D) and indirect (ID), and renewable (R) and non-renewable (NR) energies (Mohammadi et al. 2010; Mohammadi et al. 2008). The direct energy inputs involve human labour, water, and electricity, the indirect energy involves seeds, fertilizers, manures, chemicals, and machinery, the renewable energy inputs includes human labour, seeds, manure and water and the non-renewable energy accounts for chemicals, fertilizers, machinery and electricity.

Results and Discussion

The descriptive summary of inputs used for cucumber production and output are presented in Table 2. There

Figure 1. Quonset greenhouse with polyethylene covering and (a) fan-(b) pad evaporative cooling

Figure 2. The greenhouse vertical cultivation

were variations among inputs and output which implied that there was a potential efficiency improvement for cucumber greenhouse farmers. Thus, EUE of greenhouses was evaluated hereafter.

The inputs with their equivalent energy values consumed in cucumber production for the studied greenhouses are illustrated in Table 3. Average yield was 112.428 ton ha^{-1} which is equivalent to a total energy output of 89.943 GJ ha^{-1}. Results also revealed that 1171.637 GJ ha^{-1} of total energy input were needed in the production of cucumber crop. Electricity, chemicals and chemical fertilizers were calculated as 1025.352, 5.775 and 59.847 GJ ha^{-1}, respectively. Among all energy inputs, electricity was the highest energy consuming factor with a total of 88% (Fig. 3). This high electricity consumption was further analyzed and the analysis revealed that most of this electricity was consumed by the cooling system (Fig. 3) as the temperature sometimes exceeds 50°C (Al-Ajmi and Abdel-Rahman 2001; Al-Sadi

Table 5. Energy use efficiency in cucumber production in Oman.

Items	Unit	Value	SD
E_{input}	GJ ha^{-1}	1171.64	374.58
E_{output}	GJ ha^{-1}	89.94	25.75
Yield	ton ha^{-1}	112.43	32.18
EUE	unitless	0.08	0.03
EP	ton GJ^{-1}	0.10	0.04
NE	GJ ha^{-1}	-1081.69	361.78

et al. 2011). To limit this use of non-renewable energy, it is recommended to investigate the use of alternative power sources such as solar energy to operate the cooling system (fans and pumps).

The EUF, EP and NE of cucumber production were 0.08, 0.10 tons GJ^{-1} and -1081 GJ ha^{-1} respectively (Table 5). In the literature, the EUE for greenhouse cucumber production was considered inefficient when it equals to

0.017 (Zarini et al. 2013) or even as high as 0.64 (Mohammadi and Omid 2010). Therefore, the EUE of the surveyed greenhouses in this study was (0.08) should also be considered inefficient. This EUE in cucumber greenhouse production could be increased by either increasing crop yield or by decreasing inputs consumption. The average EP (0.10 ton GJ^{-1}) for greenhouse cucumber production was lower than the reported values of 0.80 (Mohammadi and Omid 2010), 0.55 (Heidari and Omid 2011) and 0.50 (Omid et al. 2011). This indicates that every kilogram of cucumber in the surveyed greenhouses in Oman consumed 5-8 times more input energy than the greenhouses cited above. The high negative value of NE (-1081.690 GJ ha^{-1}) indicates that energy being lost in cucumber production. This can be attributed to the high electricity consumption consumed by the cooling systems. In Iran, Omid et al. (2011) and (Bolandnazar et al. 2014) found that the NE for greenhouse cucumber production gave also negative values (-55.553 and -149.317 GJ ha^{-1}, respectively) due to the high fuel consumption for heating (more than 50% of the total inputs energy). Since, energy consumption for temperature adjustment (heating or cooling system) is the highest among all inputs in greenhouse cucumber

Table 4. Energy forms (D, ID, R and NR) in cucumber production in Oman.

Form of energy	Energy value (GJ ha^{-1})	% of total energy input
D [a]	1038.785	88.66
ID [b]	73.005	6.23
R [c]	19.595	3.32
NR [d]	1092.20	93.219
Total energy input	1171.637	100.00

a -involves human labour, water, electricity.
b -involves seeds, fertilizer, manure, chemicals, machinery.
c -involves human labour, seeds, manure, water.
d -involves chemicals, fertilizer, machinery, electricity.

Figure 3. The The percentage energy (GJ/ha⁻¹) inputs used in cucumber production and the percentage of irrigation and cooling from the total electricity

production; the existing heating/cooling systems need improvement in order to achieve a more efficient energy use and sustainable crop production.

The percentage of total energy inputs as D, ID, R and NR energy is shown in Table 4. It was found that the D energy inputs (e.g. human labour, water, electricity) followed by NR energy inputs (e.g. chemicals, fertilizer, machinery, electricity) were the highest energy consumers and the R energy forms (e.g. human labour, seeds, manure, water) were the lowest energy consumers. Other investigators, such as, Ozkan et al. (2007), Kizilaslan (2009), Mohammadi and Omid (2010), Banaeian et al. (2011) and Zarini et al. (2013) reported similar outcomes.

The tetra-inoculum (Tm+Tc+Gr+Gn) reported highest average of dry weight of plants which was 710 mg/plant compared with negative and positive control which was 86 and 479 mg/plant respectively, followed by the treatment of tri-inoculum (Tc+Gr+Gn) which was 602

mg/plant. The combined application of Gr+Gn exhibited significant increase in the dry weight of plant which was 559 mg/plant. Tariq and Magee (1990) found that volatile components of garlic extracts inhibited germination of the microconidia, macroconidia and mycelium of the *F. oxysporum* f. sp. *lycopersici in vitro*. Al-Rahmah et al. (2013) has been found that ginger extracts has strong fungistatic and fungicidal activities against *F. oxysporum* and *Pythium aphanidermatum* with minimal inhibitory concentration. Furthermore, Sahar et al. (2013) proved that foliar application of Topsin-M with concentration of 0.1 % was significantly reduced the disease incidence of Fusarium wilt disease on eggplant.

Conclusion

This study investigates the EUE of greenhouse cucumber production. Results revealed that the total energy output for greenhouse cucumber production was 12 times lower than the total energy inputs. The EUE in greenhouse cucumber production was inefficient and electricity was the major energy consuming factor. Fan-pad evaporative cooling system was the major consumer of the electricity and thus the existing cooling systems need be improved to achieve better efficient energy. Highest energy form in greenhouse cucumber production was the NR energy. Special attention has to be given to this form of energy in order to achieve sustainable cucumber production. Solar energy needs to be exploited to improve the sustainable cucumber greenhouse production in Oman.

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Variations in consumer's acceptability and proximate composition of yellow croaker (*Larimichthys polyactis*) with processing methods

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الاختلافات في قبول المستهلكين والتركيب الكيميائي للكروكر الأصفر (*Larimichthys polyactis*) مع طرق التصنيع

أيلويا ا.ا. وجورج ف.و.ا. وجيموح و.ا. وعبدلالسلامي س.ا.

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ABSTRACT. The effect of processing methods on the proximate composition and consumer's acceptability of yellow croakers were investigated. The experiment followed a completely randomized design with several processing methods. Frozen yellow croaker fish of 12 kg were purchased, cut into chunks, divided into 4 groups of 3 kg each, each group was salted and then subjected to different processing methods including: air drying in the sun, deep-frying, solar drying and smoking. After processing, the products were subjected to organoleptic assessment using an hedonic scale while the proximate composition was determined using standard experimental procedures. The results of this study indicated that the odour and flavour of smoked fish product were significantly (p < 0.01) preferred by consumers. Solar dried fish product had highest percentage crude protein (71.45 kg/100 kg sample) followed by smoked fish product (70.43 kg/100 kg sample) while smoked fish product had the highest crude ash (10.73 kg/100 kg sample). The study suggested that it was better to process quality yellow croaker using solar dryers and smokers to attract customers.

KEYWORDS: Consumers; proximate composition; processing methods; yellow croaker.

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المستخلص: تم دراسة تأثير طرق التصنيع على التركيب الكيميائي وقبول المستهلك لسمك الكرور الأصفر. وقد إتبعت التجربة تصميمًا إحصائيا عشوائيًا تمامًا مع طرق التصنيع المستخدمة. تم شراء أسماك الكروكر الصفراء المجمدة بوزن 12 كجم ، مقطعة إلى قطع ، مقسمة إلى 4 مجموعات كل منها 3 كجم ، وتم إضافة الملح على كل مجموعة ثم تعريضها لأساليب تصنيع مختلفة بما في ذلك: التجفيف الهوائي الشمسي ، القلي العميق ، التجفيف بالطاقة الشمسيه والتدخين . بعد عملية التصنيع ، تم دراسة المنتجات بإستخدام طرق التقييم الحسي باستخدام مقياس هيدونيك وكذلك دراسة التركيب الكيميائي باستخدام التجارب القياسية. أشارت نتائج هذه الدراسة إلى أن رائحة ونكهة منتج السمك المدخن من قبل المستهلكين كانت مفضلة بدرجة كبيرة (0.01>P). يحتوي منتج الأسماك المجففة بالطاقة الشمسية على أعلى نسبة من البروتين الخام (71.45 كجم / 100 كجم عينة) يليه منتج السمك المدخن (70.43 كجم / 100 كجم عينة) بينما يحتوي منتج السمك المدخن على أعلى نسبة رماد خام (10.73 كجم / 100 كجم عينة). أشارت الدراسة إلى أنه من الأفضل إستخدام مجففات الطاقة الشمسية و التدخين للحصول على منتج ذات جودة عالية من سمك الكروكر الأصفر لجذب المستهلكين.

الكلمات المفتاحية: المستهلكون ، التركيب الكيميائي ، طرق التصنيع ، الكروكر الأصفر

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Introduction

Consumers have become increasingly interested in fish as a source of dietary protein due to the rising cost of meat protein foods (Emere and Dibal, 2013). Fish as a whole, has a lot of food potential and can therefore be expected to provide relief from malnutrition, especially in developing countries (Ashraf et al., 2011). It constitute about 60% of the total protein intake in adults especially in rural areas (Adeleye, 1993). Foran et al. (2005) pointed that fish is a highly protein containing food consumed by a larger percentage of population because of its availability and palatability. In Nigeria, fish is eaten fresh, preserved or processed (smoked) and form a much-cherished delicacy that cuts across socio-economic, age, religious and educational

barriers (Adebayo-Tayo et al., 2008). However, the gap between demand and supply of fish is widening due to increase in population, poor postharvest handling, lack of processing and storage facilities and utilization of unconventional fish species (Ogbonnaya and Ibrahim, 2009). Different fish processing and preservation methods are used to slow down spoilage resulting in fish that are both be safer for consumption and with an increased shelf-life. Researchers have also studied the effect of processing methods on the nutritional qualities of different fish species. Nutritive and organoleptic changes of Nigerian traditionally-processed freshwater fish species were studied by Afolabi et al. (1984). The effect of traditional drying processes on the nutritional values of fish was studied by Eves and Brown (1993). Changes in chemical composition and nutritional quality of fried Sardine (*Clupea pilchardus*) produced by frozen storage and microwave reheating were reported by Castrillon et al. (1997). Ayeloja et al. (2013) reported the effect

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Table 1. Effect of processing methods on the perceived physical attributes (hedonic scale) of yellow croaker.

Fish products	Odour	Kruskal–Wallis (χ^2)	p-value	Flavour	Kruskal–Wallis (χ^2)	p-value	Texture	Kruskal–Wallis (χ^2)	p-value
Sun dried	25.65	36.836**	0.01	20.59	40.705**	0.01	36.76	3.932	0.27
Fried	53.38			52.68			28.56		
Solar dried	16.47			18.53			31.85		
Smoked	42.50			46.21			40.82		

**Kruskal Wallis test (χ^2) is significant between rows at p < 0.05

of processing methods on the nutritive value of catfish (*Clarias gariepinus*). However, there is little information on the effect of processing methods on the nutritional quality of Yellow croaker, thus the need for this study.

Schiffman and Graham (2000) reported that taste and smell played an important role in developing appetite, making food choices, and nutrient intake for the following reasons: (1) these chemosensory signals prepare the body to digest food by triggering salivary, gastric, pancreatic, and intestinal secretions, which are termed cephalic phase responses, (2) they enable us to detect and discriminate among foods in the face of fluctuating nutritional requirements. Taste and smell enable selection of a nutritious diet as there are associations between a food taste or smell and its post-ingestion effects as taste enable the consumer to modulate food intake in anticipation of its nutritional consequences. Thus, taste sensations serve as an indicator of a particular food nutritional value. In addition, taste and smell signals initiate, sustain and terminate ingestion, and hence play a major role in the quantity of food that is eaten and the size of meals. Taste sensations induce feelings of satiety and are primary reinforced by eating. Thereby, justifying the use of texture, odour and flavour (which is a combination of taste and odour) in determining consumers' acceptability of various food, in this case differently processed yellow croaker.

Materials and methods

The experiment followed a completely randomized design where the treatments were the various processing methods employed. Twelve (12 kg) frozen yellow croaker fish was purchased from the cold room of Atlantic Shrimpers limited, Ring Road, Near Challenge Roundabout, Ibadan Oyo State, Nigeria. These were transported within 17 minutes to the fish processing unit of Federal College of Animal Health and Production Technology (FCAH & PT) Moor Plantation, where these were cut into chunks and then divided into 4 groups of 3 kg each. Each chunk was washed, salted and then subjected to different processing methods including: air drying in the sun (sun drying), deep-frying, solar drying (in a solar oven) and smoking. Sun drying was done for 4 days using locally constructed sun drying rack with appropriate screen, which protected the product from direct contact

with insects. The average ambient temperature during the period of the experiment was 27ºC ± 3ºC. Deep-frying was performed in vegetable oil in a pot on a hot flame with occasional flipping in order to achieve even frying. Frying was achieved within 21 minutes at a temperature of about 240ºC. Solar drying was achieved in 4 days using locally constructed solar dryer with appropriate screen, which protected the product from direct contact with insects. Smoking was done at the temperature of 90ºC ± 10ºC for 42 hours using NIOMR (Nigeria Institute for Oceanography and Marine Research) smoking kiln installed in the fish processing unit of the Fisheries Technology Department, Federal College of Animal Health and Production Technology (FCAH & PT) Moor Plantation Ibadan Oyo State Nigeria. Charcoal was used to generate heat and smoke.

The various processed fish products were subjected to sensory quality evaluation using descriptive test based on 5-point hedonic scale modified from Tobor (1994) and Eyo (2001). Odour, flavour and texture were the sensory attributes examined. The following grades were allotted depending on their qualities: 8 − 10 = Excellent, 6 − 8 = Very good, 4 − 6 = good, 2 − 4 = bad and ≤ 2 = worst. Twelve trained panelists from the Federal College of Animal Health and Production Technology Ibadan were used for the assessment. The proximate composition of the fish samples was determined using
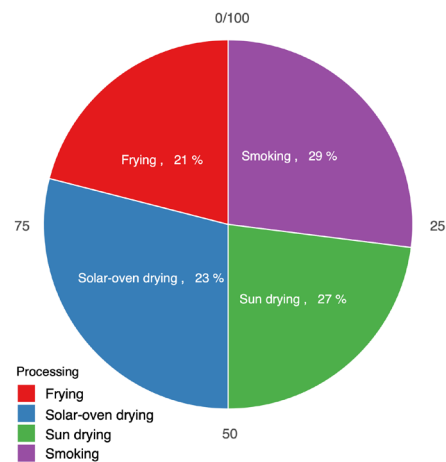

Figure 1. Effect of processing methods on texture of processed yellow croaker

Table 2. Effect of processing methods on proximate composition yellow croaker

Fish Products	Moisture + SD (g/100 g sample)	Crude Protein + SD (g/100 g sample)	Lipid + SD (g/100 g sample)	Ash + SD
Sundried	15.39 + 0.02c	69.05 + 0.05c	6.42 + 0.02b	8.15 + 0.02b
Fried	30.44 + 0.04a	47.08 + 0.04d	18.58 + 0.02a	3.55 + 0.01d
Solar dried	14.13 + 0.03d	71.45 + 0.02a	4.43+ 0.03c	5.70 + 0.02c
Smoked	16.03 + 0.05b	70.43 + 0.03b	2.72 + 0.02d	10.73 + 0.01a

Values with different letter within each column are significantly different (P<0.05)
SD: Standard deviation

the standard methods of AOAC (1994).

Statistical analysis

Data collected on descriptive organoleptic assessment using hedonic scale were subjected to nonparametric test (Kruskal Wallis test) and data collected on proximate composition were subjected to analysis of variance (ANOVA) to determine significance between means. Duncan Multiple Range Test (DMRT) was used to compare differences among means. Significant level was chosen at p < 0.05.

Results and discussion

There was a significant difference between the odour of differently processed yellow croaker (χ^2=36.836, p<0.01) with fried product, rated as having the best odour followed by smoked fish product (Table 1). There was also a significant difference between the flavour (χ^2=40.705, p<0.01) of the various processed fish products with fried yellow croaker rated as having the best flavour followed by smoked product (Table 1). Ayeloja et al. (2013) reported similar result in their study of the effect of processing methods on nutritive value of catfish (*Clarias gariepinus*). The result of this study also agrees with the opinion of Jang et al. (2009). In their study of food attributes as reported that the satisfaction levels of ethnic foods varies depending on diverse food attributes such as taste, fresh, colorful, uniqueness and healthiness. In related veins, Lin (1991) observed that oyster consumption was mainly influenced by five food attributes, including taste, nutritional value, freshness, cost and safety. However, there was no significant difference (χ^2 =3.932, p > 0.05; Table 1) in the perceived texture of the various fish products although there was a small preference for smoked fish, (29%) over sundried fish product (27%) and fried fish (21%) (Fig.1).

The results of the effect of processing methods on proximate composition of yellow croaker (Table 2) indicated that processing methods showed effect on the proximate composition of processed yellow croaker. Ogbonnaya and Ibrahim (2009) also reported that different drying methods affected the proximate compositions of catfish. The moisture content of the various fish products was significantly different (P<0.05) with fried fish having the highest moisture content (30.44 +0.04 g/100 g sample). A similar trend was observed for percentage lipid composition with fried fish product had the highest percentage of lipids (18.58+0.02 g/100 g sample). However, solar dried fish product had the highest crude protein (71.45+0.02%) which was significantly different (p<0.05) from the protein content of the other fish products, which suggests that proteins in solar dried fish product were retained and less denatured by processing method employed than in other processed fish products. Fried fish had the lowest percentage protein. This is in accordance with the findings of Gokoglu et al. (2004), Tao and Linchun (2008). There was a significant difference (p<0.05) in the percentage ash of the various fish products with smoked fish product having the highest ash content (10.73 + 0.01 g/100 g sample). Oyero et al. (2012) expressed a similar opinion that smoked fish products showed significant higher levels of ash content, which was as a result of inorganic matter which remains on it after the organic matter burnt. This signifies that the smoked fish products had high levels of mineral contents.

Conclusion

This study indicates that consumers prefer the odour and flavor of fried fish product above others followed by that of smoked fish product. On the other hand, solar-oven dried fish had the highest crude protein followed by smoked fish. Smoked fish had the highest percentage of ash which probably resulted from the burnt organic matter; this signifies that the smoked fish products had high levels of mineral matter. It is therefore better to process yellow croaker fish using solar oven or smoking as fish preserved using these preservation methods have better protein content. Consumers also preferred the odour and flavour of smoked fish above others; this additional value could increase the economic value of smoked fish and in turn increase the income of fishers.

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First report of *Dermatophilus congolensis* infections in local Omani cattle.

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التقرير الأول عن عدوي المجتلدة الكونغولية في الأبقار المحلية في سلطنة عمان

محمود النويشي[2,3] وجلندا المعولي[3] وهيثم علي[*1]

ABSTRACT. Dermatophilosis is an infectious skin disease in animals and man caused by *Dermatophilus congolensis*. **Objective**: to report the isolation of *Dermatophilus congolensis* from paint-brush matted hairs to wart-like skin lesions in 12.1% of quarantined local cattle from the Sultanate of Oman. **Methodology**: skin scrapings, blood samples and skin biopsies were aseptically collected from the affected cattle for bacteriological, hematological and histopathological examinations, respectively. **Results**: bacterial cultural and staining characteristics of skin scrapings were confined with *D. congolensis*. Neutrophilia were reported in 86.75% of the examined animals. Histologically, there was severe multifocal exudative dermatitis with hyperkeratosis. Affected animals were treated successfully with three doses of long-acting oxytetracycline (20 mg/kg; IM injection) with topical application of antibiotic spray. **Conclusion**: this is the first report of dermatophilosis in local Omani cattle, and field veterinarians should be kept up to date with this recent detected disease.

KEYWORDS: *Dermatophilus congolensis*, Oman, Pathology, Natural infection, Zoonotic disease

المستخلص. المقدمة: داء المجتلدات هو مرض جلدي معدي في الحيوانات والإنسان يسببه بكتيريا المجتلدة الكونغولية. **الهدف**: تسجيل عزل بكتيريا المجتلدة الكونغولية من أفات جلدية تتراوح بين شعر متلبد يشبه فرشة الطلاء إلى شبيه الثآليل في 12% من ابقار محلية في الحجر البيطري في سلطنة عمان. **المنهجية**: تم تجميع كشط جلدي وعينات دم وخزعات جلدية معقمة للفحص البكتيري والدموي والنسيجي، بالترتيب. **النتائج**: اظهرت الزراعة البكتيرية والصبغ للكشط الجلدي بكتيريا المجتلدة الكونغولية وكذلك زيادة العدلات في الدم في 86.75% من الحيوانات المفحوصة. لوحظ التهاب جلدي نضحي وخيم مع فرط التقرن بالفحص الهيستوباثولوجي وقد تم علاج الحيوانات المصابة بنجاح باستخدام ثلاثة جرعات من دواء أوكسي تتراسيكلين طويل المفعول (mg/kg 20 عن طريق الحقن العضلي) مع استخدام علاج موضعي باستخدام بخاخ المضاد الحيوي. **الاستنتاج**: هذا هو التقرير الأول عن داء المجتلدات في الأبقار المحلية بسلطنة عمان وينبغى إحاطة الأطباء البيطريين العاملين بالحقل بهذا المرض المكتشف حديثاً.

الكلمات المفتاحية: المجتلدة الكونغولية، سلطنة عمان، أفات مرضية، عدوي طبيعية، مرض حيواني المنشأ

Introduction

Dermatophilosis is a skin disease of cattle, sheep, camels, goats, horses and other wildlife species (Gebreyohannes and Gebresselassie 2013). The diseases is caused by *Dermatophilus congolensis*, a facultative anaerobic gram-positive branching filaments bacteria with both transverse and longitudinal fragments formed from packs of coccoid bodies arranged in parallel rows giving the characteristic 'train track' morphology (Quinn and Markey 2003). Although dermatophilosis is worldwide, more economic losses are encountered in tropical countries. Losses are mainly attributed to dam-aged hides, decreased production and culling (Stewart 1972). Skin abrasions and humid conditions triggers the dormant coccoid bodies to become motile flagellated zoospores that breakthrough the barriers of the skin and forms invasive filamentous in the hair follicle and superficial epidermis (Yeruham et al. 2003). Presence of the bacteria evokes an acute inflammation that recruits neutrophils resulting in multiple microabscesses formation in the epidermis. The characteristic crusty cutaneous lesions are the outcome of repeated episodes of the inflammatory reaction against the bacteria and the epidermal regeneration (Yeruham et al. 2003). Ticks and biting flies compromising the skin integrity might be involved in the mode of transmission (Quinn et al. 2011); moreover, various enzymes including proteases, keratinases, and ceramidase produced by the *D. congolensis* might play a key role in the pathogenesis. Lesions are frequently seen on the back of the animals, ears of nursing kids and distal extremities. The diseases is zoonotic,

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Figure 1. Dead cow with skin lesions on the neck and perineal areas (A &B); clinically ill cow with active skin lesions, focal alopecia and dermatitis on the face and neck (C); matted hair resembling paint brush pulled from the skin lesions (D & E); slightly hemorrhagic and eroded skin surface following the removal of the matted hair (F).

few human cases have been reported in the form of pustules and eczema on the hands and forearms of patients following direct contact with infected animals (Burd et al. 2007; Dean et al. 1961).

This study describes the detection of dermatophilosis infection and successful treatment in local cattle in the Sultanate of Oman.

Materials and Methods

Animal and case history

On January 2018, a dead cow was submitted to the Central Laboratory for Animal heath, Ministry of Agriculture and Fisheries, Muscat, Oman. The animal was covered with skin lesions all over its body with intense lesions on the neck and perineal areas (Fig. 1A & 1B). The dead cow and other 16 diseased animals out of 140 (12.1%) was part of a herd that was collected from differ-
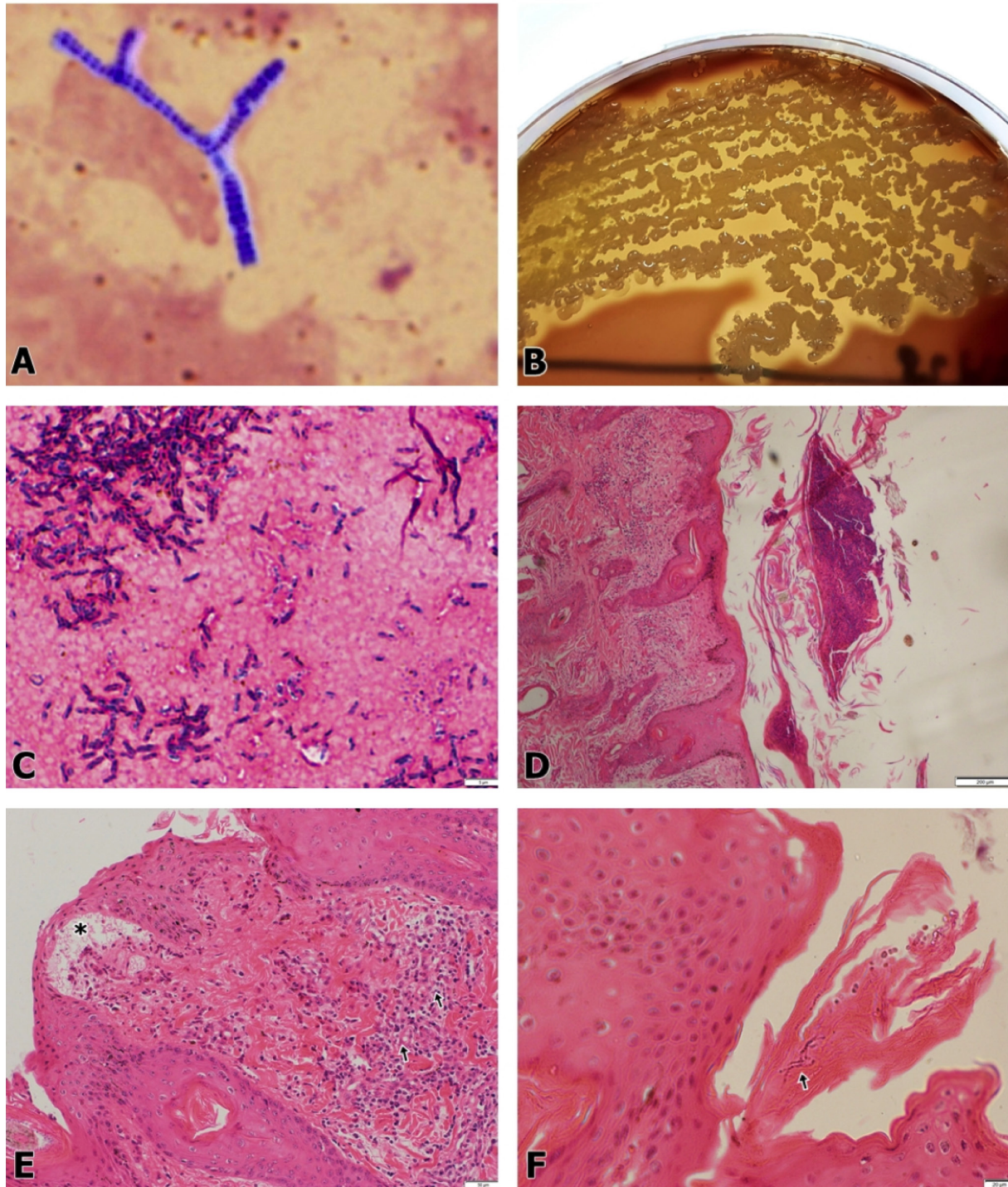
Figure 2. Direct smear from the skin lesions stained with gram stain: gram positive bacteria showing the typical train track morphology of the *D. congolensis*. 1000x (A); Cultured smear from the skin lesion showing irregular, yellowish-grey , beta hemolytic colonies on sheep blood agar (B); gram positive *D. congolensis* from culture smear 48 hours post culture (C); skin epidermis showing a multilaminated crust that are heavily infiltrated with neutrophil (D); skin dermis showing micro-abscess with clear fluid and neutrophil infiltrations (asterisk) and mononuclear cells (arrows) (E); skin epidermis showing branching filaments of *D. congolensis* bacteria arranged in parallel rows (arrow) (F).

ent governorates (Ad Dakhiliyah, Al Batinah North, Al Batinah south and Dhofar) and quarantine for exportation purpose.

Bacteriological examination

Fresh skin scraping and crusts were collected aseptically from the dead cow and the other clinically ill animals and mixed in a few drops of sterile distilled water on a glass slide then stained with gram stain. Crusts and skin lesions were inoculated in sheep blood agar plate at 37 °C for 48 hours with 5% CO_2 (Quinn and Markey 2003).

Hematology

Blood samples were collected from the clinically ill animals by Vacutainer systems from the jugular vein. Hematological parameters were measured using Vet Auto Hematology Analyzer® (model BC 2800, Mindray). The mean value was determined for following parameters: total and deferential leucocyte count, red blood cell count, platelet count, hemoglobin concentration, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, mean cell volume, and packed cell volume.

Sampling for histopathology

Skin biopsies were taken using a disposable sterile blade, then immediately fixed in 10% neutral buffered formalin. Following fixation, samples were routinely processed, trimmed, sectioned and stained with hematoxylin and eosin (H&E) for histopathological evaluation according to Bancroft (2013). Consecutive sections were stained with Giemsa stain.

Results

The necropsied cow showed severe icterus not related to the skin lesions. Clinically, the reported skin lesions from 16 affected cows consisted of exudative dermatitis, matted hair, focal areas of alopecia and crust formation as shown in Figure 1 (C, D, & E). Removal of the matted hair or crusts left an eroded skin surface that usually bleeds (Fig. 1F). Purulent exudate was noticed in active lesions. Upon bacteriological examination of skin scrapings and crusts, resulting colonies were beta hemolytic, 1-2 mm in diameter, yellowish grey in color, rough, convex and attached firmly to the blood agar surface which is consistent with *D. congolensis* (Fig. 2B). Gram staining of both direct smears (Fig. 2A), smears from the cultured colonies (Fig. 2C) revealed gram-positive, branching filaments bacteria with both longitudinal and transverse fragments, and piled with coccoid bodies.

Although, there were no significant differences (data not shown) in most of the blood parameters when compared to reference value, 86.75% of the examined cattle showed neutrophilia.

Histopathologically, there was severe, multifocal, ex-

udative and proliferative dermatitis. The epidermis was diffusely thickened by acanthosis, hyperkeratosis and multilaminated crusts that are heavily infiltrated with neutrophils (Fig. 2D). The dermis showed moderate to severe edema, hemorrhage, and was infiltrated by both neutrophils and mononuclear cells (Fig. 2E). Branching filaments of *D. congolensis* bacteria arranged in parallel rows and piled with coccoid bodies were detected in the stratified crusts of both H&E (Fig. 2F) and Giemsa stained sections.

Clinically infected animals were isolated and treated successfully with three doses of intra muscular injection of long-acting oxytetracycline 20 mg/kg every 2 days with topical application of antibiotic spray containing chloramphenicol and gentian violet and proper disinfection of the shed.

Discussion

The current study documented the first natural *D. congolensis* infections in local cattle in Sultanate of Oman. In neighboring countries, Dermatophilosis has been reported in camels in Saudi Arabia (Gitao et al. 1998), United Arab Emirates (Joseph et al. 1998), and Jordan (Tarazi and Al-Ani 2016), and in dairy cows in Iran (Jafari Shoorijeh et al. 2008), and different animals (cattle, buffalo, goat, horse and antelope) in India (Pal 1995). The high humidity, overcrowding of the animals, presence of ticks, biting flies and skin injury during animal transportations are all predisposing factors for the emergence of this infection (Zaria 1993). In the current study, the infected animals were collected for exportation purpose from different governorates after heavy rains during December 2017. The reported skin lesions were in consistent with previous reports of *D. congolensis* infection in cattle (Carter and Cole, 1990; Quinn and Markey, 2003; Quinn et al., 2011). Colony morphology and staining characteristics of the infective agent were confined with *D. congolensis* (Quinn and Markey 2003). All infected animals were successfully treated as described by Osman (2014) and skin lesions were partially healed after 10 days. Moderate economic losses are encountered to the small-scale cattle herders due to damaged hide and decreased milk and meat production. Monitoring skin lesions in Omani cattle is essential and field veterinarians should be kept up to date with recent detected disease.

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Activation of apoptotic cell death by skin mucus from Asian swamp eel (*Monopterus albus*) against human lung cancer cell line

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تنشيط موت الخلايا المبرمج بالمخاط الجلدي من ثعبان الأنقليس الآسيوي (*Monopterus albus*) ضد خط خلايا سرطان الرئة البشرية

آية ربحي حلس[1*] وسيد محمود[2,3*] ومحمد عارفين قادري[1] ورضوان هاشم[1]

Abstract. Asian swamp eel (*Monopterus albus*) is a freshwater fish which distributed widely in the East of India mainly across Malay Peninsula and Indochinese Peninsula, it is also broadly distributed in the Southern areas of East Asia including, southeastern China, Western Japanese Archipelago, and Korean Peninsula. It lives in muddy places, rice paddies, and slow-flowing currents areas. It has a unique morphological elongated body which is similar to snake and covered with a thick layer of mucus. The objective of this study is to screen the cytotoxic activity of eel skin mucus extracts and to evaluate the mechanism of cell death apoptosis or necrosis based on caspases activity assays. For this purpose eel skin mucus was collected from healthy fresh eels to prepare aqueous and methanol extracts. Preliminary cytotoxicity study was demonstrated against Non-small-cell lung carcinoma cell line (A549) using cell viability assay which revealed that methanol extract was more potent than aqueous extract based on the response of ESM methanol and aqueous extracts to the relevant IC_{50} concentrations as IC_{50} values were 621±0.09 µg/mL and 845 ± 0.25 µg/mL respectively. Subsequently cell death was induced through triggering apoptosis by the activation of caspase-3/7, 8 and 9. In conclusion, the present study has revealed the cytotoxic potentials of eel skin mucus which may lead to the development of new anticancer agents.

Keywords: *Monopterus albus*; cytotoxic activity; apoptosis; caspases.

المستخلص: أنقليس المستنقعات الآسيوية (*Monopterus albus*) هـو مـن أسمـاك الميـاه العذبة الـتي يتـم توزيعهـا عـلى نطـاق واسـع في شرق الهنـد بشـكل رئيـسي عبر شبه جزيرة الملايـو وشبه جزيـرة الهنـد الصينيـة ، كمـا أنها موزعـة بشـكل واسـع في المناطـق الجنوبيـة مـن شرق آسـيا بمـا في ذلـك ، جنـوب شرق الصـين ، أرخبيـل غـرب اليابـان ، و شبه الجزيـرة الكوريـة. تعيـش في الأماكـن الموحلـة وحقـول الأرز ومناطـق التيـارات بطيئـة التدفـق. ولهـا تركيبـة جسـم فريـدة مـن نوعهـا والـتي تشبه فيهـا الثعبـان وتغطـى بطبقـة سميكـة مـن المخـاط. الهـدف مـن هـذه الدراسـة هـو فحـص النشـاط السـام عـلى الخلايـا لمستخلصـات مخـاط جلـد ثعبـان الأنقليـس ولتقييـم آليـة مـوت الخليـة سـواء كان مـوت الخلايـا المبرمـج أو النخـر عـلى أسـاس اختبـارات نشـاط الكاسبيـزس. لهـذا الغـرض؛ تم جمـع مخـاط جلـد ثعبـان البحـر مـن ثعابـين طازجـة صحيـة لإعـداد مستخلصـات المـاء والميثانـول. تم إجـراء دراسـة أوليـة للسـمية الخلويـة ضـد الخلايـا غـير الرؤيـة الرئويـة لسرطان الرئـة (A549) باسـتخدام اختبـار قابليـة الخليـة الـتي أظهـرت أن مستخلـص الميثانـول أقـوى مـن المستخلـص المائـي حيـث أن قيـم ${IC}_{50}$ كانـت ٦٢١ ± ٠٫٠٩ ميكروجـرام / مـل و ٨٤٥ ± ٠٫٢٥ ميكروجـرام / مـل عـلى التـوالي. وفي وقـت لاحـق ، فـإن آليـة مـوت الخليـة تسـبب المـوت الخلـوي مـن خـلال التسـبب في مـوت الخلايـا المبرمـج عـن طريـق تفعيـل الكاسبيـز ٧/٣ و ٨ و ٩. في الختـام ، كشـفت الدراسـة الحاليـة عـن إمكانـات الخلايـا السـامة للخلايـا لمخـاط ثعبـان البحـر ممـا قـد يـؤدي إلى تطـور جديـد. وكـلاء مضـاد للسرطان.

الكلمات المفتاحية: *Monopterus albus* ، النشـاط السـام للخلايـا ، مـوت الخلايـا المبرمـج ، الكاسبيـزس.

Introduction

Asian swamp eel (*Monopterus albus*) belongs to the family of Synbranchidae under Synbranchiformes order (Cheng et al., 2003). Asian swamp eel skin mucus is secreted by the epidermal goblet cells in the epidermis which composed from inorganic salts, immunoglobulins, lipids and gel-forming macromolecules such as mucins, and other glycoproteins suspended in water (Bragadeeswaran and Thangaraj, 2011), which gives the mucus lubricating properties (Pearson and Brownlee, 2005).

There are two main mechanisms describing the cell death in eukaryotic cells, apoptosis and necrosis. Apoptosis is a process with well-defined key steps that mark the progress of the process in individual cells. Cells undergoing apoptosis possess distinctive morphological, biochemical and molecular features including sequence of chromatin margination and aggregation, nuclear and cytoplasmic condensation, cellular shrinkage, budding and fragmentation through the partition of cytoplasm and nucleus into the apoptotic body (Eriksson et al., 2008). these apoptotic bodies immediately recognized and phagocytized by macrophages or adjacent epithelial cells. Hence, there is no inflammatory response is elicit-

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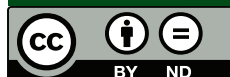
Table 1. Caspase-3/7 activity after treatment of A549 cells with ESM methanol and aqueous extracts along with positive control (Taxol) and negative control (untreated cells) for 24 hrs.

Treatment	OD1	OD2	OD3	Average	SD	Fold Change
Methanol	0.301	0.339	0.322	0.320*	0.019	3.340*
Aqueous	0.236	0.202	0.229	0.222*	0.017	2.344*
Taxol	0.351	0.332	0.347	0.343*	0.010	3.597*
Control	0.091	0.098	0.093	0.094	0.003	0.094

*Caspase-3/7 activities were determined using the CaspAce® system. Mean ± SD (n = 3 wells/treatment). *p < 0.05 compared with the untreated cells. Fold changes was calculated based on the control/untreated cells. OD is optical density.*

ed (Fadok et al., 2000). However, *in vitro*, the apoptotic bodies and residual cell fragments swell and lyse (Chang and Yang, 2000). The morphologic features that characterize apoptotic cells are the consequence of several biochemical features, which are stimulated by proteolytic destruction of cytoskeletal and metabolic proteins. Activation of the effector caspases 3 and 7 is a common step in both intrinsic and extrinsic apoptotic signal pathways, which accomplish the characteristic changes in the nuclear morphology and biochemistry, including chromatin condensation and DNA fragmentation (Fan et al., 2005).

Methodology

Materials

Cell Lines

The cell lines used in the study include human non-small lung carcinoma (A549, ATCC CCL-185) and normal mouse embryonic fibroblast (3T3-L1, ATCC CRL-3242). The cell lines were obtained from Biomedical Science Department, Kulliyyah of Allied Health Sciences, International Islamic University Malaysia.

Chemicals

Phosphate buffered saline (PBS), Fetal bovine serum (FBS), Dulbecco`s modified Eagle medium (DMEM), Penicillin-streptomycin were purchased from Gibco Invitrogen Co. (Scotland, UK). Paclitaxel (Taxol®) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Merck KGaA,

Germany. Caspase-3/7, caspase-8, caspase-9 kits were purchased from Promega, Madison, WI, USA.

Methods

Sample Collection and Extraction

The eel skin mucus (ESM) was collected from healthy eels by gently scraping the surface of the eel skin and then it was homogenized with 2 volumes of distilled water using homogenizer, followed by centrifugation at 13,000 rpm for 30 min, the supernatant was freeze-dried for 5 days. The dried substance was weighed and dissolved in distilled water to form aqueous extract and in methanol to form methanol extract, after that, the dissolved substance was filtered using 0.22 μm syringe filter to be ready for use. The extraction procedure was carried out according to the method previously described by Sadakane et al. (2007) with a slight modification.

Cell viability test (MTT-based cytotoxicity assay)

The antiproliferative effect of aqueous and methanol extracts of ESM on growth of two human cancer cell lines, i.e. human non-small lung carcinoma (A549) and normal mouse embryonic fibroblast (3T3-L1), were evaluated by MTT assay. Approximately 5×10^4 of cells were seeded into 96-well plates. after the cells reach the confluency level, they were treated with different concentrations of ESM aqueous and methanol extracts from 200 to 1000 μg/mL for 24 hrs. Then, 20 μl of MTT was added to each well and the plates were further incubated for 24 hrs. After that, 100 μl of DMSO was added to each well

Table 2. Caspase-8 activity after treatment of A549 cells with ESM methanol and aqueous extracts along with positive control (Taxol) and negative control (untreated cells) for 24 hrs.

Treatment	OD1	OD2	OD3	Average	SD	Fold Change
Methanol	0.380	0.371	0.295	0.348*	0.046	3.233*
Aqueous	0.197	0.229	0.215	0.213*	0.016	2.216*
Taxol	0.153	0.202	0.197	0.184*	0.026	1.790*
Control	0.103	0.109	0.115	0.109	0.006	0.109

*caspase-8 activities were determined using the CaspAce® system. Mean ± SD (n = 3 wells/treatment). *p < 0.05 compared with the untreated cells. Fold changes was calculated based on the control/untreated cells. OD is optical density.*

Table 3. Caspase-9 activity after treatment of A549 cells with ESM methanol and aqueous extracts along with positive control (Taxol) and negative control (untreated cells) for 24 hrs.

Treatment	OD1	OD2	OD3	Average	SD	Fold Change
Methanol	0.442	0.450	0.447	0.446*	0.004	3.937*
Aqueous	0.299	0.318	0.271	0.272*	0.023	2.725*
Taxol	0.541	0.519	0.528	0.529*	0.011	4.625*
Control	0.019	0.012	0.017	0.033	0.003	0.033

caspase-9 activities were determined using the CaspAce® system. Mean ± SD (n = 3 wells/treatment). *p < 0.05 compared with the untreated cells. Fold changes was calculated based on the control/untreated cells. OD is optical density.

and incubated for 4 hrs. Then, the absorbance was measured at 570 nm using a microplate reader. The percentage of cell survival rate was calculated using to the cell viability formula: Percentage of cell viability = (Mean of experimental absorbance / Mean of control absorbance) × 100 (Villarroel et al., 2007).

PBS was used as a negative control while 1 μg/mL of Taxol was used as a positive control as it is a chemotherapy medication used in the treatment of non-small-cell lung cancer (NSCLC) such as A549 cell line.

Determination of the mechanism of cell death by measuring of caspase 3, 8 and 9 activities

Caspase 3, 8 and 9 are members of the cysteine aspartic acid-specific protease (caspase) family which play key roles in apoptosis induction. The test was conducted according to the instructions of the kits. A549 cells were cultured in 96-well plates at the density of 1×10^4 cells per well overnight. The adherent cells were further incubated for 24 hrs with 100 μl media containing IC50 values of ESM aqueous and methanol extracts. After the treatment, the cells were harvested and centrifuged then the pellets were washed with PBS and lysis in chilled lysis buffer. The mixture was left on ice for 10 min then centrifuged at 2000 rpm for 5 min at 4°C. Then the supernatant was used for the determination of caspase activities. The results were read on a microplate reader at 405 nm (Abdullah et al., 2015).

Results

Cell viability assay

The results showed that the reduction in cell viability of A549-treated with ESM extracts was significant compared with the untreated cells. At 200 μg/mL which was the lowest concentration used, the cell viability of ESM aqueous and methanol was recorded as 87. 61% and 76.29% respectively whereas at 1000 μg/mL of ESM aqueous extract the cell viability was recorded as 46.58% while for methanol extract was 38.19%. The cell viability of ESM aqueous and methanol extracts against 3T3-L1 at 1000 μg/mL was 79.24% and 63.65% respectively while at 200 μg/mL was 92.53% and 89.21% respectively. The inhibitory concentrations of ESM extracts against

A549 which is required to reduce 50% of cell viability (IC_{50}) was calculated and it was recorded as follows; 621 ± 0.09 μg/mL for methanol extract, 845 ± 0.25 μg/mL for aqueous extract and 43.12 ± 0.6 μg/mL for Taxol.

Effects of ESM aqueous and methanol extracts on caspase 3,8 and 9 activities

A549 cells-treated with the ESM extracts exhibited a sharp increase in the activities of caspases 3, 8 and 9. The activities of the individual caspases were expressed as fold increases with respect to the untreated control. Fold change is defined as the ratio between different values, the fold change of caspase 3,8 and 9 was higher for ESM methanol extract than aqueous extract compared to the control (untreated A549 cells) as shown in table 3.1,2,3. The activities of 3, 8 and 9 caspases were increased significantly in A549 cells-treated with 600 μg/mL of ESM methanol extract and 800 μg/mL of ESM aqueous extract compared to untreated cells (the concentration of methanol and aqueous extracts was chosen based on the nearest concentration to IC_{50} value which was 621 ± 0.09 μg/mL for ESM methanol extract and 845 ± 0.25 μg/mL for ESM aqueous extract.

Discussion

Treatment of ESM aqueous and methanol extracts significantly inhibited the growth of A549 cell line compared to the normal cell line. This result agrees with what has been reported about the anticancer activities of Amphibian skin mucus (Kawasaki and Iwamuro, 2008). The present results showed that ESM extracts treatments activated 3, 8 and 9 caspases compared to the control (untreated cells), indicating that they induced A549 cell death via apoptosis as caspases test one of the biochemical markers which can be used to distinguish the mechanism of cell death, as it activated in the case of both intrinsic and extrinsic apoptotic signals (Fan et al., 2005). However, necrotic cell death does not involve caspase activation (Mansilla et al., 2006). Activation of caspases consider a hallmark of apoptosis and there are two pathways included; death receptor pathway which based on caspase-8 activation and mitochondrial pathway which based on caspase-9 activation (Leong et al., 2016). Accordingly, activities of the caspases 3, 8 and 9

Table 4. Caspase-8 activity after treatment of A549 cells with ESM methanol and aqueous extracts along with positive control (Taxol) and negative control (untreated cells) for 24 hrs.

Treatment	OD1	OD2	OD3	Average	SD	Fold Change
Methanol	0.380	0.371	0.295	0.348*	0.046	3.233*
Aqueous	0.197	0.229	0.215	0.213*	0.016	2.216*
Taxol	0.153	0.202	0.197	0.184*	0.026	1.790*
Control	0.103	0.109	0.115	0.109	0.006	0.109

caspase-8 activities were determined using the CaspAce® system. Mean ± SD (n = 3 wells/treatment). *p < 0.05 compared with the untreated cells. Fold changes was calculated based on the control/untreated cells. OD is optical density.

were measured in A549 cells-treated with ESM aqueous and methanol extracts as well as Taxol which is a chemotherapy drug use for apoptosis induction (Hu et al., 2005).

Conclusion

The activation of caspase (3, 8 and 9) only occurs as a result of apoptosis not necrosis and this parameter has been considered as one of the most reliable biochemical parameters to differentiate between apoptotic and necrotic cell death, therefore, the current study revealed that ESM extracts activate cell death via apoptosis.

Acknowledgement

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Table 5. Caspase-9 activity after treatment of A549 cells with ESM methanol and aqueous extracts along with positive control (Taxol) and negative control (untreated cells) for 24 hrs.

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Methanol	0.442	0.450	0.447	0.446*	0.004	3.937*
Aqueous	0.299	0.318	0.271	0.272*	0.023	2.725*
Taxol	0.541	0.519	0.528	0.529*	0.011	4.625*
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Caspase-9 activities were determined using the CaspAce® system. Mean ± SD (n = 3 wells/treatment). *p < 0.05 compared with the untreated cells. Fold changes was calculated based on the control/untreated cells. OD is optical density.

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Evaluation of salinity intrusion in arable lands of Al-Batinah coastal belt using unmanned aerial vehicle (UAV) color imagery

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تقييم تسرب الملوحة في الحزام الزراعي في ساحل الباطنة باستخدام الصور الملونة الملتقطة من طائرة بدون طيار

سوسنة بنت هلال الرحبي* وياسين بن أحمد الملا وهيماناثا جاياسوريا

Abstract. Salinity by seawater intrusion due to excess groundwater pumping for irrigation is a major environmental challenge in the coastal areas of the Sultanate of Oman. Increasing salinity levels moving inward the arable lands is happening in a rapid manner. Thus, salinity needs to be evaluated and quantified using a fast and accurate method. The objective of this study was to estimate salinity intrusion in Al-Batinah coastal belt using color aerial imaging. The study was conducted in five randomly selected sites at increasing distances from the seashore of Al-Suwaiq area in Al-Batinah region of northern Oman. Color aerial images were acquired for each site with an Unmanned Aerial Vehicle (UAV). Images were enhanced by orthorectification in ENVI software. A Green Leaf Index (GLI) was obtained from each site image using Matlab software. Image analysis results were compared with the results of analyzed soil and water samples taken for ground-truth verification. There was a strong negative correlation between the distance from the seashore and the soil EC of each site (R = -0.95). Similarly, the mean value of GLI increased as the salinity levels decreased, R= -0.96 and -0.92 for soil EC and water EC, respectively. We demonstrated the possibility of the use of color images taken by a UAV to accurately quantify the effect of soil salination on vegetation along the costal belt.

Keywords: Seawater Intrusion; Salinity Dynamics; UAV; Image Processing; GLI.

المستخلص: تمثل الملوحة تحديًا بيئيًا كبيرًا في المناطق الساحلية في عمان. حيث أن حركة الملوحة باتجاه الأراضي الداخلية الصالحة للزراعة تسير بسرعة عالية، لذا فهناك حاجة ماسة للكشف عن حركة التملح وتقييمها باستخدام طريقة سريعة ودقيقة. الهدف من هذه الدراسة هو تقييم حركة الملوحة داخل حزام الباطنة الساحلي باستخدام تقنيات التصوير الجوي الملون. وقد أجريت الدراسة في خمسة مواقع مختارة بطريقة عشوائية تبعد بمسافات مختلفة عن شاطئ ولاية السويق. تم إلتقاط الصور الجوية الملونة لكل موقع بواسطة طائرة بدون طيار. وتم تحسين الصور هندسيا كخطوة اولى في عملية تحليل الصور الجوية. ثم تم حساب مؤشر إخضرار الأوراق (GLI) المستنبط من صورة كل موقع. بعد ذلك تمت مقارنة تحاليل الصور مع تحاليل التربة والماء في عملية التحقق وربط المستنبط بالواقع. كان هناك ارتباط سلبي قوي بين المسافة من شاطئ البحر وملوحة التربة لكل موقع (معامل إرتباط =0.95–). وبالمثل، زادت قيمة (GLI) مع انخفاض مستويات الملوحة، معامل إرتباط = 0.96– لملوحة التربة ومعامل إرتباط = 0.92 – لملوحة المياه. أوضحت نتائج هذا العمل البحثي إمكانية استخدام الطائرات بدون طيار مثبتة بكاميرا ملونة لتقدير وتحليل تأثير البعد عن شاطئ البحر على مستويات الملوحة في التربة والمياه، وكذلك على حالة الغطاء النباتي في الأراضي الصالحة للزراعة بمنطقة الباطنة.

الكلمات المفتاحية: تسرب الملوحة، ديناميكية التملح، الطائرات بدون طيار، تحليل الصور، مؤشر إخضرار الأوراق

Introduction

The Sultanate of Oman is considered as an arid country with an average annual rainfall of about 100 mm. Although agriculture occupies about 5% of the total area of Oman distributed around eleven governorates (regions), the agricultural sector consumes more than 93% of the total water demand. Al-Batinah North governorate represents the largest area of Oman's agricultural lands (Table 1) covering 24% of the total agricultural area of the country and is considered to be Oman's most important agricultural area as it produces 65% of the Omani agricultural production with crops such as dates, fruits, vegetables and forage crops such as alfalfa and Rhodes grass (Choudri et al. 2015a; Choudri et al. 2013).

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The population in Al-Batinah has sharply increased since 2003: i.e. by more than 100,000 people within 7 years. Subsequently, the socioeconomic activities; active ports, coastal tourism projects, industrial activities, infrastructure development, intensive agriculture and urbanization have been rapidly taken place in this coastal zone (Choudri et al. 2015c). Such activities are related to population growth and have led to increasing pressures on natural resources including groundwater, agriculture and land use (Lawley et al. 2016). It also has resulted in some environmental challenges such as seawater intrusion, water and soil salinition and desertification (Choudri et al. 2015a).

The management plans to mitigate the environmental challenges are constrained by the shortage of information about the interaction between the development activities and the environment (Rishi and Mudaliar 2014). In general, lack of information about the global, national and local land resources may lead to management plans

without environmental concerns (Mulder et al. 2011). Thus, there is a necessity for accurate, cost-effective and timely monitoring method to update the information on the status changes in the arable lands of coastal area (Mishra 2014), in order to develop a framework for the decision makers to manage the environmental problems.

Bajjali (2003) has conducted a study to assess the ground water quality in Oman by analyzing 20,000 wells across different regions. The study indicated that Al-Batinah coast is the most affected area with groundwater salinity in Oman, where the water salinity ranges from 5 to 44 dS/m (Choudri et al. 2015c). As reported by Choudri et al. (2015b), Ministry of Regional Municipalities and Water Resources collected salinity data from 18 different wells in Al-Batinah region during the years 1991, 1993, 2005 and 2010 (Table 2). The collected data suggested that water salinity has increased gradually in all examined wells within the last two decades. Furthermore, water salinity is an important factor in soil salinity (Al-Belushi 2003; Hussain 2005). Approximately 52% of Al-Batinah lands are affected by soil salinity (Al-Mulla et al. 2010). Between the years 2000-2005, the percentage of the agricultural lands affected with soil salinity has increased by about 7% (Al Barwani and Helmi 2006). In addition, soil salinity is considered as one of the main reasons of desertification in arid and semi-arid regions and so in Al-Batinah coast particularly (Al-Belushi 2003; Choudri et al. 2015b). On the other hand, soil salinition is considered as one of the main reduction factors of Omani dates exportation which decreased by 2,000 MT within a 5-year period (2007-2011). Similarly, production of date palm in Al-Batinah region has steadily declined within the last few years mainly due to groundwater salinity (Al-Yahyai and Khan 2015).

Although there are many studies investigated the

Table 1. Area of the agricultural lands in each governorate of Sultanate of Oman (feddan)

Governorate	Agricultural land Area (feddan)*	Percentage %
Muscat	11,555.85	3.26
Dhofar	65,921.13	18.57
Musandum	3,242	0.91
Al Buraimi	16,123.21	4.54
Ad Dakhiliyah	45,732.97	12.88
Al Batinah North	85,118.27	23.98
Al Batinah South	48,984.53	13.80
Ash Sharqiyah South	15,206.87	4.28
Ash Sharqiyah North	27,523.27	7.75
Adh Dhahirah	33,295.08	9.38
Al Wusta	2,307.9	0.65
Total	355,011.1	100

(M.A.F 2013)

salinity levels in Al-Batinah region using the traditional field visits and lab analysis, there is no documented evidences on evaluation of salinity change inward the coastal belt, and particularly using areal imaging technique. Therefore, the objective of this study was to analyze salinity change inward Al-Batinah coastal belt using images collected from an unmanned aerial vehicle (UAV) combined with color imaging techniques.

Figure 1. The Study Area location

Materials and Methods

Study Area

The study was conducted in Al-Suwaiq area (23° 50' 58" N, 57° 26' 19" E). It is located at the south part of Al-Batinah North governorate (Fig. 1). The climate of Al-Suwaiq is characterized as dry with average annual humidity of 32% and high evapotranspiration rate. The average air temperature of the coastal area is 28.5 °C and 17.8 °C in the mountain area. The average rainfall rate in Al-Suwaiq (as a part of Al-Batinah region) is 50 mm/year, varying in time and places within the region (Kwarteng et al. 2009) .

Sites Selection

Five sites were randomly selected within 0.3 to 6 km inland distance from the seashore of Oman through the agricultural land within the study area.

Samples Collection and Analysis

In each of the five randomly selected sites, five locations were selected randomly to collect soil samples. A Global Positioning System device (Garmin eTrex Legend Cx GPS, USA) was used to register each location coordinates. At each location, three soil samples were collected from three different depths; 5 cm, 20 cm and 50 cm. Around 500 g of soil were taken with an auger to represent each depth. Each sample was kept in a clean plastic bag and annotated separately. A total of 75 soil samples were collected representing 5 sites × 5 locations × 3 depths. The saturation method was used to obtain soil extract from soil samples. Each soil extract was investigated for electrical conductivity (EC) which is expressed by deci-Siemens per meter (dS m^{-1}). In addition, water

samples (one sample from each site) were collected from the irrigation water sources (wells) of each site and were kept in a clean plastic container and transferred to the lab for analysis. The EC of each water sample were measured using EC meter.

Image Acquisition

Aerial images were taken by a digital color camera with 12.4 megapixels resolution. The camera was mounted on a quadcopter UAV (model: phantom-3-pro, DJI INC., China). Site images were taken from (130-275) m above the ground according to each site area. The captured images were saved in JPG format, which is a common format for realistic images and readable in different image processing softwares. The images were transferred to the computer to be analyzed.

Image Analysis

Orthorectification was conducted as a pre-processing technique in order to enhance the site images and to decrease image distortion. The Environment for Visualizing Images (ENVI) software (version 5.0.3, Exelis Visual Information Solutions INC., US) was used for image Orthorectification using Ground Control Points (GCPs) and Replacement Sensor Model (RSM). The GCPs were collected using Google Earth software (Version: 7.1.7.2600, Google INC.).

Several vegetation indices which depends only on color bands; Green Leaf Index (GLI), Visible Atmospherically Resistant Index (VARI) and Triangular greenness index (TGI). GLI has been commonly used in thresholding the green vegetation in aerial images of canopy scales (Chianucci et al. 2016; Hunt Jr et al. 2013; Macfarlane and Ogden 2012). Thus, GLI was computed (Eqn. 1) to determine canopy attributes within each site

Figure 2. The locations of the selected sites

Table 2. Location of salinity monitoring wells in Al-Batinah region with the observed salinity (ppm) in 1991, 1993, 2005 and 2010

Well ID	Location (E)	Location (N)	1991*	1993*	2005*	2010*
N-101	578701	2621460	1504	1632	2112	3072
N-92	582083	2620091	839	833	835	849
T-52	584162	2622750	1606	2214	8262	12288
N-79	585655	2617956	800	931	1280	1798
B-49	586184	2622605	7379	8896	8979	9126
T-30	591740	2621062	9280	9421	10682	14784
N-107	575993	2623943	1187	1112	5114	10432
B-70	571376	2627585	5440	6573	6144	11520
B-73	572962	2627174	7571	7424	8800	9728
B-83	568271	2628276	8410	6298	9600	12160
T-46	585991	2621972	6720	7507	13120	16576
N-63	590404	2619803	1382	1312	5133	14656
B-31	594298	2620548	4032	4902	11494	11514
N-53	591385	2616842	1344	672	1293	1792
N-71	587411	2619729	1427	1267	1958	3590
N-111	568658	2623958	774	833	1760	1837
N-66	588832	2617526	2138	1760	1978	2323
T-85	569008	2627063	3994	3610	6278	8896

*Observed salinity concentration (ppm#)
#ppm = dS/m x 640 (EC = 0.1 to 5 dS/m), ppm= dS/m x 800 (EC > 5 dS/m)

using MATLAB software (Version: 9.0.0.341360, Mathwork INC., USA).

$$GLI= (2G-R-B)/(2G+R+B) \qquad (1)$$

Where G, R and B are the digital values (0-255) of the green, red and blue bands of each pixel . The GLI value of each pixel in the site image were calculated using equation 1. The GLI values were reconstructed by applying the MATLAB function (inpaint_nans.m). Then, the GLI pixel values were averaged to get the GLI value of the whole image.

Statistical Analysis

Pearson correlation coefficient was calculated to compare soil and water EC of each site with site distance from seashore. Regression analysis were used to esti-

mate soil and water salinity using the distance from the seashore and the value of GLI.

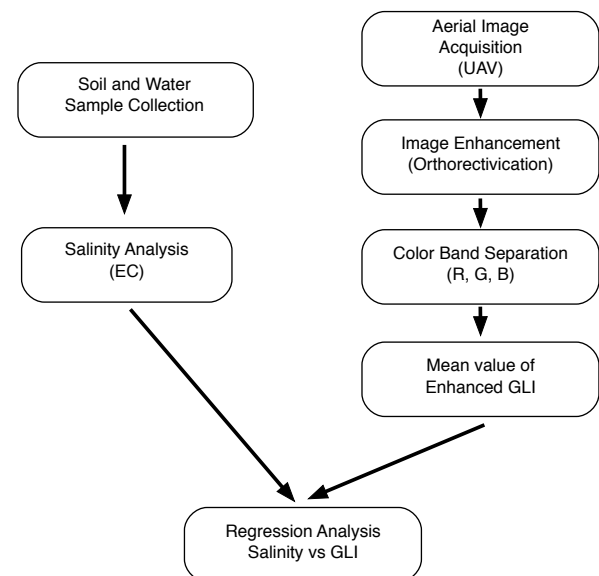The method followed in this paper is illustrated in Figure 3.

Table 3. The correlation coefficient of each salinity parameter

Salinity Parameter	Correlation Coefficient
Soil EC (5 cm)	-0.94992
Soil EC (20 cm)	-0.87461
Soil EC (50 cm)	-0.7105
Water EC	-0.48239

Figure 3. Soil EC (dS m-1) of each site (1-5) at different soil depths (5, 20 and 50 cm)

Results

Soil and Water Analysis

The level of soil EC of the collected samples at different depths of 5, 20 and 50 cm were decreased with the increase of the soil depth, as shown in Figure 4.

Effect of Seashore on Salinity Levels

The effect of site location from seashore on salinity levels is illustrated in Figure 5. As the distance between the seashore and the selected sites increased, the water EC and soil EC decreased gradually. To investigate the effect of site location on the salinity levels, the water EC soil EC at different depths (5, 20, 50 cm) were correlated with the distance from the seashore as shown in Table 3. Water EC had the lowest correlation with the distance from the seashore (R = -0.48). On the other hand, the EC of the soils in 5 cm depth had the highest correlation. Regression analysis was done to estimate the soil EC in 5 cm depth by knowing the distance from the shore using Eqn. 2 (Fig. 6).

$$y = -2.7671\,x + 22.643 \;(R^2 = 0.902) \tag{2}$$

Where y is the EC (dS/m) of the top layer of soil and x is the distance from the seashore (km) to the selected site.

Image Analysis

The image of site 2 is shown as an example in Figure 7. The averaged values of GLI had a strong negative cor-
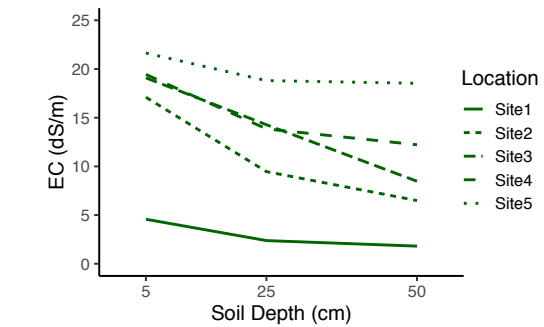
Figure 4. Steps followed to estimate soil and water salinity using GLI

relation with soil EC (R= -0.96) and water EC (R= -0.92). The GLI value of site image can be used to estimate site soil EC (Eqn. 3) and water EC (Eqn. 4) (Fig. 8).

$$y = -2.0737\,x + 16.15 \;(R^2 = 0.9128) \tag{3}$$

Where y is the soil EC (dS/m) and x is the mean value of GLI of the site image.

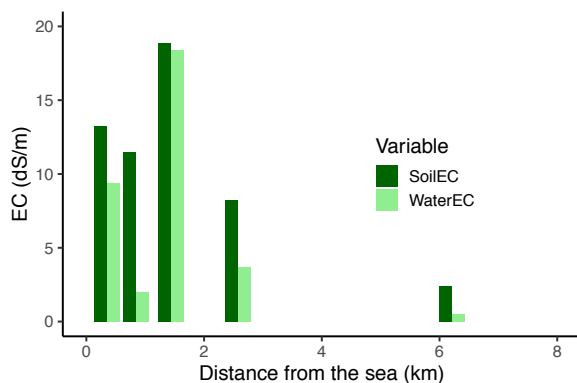$$y = -2.3241x + 13.887 \;(R^2 = 0.8429) \tag{4}$$

Figure 5. Soil and water EC (dS m-1) of each site (1-5) and site distance from the seashore
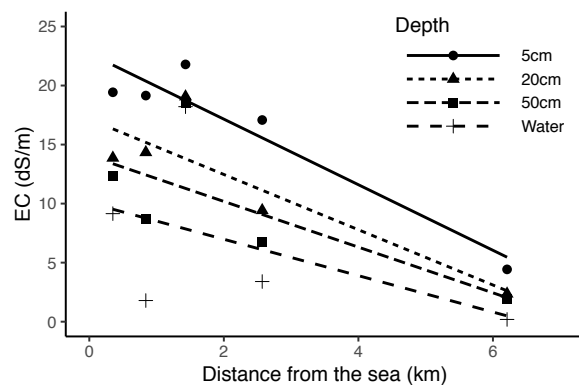
Figure 6. The regression analysis between the sites' distance from the seashore and their water EC and soil EC in different depths (5,20,50 cm)

Figure 7. Orthorectification process of site number 2

Where y is the water EC (dS/m) and x is the mean value of GLI of the site image.

Discussion

Soil and water salinity decreased as the site is located farther from the seashore (Fig. 5). Site (3) showed relatively unexpected increase in salinity levels, which could be due to the farming practices in the site. All other sites showed clear negative correlation between the distance to the site from the seashore and the salinity levels. On the other hand, an excellent correlation was observed
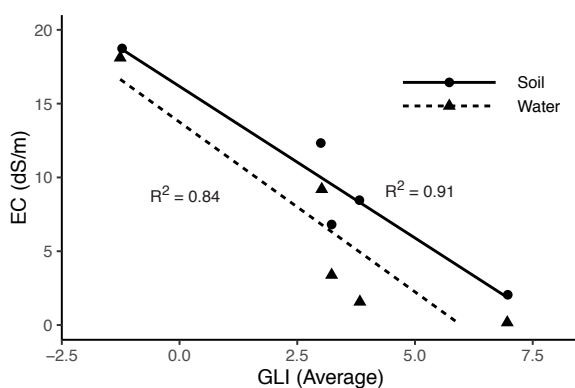

Figure 8. The regression analysis between GLI mean value of each site image and salinity levels of the site

while investigating the EC of soils from different depths with the distance from seashore (Table 3). The top soil layers showed the highest values of soil salinity, where that could be due to salt accumulation on the soil surface as reported by Herrero et al. (2003). It also had the strongest correlation (R= -0.95) with distance from the seashore.

The GLI mean values of the images ranged from -1.2 to 6.8. The positive value of GLI was assigned to the green leaves or stems while the negative value was for non-green site objects like; soils, buildings, woods and other non-living items (Louhaichi et al. 2001). In this study it was found that the lowest mean value of GLI was -1.2 for site 1 with the highest salinity level. In general, the results proved that the soil and water salinity had strongly affected the vegetation quantity and quality (greenness), where the mean green value (GLI) declined as the salinity increased. Vegetation Soil Salinity Index (VSSI) were used by Tran et al. (2018) to estimate salinity intrusion from Landsat 8 images with $R2 = 0.6957$. The salinity levels can be estimated by the mean value of GLI with relatively strong values of coefficient of determination, compared to other vegetation indices.

Conclusion

This research proved the effect of salinity intrusion on site location from the seashore. The five randomly selected sites within the agricultural land belt with different distances from the seashore showed a decline in salinity levels as the site become far from the seashore. The effect of distance on soil salinity could be represented as a regression model. Mainly, this research demonstrated the possibility of using UAV with affordable digital camera to estimate the vegetation cover. The results showed

a strong negative correlation between salinity levels and GLI as an indicator of vegetation status. Salinity assessment using UAV colour images is coast efficient, timeless and more accurate in relative to field and satellite assessments. Nevertheless, more image processing techniques may strength the possibility of aerial images in estimation of salinity effects on vegetation cover.

Acknowledgement

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RESEARCH PAPER

Improving solanine production in *in vitro* cultures of *Solanum nigrum* L. using different chemical and physical factors

Mu,ad A. AL- Kiyyam[1], Rida A. Shibli[2], Reham W. Tahtamouni[3], Tamara S. AlQudah[4], Bashaer B Abu-Iramaileh[4]

التحكم ببعض الظروف الزراعية يعزز من إنتاج السولانين في مزارع السويقات النباتية والكالوس والمعلقات الخلوية لنبات المغد الأسود *Solanum nigrum* L.: نبات طبي بري

معاذ أ. القيام ورضا أ. شبلي ورهام و. تحتموني وتمارة س. القضاة وبشائر ب. أبو الرمايلة

ABSTRACT. *Solanum nigrum* L. is a medicinal plant of solanaceae family with distinguished therapeutic properties. Traditionally, *S. nigrum.* had been used as an anti-tumorgenic, antioxidant, hepatoprotective, diuretic, and antipyretic agent. The most important alkaloid member in this plant is solanine. Therefore, this study was conducted to utilize tissue culture techniques for the enhancement of solanine production in the in vitro grown cultures of this promising neglected plant. For callus growth and development experimental part, the highest callus growth parameters (callus diameter (21.4 mm) and callus fresh weight (2202.4 mg) were obtained in callus grown on Murashige & Skoog MS media supplemented with 2,4-Dichlorophenoxyacetic acid (2.0 mg·L^{-1}) plus 1.5 mg·L^{-1} Thidiazuron. Similar trend was also obtained in cell suspension culture experiment, as maximum growth was recorded at similar hormone combination. Moreover, High-performance liquid chromatography analysis revealed that, solanine was affected by growth regulator type and concentration. The highest solanine levels were obtained when the explants were treated with 6-benzylamino-purine at level of 2.0 mg·L^{-1}, as solanine content reached up to (2.61, 1.53 mg.g^{-1}) for callus and cell suspension, respectively, while, microshoot contained the highest solanine (4.52 mg.g^{-1} DW) at 6-benzylaminopurine level of 1.6 mg.L^{-1}. Additionally, carbon source had positively affected solanine level, where 0.2 M sucrose resulted in production of the highest amounts (3.13, 2.03 and 1.20 mg.g^{-1} DW) of solanine in microshoots, callus and cell suspension, respectively. Also, exposing microshoots and callus to light intensity of (100 µmol.m^{-2}s^{-1}) yielded the highest solanine content (4.03 and 1.26 mg.g^{-1} DW, respectively),while the lowest solanine levels (1.50 and 0.48 mg.g^{-1} DW) were observed in plant material exposed to the lowest light intensity treatment (25 µmol.m^{-2}s^{-1}). Our results were promising for production of solanine especially in the microshoot (4.52 mg.g^{-1} DW) using 1.6 mg.L^{-1} of BA, as compared with previous studies which maximized production of solanine only up to 2.33 mg. g^{-1} DW using 0.5 mg.L^{-1} of cholesterol in *Solanum lyratum*.

KEYWORDS: Callus; Carbon source; Cell suspension; Light, Solanin.

المستخلص: المغد الأسود *Solanum nigrum* L. نباتٌ طبيٌّ ينتمي للعائلة الباذنجانية ويتميز بخصائصه العلاجية المميّزة، فقد استخدم تقليدياً كعامل مضاد للأورام ومضاد للأكسدة ومضاد للسمية الكبدية، وكمدر للبول وخافض للحرارة. يعد السولانين القلويد الأكثر أهمية في هذا النبات. لذلك أجريت هذه الدراسة مستخدمة تقنيات الزراعة النسيجية لتعزيز إنتاج السولانين في هذا النبات الواعد والمستحق للمزيد من الاهتمام وباستعمال مزارع النمو المختبرية. لوحظ في القسم التجريبي المتعلق بنمو الكالوس وتطوره أنّ القيم الأعلى لنمو الكالوس المزروع (قطر الكالوس: 21.4 ملم، ووزنه الرطب: 2202.4 مغ) قد تم الحصول عليها باستعمال وسط موراشج وسكوج MS مضافاً له حمض 2,4–Dichlorophenoxyacetic (2 مغ.ل–1) و(1.5 مغ.ل–1) Thidiazuron. كما تم التوصل إلى تأثير مماثل في تجربة زراعة المعلق الخلوي، حيث سُجلت أقصى قيمة للنمو باستعمال المزيج الهرموني ذاته. علاوة على ذلك، كشف التحليل اللوني السائل عالي الأداء أن السولانين قد تأثر بنوع وتركيز منظم النمو، فقد تم تسجيل أعلى مستويات للسولانين عندما عولجت العينات النباتية بـ(2.0 مغ.ل–1) من 6–benzylaminopurine، حيث وصل محتوى السولانين إلى (2.61، 1.53 مغ.غ–1) في كل من الكالوس والمعلق الخلوي، على التوالي، في حين احتوت السويقات النباتية على أعلى مستوى من السولانين (4.52 مغ.غ–1 ماء مقطر) عند إضافة (1.6 مغ.ل–1) من 6–benzylaminopurine. كما كان لمصدر الكربون أثر إيجابي في مستوى السولانين، حيث أدى استعمال 0.2 مول من السكروز إلى إنتاج كميات أعلى من السولانين (3.13، 2.03، و1.20 مغ.غ–1 ماء مقطر) في كل من السويقات النباتية والكالوس والمعلق الخلوي على التوالي. وكذلك فإن تعريض السويقات النباتية والكالوس لشدة الضوء البالغة (100 ميكرومول.متر2– ثانية 1–) قد حقق أعلى محتوى من السولانين (4.03 و1.26 مغ.غ–1 ماء مقطر(على التوالي، بينما لوحظت أدنى مستويات للسولانين (1.50 و0.48 مغ.غ–1– ماء مقطر) في المواد النباتية عند تعرضها لأدنى معالجة من شدة الضوء (25 ميكرومول.متر2– ثانية 1–).

الكلمات المفتاحية: الكالوس، مصدر الكربون، المعلق الخلوي، الضوء، السولانين.

Introduction

Plant cell and tissue culture technologies have been established from different explant types, such as plant leaves, stems, roots, and meristems under sterile conditions for the production of secondary metabolites besides micropropagation purposes. Recently, successful production of many elite chemical compounds from plant cell suspension cultures has been

Table 1. Effect of plant growth regulators type and level on callus fresh weight and diameter of *in vitro* grown *S. nigrum* in combination with (2.0 mg.L^{-1}) 2,4-D, except for (C & 0.0) concentrations.

TDZ	Plant growth regulator Concentration (mg.L^{-1})	
	Callus Weight (mg)	Callus Weight (mg)
0.0	551.7^z f	6.3 d
C^x	613.4 e	6.9 d
0.5	679.8 d	7.0 d
1.0	1729.2 b	16.1 b
1.5	**2202.4 a**	**21.4 a**
2.5	1229.5 c	10.9 c
	Kinetin	
0.0	551.7 d	6.3 a
0.5	589.8 c	6.8 a
1.0	**623.3 a**	**7.0 a**
1.5(C^x)	613.4 ab	6.9 a
2.5	601.9 b	6.9 a
	BAP	
0.0	551.7 d	6.3 d
C^x	613.4 c	6.9 c
0.5	809.9 b	8.1 b
1.0	**1137.8 a**	**10.2 a**
1.5	615.9 c	7.0 c
2.5	605.5 c	7.1 c

^{x} *C (Control treatment) represents callus establishment media consisted of MS solid media plus 2.0 mg.L^{-1} 2,4- D and 1.5 mg.L^{-1} kinetin.* ^{z} *Means within columns for each growth regulator having different letters are significantly different according to Tukey HSD at p≤0.05.*

reported in various medicinal plants by exposing the cultured explants to different growth conditions *in vitro* such as, using different types of culturing media, growth regulators, sugars and manipulating some physical environmental factors, such as, temperature, moisture

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and light (Robbins et al., 1996; Shibli and Ajlouni, 2000; Chawla, 2002; Jasmin et al., 2011).

Solanum nigrum L. (black nightshade) (Fig. 1A) is an annual herbaceous plant of 30-90 cm height with a green, smooth and semi-climbing stem. This plant grows wild in wet woods, near river, waste land, old field, ditches roadside and cultivated land (Pronob and Islam, 2012). Despite of being considered as a noxious weed that grows in many agro-climatic regions (Sundari et al., 2010), *S. nigrum* has been recently classified as a medicinal plant with distinguished therapeutic properties (Rajani et al., 2012). Traditionally, *S. nigrum* had been used in oriental systems of medicine for various purposes as, an anti-tumorgenic, antioxidant, hepatoprotective, diuretic, and antipyretic agent (Lee et al., 2003 ; Raju et al., 2003).

Solanine is considered the most important alkaloid produced in the green fruits of *S. nigrum* and many studies have confirmed Solanine activity as a cardiac anti-accelerating agent (Cham; 1994). This is in addition to its use as an antimicrobial agent against many strains of bacteria and fungi (Roddick, 1996). Moreover, Solanine is recently recommended as a new therapy for treatment of many cancer cell lines (Cham, 2008; Ji et al. 2008; Sutkovic et al., 2011).

Solanine can be found in the wild plants at high concentrations; but if we want to study how secondary metabolites are produced in plant cells in details we must adopt the tissue culture approach where the interference of all other factors such as, environmental factors is excluded. Moreover, wild plants in general are not effective sources to study secondary metabolites, as most medicinal wild plants are subjected to heavy loss due over collection; climate fluctuations; grazing ;urbanization , etc. Tissue culture is a potent technique that is used to enhance massive plant *in vitro* propagation and huge plantlets production under controlled conditions in few times and all around the year (Vinod and Dipali , 2013). Furthermore; *in vitro* culture permits better understanding of the mechanism by which plant cells synthesize secondary metabolites and the factors the can maximize their production by optimizing the cultural conditions of plant cells (DiCosmo and Misawa, 1995; Saito and Mizukami, 2002; Jasmin et al. 2011). Additionally, *in vitro* propagation of medicinal plants with enriched medicinal compounds and cell culture methodologies for selective metabolite production was found to be highly useful for commercial production of medicinally important compounds, as tissue culture techniques guarantee sustainable supply of true to name plants with high purity of the medicinally important compounds by excluding environmental factors and any possibility of genetic drift due to outcrossing (Hussain et al., 2012).

Large-scale plant tissue culture is found to be an attractive alternative approach to traditional methods of plantation as it offers controlled supply of biochemi-

Table 2. Effect of different combinations of BAP and 2,4-D on growth rate of cell suspension cultures of *S. nigrum* within different periods.

Days		Growth regulator			
0.0	C[x]	BAP 0.1 mg.L^{-1} + 2,4-D 0.1 mg.L^{-1}	BAP 0.1 mg.L^{-1} + 2,4-D 0.25 mg.L^{-1}	BAP 0.25 mg.L^{-1} + 2,4-D 0.1 mg.L^{-1}	BAP 0.25 mg.L^{-1} + 2,4-D 0.25 mg.L^{-1}
7	11.5d[z]	**39.9 a**	19.5 c	19.7 c	18.7 c	27.5 b
15	37.7d	**88.5 a**	44.9 d	59.2 c	56.1 c	68.7 b

[x] C (Control treatment) represents callus multiplication media consisted of liquid MS media plus 2.0 mg.L-1 2,4 D + 1.5 mg.L-1 TDZ. [z]Means having different letters are significantly different according to Tukey HSD at P≤0.05. Cell suspension culture growth expressed as % packed cell volume (PCV)

cal's independent of plant availability (Sajc et al., 2000). Therefore, this study was conducted to investigate the possibility of improving solanine production in *S. nigrum* in *in vitro* grown cultures by manipulating some tissue culture growth conditions (chemical and physical factors).

Materials and Methods

Establishment and multiplication of plant material

Microshoots

Mature seeds of *S. nigrum* were collected from the mother plants grown in wild in Jerash- Jordan (N: 32.27372, S: 35.89464).Then the seeds were surface sterilized before being subcultured into full strength MS (Murashige and Skoog, 1962) solid media at concentrations of 4.4 g.L^{-1} and supplemented with 1.0 ml.L^{-1} MS vitamin mixture (Sigma Aldrich Murashige and Skoog Vitamin Powder 1000X) pluse 0.1 M sucrose. Seeds were cultured and kept up in the growth room under a daily temperature of 24±1 °C under a 16/8 (light/dark) photoperiod of 45–50 µmol m-2s^{-1} irradiance, until germination. For shoot multiplication, a prelimenary experiment was conducted to determine which growth regulatores would result in best shoot multiplication (data not shown), and based on the obtained results nodal segments (2 cm) from the germinated seedlings were subcultured into MS media supplemented with (1.2 mg.L^{-1}) Thidiazuron; 1-Phenyl-3-(1,2,3-thiadiazol-5-yl (TDZ) plus 0.1 mg.L^{-1}alpha-Naphthalene acetic acid free acid (NAA) (media that was found to be best for shoot proliferation). The cultures were kept under growth room conditions described earlier.

Callus

Callus was successfully established from excised *in vitro* grown leaf discs subcultured into callus establishment media (MS solid media supplemented with 2.0 mg.L^{-1} of 2,4-dichlorophenoxy acetic acid (2,4-D) and 1.5 mg.L^{-1} of kinetin) and kept under complete dark condition for 8weeks. After callus establishment, calli with diameter

of (5.0 mm) and weight of (500 mg) were subcultured into a hormone free MS medium (HF-MS) for one week to remove the carry-over effects of plant growth regulators (PGRs.). Later, the calli were sub-cultured into fresh MS media supplemented with (2.0 mg.L^{-1}) 2, 4-D in combination with different levels (0.0, 0.5, 1.0, 1.5 or 2.5 mg.L^{-1}) of kinetin, 6-Benzylaminopurine (BAP) or TDZ. Control treatment was consisted of solid MS media plus (2.0 mg.L^{-1}) 2, 4-D in combination with 1.5 mg.L^{-1} kinetin (callus establishment media). Data were obtained after 8 weeks for callus diameter and weight.

Cell suspension cultures

Approximately 1.0 g of friable callus, which was still in its active growth phase (i.e., after the 15th day of subculture) was placed in 250 ml jar containing 50 ml liquid MS medium (Murashige and Skoog, 1962). The culture media used in this experiment were as follows: hormone free MS solid media , MS media plus hormone combination which was found to be optimum for maximum callus growth in callus multiplication experiments (2.0 mg.L^{-1} 2,4-D + 1.5 mg.L^{-1} TDZ) (control) or MS media plus (0.1 or 0.25 mg.L^{-1}) BAP in combinations with (0.1 or 0.25 mg.L^{-1}) 2,4-D. Cultures were incubated in complete darkness at 25ºC on a horizontal shaker at 100 rpm for 15 days. Growth of cells was determined by measuring packed cell volume (PCV) described by Allan (1996) under sterile conditions at 7and 15 days of which was expressed as ml cell pellet per culture.

For PCV measurements, which were repeated three times per treatment, the cell suspension in flasks was gently shaken and then 10 ml aliquots were transferred into 15ml graduated conical centrifuge tubes followed by centrifugation at 200 g for 5 min using a swing-out rotor (Allan, 1996) in order to determine the best hormonal combinations that can give the maximum cell suspension culture weight.

Effect of chemical factors and light intensities on the *in vitro* production of solanine

Effect of Cytokinins

Microshoots were grown in MS media supplemented with 0.1 M sucrose, 0.1 mg·L^{-1} NAA in addition to different concentrations of different cytokinin growth regulators: BAP, kinetin or 6-(gamma, gamma- Dimethylallyl amino) purine (2iP) at levels of 0.0, 0.4, 1.0, 1.6 or 2.0 mg·L^{-1}. The subcultured microshoots were kept under normal growth room conditions. For callus and cell sspenssion, a part of plant material was treated with the different cytokinin types and levels mentioned before and kept under dark, while the other part was cultured on a media consisted of MS media plus best hormone formula resulted in the callus multiplication experiments (2.0 mg·L^{-1} 2,4-D and 1.5 mg·L^{-1} TDZ) and maintained under complete dark conditions. After 8 weeks, plants material (microshoots, callus and filtered cell suspension) from each experiment were collected, dried in the oven (35 ºC for 2 days). Next, the dried plants material were grounded in a mortar and pestle, and pooled before being analyzed for alkaloids quantification (extraction and analysis), and the results were compared with those obtained from wild mother plant from Jerash grown in green house at Faculty of Agriculture/ University of Jordan, Amman -Jordan.

Effect of carbohydrate sources

Microshoots of *S. nigrum* (1.0 cm long) were subcultured into a hormone free MS medium for one week to remove the carry-over effects of plant growth regulatores (PGRs). Next , the microshoots were transferred into hormone free MS media supplemented with elevated concentrations of sucrose, glucose and fructose (0.05, 0.1, 0.15 and 0.2 M). For the callus and cell suspension, plant material were subcultured into callus multiplication media described earlier and supplemented with the different sugar types as mentioned above and kept under dark. After 8 weeks, the plant materials were dried and grounded as described earlier before being analyzed for alkaloids quantification (extraction and analysis) and the results were compared with those obtained from wild mother plant.

Effect of different light intensities

Microshoots and callus samples were kept at different light intensities (25, 50, 75 or 100 µmol. m^{-2}.s^{-1}) irradiance for 8 weeks. The plant material was collected, dried and grounded as described earlier before being analyzed for alkaloids quantification (extraction and analysis), and the results were compared with those obtained from wild mother plant from Jerash grown in green house at Faculty of Agriculture, University of Jordan, Amman -Jordan.

Determination of alkaloids content using high performance liquid chromatography(HPLC):

Alkaloids extraction

Samples (3 g) from each powdered plant material taken from each experiment, in addition to samples taken from the wild plants collected from the five different places in Jordan (including the wild mother plant from Jerash) were extracted thrice with 200 mL of 5% aqueous acetic acid and its pH was adjusted to 11 with ammonium hydroxide before being vacuum filtered. The alkaline extract was partitioned with water-saturated butanol and evaporated to dryness, purified and the residue was weighed and dissolved in acetonitrile and analyzed.

Preparation of solanine stock solution and working standards

Solanine stock solution at concentration of 20 ppm was prepared by weighing 2.0 mg of solanine in 100ml volumetric flask, dissolved and completed up to volume by methanol HPLC grade .The prepared stock solution was stored at 4ºC in dark. Working solutions were prepared by serially diluting stock solutions using the mobile phase at concentrations of 0.5, 1, 2.5, 5 or 10 ppm. Fresh working standards were prepared daily. About 2.0 mg (±0.01mg) of solanine reference standard was dissolved into a 100 m1 volumetric flask and about 100 ml of acetonitrile until complete dissolve. Four points were constructed for the calibration curves (Fig. 2) and HPLC chromatogram (Fig. 3) for solanine was obtained before starting chemical analysis.

Chromatographic conditions

Chromatographic separation was carried out on HPLC-Shimadzu system (Japan), equipped with (LC-10 AT) pump, a manual injector, a system control (SCL-10A),a UV/VIS detector (SPD-10A), and an analytical column – C18 (250 - 4.6 mm I.D. 5 µm, Merck, Germany). The software, Shimatzu CLASS-VP (V6.14 SP1) chromatography data system was used as the data acquisition system. An ultrasonic cleaner (JEIOTECH- US) , a VM-300 vortex (Germany industrial CORP), an electronic balance (Precisa 410AM-FR, Switzerland), a SUPRA 30K (HANIL science industrial co., Ltd., Germany) ultra-centrifuge, a microfiltration unit (Advantec, MFS, Inc) with a rotary pump (Rocher 300) were used in sample treatment.

The alkaloids were analyzed using HPLC apparatus consisting of Shimadzu LC-10A system equipped with a model LC-10AT pump, an SPD-10A variable wavelength detector, a CBM-10A interface module with class LC-10 HPLC software using a Merck C-18 column (250×4.6, i.d., 5 µm particle size).
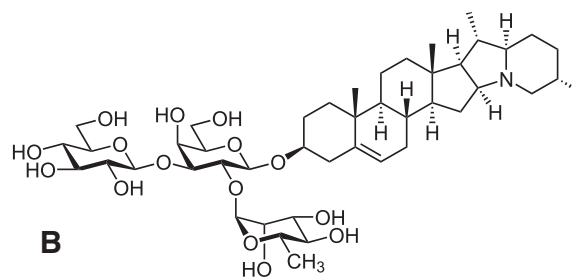
Figure 1. A: *Solanum nigrum* plant. (http://www.homeredies.com/solanum-nigrum-medicinal-uses-and-images). B: Chemical structure of solanine (Nino et al. 2009).

Solanine identification test

The basic method for solanine identification followed in this research was that set by Mohy-Ud-Din et al., (2010).

After conditioning the column and reaching equilibrium with the mobile phase, 20 μl of each of the solanine standard solution was injected at 0.5ml/min flow rate and tested at the range of detection wavelength 204 nm in order to determine solanine peak.

Method development

When the basic solanine identification method was applied, no separation between solanine peak and a matrix impurity peak was achieved. Therefore, it was necessary to develop this method, to improve the separation of solanine from the peak of impurity in plant extract matrix. The best separation of solanine was achieved on the following detection conditions: mobile phase: 25 acitonitrile (ACN), 75 potassium dihydrogen phosphate and 5ml Triethylamine at pH 7.5, the flow rate was 0.5 ml/min and UV detector wavelength set at 204 nm.

Experimental design and arrangement

All treatments in each experiment were arranged in a completely randomized design (CRD) and consisted of five replicates with 4 explants /replicate. Meanwhile, in alkaloids content determination experiment each treatment consisted of 3 replicates with 3 samples /replicate and sample dry weight of (3.0 g). The collected data were statistically analyzed using SPSS analysis system and analysis of variance (ANOVA) was used to analyze the obtained results, and mean was separated with probability level of 0.05 according to the Tukeys HSD.

Results and Discussion

Callus induction and multiplication:

Effect of TDZ

In callus multiplication experiments, the combination of 2,4,D (2.0 mg·L^{-1}) plus TDZ resulted in increasing callus growth in terms of fresh weight and diameter at all TDZ levels compared to control (C) and 0.0 mg·L^{-1} (Table 1). Similar results were reported by Sajid and Faheem (2009) who investigated the effect of Thidiazuron (TDZ) on *in vitro* micropropagation of Solanum tuberosum and they reported that, TDZ promoted callus induction and multiplication due to its biological activities in inducing the synthesis and accumulation of endogenous cytokinins which would direct cell division and callus clumps formation. The highest callus diameter (21.4 mm) and callus fresh weight (2202.4 mg) (Table 1) were recorded in callus grown in hormone combination of 2,4-D (2.0 mg·L^{-1}) plus 1.5 mg·L^{-1} TDZ. Improving callus growth using different combinations of 2, 4-D and TDZ of was also reported to be efficient in *Stephania tetrandra*, as it was found that, best callus growth and multiplication resulted on MS media plus 1.0 mg·L^{-1} 2, 4- D and 0.5 mg·L^{-1}TDZ (Chao et al., 2011). However, increasing TDZ level to 2.5 mg·L^{-1} resulted in decreasing callus fresh weight and diameter (Table 1), which might be attributed to the high TDZ concentration that might had inhibitory influence on callus growth and development.

Effect of kinetin

Callus fresh weight and diameter increased significantly with increasing kinetin level in the media up to 1.5 mg·L $^{-1}$ (Table 1). The maximum values for both tested

parameters were obtained in the combination of 1.0 mg·L^{-1} kinetin and 2.0 mg·L^{-1} 2, 4-D as it yielded the highest callus fresh weight (623.3 mg) and diameter (7.0 mm) as shown in Table (1). Generally, kinetin performance was poor in terms of callus growth compared to the results obtained in TDZ treated explants. This contrasted the results reported about callus induction and multiplication of *Securinega suffruticosa* and *Viola uliginosa*, as they were maximized when their explants were grown on media supplemented with 2,4-D plus Kinetin (Raj et al., 2015; Slazak et al., 2015; respectively). This might refer to the fact that, plant responses to the different types and combinations of the growth regulators are species dependent.

Effect of BAP

Adding BAP in combination with 2,4-D improved callus growth significantly up to BAP level of 1.0 mg·L^{-1} compared to the results recorded in control (C and 0.0 mg·L^{-1}) (Table 1). The maximum callus weight (1137.8 mg) and diameter (10.2 mm) were obtained in BAP level of 1.0 mg·L^{-1} (Table 1). These results agreed with those reported by Sridhar and Naidu (2011), Sheeba and Palanivel (2013) and Sheeba et al., (2013) through their investigation on callus induction procedure of *S. nigrum*, *Solanum surattense* and *Physalis minima*, respectively.

The obtained results from callus multiplication experiment can lead to a conclusion that, TDZ (1.5 mg·L^{-1}) and 2,4, D (2.0 mg·L^{-1}) was the best combination that gave the maximum callus fresh weight and diameter of *S. nigrum*.

Cell suspension culture

The obtained results indicated that, adding 2.0 mg·L^{-1} 2,4-D plus 1.5 mg·L^{-1} TDZ (callus multiplication hormonal combination (C)); to the culture medium had yielded the maximum cell growth rate compared to the other treatments (Table 2). Meanwhile, for BAP and 2,4-D combination treatments, the highest cell growth rate (68.7%) was recorded in BAP (0.25 mg·L^{-1}) and 2,4-D (0.25 mg·L^{-1}) treatment (Table 2), this agrees with Song et al., (2002) results, as they reported that the media containing high BAP (0.25 mg·L^{-1}) and 2,4-D (0.25 mg·L^{-1}) produced greater rates of cell division in sugar Beet (*Beta vulgaris*) than either medium with lower BAP (0.1 mg·L^{-1}) and 2,4-D (0.1 mg·L^{-1}) levels or the control (hormone free MS liquid media). This agrees with Kshirsagar et al. (2015) findings in their study on *Swertia lawii* Burkill; as the maximum growth of cells suspension cultures (fresh weight = 2.8 g and dry weight 0.6 g) was observed after 15 days of culture in MS liquid medium supplemented with 2, 4-D (2.0 mg l–1) and BAP (2.0 mg l–1). Similarly, maximum growth of cells in suspension were observed between 10–15 days were reported in *Passiflora alata* using similar combination of growth regulators (2, 4-D and BAP) (Pacheco et al., 2012).

Table 3. Effect of cytokinine type and concentration on solanine % (mg.g^{-1}) dry weight (DW) in microshoots, callus and cell suspension of in vitro and wild (in green house) grown *S. nigrum*.

Concentration (mg.L^{-1})	BAP	Kinetin	2iP
Microshoot			
W*	4.92 a^z	4.92 a	4.92 a
C^y	**1.82 d**	1.82 d	1.82 e
0.4	2.20 c	2.00 d	2.16 d
1.00	3.93 b	2.60 c	3.00 c
1.6	**4.52ab**	3.83 b	**3.87 b**
2.0	3.77 b	**4.00 b**	3.43 bc
Callus			
W	4.92 a	4.92 a	4.92 a
C^y	0.73 d	0.73 d	0.73 e
0.4	0.95 d	1.97 c	1.10 d
1.00	1.62 cd	2.13 c	1.53 c
1.6	2.01 c	2.21 c	1.62 c
2.0	**2.61 b**	**2.82 b**	**2.55 b**
Cell suspension			
W	4.92 a	4.92 a	4.92 a
C^y	0.43 c	0.43 d	0.43 c
0.4	0.63 c	0.50 d	0.42 c
1.00	1.00 bc	0.50 d	0.76 b
1.6	1.11 bc	0.98 c	**1.01 b**
2.0	1.53 b	1.33 b	0.86 b

W: represents mother plant collected from Jerash. C^Y: control treatment for microshoots consisted of hormone free solid MS media. CY: control in callus experiment consisted of callus multiplication media (MS solid media + 2.0 mg.L^{-1} 2, 4-D + 1.5 mg.L^{-1} TDZ). CY: control for cell suspension cultures experiment consisted of MS liquid media plus 2.0 mg.L^{-1} 2,4-D + 1.5 mg.L^{-1} TDZ. ^{z}Means within columns having different letters for each growth regulator type are significantly different according to Tukey HSD at P≤0.05.*

Effect of chemical factors and light intensities on the *in vitro* production of solanine

Calibration curve

A calibration curve of alkaloids (Solanine) reference standard solution was prepared (Fig. 2) in order to calculate solanine content in the microshoots, callus and cell suspension of *S. nigrum* and comparing the results to solanine content in the wild type plant as mentioned above. Figure (2) explains how solanine contents was determined and calculated in the samples (a quantification method) using different quality control points includes different concentrations of the solanine standard to build up the calibration curve of solanine. Alkaloids

compounds were eluted at 6.26 min for solanine, and (Fig. 3) represents the peak of solanine, areas and linear calibration curve (r^2= 0.9999) as they were measured in the range of 12.5-12800 $\mu g.ml^{-1}$ (ppm or $mg.L^{-1}$). Solanine was determined based on Figure 2 (calibration curve) using the following equation provided by the calibration curve: y = ax + b

Where: y = area, a = constant, x = solanine concentration and b = constant

Effect of plant growth regulator on solanine content

Effect of BAP

The obtained data in BAP experiment revealed that, solanine percentage in microshoots had increased remarkably in response to BAP level in the media to reach a maximum level of (4.52 mg.g-1 DW) at BAP concentration of 1.6 $mg.L^{-1}$ compared to (1.82 mg.g-1 DW) recorded in the control treatment (C) (Table 3). This means that BAP level of (1.6 $mg.L^{-1}$) was able to enhance solanine production in the microshoots to reach a level which was very close to solanine content obtained in Jerash mother plant grown in the greenhouse. Meanwhile, solanine tended to decrease (3.77 $mg.g^{-1}$ DW) at higher BAP level (2.0 $mg.L^{-1}$).These results fully agreed with Yogananth et al., (2009) and Bhat et al., (2010), where BAP enhanced the solanine accumulation in *S. nigrum* shoots. Also, Jayabalan et al., (2014) studied improve solasodine (another important alkaloid in Solanacea family) accumulation *in vitro* Solanum trilobatum using different concentration of BAP, and they concluded that 2.0 $mg.L^{-1}$ of BAP was the best levels that gave the maximum solasodine percentage. Additionally, Al-Hawamdeh et al., (2013) reported the effect of BAP on the content of silymarin compounds in *Silybum marianum*, as they found that Silybin, silydanin, and silymarin content increased as BAP concentration increased compared with the control.

In callus experiment, solanine was recorded to increase with increasing BAP level to reach a maximum concentration (2.61 $mg.L^{-1}$ DW) at 2.0 $mg.L^{-1}$ of BAP (Table 3). Moreover, solanine was positively influenced by BAP level in the samples taken from the cell suspension, but in lower percentages than those obtained in the microshoots and callus cultures (Table 3), as the maximum level of solanine recorded in the cell suspension was only (1.53 $mg.g^{-1}$ DW compared to 3.77 and 2.61 $mg.g^{-1}$ DW) extracted from the microshoots and callus; respectively at similar BAP level (Table 3). This could be attributed to high water content in the cells cultured in the liquid MS media, as high water content in the cell was always reported to have a negative impact on the production and accumulation of secondary metabolites including alkaloids (Morales et al., 1993; Tahtamouni et al., 2016).

Table 4. Effect of charbohydrate type and concentration on solanine % ($mg.g^{-1}$ DW) in microshoots, callus and cell suspension of in vitro grown *S.nigrum*, in addition to wild (in green house) grown *S. nigrum*.

Concentration ($mg.L^{-1}$)	Sucrose	Glucose	Fructose
Microshoot			
W*	4.92 a[z]	4.92 a	4.92 a
0.05	0.90 e	0.43d	1.68 c
0.1 (C[y])	1.82 d	2.67 c	1.85 c
0.15	2.43 c	3.00 b	2.13 bc
0.2	3.13 b	2.00 c	2.40 b
Callus			
W	4.92 a	4.92 a	4.92 a
0.05	0.21 e	0.58 c	0.58 c
0.1 (C[y])	0.73 d	0.85 b	0.61 c
0.15	1.50 c	1.03 b	0.93 b
0.2	2.03 b	0.48 c	1.05 b
Cell suspension			
W	4.92 a	4.92 a	4.92 a
0.05	0.10 d	0.33 c	0.13 d
0.1 (C[y])	0.43 c	0.38 c	0.26 c
0.15	1.16 b	0.56 b	0.50 b
0.2	1.20 b	0.40 c	0.66 b

W: represents wild mother plant from Jerash. Cy: for microshoots control treatment is consisted of hormone free MS media + 0.1 M of each carbohydrate types. CY: control in callus experiment consisted of callus multiplication media (MS solid media+2.0 $mg.L^{-1}$ 2,4- D+ 1.5 $mg.L^{-1}$ TDZ) + 0.1 M of each sugar types. CY : control in cell suspension cultures experiment consisted of MS liquid media plus (2.0 $mg.L^{-1}$ 2,4-D + 1.5 $mg.L^{-1}$ TDZ)) + 0.1 M of each sugar types. zMeans within columns having different letters for each sugar type are significantly different according to Tukey HSD at P≤0.05.*

Effect of kinetin

In kinetin experiment, the results showed that, increasing kinetin concentration positively affected solanine percentage ($mg.g^{-1}$ DW) in shoots, callus, and cell suspension at all levels. (Table 3). Similar to the results trend obtained in BAP experiment, the highest solanine level were recorded in the microshoots (4.00 $mg.g^{-1}$ DW) and callus culture (2.82 $mg.g^{-1}$ DW) while the lowest values were recorded in the cell suspension cultures at all kinetin levels (Table 3).

The positive impact of kinetin on alkaloids production was also reported by Al-Hawamdeh et al., (2013) as kinetin enhanced the content of silymarin compounds in *Silybum marianum* shoots at 1.6 $mg.L^{-1}$ kinetin. Moreover, effects of kinetin on biosynthesis of vindoline and other indole alkaloids in *Catharanthus roseus* callus cultures was investigated by Jian et al., (2001) and
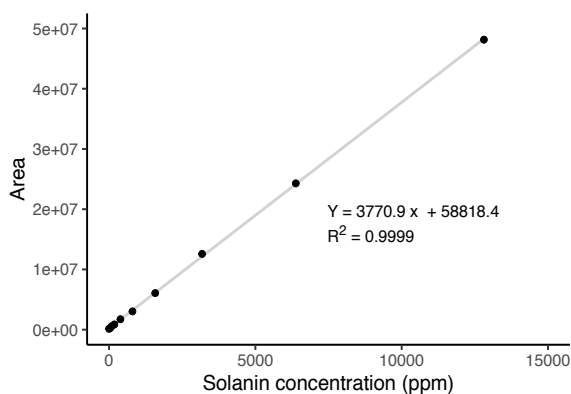
Figure 2. Standard calibration curve of solanine. Y: area, R2: retention time.

they reported that, indole alkaloids increased as BAP increased, which was attributed to enhanced peroxidase activity, reflected the pattern of alkaloid biosynthesis under the same culture conditions.

Effect of 2iP

The obtained data in 2iP experiment concluded that, solanine percentage in the microshoots had increased significantly in response to 2iP level in the media to reach a maximum level of (3.87 mg.g^{-1} DW) at 2iP concentration of 1.6 mg·L^{-1} compared to (1.82 mg.g^{-1} DW) recorded in the control treatment (Table 3). Meanwhile, solanine in microshoots tended to decrease (3.43 mg.g^{-1} DW) at higher 2iP level (2.0 mg·L^{-1}). In callus experiment, solanine was observed to increase with increasing 2iP level to reach a maximum concentration (2.55 mg.g^{-1} DW) at 2.0 mg.L^{-1} of 2iP (Table 3). In cell suspension experiment, solanine content increased as 2iP concentration increased; 1.6 mg·L^{-1} of 2iP gave the highest solanine content (1.01 mg.g-1 DW) in cell suspension after that solanine content decreased to (0.86 mg.g^{-1} DW).

Al-Hawamdeh et al., (2013) studied the effect of 2iP on the content of silymarin compounds in *Silybum marianum* shoots and they reported that Silybin, silydanin, and silymarin content increased as 2iP concentration increased compared with the control, and the maximum percentages for both silybin (0.76%) and silydanin (0.24 %) were obtained at 1.0 mg·L^{-1} 2iP and 0.1 mg·L^{-1} NAA, while higher concentrations of 2iP (more than 1.0 mg·L^{-1}) was reported to reduce the production of all silymarin compounds. Karolak et al., (2015) explained that cytokinin significantly suppresses the transport of macronutrients such as nitrate, ammonium, sulfate and phosphate, while nitrate regulates the expression of genes involved in the phenylpropanoid and flavonoid pathways.

On the other hand, the concentrations of solanine in the control (C) treatments were lower compared to solanine concentrations found in the plant materials treated

Table 5. Effect of different light intensities on solanine % (mg.g-1) in microshoots and callus of *in vitro* grown *S. nigrum*, in addition to wild (in green house) grown *S. nigrum*.

Light Intensity (μmol/m^{-2} s^{-1})	Solanine (mg.g^{-1})
Microshoot	
W*	4.92 a^z
25	1.50 b
50 (C^y)	1.82 b
75	3.53 a
100	4.03 a
Callus	
W*	4.92 a
25	0.48 d
50 (C^y)	0.73 cd
75	0.93 bc
100	1.26 b

*W: represents mother plant from Jerash grown under ordinary light intensity (50 (μmol.m^{-2} s^{-1}) . ^{y}C: control for microshoots, was microshoots grown in MS solid media + 0.1 M sucrose under normal growth room light intensity. For callus control (C^y) represents callus grown in callus multiplication media + 0.1 M sucrose under normal growth room light intensity. ^{z} Means within columns having different letters are significantly different according to Tukey HSD at P$\leq$0.05.

with the growth regulators (2iP, BAP, kinetin) (Table 3). This could refer to the fact that, C media was designed for each explants type to be optimum for cell division and growth which would direct all plant cell resources towards cell division and primary metabolites (proteins, carbohydrates …etc) synthesis rather than production of secondary metabolites, which was very obvious in the results obtained in callus and cell suspension cultures where cell division is the predominant task for the cells (Table 3). Meanwhile, the obtained data revealed that, the highest values for solanine in this experiment were those extracted from mother plant (W) collected from Jerash.

Effect of carbohydrates sources on solanine content

Sucrose

Different levels (0.05, 0.1, 0.15 and 0.2 M) of sucrose were investigated for their impact on the solanine content of *S. nigrum* microshoots, callus and cell suspension. As sucrose level increased in the media, higher solanine in the microshoots than control (C) and the maximum solanine content (3.13 mg.g^{-1} DW) at sucrose level 0.2 M (Table 4). Similarly, solanine content in the callus cultures increased significantly with increasing sucrose level in the media and 2.0 M sucrose treatment resulted in production of the highest solanine content (2.03 mg.g^{-1} DW) (Table 4). However, solanine content
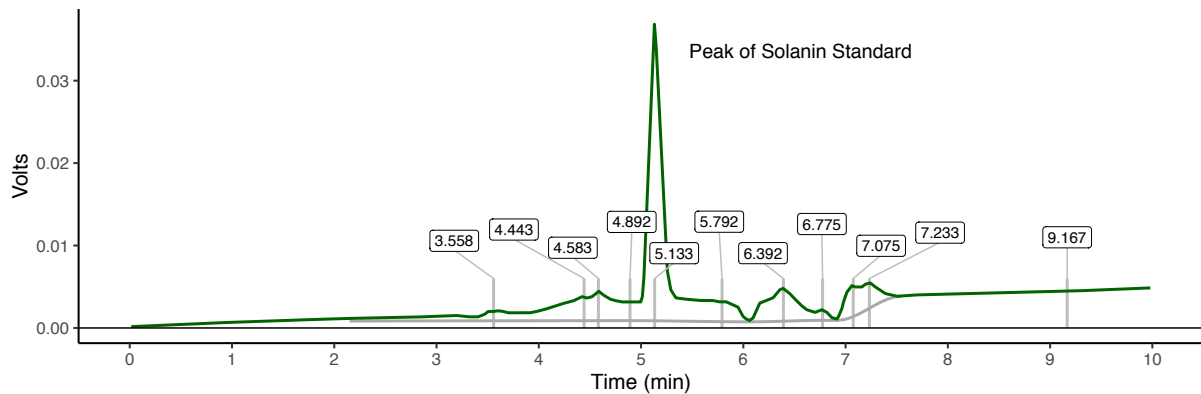
Figure 3. HPLC chromatogram for the solanine content from *in vitro* grown plantlets of *S. nigrum.*

in cell suspension culture was less than those obtained in microshhots and callus experiments, as the maximum solanine content was only (1.20 mg.g^{-1} DW) obtained at the highest sucrose level (0.2 M) (Table 4). This agrees with another research, where sucrose (60% = 0.2 M) was found to improve alkaloid content in callus culture of *Catharanthus roseus* (Ashutosh et al., 2012). Also, in cell suspension cultures of *Gymnema sylvestre*, different sugars types were tested, and sucrose was found to be the perfect carbohydrate source for biomass accumulation (11.56 g.L^{-1} DW) and gymnemic acid production (9.95 mg.g -1 DW) (Nagella et al., 2011).

High sucrose concentrations was reported as a technique for inducing osmotic stress in plants which forces plant cell to produce more electrolytes and secondary metabolites inside the cell as a defense mechanism to increase osmolarity and to decrease water loss from the cells (Shibli et al., 2006), which might explain the increase in solanine level resulted in our experiments.

Glucose

Adding elevated levels of glucose had improved solanine content in all of the experimented explants types, and 0.15 M glucose produced the highest solanine content in microshoots, callus and cell suspension culture, respectively (3.0, 1.03 and 0.56 mg.g^{-1} DW) (Table 4).

Al-Hawamdeh et al., (2013) investigated the effect of glucose on the content of silymarin compounds in *Silybum marianum* shoots and they reported that, these compounds increased as glucose concentration increased to reach the maximum at 0.1 M of glucose, while exceeding this concentration led to the reduction of secondary metabolites production.

Similarly, Wang and Weathers, (2007) investigated the effect of equal concentrations (0.1M) of carbohydrates types such as sucrose, glucose, or fructose on artemisinin production from the *in vitro* grown of *Artemisia annua,* and they concluded that a dramatic increment in the production of artemisinin in the medium

treated with glucose compared with other sugar types.

Fructose

Solanine content of *in vitro* grown *S. nigrum* was significantly and positively affected in microshoots, callus and cells suspension culture (Table 4). Maximum solanine content (2.40, 1.05 and 0.66 mg.g-1 DW) were obtained in micoshoots, callus and cell suspension; respectively, treated with 0.2 M of fructose as shown in (Table 4). This agrees with Al-Hawamdeh et al., (2013) who reported that, Silybin, silydanin, and silymarin content increased as fructose concentration increased up to 0.15 M of fructose. In general, the result obtained from our study indicated that solanine content responded positively to increasing the concentration of sucrose, glucose and fructose. However, based on current study sucrose was for the solanine production in all the tested plant materials. For all sugar types; wild collected plants from Jerash; produced the highest solanine contents.

Effect of light intensity on solanine content

Solanine content in the microshoots and callus increased significantly with increasing light intensity compared to the results obtained in C plant materials which cultured on the ordinary light intensity of 50 (μmol/m^{-2} s^{-1}) (Table 5). Beside that wild plant gave the highest content of solanin at ordinary light intensity of 50 (μmol/m^{-2} s^{-1}) . Exposing both plant materials types to light intensity of (100 μmol/m^{-2} s^{-1}) yielded the highest solanine content in shoot and callus (4.03 and 1.26 mg.g^{-1} DW, respectively) (Table 5). Meanwhile, the lowest solanine level was observed in explants exposed to the lowest light intensity treatment (25 μmol/m^{-2} s^{-1}) for both microshoots (1.50 mg.g^{-1} DW) and callus (0.48 mg.g^{-1} DW)(Table 5). This might be justified by the fact that, solanine biosynthesis in tissues is highly dependent on the presence of active chloroplast (Moreira et al., 2010). Additionally, high light intensities might resulted in building a strong stress on the cultures that might cause cell dehydration

and yet increasing cell osmotic potential as a defense response, which shifted cell mission from division and production of primary metabolites into production and accumulation of secondary metabolites (Lenore et al., 1985)

Many studies investigated the influence of light on alkaloids production, for example, Lenore et al. (1985) concluded that the light increased the alkaloids of *Heimius alicifolia* , and they explained that there was a positive correlation between chlorophyll content and lupine alkaloid formation, and suggested that the lysine branch of the biosynthesis of the *Heimia alkaloids* might also be influenced by light. Similar findings were reported by Karimi et al., (2013) as they reported that, the maximum production of flavonoids and phenolic compounds were achieved at high light intensity (630 μmol m^{-2}s^{-1}) as increasing light intensity increases primary photosynthate, which leads to an increase in phenolic concentration in the plant (Warren et al., 2003). Moreover, effects of light on the biosynthesis of vindoline and other indole alkaloids in *Catharanthus roseus* callus cultures was studied by Jian et al., (2001) and they reported that light enhance all alkaloid biosynthesis in the callus, especially vindoline and serpentine about 3–4 folds higher than that in the dark.

Conclusion

Based on the outcomes of this study, it was found that manipulating some tissue culture growth conditions (chemical and physical factors) enhanced the production of solanine in microshoots, callus and cell suspension cultures of *Solanum nigrum* L. The highest solanine level (4.52 mg.g^{-1}) DW was obtained when the plant material were treated with BAP at level of 1.6 mg·L^{-1}; this results were remarkable if compared to other previous studies on the production of solanine *in vitro* using other techniques. For example; in *Solanum lyratum* the addition of cholesterol at a concentration of 0.5 mg/l increased the synthesis of solanine (2.33 mg/g DW) compared to those extracted from the control (1.32 mg/g DW) (Lee et al., 2007) which were less than solanine level obtained in our study. Also, our data revealed that carbon source had positively affected solanine level, especially in plant material grown in media supplemented with 0.2 M sucrose. Moreover, exposing microshoots and callus to light intensity of (100 μmol/m^{-2}s^{-1}) yielded the highest solanine content compared to the other light intensities treatments. However, other culture growth conditions need to be under research for better enhancement of solanine production in *Solanum nigrum* L. *in vitro*.

Acknowledgement

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> There were no differences in the species of ticks collected on different farm animals (Bobade 2004a; 2004b)…

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> The Omani clownfish has a distribution restricted to 400 km along the Arabian Sea coast of Oman (Simpson *et al.* 2014).

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Book chapter from an edited book

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Samimi NS. 2004. Soft-corals and gorgonians of the Iranian shore of the Straight of Hormuz. Iranian Journal of Oceanography 7(2): 45-49. Farsi.

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